Phylogeography and Molecular Systematics of Species Complexes in the Genus *Genetta* (Carnivora, Viverridae)

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Abstract

The main aim of this study was to estimate phylogeographic patterns from mitochondrial DNA diversity and relate them with evolutionary structure in two species complexes of genets, *Genetta genetta* and *Genetta "rubiginosa"*, which have fluid morphological variation. Both are widely distributed in sub-Saharan Africa but whereas *G. "rubiginosa"* appears in both closed and open habitats, *G. genetta* is absent from the rainforest and occurs also in the Maghreb, southwest Europe, and Arabia. DNA sequence data, mainly acquired from museum skin samples (about 75% of the total number of samples), was analysed using methods from the fields of phylogenetics and population genetics.

The results for *G. genetta* are compatible with a scenario of allopatric fragmentation in grassland refuges during the Pleistocene climatic cycles as the main factor responsible for geographic genetic structure within Africa. The Arabian isolate showed significant genetic divergence, species-level compatible, and is probably the result of a long-distance dispersal from North Africa. Genetic diversity in Europe is a subset of that found in North Africa and shallow genetic distance is concordant with their anthropogenic introduction into Europe. North Africa seems to be cyclically connected to West and Central Africa during interglacial periods in which the Sahara recedes substantially.

For *G. "rubiginosa"*, isolated biogeographic relicts in the eastern African coast, possibly unsuspected species, were uncovered. However, the dominant pattern in the evolution of this species complex seems to be ecological differentiation in parapatry after invasion of open habitats from the rainforest ancestral habitat.

Three general conclusions may be extracted from this study. Firstly, the use of mitochondrial DNA is clearly informative, but both gene flow among parapatric populations and introgression are confounding factors. Secondly, the Pleistocene in Africa has had variable biogeographical and evolutionary consequences for different taxa, depending on their ecological breadth. Lastly, large-scale utilisation of museum samples in phylogeographies of cryptic taxa has been rarely attempted but should become a standard approach.

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Chapter 1. Introduction

1.1 Context

This thesis is the product of my personal research interests in evolution, biogeography and taxonomy, and on a molecular approach to these disciplines, applied to an available, interesting and basically unexplored animal group, the genets. It is also the natural continuation of my previous research in this cryptic taxon of mammalian carnivores and aims both to increase the knowledge about them and use them as a case study to examine questions of evolution and biogeography in Africa. It is widely accepted that morphology is unable to resolve genet taxonomy and that molecular systematics data may prove fundamental to assess putative cases of speciation and described subspecies (see Martinoli *et al.* in press, for an example of genet species identification using diagnostic genetic characters; Appendix I). On the other hand, and independently of the taxonomic questions, the genet species complexes considered in this study are, due to their continent-wide ranges, very suitable models for phylogeographic and population genetics analyses. The marker of choice is mitochondrial DNA since for several reasons it is the most appropriate molecule for addressing large-scale phylogeographic questions in unstudied organisms. Almost the whole of Africa has been, and still is, stage to the origin and diversification of the several different species that constitute this very speciose group of carnivores. Consequently, the evolution of genets has been shaped by historic events in this continent, such as climatic fluctuations, geological transformations and vegetation changes. This thesis seeks to uncover some of the possible roles that these historical processes might have played in defining the present genetic structure of two common genet taxa.

1.2 The species complexes

The viverrids (genets and civets) are considered the most generalised and primitive of living carnivores (Ewer 1973). In fact, viverrids, being at the same time conservative and versatile, are described as being to the recent carnivores what lemurs are to the higher primates (Kingdon 1997). Because all modern carnivores derived from a Miacidae stock (Colbert 1969), viverrids have a special interest for the approximation they offer to an Eocene miacid (Kingdon 1977). Although viverrids are present since the Lower Oligocene, the essentially African genus *Genetta* G. Cuvier, 1816, is only known in the fossil record from the Late Miocene onwards (Hunt 1996).

Genets are small carnivores, somewhat cat-like with retractile claws and a spotted or blotched pattern, nocturnal, semi-arboreal, and omnivorous (Ewer 1973; Taylor 1974, 1976; Waser 1980; Ikeda *et al.* 1982; Livet & Roeder 1987; Estes 1991). Genets are solitary and polygynous: adult females live and reproduce within exclusive home ranges which they defend as territories; the males have much larger ranges that include the ranges of up to several females (Carpenter 1970; Ikeda *et al.* 1983; Fuller *et al.* 1990; Palomares & Delibes 1994). Life span is about eight years in the wild, gestation about 75 days and litter size ranges from one to five offspring (Estes 1991).

Contrary to other genera of viverrids, which are either monospecific or composed of a small number of well-discriminated species, the genus *Genetta* is an assembly of numerous species distributed over all Africa with the exception of Madagascar and the Sahara desert. One of these species also occurs in southwestern Europe and in the Arabian Peninsula. As a consequence of the absence of any data other than morphological and craniometrical, together with the vast amount of geographical, ecological, and individual variation in such phenotypic characters, the systematics of this group has always been strongly debated and still remains largely unsettled (Rosevear 1974). This is true both in terms of the genus as a whole (Crawford-Cabral 1981a,b; Corbet & Hill 1986; Wozencraft 1989; Gaubert 2003b) and of the microataxonomy of certain species within (e.g. Heard & Van Rompaey 1990; Crawford-Cabral & Pacheco 1992; Van Rompaey & Colyn 1998; Crawford-Cabral & Fernandes 1999, 2001; Gaubert 2003a).

The main purpose of the present study is to investigate the molecular systematics and phylogeography of two genet species complexes, *Genetta genetta* (Linnaeus, 1758) and *Genetta rubiginosa* nec Pucheran, 1855, which are, simultaneously, the most widespread and taxonomically unresolved members of the genus. The latter, in need of a new scientific name (Crawford-Cabral & Fernandes 1999), is moreover also a member, together with *Genetta pardina* I. Geoffroy Saint-Hillaire, 1832 and *Genetta tigrina* (Schreber, 1776), of a putative superspecies, colloquially called the large-spotted genets group (Crawford-Cabral & Fernandes 2001). Nelson (1999) was followed for definitions of the taxonomic categories immediately above and below the species rank level.

In order to place this study in an evolutionary context, where for instance species boundaries may be evaluated and upon which a sampling strategy can be designed, a working taxonomic basis for the whole genus, a provisional framework for genet systematics, is needed. Such approach is the most objective possible when intending to

undertake a revision, even if partial, of the relationships within and among taxa (Mayr & Ashlock 1991).

The most recent attempt to produce a proposal for a thorough re-classification of the whole genus, the first based on morphology and molecular data, fixed provisionally as 16 the number of genet species (Gaubert *et al.* 2004; Appendix II). This research also showed that several morphological characters in genets suffer strongly of homoplasy, the multiple evolutionary occurrences of the same character that are identical in state but not by descent, due to adaptive value correlated with habitat type, preventing constant or comparable evolutionary morphological rates among lineages. Finally, the study suggested that genets are a monophyletic group, with a linsang-like ancestor adapted to tropical rainforest habitat, in which speciation was promoted by the climatic cyclic periods of the Quaternary, particularly for the most recent lineages. Nevertheless, attempts to reconstruct the details of the evolutionary and biogeographic history of the genus, or of specific lineages/radiations within, are hampered by a poor fossil record (Hendey 1974; Hunt 1996; Geraads 1997).

The putative existence of additional species and the need for confirmation of the validity of some of these 16 species are also acknowledged in the same study. Consequently, I have chosen here a provisional species list that also takes into account the most important genet classifications proposed before (Schwarz 1930; Coetzee 1977; Wenzel & Haltenorth 1972; Crawford-Cabral 1981a,b; Schlawe 1981; Wozencraft 1993) (Table 1.1).

The small-spotted genet, *G. genetta*, is recognisable by a visible dorsal hair crest, which runs from the shoulder to the base of the tail, and by a generally greyish coat with coarse long hair. The spots, comparatively small and numerous, in the coat are usually black and the tail has 9 or 10 black rings and a white tip (Fig. 1.1.A).

The populations of *G. genetta* spread out, within the African continent, across the regions outside the main deserts, in woodland-savannahs and grasslands north and south of the equator, and to those with a Mediterranean climate, either at the Cape or in northern Africa. Outside Africa, they occur in the south of the Arabian Peninsula and in southwestern Europe. The populations of genets that exist in Europe are mainly distributed through Portugal, Spain, and France, in areas of macchia and holm/cork oak, but may appear in certain countries of Central Europe, where nevertheless these animals are rare (Saint-Girons 1973; Meester *et al.* 1986).

Table 1.1 List of *Genetta* species with distribution areas (Sources: Crawford-Cabral 1969, 1970, 1973, 1981a.b; Schlawe 1980; Kingdon 1997; Gaubert 2003b).

Species name	Common name	Distribution area
Genetta victoriae Thomas, 1901	Giant genet	North of Democratic Republic of Congo and extreme west of Uganda
Genetta johnstoni Pocock, 1908	Johnston's genet	Guinea, Liberia, Ivory Coast and Ghana
Genetta thierryi Matschie, 1902	Hausa genet	From Senegal to Cameroon
Genetta abyssinica (Ruppell, 1836)	Abyssinian genet	Djibouti, Somalia and Ethiopia
Genetta piscivora (Allen, 1919)	Aquatic genet	NE Democratic Republic of Congo
Genetta cristata (Hayman, 1940)	Crested genet	South-eastern Nigeria and adjacent southern Cameroon
Genetta servalina Pucheran, 1855	Servaline genet	Gabon, Congo Republic, Democratic Republic of Congo, SW Kenya and NW Tanzania
Genetta pardina I. Geoffroy, 1832	Pardine genet	From Gambia to the Dahomey Gap
Genetta poensis Waterhouse, 1838	King genet	Liberia, Ivory Coast, Ghana, Congo Republic
Genetta bourloni Gaubert, 2003	Bourlon's genet	From Guinea to Ivory Coast
Genetta angolensis Bocage, 1882	Miombo genet	Angola, Zambia and south of Democratic Republic of Congo
Genetta mossambica Matschie, 1902	Mozambique genet	Northern Mozambique and southern Tanzania
Genetta tigrina (Schreber, 1776)	Blotched genet	Coastal areas of Cape province and Pondoland, South Africa
Genetta genetta (Linnaeus, 1758)	Small-spotted genet	South-western Europe, south of Arabian Peninsula, Maghreb and sub-Saharan Africa
Genetta "rubiginosa" nec Pucheran, 1855	Rusty-spotted genet	From the Dahomey Gap to the Horn of Africa and to the Natal Province in South Africa

So, the range is presently discontinuous, consisting of five to six isolates or regional groups depending on the uncertain distribution continuity from West to East Africa and from East to South Africa (Crawford-Cabral 1981b; Kingdon 1997) (Fig. 1.2).

The details of how this complex should be taxonomically regarded, both at specific and subspecific levels, is still presently unresolved after a history of strong debate. Schwarz (1930) and Wenzel & Haltenorth (1972) defended the subdivision, which has become traditional, of the complex into three groups of subspecies.

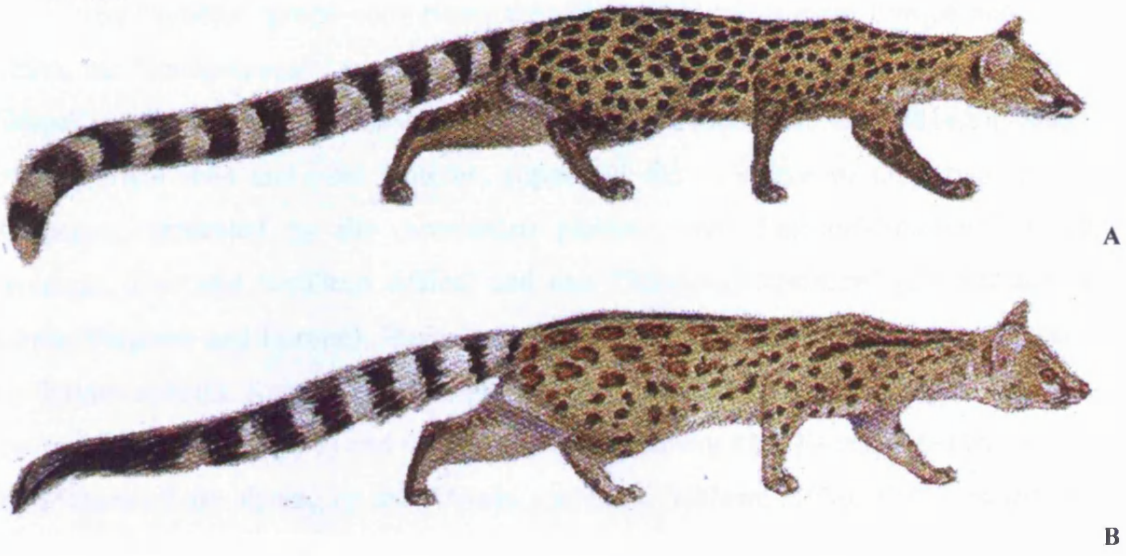

Figure 1.1 *Genetta genetta* (Linnaeus, 1758) (**A**) and *Genetta rubiginosa* nec Pucheran, 1855 (**B**).

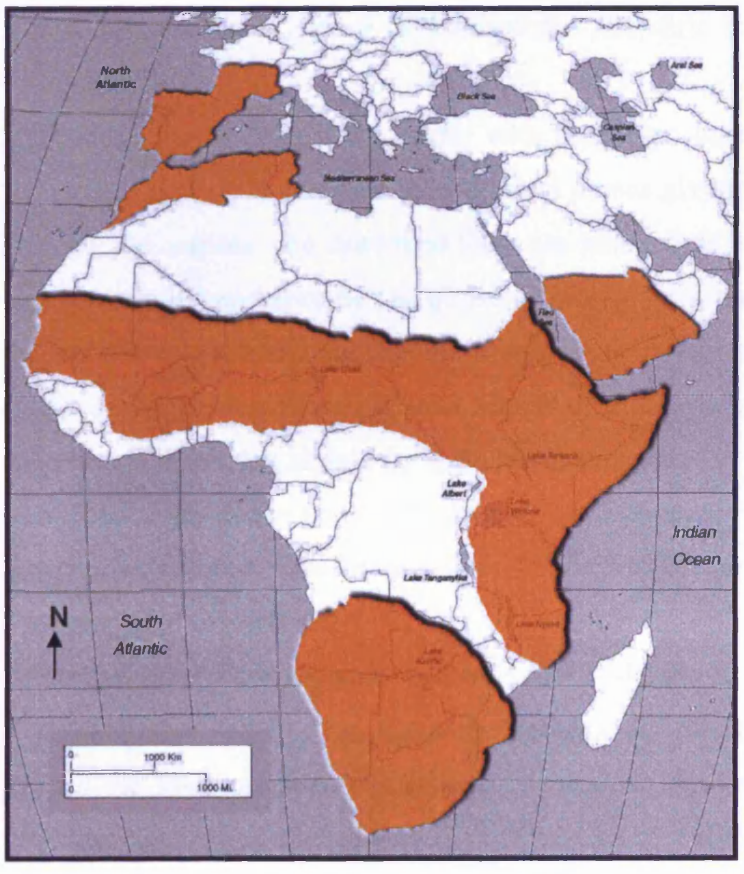

Figure 1.2 Map showing the approximate distribution area of *G. genetta*. The exact shape and extent of the range is not very well known for the Sahel region, Sudan, and the Tanzania-Zambia-Mozambique triangle.

The "genetta" group comprising the populations of southern Europe and northern Africa, the "senegalensis" group ranging coast to coast along a sub-Saharan strip, and the "felina" group for the Southern African forms. Crawford-Cabral (1981a,b), based on craniometrical data and coat patterns, supported the existence of only two groups of subspecies, separated by the Abyssinian plateau: one "East-and-Southern" (Arabian Peninsula, East and Southern Africa) and one "West-and-Northern" (Central and West Africa, Maghreb and Europe). However, other authors saw this complex as more than just a polytypic species. Rosevear (1974) proposed a set of three species - *G. genetta*, *Genetta senegalensis* (Fischer 1829) and *Genetta felina* (Thunberg 1811) – separated by vast areas, from where all are absent, on the African continent. Schlawe (1980; 1981) argued for the existence of only two species, *G. genetta* and *G. felina*, and included in the latter the forms usually placed in the "senegalensis" group, a classification followed by Harrison & Bates (1991). The most recent study (Gaubert *et al.* 2004), using cytochrome *b* sequences and morphology, hypothesises *G. felina* as a valid species, but restricted to the west coast of Southern Africa, and the putative existence of unsuspected allopatric species within the remaining *G. genetta* complex.

Table 1.2 lists the forms described to date, with their type localities, which are presently included in the *G. genetta* complex. The original names given to the specimens or local populations by the authors who described them are retained. It is clear that many of them may represent only infrasubspecific categories or individual variation, products of the species typological view, and, consequently, mere synonyms. In any case, such a list is useful as a guide for a comprehensive and correct sampling strategy, for instance in the choice of the geographical areas that should be included in the analysis. Although such a list does not need to follow any of the proposed schemes, the traditional nomenclature and subdivision of the species complex into three groups is adopted because it corresponds roughly to a real geographical disjunction.

It is now widely accepted that the small-spotted genet – like the Algerian hedgehog, *Atelerix algirus* (Lereboullet, 1842), the Egyptian mongoose, *Herpestes ichneumon* (Linnaeus, 1758), and the Mediterranean chameleon, *Chamaeleo chamaeleon* (Linnaeus, 1758) – was introduced in Europe by humans (Crawford-Cabral 1981b; Dobson 1998; Paulo *et al.* 2002a).

Table 1.2 List of the morphological and/or geographical forms presently included in the *G. genetta* complex and respective type localities (Source: Crawford-Cabral 1981b).

Geographic group	Morphological and/or geographical form	Typical locality/area
"Genetta"	*Viverra genetta* Linnaeus, 1758	Spain
	Genetta afra F. Cuvier, 1825	Morocco
	Viverra genetta var. *barbar* Wagner, 1841	Algeria
	Genetta Bonaparti (sic) Loche, 1857	Algeria
	Genetta melas Graells, 1897	Serra Morena, Spain
	Genetta rhodanica Matschie, 1902	France
	Genetta terraesanctae Neumann, 1902	Mt. Carmelo, Palestine (?)
	Genetta genetta balearica Thomas, 1902	Inca, Majorca, Balearics
	Genetta peninsulae Cabrera, 1905	El Pardo, Madrid, Spain
	Genetta genetta lusitanica Seabra, 1924	Ribeira do Papel, Portugal
	Genetta genetta pyrenaica Bourdelle & Dezillière, 1951	Pyreneans, France
	Genetta genetta isabelae Delibes, 1977	Ibiza, Spain
"Senegalensis"	*Viverra senegalensis* Fisher, 1829	Senegal
	Viverra dongolana Hemprich & Ehrenberg, 1832	Dongola, Sudan
	Genetta neumanni Matschie, 1902	Irangi, Kondoa, Tanzania
	Genetta hararensis Neumann, 1902	Harar, Ethiopia
	Genetta guardafuensis Neumann, 1902	Cape Guardafui, Somalia
	Genetta Grantii (sic) Thomas, 1902	Azraki, Haushabi, Yemen
	Genetta dongolana tedescoi de Beaux, 1924	Tigiglo, Somalia
"Felina"	*Viverra felina* Thunberg, 1811	Cape of Good Hope, South Africa
	Genetta pulchra Matschie, 1902	Cubango, Angola
	Genetta bella Matschie, 1902	Luanda, Angola
	Genetta ludia Thomas & Schwann, 1906	Klein Letaba, South Africa

The Strait of Gibraltar, although only 14 Km wide at its narrowest point, exceeds 200m in depth and represented an effective marine barrier, at least for non-flying terrestrial mammals, during all sea level fluctuations since the early Pliocene (Thomas *et al.* 1982; Schule 1993). Schauenberg (1966) proposed the passage of genets across a temporary land bridge connecting North Africa and Iberia, during the Messinian Salinity Crisis (Hsu *et al.* 1973) and before the opening of the Gibraltar Strait, but such an early invasion of Southern Europe is incompatible with the palaeontological record. Genet fossils are only known

from the Late Pliocene onwards in North Africa (Geraads 1997) and absent from the European record before the Holocene (Saint-Girons 1973; Morales 1994).

The entry of genets into Europe is more likely a case of anthropogenic colonisation whereby they, as pets and/or domestic rat-catchers in a role nowadays carried out by the domestic cat, were brought from Africa less than 2,000 years ago. Genets were kept by the ancient Egyptians and are still kept by the Berbers (Kingdon 1977, 1997) and, most likely, their arrival is contemporary with the Arab invasions (Perez & Gutierrez 1990). Their absence in Italy and Greece is another argument in favour of an introduction of Arabic origin (Livet & Roeder 1987). Although it is established that the origin of the European populations traces back to northern Africa (Crawford-Cabral 1981b), the precise geographic location(s) of the founder stock(s) remains to be determined. Fossils of *G. genetta* have been found in East Africa from the Early Pleistocene and in North Africa from the Late Pleistocene (Lariviere & Calzada 2001).

Concerning the populations in the Arabian Peninsula, the available information is more scarce and there is no certainty about when the genets arrived to the area, putative invasion routes, and if they were introduced by humans or not. The available data suggests that the *G. genetta* lineage arose in Central-East Africa (Crawford-Cabral 1981b) around three to four million yr BP (Gaubert *et al.* 2004).

The separation of the Arabian Peninsula from Africa, which started the formation of the Red Sea, began in the Late Oligocene and was followed by seafloor spreading during the Miocene (Girdler & Styles 1974; Bonatti 1985; Bohannon *et al.* 1989). At the onset of the Pliocene the Red Sea basin was already a wide and deep marine barrier to the dispersal of terrestrial vertebrates (Bohannon 1986). There is evidence that at least for the last 500,000 years, even at the most severe glacial maxima, no landbridges existed across the Red Sea (Rohling *et al.* 1998). Furthermore, considering the absence of evaporites younger than ten million years old, connections by land between the Horn of Africa and Arabia were probably not present after the Late Miocene (Rohling *pers. comm.*). This seems to indicate that during the Pleistocene, migration through the Red Sea would have been a likely scenario mainly for human dispersal. Although nowadays genets, in what concerns their eastern African range, have their northern distribution limit in Sudan with occasional records for southern Egypt, this was probably not the case in the past. There are several reported warm and wet climate episodes for the last two million years in Africa associated with apparently significant contractions of desert regions (deMenocal 1995; deMenocal *pers. comm.*). For some of the climate optima it is known, for instance, that

vegetation covered large areas of the Sahara and steppe, now typical of some regions in East Africa, may have spread up to the Middle East (Jolly *et al.* 1998). An invasion of Asia across the corridor provided by the Sinai region during one or more of these periods, followed by range contraction to the limits we see today, in response to the return of cooler and drier conditions, would explain the present disjunct distribution. One of the results of this study may be a suggestion to how and when genets colonised the southern Arabian Peninsula.

The rusty-spotted genet, *G. "rubiginosa"*, has a shorter dorsal crest and fur than *G. genetta*, larger and less numerous spots, usually of a rusty colour, and a dark-tipped tail with 8 or 9 rings (Fig. 1.1.B). They display individual, ecological, and geographical variation in external morphology without parallel in any other genet species (Roberts 1951; Coetzee 1977; Crawford-Cabral & Fernandes 2001; Gaubert 2003a). Small and rusty-spotted genets are sympatric in many areas without any sign of interbreeding (Smithers 1971) and they are ecologically distinct: the first is a typical inhabitant of the dryer bush and woodland and tolerates severe dry seasons, occurring in escarpments, rocky outcrops and other hills, while the second is more associated with mesic habitats (Kingdon 1977, 1997). Hybridisation seems to occur however between the closely related *G. "rubiginosa"* and *G. tigrina* in South Africa, as indicated by a recent study (Gaubert *et al.* in press; Appendix III) which detected a narrow hybrid zone at the border between the Natal and Eastern Cape provinces, in contrast to the suggestion by Pringle (1977) of a broad hybrid zone. Further elucidation of this and as well of other suggested instances of hybridisation in Southern Africa, between genet species for which reproduction in captivity has been already observed (Gray 1971), are also potential outcomes of the present study.

G. "rubiginosa" is the classical name for a very polymorphic complex of forms, distributed from the Dahomey Gap eastwards to the Abyssinian plateau and southwards down to the Natal Province in South Africa (Fig. 1.3), commonly called rusty-spotted genets (Roberts 1951). Schwarz (1930) regarded them as subspecies of *G. tigrina*, the blotched genet, a classification followed by several authors (Ansell 1960; Wenzel & Haltenorth 1972; Coetzee 1977; Kingdon 1977; Smithers 1983; Meester *et al.* 1986). In contrast, they were considered conspecific with *G. pardina*, the pardine genet, by Schlawe (1980, 1981) and Wozencraft (1993). Finally, some other authors (Hill & Carter 1941; Ellerman *et al.* 1953; Crawford-Cabral 1966, 1981b; Ansell & Dowsett 1988) deemed the rusty-spotted genets as distinct from both these sister species and constituting a taxon on

their own to which the name *Genetta rubiginosa* Pucheran, 1855 was given (Roberts 1951).

The separation of the large-spotted genets group in at least three valid species (*G. tigrina*, *G. pardina*, and the rusty-spotted genets) is now apparently established (Crawford-Cabral 1981b; Crawford-Cabral & Pacheco 1992; Gaubert 2003b). Although the status of the rusty-spotted genets as a monophyletic clade seems undisputed, their microtaxonomy is very unclear, since they are polytypic through a vast range of heterogeneous habitats separated by several types of physical barriers across Africa (Crawford-Cabral & Fernandes 2001).

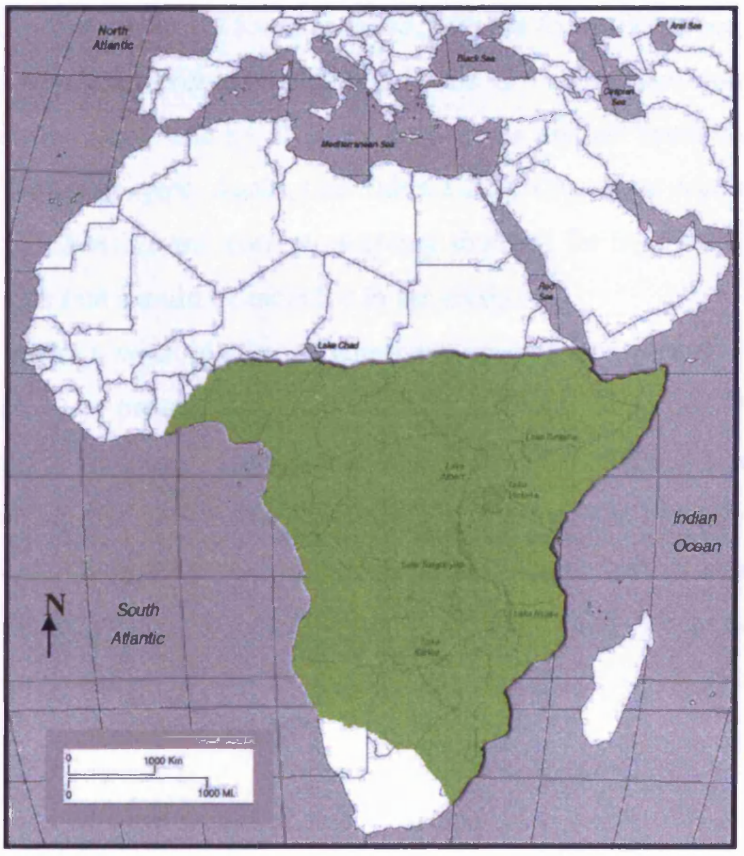

Figure 1.3 Map showing the approximate distribution area of *G.* "*rubiginosa*". The exact shape and extent of the range is not very well known for the Sahel region, Sudan, and the Dahomey Gap.

An additional issue, originally noticed by Schlawe (1980), in relation to the rusty-spotted genets is the fact that they need a new scientific name since the type specimen of *G. rubiginosa* Pucheran, 1855, is an individual of a completely different species, *Genetta thierryi* Matschie, 1902. For this reason, *G.* "*rubiginosa*" or *G. rubiginosa* nec Pucheran, 1855 are used here to designate the rusty-spotted genets, underlining the problem with their current scientific name. There is, at the moment, a rising controversy about the scientific

name that should be given to this taxon (Gaubert *et al.* 2003a,b; Grubb in press). However, it is evident that any decision over this issue is premature before determining if the rusty-spotted genets are a single species or a set of sibling cryptic species (Fernandes & Crawford-Cabral in press; Appendix IV). The present study aims to produce molecular data, to join the vast amount of morphological information already available, relevant to the issue of putative speciation within the rusty-spotted genets and hence to contribute to a sounder decision on this taxonomic and nomenclatural problem. Crawford-Cabral & Fernandes (1999) proposed that if the rusty-spotted genets are a single entity, then the valid name should be the oldest available synonym of *rubiginosa*, *Genetta fieldiana* Du Chaillu, 1860.

Table 1.3, like Table 1.2 for *G. genetta*, lists the forms described to date, with their type localities, which are presently included in the *G.* "*rubiginosa*" complex. They were regarded at different times and by different authors, as distinct species, subspecies, races, ecotypes, or just as synonyms. Again, like Table 1.2 for *G. genetta*, such a list is useful as a guide for a comprehensive and correct sampling strategy, for instance in the choice of the geographical areas that should be included in the analysis.

When dealing with highly polymorphic species complexes, in which putative cryptic species may be present, sampling intensity is clearly important (Funk & Omland in press). For a given sampling effort, to include all species believed a priori to be closely related, maximising the geographic diversity and the number of samples from areas of sympatry between study taxa, and to sample broadly from known sources of biological variation (subspecies, ecotypes, morphotypes) is recommended (Wiens & Servedio 2000).

Table 1.3 List of the morphological and/or geographical forms presently included in the *G. "rubiginosa"* complex and respective type localities (Source: Crawford-Cabral 1981b).

Morphological and/or geographical form	Typical locality/area
Genetta rubiginosa Pucheran, 1855	Cape, South Africa
Genetta fieldiana Du Chaillu, 1860	Central Gabon
Genetta aequatorialis Heuglin, 1866	Bahr-el-Abiad, Sudan
Genetta schraderi Matschie, 1902	Massaua, Eritrea
Genetta zambesiana Matschie, 1902	Boror, Mozambique
Genetta gleimi Matschie, 1902	Luanda, Angola
Genetta stuhlmanni Matschie, 1902	Bukoga and Mengo, Uganda
Genetta suahelica Matschie, 1902	Tanga, Tanzania
Genetta erlangeri Matschie, 1902	Kitui, Kenya
Genetta matschiei Neumann, 1902	Harar, Ethiopia
Genetta letabae Thomas & Schwann, 1906	Klein Letabae, South Africa
Genetta pumila Hollister, 1916	Mt. Gargues, Kenya
Genetta insularis Cabrera, 1921	Rebola, Fernao do Po (Bioko)
Genetta rubiginosa zuluensis Roberts, 1924	Umfolozi, South Africa
Genetta tigrina soror Schwarz, 1929	Mt. Kundilungu, SW Lake Mweru, Democratic Republic of Congo
Genetta rubiginosa albiventris Roberts, 1932	Maun, Botswana
Genetta deorum Funaiolo & Simonetta, 1959	Duco degli Abruzzi, Somalia
Genetta pardina schoutedeni Crawford-Cabral, 1970	Bokungu, Democratic Republic of Congo

1.3 Species concepts, speciation and genetic markers

The "species problem" is the persistent biological and philosophical debate on the meaning of the word "species" and the methods of species identification (Mallet 1995). Hey (2001) proposed that since it seems a consensus about species definitions is unlikely in the near future, authors should always state which species concept(s) they are adopting.

In deference to the above, I present in this section an overview of the main species concepts proposed so far and I refer the one(s) I will consider and apply in this work, the reasons for such a choice, and their relation to the use of molecular markers.

Species have long been assumed as representing a distinct level of biological organisation with a unique role in evolution (Dobzhansky 1951; Mayr 1963; Hull 1976) but this view has been often questioned in more recent times (Mishler & Brandon 1987; Nelson 1989; Pleijel & Rouse 1999).

Although above-species taxon categories lack objective recognition criteria (de Queiroz & Gauthier 1992), the Biological Species Concept (BSC) (Mayr 1963, 2000) states that for the attribution of specific rank to a taxon there is a biologically meaningful, comparable, and objective test, based on the phenomenon of interbreeding. This measure is usually associated with the BSC but is also part of other reproduction-based concepts (Ghiselin 1974; Paterson 1985; Templeton 1989). The interbreeding criterion is the only one, among several other methods of species determination associated with alternative species concepts, that aims to be solely based on the observation of a biological reality (reproductive isolation or not) instead of on the subjective delimitation of the meaning of taxonomic measures (Coyne 1994). Moreover, it intends to provide ontological validity to the functional individuality of species, being the largest biological entities exhibiting reproductive isolation of its members (Baum 1998).

Over time, objections to the merits of the BSC have arisen and many other alternative species concepts have been proposed, in general associated with specific criticisms to the BSC and aiming to overcome its apparent problems (e.g. Mayden 1997). However, all of them also have weaknesses of one kind or another, as revealed sometimes blatantly by defenders of interbreeding concepts (Noor 2002). For instance, Lee (2003) describes similarity concepts (e.g. Sokal & Crovello 1970; Nixon & Wheeler 1990) as missing objective criteria to relate species recognition with divergence levels or to define universal methods allowing detection of a species-diagnostic "unique combination of character states" (Wheeler & Platnick 2000).

Concepts such as the ecological (Van Valen 1976), cohesion (Templeton 1989), evolutionary (ESC: Simpson 1961; Wiley & Mayden 2000) and genotypic cluster (Mallet 1995), regard species as a group of organisms sharing features due to different cohesion mechanisms (e.g. common ecological niche; common evolutionary fate; common selective regime). If cohesion is because of gene flow (Templeton 1989), the concept becomes close to the BSC in meaning and consequences for species definition. However, if cohesion does not reflect reproductive continuity then both types of concepts diverge significantly. The problem is that, under strong divergent or disruptive selection, the individual demes of a structured population may each exhibit differentiating cohesion from the others but still be all connected by gene flow. On the other hand, under strong stabilising selection, isolated lineages will loose cohesion only after an appreciable length of time. This leads to the absence of an objective criterion to assign species status to a certain level of shared cohesion (Lee 2003).

Monophyly concepts (e.g. Mishler & Theriot 2000) only accept monophyletic clades as species. This becomes problematic in cases of reticulate relationships and mixed ancestry, which are frequent among populations (Avise 1994). Recognition of species-level monophyly is based on features such as the number of synapomorphies, geographical distribution, and geological age, the problem with these being that they are also used to determine above-species clades (Hudson & Coyne 2002). Indeed, as acknowledged by Pleijel & Rouse (1999), there are no biologically objective criteria associated with the monophyly concept of how to rank clades at the species level.

The school of phylogenetic systematics has produced several alternative and independent species concepts (reviewed in Baum & Donoghue 1995 and Wheeler & Meier 2000). For some, the species is the smallest unit suitable for phylogenetic analysis (Nixon & Wheeler 1990; Brower *et al.* 1996) but this is criticised by authors arguing that within-species hierarchical patterns are still approachable with phylogenetic methods (de Queiroz & Donoghue 1990b; Shaw 2001). Common to all the different phylogenetic species concepts (PSCs) is the attempt to identify species as the irreducible biological entities that are diagnosable and/or monophyletic (Mayden 1997). The diagnosable versions of the PSC (Cracraft 1989; Wheeler & Platnick 2000) are criticised both in terms of not relating a unique biological property with the species category and of ambiguity in rank assignment, as referred earlier. Pure monophyly versions (de Queiroz & Donoghue 1988; Mishler & Theriot 2000) or those with an added diagnosable component (McKitrick & Zink 1988) face similar objections. The main positive aspect of the PSC is clearly its operationality for

the discovery of biodiversity. The additional strengths of being a lineage concept without assumed models of selection or speciation behind it are also significant. The PSC is empirically useful in identifying recently diverged or otherwise cryptic species and provides a framework for the use of phylogenetic methods to complement population genetics tools in the study of populations and species (Goldstein & DeSalle 2003). Because it has associated with it procedures that are operational, testable and powerful, such as parsimony and population aggregation analysis, the phylogenetic approach has been used, for instance, in the detection of relevant populations for conservation (Vogler & DeSalle 1994; Goldstein *et al.* 2000). The recognition however that phylogenetic species may not represent equivalent evolutionary units or reproductively stable and cohesive lineages has led some of its recent proponents to redefine the PSC basically as an operational standard (Goldstein & DeSalle 2000).

The evaluation of evolutionary significant units (ESUs; Ryder 1986) by using proxies of the interbreeding criterion (O'Brien & Mayr 1991) or phenetic distances of genetic data (Dizon *et al.* 1992) has difficulties in terms of applicability and accuracy, respectively. Moritz (1994a) suggested that ESUs could be identified as groups whose mitochondrial DNA (mtDNA) lineages were reciprocally monophyletic and with significant divergence in allele frequencies for nuclear loci, and coined the term "management unit" (MU) for populations currently demographically independent. Criticisms to these operational definitions include the facts that they only apply when the populations under study are subdivided, that mtDNA reciprocal monophyly may not be attained between otherwise reckoned ESUs if the time of the split is recent, and that the criterion for MU recognition is vague (Paetkau 1999). More recently, Crandall *et al.* (2000) even defended a "return to the past" (e.g. Ryder 1986) by arguing that ESUs should be classified taking into account both ecological data and genetic variation, not only neutral but also with adaptive significance. They propose a new criterion based on concepts of ecological and genetic exchangeability related with Templeton's cohesion view on population differentiation (e.g. Templeton *et al.* 1995). Updates on the status of the ESU debate and comprehensive reviews can be found in Fraser & Bernatchez (2001) and Moritz (2002).

Interbreeding concepts have in common the identification of species as the largest biological entities undergoing regular or significant genetic recombination. The BSC emphasises overall reproductive isolation, the recognition concept (Paterson, 1985) the shared mate recognition systems within a species, the reproductive competition concept

(Ghiselin 1974) the common reproductive resources within a species, and the genic view (Wu 2001) the existence of adaptive genes linked pleiotropically to reproductive incompatibility. These concepts are all designed to delimit species at a given point in time. The cladistic species concept (Meier & Willman 2000) also uses interbreeding ("tokogenesis"), defining species as the branches between successive cladogenetic events (i.e. successive origins of reproductive isolation), but is largely a diachronic concept for delimiting lineages through time.

The reconciliation between contemporaneous concepts, like the BSC, and historical viewpoints, such as the PSC and the ESC, is possible if we consider them both as applicable in different contexts and inter-convertible (Endler 1989; Avise 2000a). Species are the upper-most limit to involve tokogenetic relationships and the lower-most level participating in phylogenetic relationships (Mayden 1997) and the phylogeographic approach bridges the gap between gene trees and species trees (Avise & Wollenberg 1997).

Similar to other criteria (similarity, monophyletic inclusiveness) for the recognition of species rank, interbreeding may also be a matter of degree, in this case between panmixia and total isolation. But an important difference is that interbreeding tends to be the least clinal of all the criteria and the hazy borderline of partial and irregular gene flow (the "species boundary") can be at least restricted to the particular regions in the phylogenies in which the relationships change from hierarchical to reticulate (Lee 2003). Consequently, interbreeding concepts are often the least ambiguous in determining species-level clades and, hence, the most successful in avoiding futile "splitting" versus "lumping" debates.

Since interbreeding concepts apparently have their strengths in terms of ontology and objectivity, the arguments against them have been generally on empirical grounds. The argument that interbreeding is gradual and then species boundaries based on it are fuzzy does not hold, as described above, as all other criteria are generally worse in this regard. The objection that interbreeding needs to be almost always inferred from phenotypic or genetic traits is also not very relevant. Cohesion and monophyly are also diagnosed by character distributions (Lee 2003). For instance, allopatric populations have been regarded as a problem for reproductive species concepts (Cracraft 1989; Mallet 1995) because of the difficulty in determining whether they would viably cross if brought into contact. However, even if a direct test of reproductive isolation is unfeasible or inconclusive, as it is often the case, the need of additional criteria for species diagnosis does not imply that the concept is non-operational; other traits are proxies for the variable that matters, gene flow.

Furthermore, the evaluation of natural allopatric populations as being isolated may be sometimes misleading since it depends on the time scale we consider and how it compares with speciation times in the species in question (Losos & Glor 2003).

Another criticism of interbreeding concepts is the fact that a rigid application would recognise many cases of overly "large" or "small" species, while overturning traditional taxonomies in the process (Wheeler & Meier 2000). Many highly distinct plant species show some levels of interbreeding whereas in some invertebrates gene flow may be so narrow as to promote sympatric radiations of geographically very restricted species (Dowling & Secor 1997). However, these cases may be regarded, even if resulting in new and counterintuitive taxonomies, as illuminating differences in biological patterns between higher levels of biological organisation. The invaluable gain of the consistent application of a single criterion is that species are then truly equivalent and comparable, and differences in diversity will reflect specific biological processes. If a pluralistic approach (Mishler & Donoghue 1982) is adopted, the recognised species will not be comparable, since they are delimited with different criteria, and differences between species may not be biological but due to artefacts derived from the usage of different concepts. Then, a more monistic view of species that only uses concepts directly involving gene flow saves the individuality of the species category (Lee 2003).

The best compromise seems to be the recognition that the BSC is the most comprehensive concept for sexual animal populations and supports testable hypotheses, but even then has limitations, some of them irresolvable (Avise & Ball 1990; Barton 2001). Under a monistic interbreeding concept, self-fertilising hermaphrodites, unisexual, and asexual organisms do not constitute true species but simply clades (Ghiselin 1987). It has practical difficulties in delimiting introgression from gene flow, in the diagnosis of allopatric species, and in dealing with "incipient" species (Turner 1996) or races (Turelli *et al.* 2001). The apparently impossible universal use of the BSC (or of any other species concept) has led Hey (1997) to propose the "genetic" concept, where the criterion for the recognition of evolutionary units is the sharing of genetic drift, which makes this concept analytically very powerful, but these units may not necessarily be species. It is operationally a "cohesion" concept, very similar to the one of Templeton (1989), but it is also a reduced view of the species category, a negative solution to the "species problem".

When reconcilable, the joint consideration of species concepts, by providing complementary views and tools, may be decisive for species recognition (Hoelzer & Melnick 1994; Turner 1996). Here, a reconciliation of two "gene flow" concepts, the BSC

and the cohesion concept (Templeton 1989, 2001), with the PSC, as an operational criterion, is followed in order to justify the use of both population genetics and phylogenetics methods in a study of microtaxonomy. Species complexes lie in an evolutionary interface that is well investigated with the dual use of population genetics and phylogenetics (e.g. Wyner *et al.* 1999) and the BSC and the PSC, although with a history of debate, should be jointly considered when tackling speciation analyses (Cracraft 1997; Avise 2000b).

The birth of the cohesion species concept is essentially contemporaneous to the appearance of phylogeography (Avise *et al.* 1987), the study of the geographic distributions of genealogical lineages within and among closely related species. They share many theoretical and analytical bridges since both rely on gene trees and networks (Templeton *et al.* 1992; Templeton & Sing 1993; Posada *et al.* 2000), gene flow estimates (Slatkin 1985a; Slatkin & Barton 1989), and genealogical tools like the coalescent (Felsenstein 1971; Tavare 1984; Hudson 1990; Kingman 2000). The cohesion concept accommodates better than the BSC cases of complicated population structure (e.g. in which a hierarchy of multiple nested genetic drift patterns is present), hybridisation/introgression, and isolation by distance (Wright 1943). Marquez *et al.* (2002) is a good example of when the cohesion approach together with the phylogeographic framework can be very powerful for species recognition; see Templeton (2001) for a review of other cases.

A recent review of speciation studies by Ferguson (2002) underlined that measures of gene flow and population differentiation (e.g. Excoffier *et al.* 1992; Raymond & Rousset 1995) are potentially informative. In contrast, it is known that genetic distances on their own are not good indicators of speciation (Meyer *et al.* 1990; Moran & Kornfield 1993) unless the phenetic clustering matches other methods of phylogenetic reconstruction (e.g. maximum parsimony or maximum likelihood) as described in Bradley & Baker (2001).

The ability of molecular phylogenetics tools in recovering at least the same microtaxonomic discontinuities as traditional morphological appraisals has been demonstrated (Hillis *et al.* 1996). Graur & Li (2000) present several reasons why molecular data, particularly from DNA sequences, may be much more suitable for evolutionary studies than morphological data, by being strictly heritable, unambiguous, homologous, and more amenable to quantitative treatments. Regardless of this potentially controversial view, it is becoming evident that molecular markers are probably the only

possibility for solving taxonomic issues related with cryptic species, sibling species and species complexes (e.g. Zink *et al.* 2002; Kai *et al.* 2002; Starkey *et al.* 2003).

Even if natural selection can confuse a historical legacy, molecular phylogenetics is still often a relevant contribution for the understanding of geographical variation in morphology (Gubitz *et al.* 2000). It provides an independently derived hypothesis against which it is possible to assess the relative importance of, for instance, selection and divergence in allopatry as causes of geographic variation in morphology (Malhotra *et al.* 1996; Puorto *et al.* 2001). Concordant patterns of morphological and molecular variation are usually taken as providing support for a historical cause (e.g. Pestano & Brown 1999), while discordance in these, together with closer association between morphological variation and ecological patterns, may indicate clines mediated by natural selection (Thorpe *et al.* 1996). Cryptic species might reflect both the retention of ancestral morphology, or convergent evolution due to similar selective pressures, and the dangers of using selected traits in systematics (Richmond & Reeder 2002).

Avise & Wollenberg (1997) demonstrated how lineage sorting patterns in gene trees, the evolutionary reconstruction of the genealogical history of the genetic variation found in a sample of homologous genes, can be particularly informative with respect to speciation mode. Different gene trees are expected to have variable depths and branching patterns, simply in consequence of stochastic lineage sorting (Nei 1987; Hudson 1990). Differences between loci can anyway be informative concerning the demography and structure of past populations (Takahata *et al.* 1995). Lineage sorting rates for neutral markers (Kimura 1983) are mainly influenced by demographic factors such as geographical structure and effective population size (N_e; Wright 1931). For any non-ideal population (i.e. departing from the Wright-Fisher model for populations), N_e is the size that an ideal population would be that behaved demographically and genetically in the same way (Neigel 1996). Real populations depart from the ideal model due to several causes, such as, for instance, unequal sex ratios, overlapping generations, differential reproductive success, population subdivision and changes in size through time (Hartl & Clark 1989).

When speciation involves two large geographic populations most gene trees will initially appear polyphyletic in the new sister species. Then, through time, lineage sorting converts these gene trees first in paraphyletic and finally in reciprocally monophyletic, "exclusive" for each species (de Queiroz & Donoghue 1988; Shaw 2001). If speciation involves a small population isolated from a larger ancestral stock (e.g. founder effect) the polyphyletic phase is usually absent (Avise 2000a). In the case of peripatric speciation the

peripheral isolate will possess a restricted subset of parental alleles and loose lineages under drift at a faster rate than the parental population; this might be phylogenetically reflected in a monophyletic daughter species diverging from a paraphyletic parental clade (Olmstead 1995). Indeed, when one or more infraspecific units within a nominal species prove to be monophyletic, a substantial history of genetic isolation is indicated, but if the clades are geographically separated, to infer reproductive isolation and specific status from such evidence is nevertheless problematic (e.g. Riddle *et al.* 2000).

Although allopatry and founder effects associated with clear gene flow interruption are the intuitive and traditional scenarios for speciation, it is now well established that differentiation of the same level may occur in sympatry and parapatry (Bush 1975; Barton & Charlesworth 1984; Bush 1994; Gavrilets *et al.* 2000; Via 2001). Allopatric speciation requires only geographical isolation and time, although it can be accelerated by divergent selection (Schluter 2001), and if driven by drift there must be no opposition from selection (Barton 1996). Parapatric speciation requires some degree of isolation by distance, which depends on the strength of selection during population divergence, regardless if driven by drift or selection (Slatkin 1973). If selection is strong this is reflected in narrow clines and hybrid zones; if divergence is "quasi-neutral" then a broader cline might be involved (Turelli *et al.* 2001). Divergence across a narrow hybrid zone can be slowed by gene flow and resulting introgressions may confound genealogical assessments based on single genes (Harrison 1989). Sympatric speciation is driven by disruptive selection (selection against intermediate phenotypes), in the form of ecological competition, sexual selection or differential habitat adaptation (Via 2001), and may involve reproductive character displacement (Van Doorn 1998; Noor 1999). However, reproductive character displacement by reinforcement of premating isolation is usually more associated with secondary contact of incipient species after some divergence in allopatry (Butlin 1995).

In cases of recent speciation, where monophyly fixation is not yet reached, paraphyletic gene trees are expected and this has been confirmed empirically (Talbot & Shields 1996; Sullivan *et al.* 1997). However, attention needs to be paid to the fact that introgression through hybridisation can cause exactly the same pattern (Avise 2000a). Introgression yields polyphyly by introducing phylogenetically divergent allelic lineages across species boundaries (Shaw 2002). Closely related taxa may also exhibit homoplasic similarity in gene trees due, for instance, to stabilising selection, retention of ancestral polymorphisms, and extreme rate heterogeneity among lineages (Patterson 1988; Wu 1991; Avise 1994). The pattern of genetic drift is indeed significantly affected by selection

if the markers are in linkage with functional DNA sequences (Hudson & Kaplan 1988). Divergent selection pressures may accelerate lineage sorting and balancing selection can slow down the process relative to neutrality expectations (Takahata 1990; Charlesworth *et al.* 1993; Clark 1997). For example, separated demes may seem connected by abundant gene flow through the analysis of selected markers but further analysis may demonstrate the real structure and unmask the effect of balancing selection on population patterns of selected genes (Neigel 1997). Also, if the chosen marker evolves too slowly (or if too small a gene fragment is analysed) or too fast relative to the speciation rate, this may result on weak phylogenetic signal and paraphyly/polyphyly as artefact of phylogenetic reconstruction. Finally, observed paraphyly/polyphyly in a gene tree may result from incorrect taxonomy, when named species do not correspond to genetic limits of separate evolutionary entities, or from sample misidentification by collectors or curators (Funk & Omland in press).

Genealogical methods that take into account the effects of demography, selection, and drift, are useful in the taxonomic evaluation of genetic structure independently of the geographic scenario (Shaw 2001). The two species complexes here under study, due to their vast distributions either in allopatry or across a wide parapatric mosaic of heterogeneous habitats, are suitable to be analysed for population differentiation and speciation using such population genetics tools.

Phylogenetic methods can also give information on speciation durations (Avise *et al.* 1998; Barraclough & Nee 2001). Separation times inferred from DNA sequence divergence and a molecular clock for intraspecific phylogeographic groups and for sister species place lower and upper bounds on the duration of speciation. The accumulating data suggests that sister species-level divergences often reflect biogeographic influences on the genealogy going back to the Pliocene, even if additionally sculpted afterwards, whereas intraspecific phylogeography in mammals is mainly consequence of the Pleistocene history (Avise & Walker 1998).

The more the time intervals between species-branching events are greater than time intervals between lineage-branching events in each species, to allow sorting of the intraspecific diversity, the more gene and species divergences are likely to be concurrent (Wollenberg & Avise 1998). Pamilo & Nei (1988) showed that, under ideal conditions, the probability of topological identity between a gene tree and a species tree only depends on N_e and generation time. More specifically, the topological deviation of a gene tree, introduced by sequence polymorphism in ancestral species, from the species tree is only

substantial when the evolutionary time considered is short and the effective population size and generation time are both large. This is, when $N_e>>T$ where T is the internodal span between the birth and the split of the ancestral species. This potential incongruence must then be considered when addressing divergence within closely related and abundant species, which is exactly the case of the genets. However, as suggested by Avise *et al.* (1988) and confirmed by many empirical studies afterwards, even widespread and numerous species tend to possess much smaller N_e values than expected considering census sizes. Consequently, since $N_e<T$ is probably a fairly common instance, the likelihood of recovering a species or population tree from a gene tree may not be as low as predicted by, for example, a Wright-Fisher equilibrium model (Avise 2000a). Nevertheless, when historical demography for the taxa under study is unknown, to increase the number of individuals per population/species (Takahata 1989), which may be even more important than to increase the number of characters (Smouse 1998), and to strive for symmetric sampling is advised (Rosenberg 2002). Additionally, to use if possible more than one locus is certainly the soundest strategy (e.g. Ruvolo 1997).

1.4 Phylogeography and mitochondrial DNA

The phylogeographic approach can be useful as an analytical tool in the uncovering of cryptic speciation, but its main focus is generally on intraspecific genealogies and their relation with geography, history and demography (Avise 1994). Phylogeography is a branch of biogeography and, in its narrowest sense, is concerned with the spatial distribution of alleles whose phylogenetic relationships are known or can be estimated (Avise 1998). By explicitly focusing on the species' history, in particular the biogeographic past, when studying patterns of genetic variation through inferred gene trees, phylogeography departs from classical population genetics, but still owns to it the statistical and mathematical foundations (Knowles & Maddison 2002).

Mitochondrial DNA (mtDNA) is by far the most widely used marker in phylogeographic studies of animal species since, in general, its merits outweigh its weaknesses (Bermingham & Moritz 1998). Several factors have contributed to this popularity, such as the non-recombining uni-parental pattern of inheritance, few insertion/deletion/duplication events and a relatively conserved gene order and number (Avise *et al.* 1987). A typical vertebrate mtDNA contains 13 protein-coding genes, 22 tRNA genes, two rRNA genes, and a noncoding segment about 1000 bp long called the control region that initiates replication and transcription (Harrison 1989). But probably the

most important reasons for its widespread use are its rate and mode of evolution (Aquadro *et al.* 1984; Moritz *et al.* 1987; Kocher *et al.* 1989). High levels of genetic variation, a consequence of either a low selective pressure or high mutation rate (Gillespie 1986; Richter 1992), are a prerequisite for the utility of mtDNA as a microevolutionary phylogenetic marker (Avise 1994). However, this also implies that, when analysing the data, attention must be paid to homoplasy due to recurrent mutation (Smouse 1998).

Differential rates of nucleotide substitution among different gene segments and between distinct evolutionary lineages is well-documented for mitochondrial genes and is likely to be a consequence of locus-specific selective constraints that delimit sequence divergence over evolutionary time (Lopez *et al.* 1997). The sequence data from complete mitochondrial genomes (Anderson *et al.* 1981; Bibb *et al.* 1981) revealed three main variables in the evolution of mtDNA (Brown *et al.* 1982). Individual genes in the mitochondrial genome evolve at different rates, as do each of the three positions in the codons, and there is an overall bias of transitions over transversions of about 15-20:1. The wide range of possible combinations of these three parameters within mtDNA offers different levels of resolution suitable for most taxonomic levels (Aquadro *et al.* 1984). Population level studies exploit the genome's rapid evolutionary rate in third codon positions of coding genes and in the non-transcribed control region, while protein-coding genes may be particularly useful bridging the intra and interspecific levels (Avise *et al.* 1987; Villablanca 1994).

The essentially haploid inheritance of mtDNA, which reduces its N_e and hence increases its sensitivity to genetic drift, also contributes to turn it in a suitable marker to reveal phylogenetic structure between populations or species (Moritz 1994b). By recording the species' matrilineal history, mtDNA provides more information than any other gene genealogy with regard to population demographic history (Avise 1995). Another important virtue of mtDNA as a marker is that it allows the application of phylogenetic tools to intraspecific evolution (Hillis *et al.* 1996). According with Hennig (1966), phylogeny had no meaning at the intraspecific level because, for sexually reproducing organisms, conspecific lineages are anastomic rather than hierarchically branched (i.e. phylogenetics has no application in tokogenetic associations). However, due to its haploid nature, mtDNA occurs in hierarchical trees even within recombining organisms.

Some mitochondrial loci have become the markers of choice for certain applications and have been widely used in several studies in the past. The control region, since it contains variable blocks that evolve about 4-5 times faster than the entire mtDNA

molecule (Brown 1993; Taberlet 1996) is usually employed in studies of intraspecific phylogeography and population structure (Norman *et al.* 1994; Goldberg & Ruvolo 1997; Bensch & Hasselquist 1999; Liebers *et al.* 2001). The cytochrome *b* gene is the typical mitochondrial marker for the study of close to moderately deep interspecific relationships (Avise 1994; Avise & Walker 1999), although still sometimes informative for wide-range intraspecific analyses (Wettstein *et al.* 1995; Santucci *et al.* 1998; Fedorov *et al.* 1999).

The advantages of using simultaneously a hypervariable marker like the control region and selected protein-coding genes, with a lower evolution rate, are well documented (Lau *et al.* 1998; Cook *et al.* 1999). A wealth of information is now available, in terms of molecular structure, selective constraints, and mechanisms and rates of evolution for such loci (Irwin *et al.* 1991; Sbisa *et al.* 1997; Griffiths 1997; Pesole *et al.* 1999). Particularly, it has been shown that the cytochrome *b* and the ND5 genes are among the most reliable and efficient coding mitochondrial genes for phylogenetic reconstruction (Zardoya & Meyer 1996; Russo *et al.* 1996).

Soon after the initial steps in the use of mtDNA for phylogenetic studies, a molecular clock was claimed for animal mtDNA, which has been described as evolving at about 2% sequence divergence per million years between pairs of taxa (Brown *et al.* 1979; Wilson *et al.* 1985). However, many later studies showed strong departures from this general mtDNA clock, in several animal groups, either within or among taxa (Hasegawa & Kishino 1989; Martin *et al.* 1992). This is hardly surprising since evolution rate heterogeneity across different nucleotide positions, different genes, and different genomes, is now evident from the vast available data (Graur & Li 2000). The fact that different sites and genes evolve at varying rates both within and among lineages renders the calibration of mitochondrial molecular clocks problematic (Gibbons 1998), but if phylogenetically local in their application (Li 1993a) they may provide reliable estimates of divergence times (Yoder & Yang 2000).

In spite of its many positive attributes there are also several drawbacks to consider when planning the use of mtDNA markers in a phylogeographic study. Even when trying to explore different evolutionary rates by analysing more than one mitochondrial locus, it is important to note, since they are linked, that from a genealogical view the entire mitochondrial genome constitutes a single locus (Avise 1994). Another issue is the common presence of heteroplasmic mtDNA variants within an individual (Hayasaka *et al.* 1991; Stewart & Baker 1994; Douzery & Randi 1997), which may constitute both a technical problem and a threat to the phylogenetic utility of the marker. Paternal "leakage",

on the other hand, seems to be fortunately rare but in taxa where it is widespread it prevents traditional mtDNA applications (Avise 2000a). The use of mtDNA sequences retrieved by using versatile Polymerase Chain Reaction (PCR; Saiki *et al.* 1985) primers may also raise some problems if part, or the whole, of the sequence is also present as an mtDNA-like copy in the nuclear genome. The apparent continuous transfer of mitochondrial genes into the nuclear genome is often overlooked and may potentially lead to highly erroneous conclusions (Arctander 1995; Zhang & Hewitt 1996; Bensasson *et al.* 2001). Nuclear copies of mitochondrial genes are common in mammals (Fukuda *et al.* 1985; Smith *et al.* 1992; Lopez *et al.* 1994; Collura & Stewart 1995).

Besides practical or technical problems associated with the recovery of mtDNA sequence information, there are also some theoretical concerns. As referred to earlier, there is always a potential discrepancy between the coalescence times of a gene tree and the timing of the split of an ancestral population or species. That discrepancy depends, among other factors, on the past and present N_e, and is a consequence of in many instances coalescence times being longer than ancestral population splits. MtDNA has essentially one quarter of the N_e of an autosomal locus, a result of being both haploid and uniparentally inherited, and as a consequence it is expected to have shorter coalescence times than nuclear genes. Indeed, when discussing the coalescence of mtDNA lineages or matrilines, N_e relates only with the number of breeding females replacing themselves each generation: $N_{f(e)}$ (Avise 2000a). This may allow mtDNA to provide more accurate divergence times for populations and species than diploid markers (Slade *et al.* 1994; Moore 1995; Maddison 1997), but still can lead to errors on divergence time estimates (e.g. Horai *et al.* 1992). In fact, coalescence times for mtDNA can exceed those for nuclear genes under some particular combinations of demographic parameters related with spatial structure, mating systems and asymmetry in reproductive success among sexes (Hoelzer 1997; Moore 1997). If the time since the split is less than $N_{f(e)}$ to $2N_{f(e)}$ generations then the daughter populations are likely to contain alleles older than the branching event (Avise *et al.* 1988). To overcome the estimate variance introduced by each single gene tree, the only option is to base inference on comparisons of multiple loci (Knowlton *et al.* 1993; Nichols 2001).

MtDNA, since it has little or no recombination (McVean 2001), is sensitive to selective sweeps and, on the other hand, when introgressing is less affected by selection, which may cause a bias for the recognition of paraphyletic species (Hudson & Coyne 2002). MtDNA introgression can occur between related species or divergent conspecific

populations in secondary contact and hide their true phylogenetic relationships (Carr *et al.* 1986; Tegelstrom 1987; Lehman *et al.* 1991). The smaller N_e of mtDNA compared with nuclear loci may permit even low levels of introgression to establish haplotypes in a foreign population, which in the case of becoming fixed prevent us to trace them as heterotypic (Takahata & Slatkin 1984).

There is now clear evidence that mtDNA sequence evolution can depart from neutrality (Ballard & Kreitman 1995; Rand 2001), even if we consider the nearly neutral model of molecular evolution (Ohta 1995), and that natural selection can be responsible for shaping mtDNA variation (Mishmar *et al.* 2003). Consequently, and although mutation/drift still are the main driving evolutionary forces involved in defining genetic patterns in structured populations, neutrality tests are crucial for the use of mtDNA in genealogical studies (Gerber *et al.* 2001).

Finally, in species with male-biased dispersal some major mtDNA phylogroups may not register genome-wide population subdivisions (e.g. Palumbi & Baker 1994; but see Avise 1995); this is less likely to occur in genets since dispersal levels are here similar between sexes (Palomares & Delibes 1994).

1.5 African biogeography and palaeoclimate

It is now well accepted, after several phylogeographic and phylogenetic studies and divergence time estimates using molecular clocks, that sister species-level splits and intraspecific population structure in mammals generally reflect historical influences going back, at the most, no further than the Pliocene (Avise *et al.* 1998; Taberlet *et al.* 1998). However, detailed records of the African climatic and landscape changes during the last six million years are still fragmentary and several gaps remain in our knowledge for this period in large parts of Africa (deMenocal 1995). The Pliocene in Africa, more or less since its beginning around six million yr BP, was characterised by warmer and moister conditions than in present times, with greater tropical forest and woodland cover and less desert. Although it is uncertain if at the time there was a continuous east-west forest belt across Africa, the region corresponding today to the Sahara desert would have been covered with semi-arid vegetation (Hamilton & Taylor 1991). Around three million yr BP African environments shifted toward more arid and cooler conditions, consequence of the onset of the glacial cycles in the northern hemisphere (deMenocal 1995). The more reduced and seasonal annual precipitation pattern led to a substitution of large stretches of lowland forest by dry savannah grasses and shrubs, particularly in East Africa, and to an

increase of desert areas in northern Africa (Leroy & Dupont 1994). The amplitude of ecological changes at the boundary Plio-Pleistocene has been considered a cause of some major mammalian fauna turnovers (Beherensmeyer *et al.* 1997) and also of hominid evolution (Cerling 1992).

Globally, the Pleistocene is characterised by glacial-interglacial cycles, which had started already in the Late Pliocene, that switched from a dominant 41,000-year frequency to a 100,000-year frequency about 700,000 yr BP (Webb III & Bartlein 1992). There is evidence indicating that environmental change in Africa during the Early Pleistocene did not follow the glacial-interglacial rhythm; only after the mid-Pleistocene peak of global aridity one million yr BP the shifts became concordant (Dupont *et al.* 2001). Nevertheless, the main picture that derives from many palaeoclimatic and palaeoecological studies covering the Pleistocene in Africa is one of several climatic cycles with very marked impacts on the landscape profiles across the continent (Dupont *et al.* 2001). Interglacial periods were warmer, wetter and with expansion of the forest biome, whereas glacial periods experienced cooler and drier climates, with forest regression (Deacon 1983; Partridge *et al.* 1990). That these repeated episodes of range contraction/expansion of the main types of biotope in Africa are a likely scenario for events of dispersal, speciation, and extinction of both flora and fauna taxa, is now well documented (Diamond & Hamilton 1980; Fjeldsa & Lovett 1997; Dupont *et al.* 2000).

Details about the climate and biome dynamics in Africa are, as expected, much better known for the Late Pleistocene and Holocene (Fig. 1.4). Vegetation in Africa at the last glacial maximum (18,000 yr BP) was characterised by a reduction of tropical rainforest and seasonal forest, with the lowland tropical rainforests encroached from above by montane evergreen and laterally by savannah and grassland (Maley 1991). In East Africa, the predominant biome was xerophytic woods/scrub and steppe, which also dominated in Southern Africa with the exception of the southwestern Cape Province where fynbos (South African Mediterranean-type xerophytic scrub) persisted as today (Elenga *et al.* 2000). After these full glacial hyperarid conditions, humid conditions returned on what is called the Holocene African Humid Period, which lasted from ca. 15,000 yr BP to 6,000 yr BP (deMenocal *et al.* 2000). At the mid-Holocene (6,000 yr BP) the Maghreb was characterised by temperate xerophytic woods/scrub and the Sahara was extremely reduced. Enhanced monsoons extended Sahelian vegetation into the Sahara with the Sahelian vegetation belts (i.e. steppe, xerophytic woods/scrub, tropical dry forest) shifting northwards and turning the Sahara into a verdant landscape (Sarnthein 1978). Tropical

seasonal forest dominated in Central Africa and the tropical rainforest belt was reduced, coinciding with a more seasonal climate in the equatorial zone. Africa was not uniformly wetter than present at 6000 yr BP and the area of the tropical rainforest biome may be greater now. In East and Southern Africa the biome distribution was similar to today, with warm mixed forest on the mountains, temperate xerophytic woods/scrub on the high-plateaux and in the Cape (Jolly *et al.* 1998).

The sequence of biome shifts during a glacial-interglacial cycle may help to account for some currently disjoint distributions. For example, the now-arid Saharan mountains may have linked Mediterranean and African tropical montane biotopes during enhanced monsoon regimes. The Sahara mountains supported woodland or scrub like *Quercus ilex*, *Pistacia* spp. and *Erica* spp. in humid periods of the Pleistocene (Prentice *et al.* 2000). In general, favourable conditions for the existence of broadleaved evergreen trees in the present Sahara desert could have existed for millennia during interglacial periods, and driven by climatic oscillations on the Milankovitch time scale (Prentice *et al.* 2000).

Several recent phylogeographic studies concerning mammals with Pan-African ranges have attempted to reconcile geographic patterns of genetic variation with the type of historical information above. Jensen-Seaman & Kidd (2001) defended that the split of the ancestral population of eastern gorillas into the two present clades may have coincided with an earlier Pleistocene arid phase or a vicariance event associated with tectonic or volcanic activity along the East African rift. Pitra *et al.* (2002) also relate the genetic subdivision observed in present-day sable antelope populations to allopatric fragmentation promoted by events associated with activity of the East African rift and superimposed range oscillations driven by the Pleistocene climate cycles. Birungi & Arctander (2000) linked evolution in allopatry of two kob lineages with range contraction during arid conditions. Flagstad *et al.* (2001) proposed an eastern Africa origin for the hartebeest followed by allopatric diversification promoted by cycles of savannah versus rainforest belt contraction/expansion, and lineage survival in refuges, during the Pleistocene. Van Hooft *et al.* (2002) referred to a Pleistocene origin for the African buffalo, with a Late Pleistocene expansion of the Cape subspecies to all Southern Africa. Nersting & Arctander (2001) highlighted the importance of the Kalahari Desert area as a woodland-savannah refuge during interglacial periods for arid adapted species. Eggert *et al.* (2002) attribute complex patterns of population differentiation and putative speciation in the African elephants to the effect of a long history of range alterations in the last six million years.

Finally, Telfer *et al.* (2003) reported a case of allopatric divergence in mandrills which fits well with previously described forest refuges in western central Africa during Plio-Pleistocene dry periods.

The climatic and biome dynamics in Africa during the Pleistocene, considering the estimated age of the genet taxa here under analysis (Gaubert *et al.* 2004), are also very likely to be of central importance for the interpretation of the results of the present study.

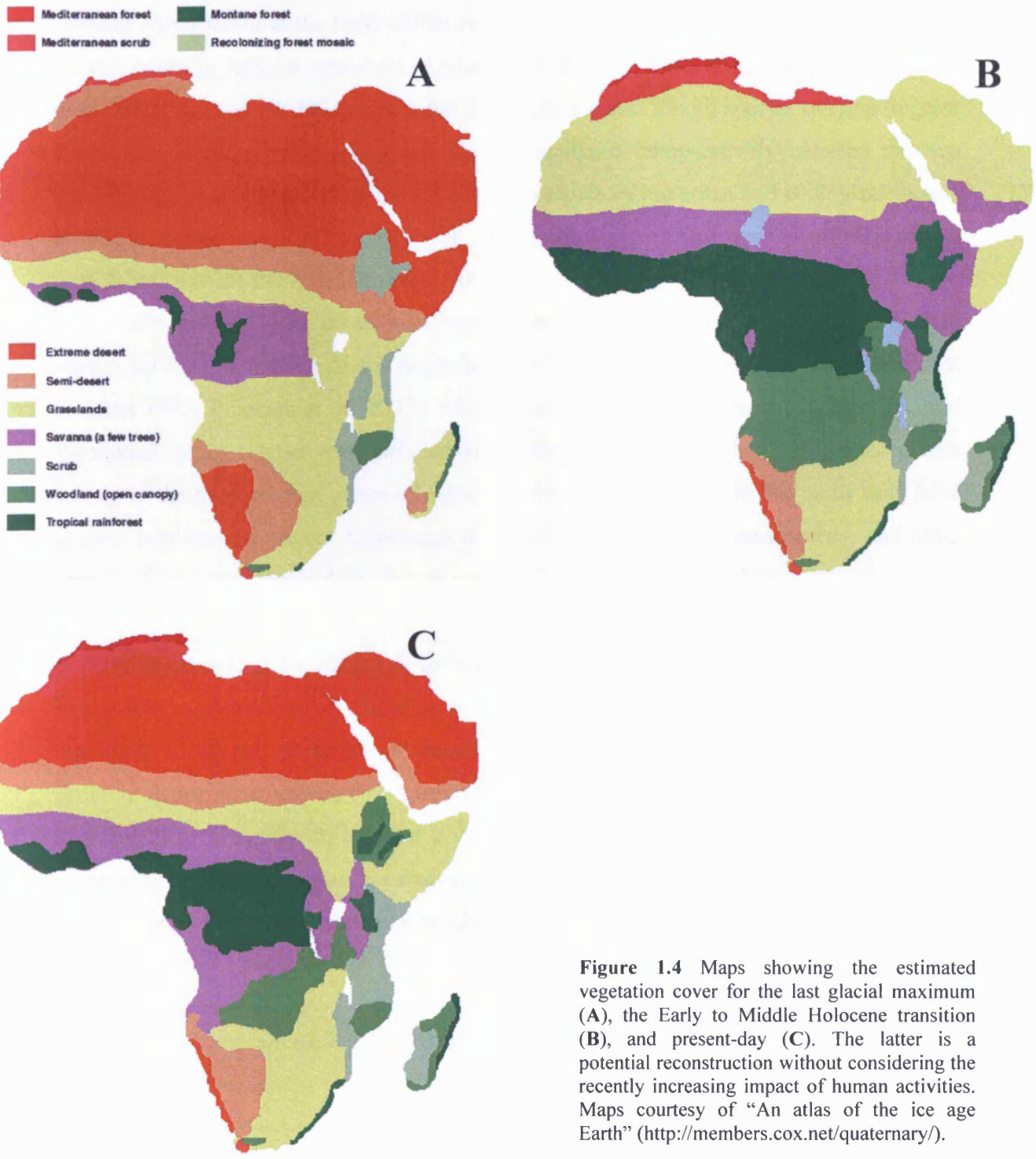

Figure 1.4 Maps showing the estimated vegetation cover for the last glacial maximum (**A**), the Early to Middle Holocene transition (**B**), and present-day (**C**). The latter is a potential reconstruction without considering the recently increasing impact of human activities. Maps courtesy of "An atlas of the ice age Earth" (http://members.cox.net/quaternary/).

1.6 Aims

The main objective of this study is to investigate the taxonomy, biogeography and evolutionary history of two genet species complexes, with different mitochondrial genes in a phylogeographic and phylogenetic framework. In order to achieve this, a detailed sample set for both groups in terms of geographic origin and representing almost all the morphologically recognised taxonomic units was analysed.

Considering the vast ranges of both species across Africa, the results are probably a relevant contribution to the field of Pan-African biogeography, joining in this regard other similar works in African mammals (Arctander *et al.* 1999; Uphyrkina *et al.* 2001; Girman *et al.* 2001; Roca *et al.* 2001). Since the distribution areas of both species overlap in great extent, the phylogeographic data can also be analysed comparatively between the two species. This may allow testing the relative importance of historical and ecological factors in shaping independent genealogies with similar life histories and genomic characteristics (e.g. Johnson *et al.* 1999; Seddon *et al.* 2001).

The present work takes advantage of the accumulated information from several studies on other members of the Superfamily Feloidea (Hoelzel *et al.* 1994; Johnson & O'Brien 1997; Freeman *et al.* 2001), when considering molecular methods, markers, and theoretical issues related with the analysis of the data. On the other hand, since genets belong to the most ancient group of extant carnivores it is likely that this study may have general implications on our knowledge about evolutionary rates, pseudogenes, and other aspects of genome organisation, function and constraints, in the Carnivora.

For this study museum skins are the main type of sample, highlighting the opportunity provided by museums to perform genetic studies in taxa from which it is impossible to obtain a good sampling by relying only in freshly collected material. This can apply to several situations of sample rarity or sampling incompleteness caused by different reasons (e.g. endangered species, species with cryptic behaviour/ecology, patchily distributed, or occurring throughout a very broad distribution area). Besides, the use of museum material also allows the analysis of the temporal component of genetic variation (Thomas *et al.* 1990; Villablanca 1994; Gravlund *et al.* 1998; Hofreiter *et al.* 2001).

Chapter 2. Concepts and methods in phylogeography

This chapter provides a survey of the main theoretical issues and associated analytical tools that were relevant to the ways the raw data was approached in the present study. Both population genetics and phylogenetics underlie the statistical treatment of DNA data within a phylogeographic framework and, accordingly, they make up for the primary partition of the chapter. With the exception of gene trees, which are the unifying theme of the two disciplines and thereby the focal point in phylogeographic research, none of the addressed subjects were mentioned, or at least more than briefly, in the previous chapter; the overview they deserve is given here.

2.1 Population genetics

2.1.1 Coalescent

Gene trees (Griffiths 1980), also known as gene genealogies (Tajima 1983) or allelic genealogies (Takahata 1993), can be approached with a neutral stochastic population genetics model called the coalescent (Kingman 1982; Tavare 1984), particularly powerful for inferences about demographic history and population mutation rates. Coalescent theory relates the age of a gene clade to its degree of genetic diversity, given a stochastic mutation process and temporal constancy of a series of factors that can affect the rate of diversification, such as N_e, selection, recombination, linkage, geographic structure and migration (Donnelly & Tavare 1995). In practical terms, the coalescent may be seen as a method of reconstructing genetic drift history that can be used to derive estimates of past demographic events from the impact they had on the drift pattern (Slatkin & Hudson 1991). It provides the field of phylogeography with an explicit historical demographic model based in random genealogies, which reflect the truly stochastic nature of evolution, that otherwise would be missing (Hey & Machado 2003).

2.1.2 Demographic history

With genetic distances between two sequences and an assumed evolutionary rate it is possible to estimate the time to the most recent common ancestor of two sequences. Such estimates accumulated for many individuals yield pairwise frequency distributions of coancestry times, or "mismatch distributions" (Rogers & Harpending 1992), which summarise the mutational history along branches of a gene tree and can be contrasted with

theoretical expectations. The pairwise differences between alleles are affected by the past population trajectories across time and space. Bimodal or even multi-peaked ragged distributions of pairwise times to coancestry often appear in the gene trees of stable-sized populations, whereas they are smooth and usually unimodal in expanding populations (Slatkin & Hudson 1991). Because of increased retention of gene lineages, rapidly expanding populations are expected to contain DNA sequences with a star-like phylogeny and a Poisson distribution of pairwise differences between alleles in which genealogy-induced correlations are of small impact (Slatkin & Hudson 1991). Rapidly growing populations, typically with low levels of genetic diversity, are then a case where a frequency histogram of coancestry times may be more informative than gene trees, which will lack significant clade structure or lineage sorting (Rogers & Jorde 1995; Harpending *et al.* 1998).

Stable populations are more likely to have strongly structured allele phylogenies with non-Poisson (e.g. geometric or multimodal) distributions of pairwise differences. Growing populations typically display "waves", travelling at rate $2u$ (u is the mutation rate for the entire region of DNA under study), whose crests fall to the right of the modes in the corresponding geometric curves of constant-sized populations and may indicate the time of expansion (Harpending 1994). New pairs of sequences with no differences appear at the left of the wave at a rate inversely proportional to the "new" N_e, while the leading edge preserves the form of the ancient distribution. Because of their slow rate of change, $1/(2u)$ per generation, pairwise difference frequencies are useful for studying events that happened well back into the Pleistocene (Rogers & Jorde 1995). A short bottleneck may be detected by generating a wave with an extremely steep leading face or, even more often, ragged distributions with many peaks at large pairwise-difference values (Rogers & Harpending 1992).

A leading wave of differences between populations ("intermatch distributions") in front of the wave within populations usually indicates an ancient split followed by expansion of the daughter populations, which are isolated or weakly connected by gene flow since then. When both intermatch and mismatch distributions are coincident this indicates that an expansion either preceded or was synchronous with population subdivision. Ancestral population structure seems to be reflected upon later structure mainly if ancestral levels of gene flow connecting demes were significantly reduced (Harpending *et al.* 1993). Marjoram & Donelly (1994) described how very high or very low levels of gene flow among demes result in unimodal mismatch distributions, whereas

an intermediate range of migrants may lead to multimodal curves and gene trees with a mixture of both very short and very long branch lengths. A subdivided population with demes connected by many migrants per generation usually present mismatch distributions and gene trees very similar to an expanding continuous population (Neigel 2002; Ray *et al.* 2003).

Although intuitive and straightforward, mismatch distributions of lineage divergence times should not be used as a sole source of inference about demographic history since early splits in the gene tree have a disproportionate effect on these distributions (Felsenstein 1992). Indeed, deviations from theoretical expectations for mismatch distributions are possible simply because lineages in a tree are correlated, both genealogically and by being jointly affected by ecological and historical factors (Ball *et al.* 1990). For instance, the signatures arising from a rapid demographic expansion after a substantial bottleneck, exponential growth *per se*, and the effect of a selective sweep, or hitchhiking event, mediated by positive selection, in a constant-size population, can be indistinguishable pasts to mismatch analysis (Excoffier 1990; Rand *et al.* 1994).

Populations that had a recent and rapid demographic growth could also be theoretically detected with Tajima's test (1989a), which relates the number of segregating sites with pairwise differences. However, this test is rapidly affected by departures from the infinite-sites assumption (Bertorelle & Slatkin 1995). On the other hand, since this test is sensitive to demographic fluctuations, when applied as a neutrality test its results are only meaningful for stationary populations (Aris-Brosou & Excoffier 1996).

Another strategy to infer the demographic history of a population/species from mtDNA data is to compare haplotype diversity (h) with nucleotide diversity (p) (Nei *et al.* 1975; Grant & Bowen 1998). The statistics h condenses information on the number and frequencies of different alleles at a locus regardless of their sequence relationships whereas p quantifies the weighted sequence divergence between individuals in a population regardless of the number of different haplotypes.

2.1.3 Population structure and gene flow

Spatially structured populations, in which migration levels are low, tend to be connected by deeper coalescence times in gene trees than expected in a single unit with the same census size (Slatkin 1991; Nei & Takahata 1993). In contrast, when populations are subdivided but connecting gene flow is still significant, for instance in the form of multiple extinction/recolonisation events, the total N_e may be even larger than in a continuous

population (Slatkin 1987; Strobeck 1987). In these instances genetic divergence among demes is slower and gene trees exhibit shallow coalescence times; this is the typical outcome when populations are integrated in a metapopulation framework (Slatkin 1985a; Hanski & Gilpin 1991). Extant mtDNA genotypes often represent ancestral conditions retained across colonisation or expansion episodes, rather than recent markers of local phylogeographic clades. In attention to this, the geographic source and number of colonisation events consistent with an observed distribution of mtDNA haplotypes may be inferred in suspected source and sink systems of demes (e.g. Ward *et al.* 1993; Horai *et al.* 1993; but see Harpending *et al.* 1993). However, levels of gene flow must be high in order for old lineages to be present in sampling locations far from the origin of the expansion (Ray *et al.* 2003).

The estimation of levels of gene flow among populations is thus central for the understanding of the hierarchy and age of an observed current genetic structure and also for inferring potential past demographic and spatial patterns (Neigel 1997). Gene flow is the movement of genes in populations that have an impact in changing the spatial distributions of genes (Slatkin 1985a). The majority of the available estimators are based on equilibrium expectations derived from models of population structure under neutrality theory (e.g. Kimura & Weiss 1964). From observed geographic variation in population allele frequencies the parameter $N_e m$ is estimated, where m is the migration rate (proportion of migrant lineages per generation in a population), to measure the mean absolute number of migrants exchanged among populations per generation (Birky *et al.* 1989). $N_e m$ is also known as "the amount of gene flow" (M) (Slatkin 1993). Wright (1931) related $N_e m$ with the standardised variance in allele frequencies across populations (F_{st}), which can be calculated from empirical genetic data (e.g. Lynch & Crease 1990), through the equation $F_{st} \approx 1/(4\ N_e m+1)$. The F-statistics are a set of correlation coefficients to partition departures from panmixia due to within population processes and to population subdivision (Wright 1951). It is possible to estimate F_{st} from allele frequencies, but F_{st} is not necessarily defined by allele frequencies only (Neigel 2002). Definitions of F_{st} that mathematically differ from Wright's have been given distinct names, including G_{st} (Nei 1973), N_{st} (Lynch & Crease 1990), and ϕ_{st} (Excoffier *et al.* 1992). The latter is an index of population genetic structure, which considers both the allelic frequencies and allelic nucleotide content, estimated within an analysis of molecular variance framework (Weir & Cockerham 1984; Excoffier *et al.* 1992).

As referred above, these gene flow estimators rely on the assumption that equilibrium between genetic drift and gene flow for neutral markers has been reached among the populations. However, this may not be the case when m is small and N_e is large since t, number of generations for F_{st} to be near an equilibrium value, is $\approx 1/[2m+(1/2N_e)]$ (Crow & Aoki 1984). In such instance, the N_em values relate with historical, not contemporary, levels of gene flow, and a large N_em value may indicate recent historical associations with absent migration at the present, populations in equilibrium with high contemporary gene flow, or a situation where these two extremes are somehow combined (Nielsen & Slatkin 2000). Nevertheless, this is a problem not only for gene flow estimation methods based on summary statistics but also to the genealogical ones, as both have difficulties in separating historical effects and gene flow (Barton & Wilson 1996).

2.1.4 Intraspecific networks and Nested Clade Analysis

Intraspecific gene genealogies are often better estimated using networks rather than trees. Traditional phylogenetic methods have difficulties in coping with consequences of tokogeny, such as low divergence among alleles, persistence of ancestral haplotypes, hard polytomies, reticulations due to lineage hybridisation and homoplasic recurrent mutations, and (computationally demanding) large sample sizes (Posada & Crandall 2001). In contrast, network methods are especially designed to account for these processes, portraying information present on the data that phylogenetic algorithms are forced to ignore, and to incorporate consequences of population genetics theory. The majority of the network methods are distance methods, with the aim of minimising the number of mutations among haplotypes, but some are based on optimality criteria (e.g. likelihood and least squares), which may be statistically more robust. Among the several methods for network reconstruction, two are worth of note due to their popularity and by being implemented in accessible software packages. The molecular-variance parsimony technique (Excoffier & Smouse 1994) is an optimisation strategy to find a solution among equally parsimonious minimum spanning trees (MSTs); when more than one solution is present this is portrayed by the presence of alternative links in the network. The selected MST is the one from which optimum estimates of populations statistics are obtained, given the data, and that least increases the variation among haplotypes due to translating phenetic distances into phyletic distances and hence that represents minimal extrapolation from the data (Smouse 1998). This method makes explicit use of sampled haplotype frequencies and geographic subdivisions, predefined accordingly with a genetic structure being tested,

since such information is summarised in the estimates against which the MST is optimised, the ϕ-statistics.

The statistical parsimony algorithm (Templeton *et al.* 1992) estimates first the parsimony connection limit of the network, which is the maximum number of differences among haplotypes as a result of single substitutions, with a 95% statistical confidence and then reconstructs the unrooted cladogram. To decide between equally parsimonious alternatives, haplotype frequencies are converted into topological probabilities of being either tip or interior and rare or common (Crandall & Templeton 1993). Predictions and results from the coalescent theory (e.g. Excoffier & Langeney 1989) are used in the resolution of ambiguities. Castelloe & Templeton (1994) provided a method to estimate relative ages of haplotypes and to root the cladogram: interior and frequent haplotypes generally show high outgroup weights whereas tip haplotypes exhibit low outgroup weights. However, Cassens *et al.* (2003) demonstrated that such method is sensitive to the frequency and non-homogeneous distribution of missing intermediates.

The network estimation through the statistical parsimony algorithm (Templeton *et al.* 1992) is often just the first step of a widely applicable and popular method in phylogeographic analysis, the nested clade analysis (NCA) (Templeton 1998). The cladogram can be statistically nested for testing hypothesis of associations between haplotypes and phenotypes (Templeton & Sing 1993). Since the nesting levels are directly correlated with evolutionary time they can be used to partition effects of contemporary population structure from those of population history, this alleged ability being one of the most valued merits of NCA (Templeton 1998). Even when there are large amounts of tree ambiguity, the nesting ambiguity is usually little and can be resolved (Crandall & Templeton 1996).

To separate population history from population structure, two measures of geographic distance are used in NCA. The first distance is from the geographic centre of all individuals bearing haplotypes that fall within a given clade. This distance is the clade distance (D_c) and measures how geographically widespread the individuals are from that clade. The second distance is the nested clade distance (D_n), which measures the distance of clades from the geographic centre of their nesting category; it measures how far individuals within evolutionarily closely related clades are located from one another (Templeton *et al.* 1995). Also calculated are the mean differences between D_c and D_n for tip clades versus interior clades. Tip clades are those connected to the rest of the cladogram by only one mutational pathway, whereas interior clades by definition do so by two or

more mutational paths; tip clades strongly tend to be younger than interior clades (Castelloe & Templeton 1994). Under a restricted gene flow model the geographical extent of a haplotype is strongly correlated with its age (Watterson & Guess 1977) and evolutionarily neighbouring clades remain geographically close (Neigel & Avise 1993). Here D_c and D_n are concordant, whereas a history of long-distance colonisations or founder events, even if rare, will be reflected on much larger D_n than D_c (Templeton *et al.* 1995). The resulting nested clades are designated by "C-N" where "C" is the nesting level of the clade and "N" is the number of a particular clade at a given nested level (Templeton 1998). NCA distances summarise spatial dispersion patterns and may be used to test alternative hypotheses of patterns of gene flow and population structure derived from other population genetics methods (e.g. Slatkin 1993).

NCA attempts to determine the relative contributions, and their localisation both temporally and geographically, of restricted gene flow and historical events (e.g. past fragmentation, long-distance colonisation, or contiguous range expansion) that interacted in yielding the current spatial patterns of haplotype distribution (Templeton 1998). It is complementary to other cladistic approaches to the study of gene flow, like Slatkin's statistics (e.g. Slatkin & Maddison 1989, 1990). While these procedures assume that the geographic partition pattern in the haplotypes is due to restricted gene flow and try to measure it given this assumption, NCA is designed to also contemplate the effect of history and then is a test to assumptions on gene flow. Contrasts in the distance measures between tip and interior clades are important in discriminating among potential causes of the geographical structuring of the genetic variation. The statistical significance of the different distance measures and contrasts is determined by random permutation testing which simulates the null hypothesis of a random geographical distribution for all clades within a nesting category given the marginal clade frequencies and sample sizes per locality (Templeton 1998). For instance, restricted gene flow, due for example to isolation by distance, among populations should produce significantly small D_c values for tip clades, whereas recent large range expansions should be reflected in significantly large D_n and D_c values for tip clades. Heterogeneous D_c estimates for tip clades may indicate isolated founder events in a species otherwise geographically structured. Templeton *et al.* (1995) list several predictions like these about historical demography in function of the behaviour of the NCA statistics. However, Templeton (1998) recognised that criteria for colonisation may not be satisfied in the case of a recent and extreme founder effect, so that neither new mutations had time to arise in the colonised area nor tip haplotypes were included among

the founders. Paulo *et al.* (2002b) provided an illustration of this case when NCA failed to detect a range expansion, through long distance colonisation, when the founders, due to a bottleneck followed by rapid growth of the ancestral source population, were mainly ancient lineages. NCA may also be unable to detect range expansion when, due to several cyclic range contractions, the rate of lineage extinction within the ancestral refuge areas is high or the colonisation process is recent relative to the mutation rate (Seddon *et al.* 2001; Alexandrino *et al.* 2002).

One of the merits of the NCA is that it can be used in speciation analysis and testing the taxonomic rank of populations, in the context of Templeton's cohesion species concept (1989), as illustrated by Templeton *et al.* (1995), and also in the study of hybridisation (e.g. Pfenninger & Posada 2002). For the problem of inferring species, the relevant biological inference from the NCA key is the inference of significant fragmentation events; hybridisation is detected through the common presence in a single population of haplotypes from clades marking different population fragments (Templeton 2001). When separated populations have large N_e they may share ancestral polymorphisms (Hey & Kliman 1993) due to incomplete lineage sorting, but NCA allows the testing of this scenario against hybridisation in cases of secondary contact between diverging populations. Areas of secondary contact may be detected in a NCA by large pairwise distances between the geographical centres of the haplotypes or clades found at the area (Templeton 2001). When hybridisation occurs between previously fragmented populations, haplotypes or clades with very distant geographic centres are more likely to occur in the same location, the area of admixture. As one moves from haplotypes to higher-level clades, the average pairwise location distances at sites under isolation by distance approaches zero whereas they stay large or even increase for sites of admixture. It is important to note that the above observations allow us to either detect divergent evolutionary lineages due to past fragmentation or their admixture after secondary contact. To evaluate if these evolutionary lineages are the same or different cohesion species we need additional information about genetic and ecological exchangeability, which may also be tested using the NCA approach (Templeton 2001).

The NCA allows, through random permutation tests ("nested contingency analysis"), to evaluate the null hypothesis of no association between geography and the inferred gene cladogram. Failure to reject the null hypothesis may indicate high contemporary gene flow or recent historical associations among demes, or insufficient power of the tests due to sampling weaknesses (Avise 2000a). When sample sizes per

location are small, geographically close sites may be pooled together and the geographic centre of the pooled sites is used as the location for the pooled sample (Templeton 2001). If still for some nesting clades, usually at level "1-N", the null hypothesis of no association between the position of a haplotype in the cladogram with geographical position fails to be rejected, this indicates lack of statistical power due to small sample sizes or low genetic variation. Fortunately, the nested design allows the recovery of statistical power at higher levels of analysis (Templeton *et al.* 1995). Poor sampling continuity may lead to misleading interpretations of the causes for geographical associations as, for example, when reciprocal monophyly between geographically distant demes is seen as evidence of past fragmentation but the absence of samples for intermediate locations prevents to rule out a scenario of isolation by distance (Templeton 1998). It is then essential to be able to assess the statistical significance of both sample sizes and sampling of geographical locations for distinguishing potential causes of geographical associations. However, if it is true that in NCA both the test for population structure and the rejection of the null model of no geographic structure are statistical, the same cannot be said of the inference key. This inference key to distinguish different historical scenarios, potentially explanatory of the observed geographic structure in the data, is controversial because it is not based on analytical models, interpretations can not be statistically assessed and this may lead to the risk of either inaccurate inference or over-interpretation (Knowles & Maddison 2002). Paulo *et al.* (2002b) also refer the absence of alternative scenarios in the inference key that can generate the same phylogeographic pattern. Furthermore, Rogers & Jorde (1995) argue that in cases of recent rapid population growth the NCA approach may be mislead by wrong inferences of haplotype and clade ages. This can indeed be true since the assumption that the oldest haplotypes are the most frequent may not hold (Smouse 1998).

The popularity of NCA stems greatly from the fact that it does not make a priori assumptions about the past, being essentially model free (Emerson *et al.* 2001), but this can also be seen as a weakness since its statistical power has not been proven by analytical derivation from an explicit stochastic model (Knowles & Maddison 2002).

2.2 Molecular phylogenetics

As discussed earlier, the application of molecular phylogenetics tools of analysis, traditionally reserved to studies at the interspecific level or higher, to within-species sequence data is relatively controversial. However, it is also clear that in the case of the non-recombinant mtDNA and for evolutionary research simultaneously addressing

questions at the species level and immediately below, phylogenetic methods are widely and successfully applied (e.g. Rodriguez-Robles & De Jesus-Escobar 2000; Burbrink 2002; Burbidge *et al.* 2003). Here, phylogenetic tools can be very valuable in depicting distinct patterns of cladogenesis, that may reveal unsuspected speciation events, or providing support for monophyletic groups, the natural clades with taxonomic value (Graur & Li 2000).

Molecular phylogenetics is the branch of molecular evolution that is concerned with trying to infer the evolutionary history of genes and organisms from molecular data (Li 1997). There are many alternative methodologies available in this research area to attempt achieving a reliable reconstruction estimate of the phylogeny of the taxa under study. All of them are based on criteria to select the best reconstruction from among the possible phylogenetic trees for a given data set. Some approaches are strictly algorithmic and when building the tree they are implicitly selecting the preferred tree according to their criteria; examples are distance methods such as neighbour-joining (NJ) and unweighted pair-group method using arithmetic averages (UPGMA). Other methods use an optimality criterion or objective function to evaluate a given tree, and the procedure consists in assigning scores to competing hypotheses and determine the tree(s) with the best values according with the criterion (Swofford *et al.* 1996). Although the same algorithm (e.g. NJ) can be used in both types of methodologies, in a criterion-based method it is just a straightforward way for comparing scores and searching more trees potentially optimal.

Different phylogenetic methods reflect the larger or smaller constraints of the theoretical framework behind each of them. Typically, methods based on more complicated models and with more explicit assumptions strive for a more faithful portrait of evolutionary intrinsic complexity, but they also incur in higher risks of being misleading if the assumed model is incorrect (Farris 1986).

2.2.1 Parsimony methods

Historically, maximum parsimony (MP) is the most widely used method with an optimality criterion to estimate phylogenetic trees directly from unordered multistate character data such as DNA sequence data (Kitching *et al.* 1998). The optimality criterion is usually minimum tree length, so the preferred trees are the ones presenting the smaller number of evolutionary steps required for explaining a given data set, hence aiming to minimise the effect of putative homoplasy (Farris 1970). Refinements of this optimality criterion usually include restrictions or weightings of the several possible character state

transformations and define alternative, but related, parsimony algorithms. The two most known and heavily used are probably the Wagner method (Kluge & Farris 1969) and the Fitch method (1971), which is a generalisation of the former to make it applicable to unordered multistate character data. Fitch parsimony allows any state to transform directly to any other state and free reversibility in character state changes. To be able to assign costs for different character state transformations, according with expectancies from extrinsic or general biological information (e.g. to attach greater weight to transversions than to transitions), may be both appealing and relevant. The problem lies in determining how to choose the relative costs for different kinds of transformations. Here, both an *a priori* weighting, even if uncertain of the exact matrix of costs, and to approximate the appropriate weights by estimation from the data are valid strategies (Swofford *et al.* 1996). In the case that the data set is convertible to protein sequences this problem can also be tackled with a matrix of transformation costs for amino acids derived from taxon-wide sequence alignment comparisons (Henikoff & Henikoff 1992).

The fact that parsimony methods do not require explicit models of evolutionary change can be seen as strength or as weakness depending on essentially to which school of systematics a researcher is affiliated. However, Felsenstein (1978) demonstrated that parsimony methods can make inconsistent estimates of the true phylogeny, by using a simple evolutionary model, under certain sets of conditions, related with rate heterogeneity among lineages, that is known as the "Felsenstein zone". Hendy & Penny (1989) presented additional situations, other than unequal rates of change throughout the tree, that result in inconsistency when using parsimony and posted the term "long-branch attraction" for this general phenomenon. This shows the importance of accounting for unobserved as well as observed substitutions using "multiple-hit" corrections, and of using models of evolutionary change already including such corrections (Hall 2001).

2.2.2 Maximum likelihood methods

The optimality criterion here is to maximise the probability of observing the data we have obtained (i.e. the tip sequences), and therefore the selected tree(s) will be the one(s) with the highest scores (likelihoods) in this respect. However, in order to evaluate every candidate tree in terms of this probability we need a model of evolutionary change, which is also evaluated for its fit to the data. A central component of such a model for maximum likelihood (ML) methods of tree inference is the specified rate of change, this is how branch length relates with evolutionary time, since for instance ML considers changes

to be more likely along long branches than short ones. A model may be fully defined or contain many parameters that are estimated from the data. The evolutionary models currently in use are Markov-type, where previous mutational history for each site does not affect present probabilities and changes along different branches in a tree are assumed to be independent (Rodriguez *et al.* 1990). This is central for the calculation of the overall tree-model likelihood, the product of all the individual-site likelihoods, as a specific case of the probability of any single scenario to be equal to the product of the probabilities of the changes required by that scenario. As the individual probabilities are generally very small, for each tree what is evaluated is the log of the likelihood, which is given by the sum of the logs of the single-site likelihoods (Goldman 1990). Furthermore, the fact that the evolutionary models are time-reversible allows the calculation of the likelihood of a tree regardless of the root location.

ML and MP share several parallelisms clearly summarised in the observation that the total cost of the tree under parsimony is given by the sum of the costs at each position, whereas the net log-likelihood of a tree is the sum of the log-likelihoods of evolution at each site. The main differences are that the cost of change under parsimony is not function of branch length and that MP looks only at the lowest cost solution(s), whereas ML looks at the combined likelihood for all solutions consistent with the tree and branch lengths (Goldman 1990).

Since their introduction (Cavalli-Sforza & Edwards 1967; Felsenstein 1981) ML methods have grown in popularity, particularly in the last decade. This is due to several factors such as their consistency properties, robustness to model' assumptions violations, and higher efficiency than competing methods, being frequently the least affected by sampling weaknesses (Kuhner & Felsenstein 1994; Huelsenbeck 1995a). Consistency is a measure of the capacity of a method to recover the correct tree, as the available data becomes infinite. Efficiency measures how fast a method converges on the correct solution, as more data becomes available to the method (Hillis *et al.* 1996).

2.2.3 Models of sequence evolution

An underlying model of evolution is essential to phylogenetic estimation with a ML approach since it provides the weights to the various possible site changes across the tree and therefore determines the likelihoods of those changes. A substitution evolution model for DNA sequences is mathematically defined by a table or matrix of instantaneous rates for the inter-conversion between the four nucleotide types; rates (r) are in substitutions per

site per unit of evolutionary time. Besides information for the rate parameters, the matrix has also information concerning the frequencies of the nucleotides, assumed to remain constant over time (i.e. in equilibrium), to which rates of change are proportional. The vast majority of the substitution models described so far are time-reversible and, more specifically, special cases of the general time-reversible model (GTR) (Lanave *et al.* 1984). Among those, the models proposed by Jukes & Cantor (1969) (JC), which assumes equal base frequencies at equilibrium and equal rates for all substitution types, and Kimura (1980, 1981) (K2P, K3ST), which assumes equal base frequencies at equilibrium but a transition bias in substitution rates, are the simplest. Other relevant models were proposed by Tamura & Nei (1993) (TrN), with unequal base frequencies at equilibrium and two classes of transition rates, Hasegawa *et al.* (1985) (HKY85), a "K2P" but with unequal base frequencies, and Felsenstein (1984) (F84), similar to TrN but with a two-parameter approach to the transition bias.

The TrN model is particularly worth of consideration in mtDNA studies since it was developed as an attempt to characterise the control region evolution in primates (Nei & Kumar 2000). Tamura & Nei (1993) found that the relative frequency of transition between pyrimidines (T-C) is almost twice as large as that between purines (A-G), a low frequency of guanines in the light strand, and suggested that the rates and patterns of mutation may be different between the two strands.

From the substitution model instantaneous rate matrix it is possible to derive a matrix of change likelihoods along a tree branch, the substitution probability matrix used by ML methods (Yang 1994a). When working with protein-coding sequences, it is recommended to analyse both the original DNA sequences, with a model allowing rate heterogeneity among codon sites, and the translated proteins, to account for the effect of selection at the amino acid or codon level (Reeves 1992). The issue is that selection upon protein variation, either at the amino acid or codon level, may represent a violation of the assumption of independence among sites (Goldman & Yang 1994). The impact of selection will be reflected in the frequency disparity between silent and replacement amino acid substitutions and in the increasing randomisation of third codon position with evolutionary divergence. Because of the structure of the genetic code, synonymous changes occur mainly at the third position of codons, whereas almost all substitutions in the first two positions of codons are nonsynonymous (Graur & Li 2000). The rate of nonsynonymous substitutions depends on the intensity of purifying selection determined by functional constraints (Jukes & Kimura 1984). To restrict the analysis to the first two

nucleotides of each codon is appropriate when reconstructing deep phylogenies; a more general approach is to infer the protein sequences from the gene sequence and estimate synonymous versus non-synonymous substitutions (Nei & Gojobori 1986; Li 1993b).

For all time-reversible models, the probability of change in state of a character is related with branch length, which depends on the interaction μt, where μ is the substitution rate and t is time (Felsenstein 1981). A branch can be "long" if it represents a long period of evolutionary time or if the mutation rate is high. Without assuming a perfect molecular clock, the length of a branch just represents the expected number of substitutions per site along the branch and it cannot be converted on an estimate of actual evolutionary time (Swofford *et al.* 1996). In contrast, if we assume that the substitution rate is approximately homogeneous across lineages, the likelihood can be estimated from branching lengths as evolutionary times. Such a molecular clock model evaluates rooted, rather than unrooted, trees and it is more efficient if the clockwise assumption is valid; a likelihood ratio test (LRT) to compare the likelihoods of a clock model (ultrametric tree) versus a branch-length model (metric tree) was outlined by Felsenstein (1981).

The selection of the model of evolutionary change, at the basis of the likelihood estimation of each candidate tree in ML methods, is usually accomplished by how well it fits with the data, using for this purpose a goodness-of-fit statistic (Huelsenbeck & Rannala 1997; Goldman & Whelan 2000; Ota *et al.* 2000). Two of the most widely used such statistics are the LRT, which assumes independent evolution among sites (Navidi *et al.* 1991), and the Akaike information criterion (AIC) (Akaike 1974), which imposes a penalty for unnecessary parameters in the model (Hasegawa 1990).

All the evolutionary models considered above assume rate homogeneity among sites and it has been demonstrated that ML is very sensitive to violations of this assumption (Gaut & Lewis 1995). Basically, in the case where rate heterogeneity across sites exists in the data, but it is not incorporated in the substitution model, ML estimates are as vulnerable as MP ones to the Felsenstein zone. The number of multiple substitutions is underestimated and this becomes much more serious when lineages are highly divergent or the mutation rate is high (i.e. for longer branches).

Rate heterogeneity can be incorporated, through an additional relative rate parameter, into the probability matrix for ML analysis. To assign a relative rate parameter value to each site, the values may be estimated from the data or *a priori* information about the sites in terms of function (e.g. first, second, and third positions of a protein-coding gene) is used. Several stochastic models that explicitly incorporate rate variation among

sites are available, where the probability of each site evolving at any rate is contained in a probability distribution, discrete or continuous. Respectively, site likelihoods are calculated by summing or integrating, over rate categories, the likelihoods of the site given each rate, weighted by the probability that the site is drawn from each category (Felsenstein 1981). The simplest model is an invariable-sites model, based on a discrete distribution with only two categories, assuming a fraction of sites as invariant and the remaining sites as mutating at the same rate (Hasegawa *et al.* 1985). The proportion of invariable sites can be estimated from the data or treated as a parameter that is optimised for each tree. The gamma (Γ) distribution (Yang 1993) is the most widely used continuous distribution for modelling rate heterogeneity across sites and it is characterised by two parameters, a shape parameter α and a scale parameter β. By setting β to $1/\alpha$, a distribution with a mean rate of 1 is obtained, which has the advantage of creating a correspondence between branch length and expected number of substitutions per site. Since α is equal to the inverse of the coefficient of rate variation, the equal rates among sites models are special cases of the Γ model when α is infinity and hence all sites have relative rate of 1. Because the calculation of site likelihoods by integration over a continuous distribution like Γ is computationally demanding, Yang (1994b) developed a "discrete Γ" model, also α-characterised, where the means of few representative rate categories provide a good approximation to the whole spectrum of rates in a continuous Γ (Felsenstein & Churchill 1996). Finally, it is becoming very common to use mixed models with both an invariable fraction of sites and a fraction with Γ-distributed substitution rates, "I+Γ" models (e.g. Gu *et al.* 1995).

The several parameters incorporated in a model of evolution must be estimated from the data or supplied on the basis of external evidence. These parameters include the tree topology, the branch-length estimates (specific to each topology), relative rate parameters and base frequencies, the gamma shape parameter, and the proportion of invariable sites. Ideally, the search for globally optimal values of these parameters would imply evaluating every possible tree and optimising jointly all parameters of the model for each tree, selecting the tree(s) with the highest likelihood. Because this procedure can be computationally intractable, a heuristic alternative must be used even if this does not guarantee a globally optimal solution. A possible strategy is to estimate the model parameters on some reasonably good tree for the data (e.g. a parsimony tree or a ML tree under the JC model) and then use these estimates to search for better trees under a desired model. Such successive approximations strategy would continue until the same tree is found in consecutive iterations (Swofford 2002).

2.2.4 Distance methods

As explained above, one of the potential problems with parsimony methods is their difficulty in coping with cases of rate heterogeneity, substitution saturation, and homoplasy. Such homoplasy may take the form of parallelism (non-homologous similarity acquired from the same ancestral condition), convergence (non-homologous similarity stemming from distinct ancestral states), or reversal by secondary loss across lineages (Page & Holmes 1998). The net consequence is that MP usually underestimates the amount of evolutionary change unless the substitution rate is extremely small (Swofford *et al.* 1996). Similarly to ML character methods, distance methods that adequately account for unobserved substitutions, by using corrected distances, will also perform better than MP in cases where high levels of homoplasy are likely. The corrected distances, estimates of the true evolutionary distance between sequences, are calculated using the same type of evolutionary models employed in ML analyses.

However, it has been shown that phylogenetic reconstruction based on these estimates of the mean number of pairwise substitutions per site since divergence from a common ancestor perform worse than ML methods (Hasegawa & Fujiwara 1993; Yang 1994c). In particular, distance methods require more data (e.g. sequence length) to achieve the same level of accuracy since their variance is inversely proportional to sequence length. In any case, distance methods, even if a less desirable approximation to likelihood (Cavalli-Sforza & Edwards 1967), can be useful when ML analyses are intractable due to data set sizes.

If two sequences of length N differ from each other at n sites, then the proportion of differences n/N is the degree of divergence, also called Hamming distance (D), usually expressed as a percentage (n/N x 100%). The number of nucleotide substitutions between two sequences is generally expressed in terms of the number of substitutions per nucleotide site (K), directly proportional to the mutation rate (μ) and inversely proportional to the selective constraint (Kimura 1983), rather than the total number of substitutions between the two sequences. This facilitates comparisons among sequence pairs that differ in length when comparing or discussing, for instance, rates of nucleotide substitution: $r = K/2T$, where T is the time span since the divergence of the two taxa (Graur & Li 2000). Only by correcting the dissimilarity (D), or uncorrected distance (p-distance), between two sequences, either for multiple unobserved substitutions at the same site or for rate heterogeneity among sites, under the assumption of a particular evolution model will the

pairwise distances conform to an additive-tree model (Zharkikh 1994). A particular example of distance correction concerns the separate estimation of transition and transversion substitutions when divergence is high and a transition bias is apparent, since in such cases transitional substitutions are likely to be saturated (Tajima & Takezaki 1994). Transition biases are a clear proof that, although mutations are random with respect to their effect on the fitness of the organism carrying them, the direction of mutation is constrained, and therefore non-random (Graur & Li 2000). When not addressed, substitutional saturation, like any other homoplasic instance, will only constitute phylogenetic noise and contribute to an increase of the variance of any evolutionary distance estimate.

Pairwise distances can be additive, which assumes that the evolutionary distance between two taxa equals the sum of the lengths of the branches forming the path connecting the two taxa, or ultrametric, when besides additive it is assumed that every common ancestor is equidistant from all its descendants. It is important to note that, in an additive tree, the most similar sequences/taxa may not be the most closely related, whereas similarity and evolutionary relationship will only coincide exactly in an ultrametric tree (Page & Holmes 1998). Stochastic and sampling errors, such as missing data distances between sister taxa, will cause deviation of the estimated evolutionary distances from perfect tree additivity even if evolution proceeded exactly according to the model used for distance correction. Although the internal consistency of additive-tree techniques must be seen undoubtedly as meritory, this places extra weight in the decision of which model to transform the distance data should we choose to turn the matrix additive-tree compatible (Huelsenbeck 1995b).

Additive-tree, or metric-tree, methods attempt to fit the data into an additive tree by finding the optimal values for the adjustable parameters: the split pattern and the branch lengths, or edge lengths (Page & Holmes 1998). In some methods, such as the one described by Fitch and Margoliash (FM) (1967), the optimality criterion, or objective function, is to minimise the deviation of the path-length distances from the distance estimates; deviation evaluations are mathematically accomplished using either squared differences, usually weighted, or absolute differences. These methods assume that each pairwise distance measurement is independent, but this usually does not hold since all the lineages have a common evolutionary history. So, trees will be less resolved than they would be if the samples were in fact independent and systematic errors in the distance estimates can be multiply sampled; neither MP nor ML are affected by these problems. A

particular type of additive-tree approach is the Minimum Evolution (ME) method (Kidd & Sgaramella-Zonta 1971; Rzhetsky & Nei 1992) since here the criterion to fit the branch lengths and the criterion to evaluate and compare trees are not the same. The first is an unweighted least-squares criterion, similar to some that can be found in older additive-tree methods (Cavalli-Sforza & Edwards 1967), whereas the second seeks the tree with the sum of branch lengths absolute values that minimises the value of the first criterion. As long as negative branch lengths are not allowed when computing deviations using single-criterion methods (e.g. FM), it is unclear if the more sophisticated ME method is at all superior in performance (Kuhner & Felsenstein 1994). Ultrametric methods, which assume a molecular clock running at the same rate in all lineages, have the advantages of being fast and efficient but they are essentially unrealistic (Sourdis & Krimbas 1987).

A straightforward alternative to distance corrections under an explicitly assumed evolutionary model is to estimate pairwise distances using maximum likelihood. This has the advantage of allowing model parameters, such as the transition:transversion ratio, to be maintained at a consistent value across all pairwise comparisons. To estimate the model parameters we can perform several phylogenetic analyses with different ranges of parameter values, and then choose the ones that most improve the optimality criterion in question. Another approach is to estimate parameters using ML on trees obtained using simpler distances and then apply their mean value (if the values are similar) or the value from the tree with the highest likelihood (if the values are disparate) to calculate the input distances in a distance-criterion tree search. These procedures seek to produce an advantageous mixed strategy where the choice of the pairwise distance is ML-guided, instead of arbitrary, and tree search is based on a computationally easy and fast objective function such as ME.

A special group of distances, the log-determinant (LogDet) (Lockhart *et al.* 1994) and paralinear (Lake 1994) distances, yields additive pairwise distances even without assuming base frequencies at a constant, equilibrium, value and in cases of changing base composition, if not too extreme, among the taxa under comparison (Nei & Kumar 2000). Additionally, they are robust to inflated sampling errors due to small size sequences and they can be applied to protein data. However, they possess the drawback of assuming equal substitution rates across sites in the sequences. In order to correct for this, sites should be first grouped in distinct rate classes, to which LogDet transformations are applied, and the separate estimates then summed in a final distance matrix that is used for the tree search.

In conclusion, when selecting a transformation operator for a matrix of pairwise sequence dissimilarities, we need to consider the trade-off between robustness, provided by the most general models, and smaller variance, usually better achieved through simple models with unsophisticated assumptions. Considering the fact that if two transformation methods give similar distance values for a set of data, to use the simpler one, with smaller variance, is recommended, a comparison of pairwise distance matrixes for several estimators is an advisable preliminary step. This is a similar strategy to the one we should conduct when, under the ML criterion, we compare simple and complex models for detection of differential impact upon them of sample-size-dependent bias (Goldman 1993a,b; Rzhetsky & Nei 1995; Whelan & Goldman 1999). This is particularly true when sequence length is below the order of magnitude of 1000 base pairs (1 Kb).

It is also important to note that for closely related sequences, the estimates obtained by the different methods are quite similar to one another (Tateno *et al.* 1994).

2.2.5 Tree searching

As stated earlier, methods of phylogenetic reconstruction with an explicit optimality criterion separate the operations of tree evaluation under the objective function in question and of exploration of the tree space in search of potentially more optimal trees. When working with data sets of small to moderate size it is computationally feasible to resort to exact algorithms that guarantee the recovery of all optimal trees. One of such ideal tree-search methods is the exhaustive search on which, as implied by the name, the scoring of every single possible tree is involved and computing times are prohibitive for phylogenies with more than ten taxa (Felsenstein 2003). An alternative exact algorithm that does not require exhaustive evaluation is the branch-and-bound method (Hendy & Penny 1989) which still calculates scores for every new added node but saves computation time by discarding all the parts of the search tree that only contain suboptimal solutions. Suboptimal regions are those that confer a higher (worse) score than the chosen optimality value (the upper bound), at a given node, to the search tree even without further investigation of branches within them. The branch-and-bound method is usually very effective, except if phylogenetic noise is abundant in the data set, and allows recovery of the best trees within acceptable computation times for phylogenies with twenty or more taxa. Only in the worst-case scenario, the one in which the random starting tree is the best possible tree, does branch-and-bound search as many trees as the exhaustive procedure (Hall 2001). In order to avoid an arbitrary choice of the upper bound it is advisable to

obtain an estimate of a near-optimal score by using a non-exact algorithm of tree search, for instance from the family of heuristic methods.

Heuristic approaches are the preferred ones when data set sizes are large and the only solution is to sacrifice optimality for computation time. Heuristic tree search operates through hill climbing methods where, instead of seeking for a global optimum in the tree space, an initial tree is rearranged until it becomes a local optimum impossible to improve in relation to the selected optimality criterion. Stepwise addition of the taxa to an initial tree is the most common starting point for further rearrangements in heuristic methods; the way such stepwise addition is performed can vary from random to strictly following the order in the data matrix and it is yet another parameter for test trials. The random addition of sequences it is an attempt at providing different starting points to the heuristic search that simultaneously aims to increase the chance of finding a global optimum and to assess the convergence of independent replicates' outcomes. Since stepwise addition usually does not find optimal trees unless the number of taxa is small and/or the data is free of phylogenetic noise, heuristic searches are usually improved by branch-swapping rearrangements. These, through exploration of the adjacent tree space, aim to improve the likelihood of finding the globally optimal tree or, at least, a less local optimum. However, the chance of success of this strategy is also dependent on the existence of a climbing route from the initial tree to the global optimum and heavily affected if large valleys or plateaus in terms of score exist in the surrounding tree space. Branch swapping rearrangements can be conducted accordingly with several different techniques (see Felsenstein 2003 for a review). The most widely used rearrangement strategies, and in increasing order of complexity and inclusiveness, are the nearest-neighbour interchanges (NNI), subtree pruning and regrafting (SPR), and tree bisection and reconnection (TBR).

An alternative to stepwise addition is the star decomposition family of divisive clustering methods, of which the most employed member is the neighbour-joining algorithm (Saitou & Nei 1987). It is important to note that both stepwise addition and star decomposition evaluate only the implications for tree scoring of each new node/connection at the time and disregard if the steps taken, which are irreversible in these types of algorithm, may eventually lead to entrapment in a local optima.

Heuristic methods are not the only options when the data set size and noisiness are not compatible with exact procedures. Several non-hill climbing algorithms, less susceptible to irreversible attraction to local optima, are available but they have the disadvantage of lacking yet a solid base of testing with real data in order to allow their

behaviour and performance in different situations to be known. Simulated annealing using Metropolis algorithms and several different versions of genetic algorithms, some of them also including tree rearrangements (Goloboff 1999), are good examples of such promising but not widely experimented techniques (Felsenstein 2003).

Exhaustive, branch-and-bound, and heuristic methods are tree search strategies suited to use as standard for tree evaluation of the value of the objective function from any optimality criterion of phylogenetic reconstruction. However, as mentioned earlier, some methods for phylogenetic inference concurrently build and select the optimal tree in accordance with a predefined set of steps. Clustering methods, such as the UPGMA and WPGMA (weighted PGMA), for representing distance data in the form of an ultrametric tree, and additive-tree methods such as the NJ and distance Wagner method (Farris 1972; Tateno *et al.* 1982), are the most popular among such strictly algorithmic approaches. Cluster analysis it is fast and can be easily applied to very large data sets but usually it is unable to cope with noisy data for delivering a best tree, which will be quite divergent from the optimal tree of the ultrametric assumption. Additive-tree algorithms are clearly less problematic since their assumption is less stringent and still allow very rapid computation times. Nevertheless, as seen above, they still require correction of the dissimilarity matrix for superimposed substitutions in order to fit the data to an additive tree. The discrepancy between observed distance and branch lengths (patristic distances) can be used to measure how good the fit is between the data matrix and the selected tree (Page & Holmes 1998). It is important to keep in mind that when applying a popular technique such as the NJ we obtain a single tree that, besides not being explicitly optimised for any evolutionary criterion, will not give us any information about how much really is our data tree-compatible. In contrast, optimality criterion-based methods of phylogenetic inference tend to display a diagnosable behaviour when a tree-like diagram does not adequately portray the data, by rendering alternative optimal topologies quite distinct of each other (Hillis *et al.* 1996).

2.2.6 Statistical assessment of trees

Similarly to any other type of statistical analysis of genetic data, phylogenetic inference may suffer of random and systematic errors. Random errors are deviations between a population parameter and its estimate caused exclusively by a limited sample size on the basis of the estimate. Systematic errors are deviations between a population parameter and its estimate due to incorrect assumptions in the estimation method. To

increase sample sizes allow us to gradually attenuate the impact of random errors but also has the undesirable effect of amplifying the effects of systematic errors, which will tend to infinite. It is therefore critical, in order that one takes full advantage of comprehensive sample sizes, that the assumptions of a selected model to describe the evolutionary patterns of our data are not violated in a critical way. Otherwise, our model can be inconsistent or "positively misleading" (Felsenstein 1978) and lead to a situation where support for the wrong conclusions is reinforced when additional data is considered, as in the case of inference under parsimony when rate heterogeneity among lineages is present. On the other hand, sample-size-dependent bias will always affect the selected method and prevent it to deliver the optimal result, although it should be noticed that random errors affect differently the several assumptions of a model (Hillis *et al.* 1994). In summary, when carrying out phylogenetic inference, both the sensitivity of the results to random errors and the consequences of violations on our model assumptions need to be assessed.

A potential source of systematic error is the existence of asymmetrical base composition among lineages when the model assumes equal equilibrium base frequencies throughout the tree. In such cases, clades may result of convergence in base composition and not of a close evolutionary affinity, although this problem is less likely when our data set only includes closely related taxa (Rzhetsky & Nei 1995). A χ^2 test for base frequency equilibrium between lineages can be performed to assess this putative source of bias (Swofford 2002). Also, as referred above, to compare results of phylogenetic inference using distance data corrected with the LogDet transformation against results obtained with a distinct distance-criterion method, or with a ML approach, may reveal the net effect of unequal base composition among taxa.

When more divergent taxa are included into the analysis, as in the case of tree rooting with the outgroup method (Lions-Weiler *et al.* 1998), the addition of both random and systematic error are likely. To gain some idea of the effect of the systematic component of error, it is recommend to investigate how incongruent are inferred trees with and without the presence of the distant taxa.

Tree congruence should be also assessed when different gene data sets are used for phylogenetic inference. One strategy for this to be carried out is by fitting each data set to the tree(s) derived from the other data set(s); the measure of this cross-fitting will be expressed on the tree scores for each data-tree combination, which if not congruent may indicate significant evolutionary differences among genes. This issue of tree congruence falls into a more general question in phylogenetic analysis of how and whether multiple

data sets should be combined (Hillis 1995). It is most likely that the best estimate of phylogeny is derived from a combined analysis of all relevant data (Hillis 1987), but combination should be conditional upon congruence evaluation (i.e. that differences between data sets are only due to stochastic variation) (Huelsenbeck *et al.* 1996). This is the most balanced approach since it lies between the two diametrally-opposing positions of the total evidence approach (Kluge 1989) and the consensus approach (Miyamoto & Fitch 1995). The total evidence approach, or character congruence, advocates combining all characters in a single analysis regardless of congruence of the individual data sets. The consensus approach, or taxonomic congruence, argues that different data sets should always be analysed separately, as long as they have different biological properties.

A combined phylogenetic analysis makes two important assumptions: first, that the same underlying tree would be obtained using each data set separately; and second, that the selected method of analysis for the combined set is adequate for each of the individual data sets (Bull *et al.* 1993). However, a violation of the first assumption can occur when individual genes present, even if tightly linked as in the mtDNA, variable lineage sorting patterns simply due to different selective pressures. Likewise, a violation of the second assumption may occur because a given phylogenetic method is inconsistent or biased for some data sets, for instance in the case where the method is not robust to rate heterogeneity among nucleotides. The advantage of the conditional combination approach seems intuitive: if different data sets are congruent, to combine them makes the most use of the total information; if incongruence goes beyond the one expected by random heterogeneity then separate analyses avoid risks of widespread assumptions violation. Among the several partition homogeneity tests available, the incongruence length difference (ILD) test (Farris *et al.* 1994), which measures the extra homoplasy entailed by combining data sets, seems to be one of the most useful (Cunningham 1997), at least if the data is not very noisy. The ILD test possesses the advantage over other, also powerful, methods, such as the likelihood heterogeneity test (Huelsenbeck & Bull 1996), of being computationally much faster.

There are probably more ways of determining the sensitivity of results from a phylogenetic inference exercise to the stochastic error associated with the sample size of a data set than to investigate assumption violation implications (Li & Zharkikh 1995). When addressing sample-size-dependent bias, the first issue worth investigation should be to know if our data has a clearly, in statistical terms, stronger phylogenetic signal than just random noise. An effective way of examining if the data set contains more hierarchical structure than could be conveyed by chance alone is to perform permutation tests (Archie

1989; Faith & Cranston 1991). The null hypothesis, of absence of significant phylogenetic signal, is recreated by randomization through permutation of the character states among taxa while holding constant, in the case of DNA data, the base frequencies. An alternative method to permutation tests is to examine the shape of the tree length distribution for either all possible trees or a random sample of them (Huelsenbeck 1991). Hillis & Huelsenbeck (1992) demonstrated that in data sets with true hierarchical structure the shape of these distributions is markedly left-skewed in a degree that can be quantified with a statistic (g_1).

There are many circumstances when we want to compare trees, for instance obtained with different algorithms, and possess some kind of statistical support to make a decision of which of them describes best the data. Templeton (1983) developed a non-parametric test where the null hypothesis to explain differential statistical support between trees is random error, and Kishino & Hasegawa (1989) devised a parametric test that uses tree length differences as one statistic expected to be zero under the null hypothesis. The standard LRT (Muse & Weir 1992) can also be used for tree comparison, but only if one tree is a subset of a second tree. Here, the statistic δ is compared against the χ^2 distribution, with degrees of freedom equal to the additional number of branches in the more fully resolved tree. The strict LRT is invalid if the trees are not nested since, in such case, the number of parameters in the two trees is equal and, consequently, the degrees of freedom are zero. The only way to surpass this restriction is to generate an expected distribution of δ through simulation of the null hypothesis, instead of assuming a χ^2 distribution, but this can be infeasible in terms of computation time for large data sets and under the ML criterion.

One process of summarising information common to two or more trees is to derive a consensus tree (Margush & McMorris 1981). The different methods of computing consensus trees vary essentially in the level of how frequently the same information must be shared among the trees in order to appear in the consensus. A strict consensus tree includes only those groups that occur in all trees being considered, an approach only useful if not very different trees are being compared, whereas a majority-rule consensus is more relaxed and will retain those splits found in a majority of trees (Page & Holmes 1998).

Since phylogenetic inference is very often a support tool for interpretations in molecular taxonomy, tests for evaluation of the validity of monophyletic groups in a tree are of central importance. One of such tests, applicable in the context of parsimony, is the decay or Bremer support index (Bremer 1994), obtained by calculating the tree length difference between the shortest trees that contain versus lack the group in question. The

problem with this index resides in how to statistically assess the significance of its values in terms of a threshold level to accept monophyly.

Still in the context of the parsimony criterion as objective function for tree scoring, and considering the central role of homoplasy in affecting how able a parsimony tree is to depict the true phylogeny, several indices have been proposed to quantify the homoplasy of a given tree. The consistency index, C (Kluge & Farris 1969), measures the ratio between the minimum number of changes that an informative character could show on any tree and the actual number of changes for the tree in question. The retention index, R (Farris 1969), evaluates the proportion of apparent synapomorphy (a derived state that is shared by several taxa) that can be accepted as true synapomorphy, and not as homoplasy. Values closer to one for both C and R are indicators that the tree is little affected throughout by homoplasic changes of characters between nodes.

To assess the statistical support for all the branches in the topology of a given tree the most widely used methods are resampling techniques, such as the bootstrap and the jackknife, which estimate the sampling variance through resampling replications of the original data set (Felsenstein 1985; Penny & Hendy 1985). In non-parametric bootstrapping data points are resampled randomly with replacement and the proportion (P) of pseudosamples, which in total should be preferentially of several hundreds (Graur & Li 2000), supporting a given internal branch on a tree is recorded. P has been interpreted either as a measure of repeatability (Felsenstein 1985) or as a measure of accuracy (Felsenstein & Kishino 1993) of a given branch in the true tree. However, both of these ascribed attributes to the bootstrap proportions are now known to be unreliable (Hillis & Bull 1993; Li & Zharkikh 1995). Depending on the number of taxa, characters, and location of the branch, bootstrapping gives underestimates of accuracy at high bootstrap values and overestimates of accuracy at low bootstrap values. Low bootstrap values (50-90%) need to be strictly considered, as they mean that there is a good chance of getting a bootstrap value that high or higher even if the clade is wrong (Berry & Gascuel 1996; but see Mason-Gamer & Kellogg 1996). The bootstrap procedure assumes that every position evolves independently and that the distribution of sampled sites reflects the distribution of all the sites. Statistical tests indicate, however, that very often the four nucleotide types and variable sites are not evolutionarily independent and identically distributed across the genome (Cummings *et al.* 1995). Unless modified in order to minimise somehow the above drawbacks (Hall & Martin 1988; Zharkikh & Li 1995), it is apparently more correct

to see bootstrap values of branch support as an index rather than as a thorough statistical assessment (Swofford *et al.* 1996).

2.2.7 **Tree rooting**

A rooted tree is often desired since by rooting a tree, with for example the addition of outgroup taxa, we can gain information about character polarities, which may anyway be obscured by homoplasy along the evolutionary history, and the ancestor-descendant relationships between nodes (Page & Holmes 1998). To include more than one outgroup taxon is recommended to test the assumption of ingroup monophyly, but since such test is one-tailed the guarantee that the root does not lie somewhere within the defined ingroup must come from extrinsic evidence.

Considering that the majority of the evolutionary models currently in use are time-reversible, the likelihood of a tree is generally independent of the location of the root or of how the tree is rooted. Consequently, as for most of the parsimony methods, ML estimation is also usually limited to the inference of unrooted trees and other assumptions must be invoked to root such unrooted estimates.

How to root a tree is one of the most difficult decisions in phylogenetic analysis, even more so because the choice of outgroup taxa can exert a strong effect on the analysis, particularly when using distance methods (Hall 2001). Sister-group lineages should be preferred than more distant clades in order to avoid randomisation of ingroup sequences and "long-branch attraction" effects (Wheeler 1990). Nevertheless, multiple and carefully chosen, in terms of gradual increasing divergence, outgroups may improve MP analysis by diluting the potentially negative effects of the longest branch in the tree (Smith 1994). In general, tree reconstruction for data sets with large numbers of taxa it is usually extremely difficult due to the challenge of correctly inferring every single section of the true topology. However, when aiming to discover the relationships of a smaller number of taxa it is often rewarding to include many other interspersed additional taxa, in order to decrease the number of sparse phylogenetic regions in the tree, and prune them from the optimal tree afterwards.

In conclusion, it can be said that tree rooting should be only performed when unavoidable for the end in target, as for example when trying to determine the evolutionary sequence of branching/divergence among a group of taxa. Unrooted trees are all that is necessary when trying to answer specific phylogenetic questions concerning the monophyly or paraphyly of certain clades. Besides, unrooted strategies allow much

reduced computation times when searching and optimising trees (Graur & Li 2000), which indirectly also makes more likely the recovery of a better tree (Page & Holmes 1998).

2.2.8 Sampling in phylogenetics

Considering that the underlying processes of molecular evolution vary across the genome, reflected in different mutation and selection patterns, the extent to which phylogenetic inference depends on the particular sample of DNA sequenced is critical to be evaluated. Base composition and nucleotide variability are extremely heterogeneous across the mitochondrial genome (Churchill 1989; Cummings *et al.* 1995). Otto *et al.* (1996) showed, using mtDNA, that a gene tree recovers less than 50% of the times the genome tree, which is likely to represent the true species tree if the same tree is produced by several different methods of phylogenetic reconstruction. However, other conclusions from the same study are also relevant if we were to try being the most accurate possible when making phylogenetic inference from gene sequences, either complete or even if only partial. Maximum likelihood reconstruction was the best method (comparing with maximum parsimony and neighbour-joining) in yielding the genome tree from gene sequences; neighbour-joining was second best. When using small sequences, which should be always avoided whenever possible (Saitou & Nei 1986), only clades with 95% bootstrap support should be accepted as probably reliable estimates. For the same number of sites, sequences from scattered regions inferred the whole genome tree significantly more often than sequences from contiguous segments. By producing sequences from scattered blocks, one can also gain a more complete picture of the genome evolution. Finally, although we are unlikely to obtain the whole genome tree using short sequences, the recovered tree will, on average, be fairly similar to the genome tree. This is even much more so if the regions are carefully chosen in order that the evolutionary rate is high enough to segregate the taxa of interest but not so high that signal is lost to noise (e.g. Graybeal 1994).

Chapter 3. Mitochondrial DNA phylogeography of the small-spotted genet (*Genetta genetta)*

3.1 Introduction

Phylogeography is a powerful discipline that links biogeography, population genetics and molecular phylogenetics (Avise 1994). This now widely used approach has provided valuable insights into numerous evolutionary questions (Avise 2000a), particularly towards a better understanding of general biogeographic history and taxonomic relationships of morphologically and/or geographically designated populations (e.g. McKnight 1995; Wake 1997; Zamudio *et al.* 1997).

The number of studies that attempted to relate present-day genetic structure of species in the northern hemisphere with historic events, such as changes in geographic distribution during their evolution, and current levels of migration versus genetic drift is now quite extensive (Avise 1998; Taberlet *et al.* 1998; Hewitt 2000). In contrast, although the intraspecific molecular phylogeography has been recently characterised for some pan-African vertebrates (Simonsen *et al.* 1998; Uphyrkina *et al.* 2001; Eggert *et al.* 2002), there is still a relative paucity in the number of similar studies concerning African taxa. Consequently, detailed knowledge of the general biogeographic patterns and their relation with past climatic and geologic processes remains elusive for large areas of the continent.

Small-spotted genets (*Genetta genetta* Linnaeus, 1758), also known as common genets, provide a potentially excellent system to evaluate phylogeographic patterns across Africa and historical relations between populations on this continent and the adjacent land masses of Southern Europe and the Arabian Peninsula. This species has a broad but disjoint distribution within Africa, ranging continent-wide outside the main deserts in wooded dry savannahs north and south of the equator and in regions with a "Mediterranean-like" climate, such as the Cape and North Africa (Lariviere & Calzada 2001). It is essentially absent from the areas within the tropical rainforest belts of Central and West Africa (Gaubert 2003b). Outside Africa they occur in the south of the Arabian Peninsula and in southwestern Europe. The populations of genets in Europe are mainly distributed through Portugal, Spain, and France, in areas of macchia and holm/cork oak, but they also appear in certain countries of central Europe, where they are nevertheless rare (Leger *et al.* 1998). Presently available evidence seems to suggest that the genet, as for some other terrestrial vertebrates of African origin (Dobson 1998; Paulo *et al.* 2002), was

introduced in Europe by humans (Crawford-Cabral 1981b; Morales 1994; Geraads 1997). Although it has been proposed by some authors (Livet & Roeder 1987; Perez & Gutierrez 1990; Kingdon 1997) that genets were brought into Europe as pets and/or domestic rat-catchers with the Arab invasions, the timing of the colonisation and the geographic origin(s) of the founder stock(s) are still undetermined. Concerning the populations in the Arabian Peninsula, the available information is even more scant and there is no certainty about when genets arrived to the area, their putative invasion routes, and if they were introduced by humans or not. The recent estimate (Gaubert *et al.* 2004) of the Middle Pliocene as the period for the appearance of the *G. genetta* lineage, based on a substitution rate of about 1% per million years, may bear some relevance when deciding upon alternative scenarios for this region.

The taxonomy of *G. genetta* is still debated and the specific or subspecific status of the main geographic partitions has a history of controversy, so far without a clear consensus (Schwarz 1930; Wenzel & Haltenorth 1972; Rosevear 1974; Schlawe 1980, 1981; Crawford-Cabral 1981a). The most recent review of the genus *Genetta* phylogeny (Gaubert *et al.* 2004) using cytochrome *b* sequences and morphology raised, due to the estimated very high levels of intraspecific genetic divergence, the hypothesis of the existence of several allopatric species within *G. genetta*. This is just the most recent confirmation of a dilemma first faced by Matschie (1902) and Neumann (1902) when describing *G. genetta* specimens from disparate geographic origins. All these studies point to the conclusion that, due to significant ecological and geographical variability in morphological traits, which can be misleading when deciding true evolutionary affinities, and the disjoint distribution of several populations, it is probably more advisable to regard *G. genetta* as a species complex. In the most detailed analysis carried so far on the taxonomy, biogeography and evolution of common genets, Crawford-Cabral (1981a,b) discussed all the previously described forms, highlighted by different authors at different times, as species, subspecies, races/ecotypes, or just synonyms. In a study essentially of linear craniometry, but in which coat patterns were also considered even if only qualitatively, the author interpreted the results as evidence that *G. genetta* is a single species but structured in subspecies across its range. In some cases the discussed subspecies have an obvious craniometrical and/or coat pattern basis, but in others they correspond more to the recognition of the potential for evolutionary divergence associated with allopatric distribution, even if clear-cut differentiation was not detected with the analytical techniques employed.

The author includes the European populations and the ones in North Africa, craniometrically similar but highly variable in coat pattern, together within the subspecies *genetta*. As the colonisation of Europe is apparently a very recent event, and assuming North Africa as the area of origin of the founders, to include both sides of the Mediterranean as members of the same subspecies seems to be the most appropriate taxonomic decision. However, a possible differentiation, suggested by the coat pattern, within North Africa associated with the Atlas Mountains is also referred to in the study (*afra* in Morocco and *bonapartei* in Algeria and Tunisia); such a biogeographic pattern caused by the Atlas-Tell boundary it is found in other vertebrates (Steinfartz *et al.* 2000). The populations from Senegal to Niger and Nigeria were included in the subspecies *senegalensis*, different in terms of coat pattern from the ones occurring between Niger and Sudan, the subspecies *dongolana*. In East Africa, he considered a subspecies in Ethiopia and northern Kenya, *hararensis*, and one in southern Kenya, Tanzania, northern Zambia and northern Mozambique, *neumanni*. These two subspecies are well differentiated in skull measurements, but since the same does not occur between *hararensis* and specimens from Somalia, although they differ in coat pattern, the subspecific status of the Somalian population (*guardafuensis*) was left conditional upon additional evidence. In the Arabian Peninsula, the subspecies *granti* is craniometrically distinct and easily diagnosable due to a small body size. In Southern Africa, two subspecies, different in the coat pattern but not in skull measurements, are recognized: *pulchra* in a vast area from Namibia to the Transvaal and *felina* in the Cape province and in the Orange Free State, the Orange River being the putative boundary between the two forms.

It is suggested that the Abyssinian plateau had a role in shaping how the subspecies are related to each other, relationships that can be summarised by considering two groups of subspecies, one including Europe, North, West and Central Africa, and another one including Arabia, East and Southern Africa. This was in contrast with the traditional view that clustered the *G. genetta* subspecies in three groups, one containing Europe and North Africa, a second one with Arabia and all the subspecies below the Sahara except Southern Africa, and a third one including Southern Africa (Schwarz 1930; Wenzel & Haltenorth 1972). Finally, the study described cases of convergence in the coat pattern, both in terms of hair length and coloration and apparently correlated with climate and habitat type, between distant populations occurring in biotopes with similar characteristics. However, when discussing the above list of possible subspecies the author attempted to avoid such homoplasic instances.

The subspecies concept is highly controversial, and depending on the authors, is regarded as a useful and quantifiable category (Avise & Ball 1990), epistemologically untenable (Frost & Kluge 1994), inconvenient by often covering true specific status (Burbrink *et al.* 2000), or of importance conditional upon the species concept considered (Kvist *et al.* 2003). Although subspecies can apparently be objectively determined (O'Brien & Mayr 1991; Patten & Unitt 2002), since in the proposition of *G. genetta* subspecies no such criteria were strictly followed, to designate them as forms or units it is probably the best compromise. The precise distribution areas and boundaries of several of these units are uncertain but Fig. 3.1 attempts to represent their approximate ranges. In appendix, at the end of the chapter, Figs. 3.I to 3.IX show museum skins photographs representing all but two (*bonapartei* and *neumanni*) of these morphological and/or geographical forms.

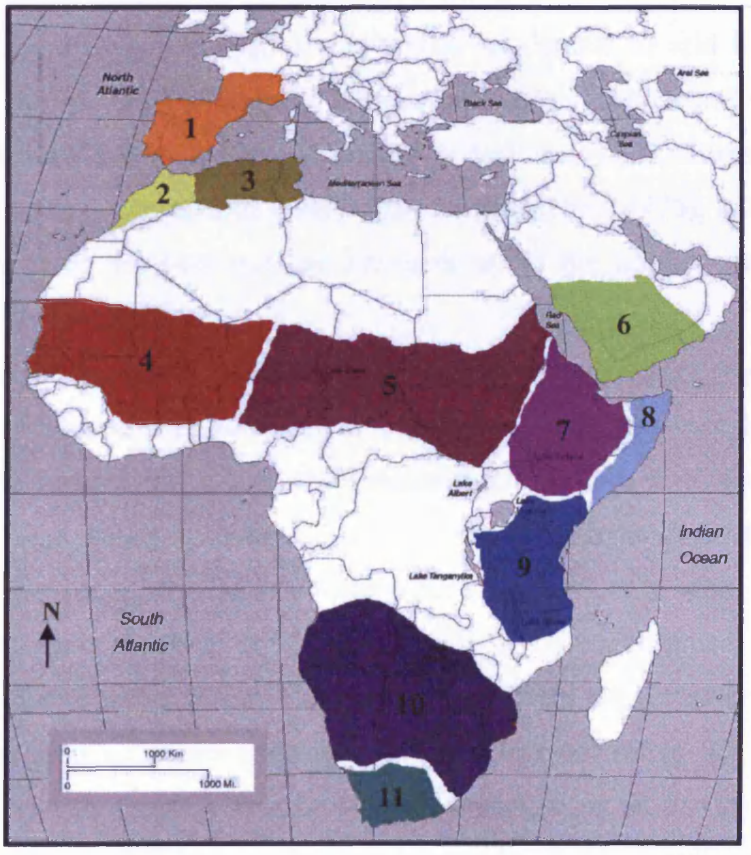

Figure 3.1 Map showing the postulated ranges of *G. genetta* forms that, among several others described up to date, may represent distinct evolutionary units of the *G.* genetta complex worth formal taxonomic recognition (Crawford-Cabral *pers. comm.*). 1- *genetta*, 2- *afra*, 3- *bonapartei*, 4- *senegalensis*, 5- *dongolana*, 6- *granti*, 7- *hararensis*, 8- *guardafuensis*, 9- *neumanni*, 10- *pulchra*, 11- *felina*.

More recently, Gaubert (2003b) has extended the previously described range of *felina* to Namibia, Angola and Zambia, and constrained the range of *pulchra* in South Africa to its northeast (Mpumalanga and Limpopo Provinces). However, the author acknowledges that in the three countries where, in consequence of his proposed extension of the *felina* range, the morphotypes are broadly sympatric, morphological differentiation is less obvious. The detection that some *felina* specimens possess significantly divergent mitochondrial haplotypes from any other *G. genetta*, and more similar to the ones of a separate genet species, *Genetta angolensis* Bocage, 1882, has led to speculation about a possible specific status for *felina* (Gaubert *et al.* 2004).

Genets are usually characterised by habitat specificity, and this ecological distinctiveness certainly allows the reduction of interspecific competition and probably has a role in preventing hybridisation (Kingdon 1977). In fact, the two most widely distributed genet species, *G. genetta* and *Genetta rubiginosa* nec Pucheran, 1855, which occupy a broad spectrum of biotopes, are sympatric in many areas without any sign of interbreeding (Smithers 1971). *Pulchra* and *felina* are typical inhabitants of arid bush and woodland areas and tolerate severe dry seasons, whereas all other genet species in Southern Africa are associated with mesic habitats (Skinner & Smithers 1990; Stuart 1991). However, hybridisation between *felina* and *Genetta tigrina* (Schreber, 1776), a species endemic to the coastal areas of the former Cape Province up to the border with Natal, has been detected (Gaubert *et al.* in press).

Taking into account the information above, and using mitochondrial DNA (mtDNA) data of specimens from almost the whole range of the species complex, several issues were addressed using the phylogeographic approach and analytical tools now available to it. The main evolutionary questions were: (i) are the currently described forms of *G. genetta* genealogically closer to each other than they are to other genets; (ii) is there any correspondence between how genetic variation is partitioned and the observed geographic structure and, if so, to which extent; (iii) what are the relative contributions of the present geographic distribution and of historical events to the estimated genetic structure; (iv) can the genetic data be helpful in suggesting an origin and timing for the foundation of the non-African populations; (v) are the genetic results compatible with the recognition of distinct evolutionary lineages and, if so, do they support previously suggested taxonomic units or indicate different arrangements.

In addition to this investigation of the phylogeography of *G. genetta*, the present study examines in a broader phylogenetic context the two most recently raised

evolutionary issues for the small-spotted genets in Southern Africa. They are the hypothesised species status of *G. felina* and its close phylogenetic association with *G. angolensis* (Gaubert *et al.* 2004), and the suggested occurrence of interspecific gene flow involving the *G. genetta* complex and other species in Southern Africa (Gaubert 2003b). A multispecies phylogenetic analysis was carried out including, besides *G. genetta*, all the other consensual genet species in Southern Africa: *G. tigrina*, *G. angolensis*, and *G. "rubiginosa"*. The name "*rubiginosa*" it is used here, although invalid (Schlawe 1980; Crawford-Cabral & Fernandes 1999), as a provisional designation since a new name has still to be ascribed to this species (Gaubert *et al.* 2003a.b; Grubb in press; Fernandes & Crawford-Cabral in press). *G. tigrina* (Fig. 3.X in the appendix of this chapter), the blotched genet, has a restricted distribution comprising the coastal areas of the Western and Eastern Cape Provinces, up to the border with the Natal Province, in South Africa; regions where a mixture of evergreen temperate forests and the presence of Mediterranean xerophytic hard leaf scrub ("fynbos") shapes a unique type of habitat in Southern Africa. *G. "rubiginosa"* (Fig. 3.XI in the appendix of this chapter) is a very polymorphic complex of forms, distributed from Ghana eastwards to Eritrea and southwards down to Namibia and the Natal Province in South Africa, commonly called the rusty-spotted genet (Roberts 1951). It is the most ubiquitous genet, occurring in a diversity of habitats ranging from tropical rainforest and woodland savannahs to steppe and montane biotopes (Crawford-Cabral 1981a,b; Crawford-Cabral & Fernandes 2001). It is closely related to *G. tigrina*, with both sometimes collectively designated as large-spotted genets, and hybridisation between the two species at their contact zone in the Natal Province has been hypothesised (Pringle 1977; Gaubert *et al.* in press). Although morphologically distinct, their level of genetic divergence is low and coalescence is estimated to have occurred during the Late Pleistocene (Gaubert *et al.* 2004). *G. angolensis* (Fig. 3.XII in the appendix of this chapter), the Miombo genet (Kingdon 1997), is distributed from Angola, through Zambia, southern Democratic Republic of Congo and Malawi, to Mozambique and Tanzania (Crawford-Cabral 1981b). The preferred type of habitat of this species is woodland forest of *Adansonia* and *Brachystegia* (Crawford-Cabral 1981b). Gaubert (2003b) reported isolated specimens collected from northern Botswana and Kenya (Mount Elgon). According with the most recent study on genet divergence times, this species appeared sometime between the Late Pliocene and the Pliocene-Pleistocene boundary (Gaubert *et al.* 2004).

3.2 Material and methods

3.2.1 DNA samples

For the phylogeographic analyses 96 *G. genetta* samples were used, of several different biological types, representing almost all the main geographic subdivisions within its range. To address the status of *felina* and the hypothesised gene flow between genet species in Southern Africa, 15 samples of *G. tigrina* from the Cape, a set of 16 samples of *G. "rubiginosa"* representative of all Southern Africa, and 5 samples of *G. angolensis* from Zambia were included. Table 3.I (in appendix at the end of the chapter) provides detailed information about the samples. Species or subspecies identification of samples by collectors or museum curators was assessed by comparison of the respective DNA sequences with a comprehensive, taxonomic and geographic, database of genet sequences that had been previously generated exclusively from specimens for which both the phenotype and sampling locality were known.

3.2.2 Laboratory techniques

3.2.2.1 DNA extraction

Genomic DNA was isolated from the samples using protocols in accordance with their biological type. For tissue biopsies, originating either from live-trapped or road-killed specimens and stored in suitable solutions [e.g. 95% ethanol with 100 μM EDTA or NaCl-saturated 25% DMSO (Dimethylsulphoxide)] immediately upon collection, both a "salting-out" protocol (Bruford *et al.* 1992) and a standard phenol-chloroform method (Sambrook *et al.* 1989) were successfully applied. The method described by Walsh (1991), which takes advantage of the nuclease-inhibiting chelating properties of the Chelex 100 resin, was used for DNA extraction from plucked or shed hair samples, whereas blood samples were extracted using a commercial kit (DNeasy Tissue Kit, catalogue #69506, Qiagen) and following the manufacturer's recommendations. Finally, the main source of DNA, samples from museum skins, were extracted with the same kit, but this time following the modifications described by Mundy *et al.* (1997) and using separate facilities and lab materials in an ancient-DNA-dedicated room isolated from the main lab. DNA extractions from ancient-DNA samples (e.g. museum skins and hair) were carried out in rounds, in such a way that the next round always included a set of randomly chosen samples from the previous round in order to authenticate ancient DNA results through independent events (Hummel & Herrmann 1994).

3.2.2.2 Preliminary study of common genet mtDNA

The dominant type of sample (museum skin; N=76) determined the size of the mitochondrial fragments examined for all samples, as it is unlikely to obtain, in a regular fashion, contiguous nucleotide information for more than 200 base pairs (bp) from typical museum skins (Hofreiter *et al.* 2001). Since this is the first population-level analysis of a genet species with molecular markers, no previous information about genetic variation patterns in the genet mtDNA that could be helpful for selecting suitable 200 bp blocks for this study was available. Using DNA extracts with high molecular weight from specimens representing all the main geographic populations of the species complex, levels of genetic variation were assessed for the two mitochondrial regions most widely used in phylogeographic studies of vertebrates, the control region and the cytochrome *b* gene (reviewed in Avise 2000a). Complete DNA sequences for the cytochrome *b* gene (1140 bp) and the control region (≈ 1430 bp) were produced via polymerase chain reaction (PCR) with versatile primers, and respective PCR conditions, as described by Kocher *et al.* (1989), Shields & Kocher (1991), Irwin *et al.* (1991) and Palumbi (1996). In addition to those, Carnivora-specific primers, provided by Stephan Funk (*pers. comm.*), were also used and allowed a more straightforward sequencing of internal regions of the two genes for which no general primers are described.

The presence of more than one type of fragment in the PCR products yielded by the versatile primers, which was resistant to exhaustive PCR optimisation attempts, was detected both by multiple bands in 1.5% agarose tris-borate-EDTA (TBE) gels (Fig. 3.2) and by ambiguities and high background in sequence reads. This was interpreted as indicating the presence of mtDNA-like nuclear copies of the targeted fragment (*Numts*; Lopez *et al.* 1997) and/or extensive VNTR heteroplasmy in the left and right domains of the control region, which has been described as widespread in Carnivores (Hoelzel *et al.* 1994). Both instances were confirmed through sequencing of cloned PCR products, cloned with the CopyControl™ cloning kit (Epicentre) following the manufacturer's protocol, from individuals from different geographic populations; five clones per individual were sequenced. Figure 3.3 shows a schematic representation of the detected length heteroplasmy of a VNTR with an 81 bp motif in the left domain of the control region. To avoid the effect of heteroplasmy in the sequence data output a simple solution exists, which is to exclude the heteroplasmic region from the fragment targeted by the PCR primers, avoiding the costly alternative of only sequencing the cloned PCR products of

every single analysed sample. In contrast, for eliminating co-amplification of *Numts*, to use PCR primers not only taxon-specific but also, by being designed over sequences known with certainty to be of mitochondrial origin, likely to be mtDNA-specific, it is one of the best approaches (Bensasson et al. 2002). In order to obtain a reliable mtDNA sequence a fresh extract of total genomic DNA was used and the whole mtDNA (≈ 17,000 bp) was amplified by Long PCR using the Expand™ Long Template PCR system (Roche) and a single set of primers suggested by Stephan Funk (*pers. comm.*). This enzymatically-driven mtDNA isolation strategy is a valid alternative to the traditional physical separation of genomes using ultra-centrifugation gradients as described by Dowling *et al.* (1996). A similar procedure has been applied successfully to isolate mitochondrial genomes (*Cymts*; Lopez *et al.* 1997) in arthropods (Hwang *et al.* 2001) and edentates (Nelson *et al.* 1996). Long PCR was conducted in 50-µl reaction volumes with 1x Buffer 3 [10x stock contains 22.5 mM $MgCl_2$, 500 mM Tris-HCl pH 9.2, 160 mM $(NH_4)_2SO_4$, 20% v/v DMSO, 1% v/v Tween 20], 2.5 units of enzyme mix, 250 ng of genomic DNA, 1.4 mM dNTPs, and 0.5 µM of each primer. Cycling parameters consisted of initial denaturation at 94° C for 2 min; 10 cycles at 92° C for 10s, annealing at 63° C for 30 s, and elongation at 68° C for 13 min; another 15 cycles with 20 additional seconds for the elongation step per cycle; and 7 min at 68° C for final extension.

Besides isolation of the entire mtDNA molecule from a single sample, around 5 Kb of mtDNA encompassing the genes ND-5, ND-6, cytochrome *b*, and the control region was amplified, using a single primer pair, for two samples per each of the main geographic populations. Cracraft *et al.* (1998) were able to separate *Cymts* from *Numts* in tigers by resorting also to amplification of longer fragments than the ones initially elected as targets. For this PCR, the forward primer L12603 Genetta [the number identifies the 3' end of the primer following the *Felis catus* mtDNA numbering (Lopez *et al.* 1996)] was designed, based on an alignment of whole-mtDNA Carnivora sequences, and the reverse primer 12SAR-3' is listed in Palumbi (1996). Amplification was performed in 50-µl reactions with 0.8x PCR Buffer (Invitrogen; whose 10x stock contains 200 mM Tris-HCl pH 8.4 and 500 mM KCl), 2 units of *Taq* DNA Polymerase (Invitrogen), 1.75 mM $MgCl_2$, 1 mM dNTPs, 100-150 ng of genomic DNA, and 0.5 µM of each primer. The thermocycling profile consisted of initial denaturation at 94° C for 2 min, followed by 40 cycles in 94° C for 10s, 58° C for 30s and 72° C for 4 min. The final extension was 7 min at 72° C. Negative controls were carried out alongside all PCR reactions. All the genet sequences,

either derived directly from the cytochrome *b*-control region, 5 Kb, and whole-mtDNA amplifications, or from cloned PCR products, were aligned with available Carnivora sequences in the GenBank database. From this alignment genet mtDNA specific primer pairs were designed for three substitution-rich fragments, smaller than 200 bp and each belonging to a different gene, to be screened in all samples. The sequences of these primers for the genes ND-5, cytochrome *b*, and the control region, are listed in Table 3.1.

3.2.2.3 PCR and sequencing

PCRs of all samples for the three gene fragments were carried out in 25-µl reaction volumes. For museum skin or hair samples and to each PCR reaction, 1.2x PCR Buffer (Invitrogen), 1.25 units of *Taq* DNA Polymerase (Invitrogen), 3 mM $MgCl_2$, 0.8 mM dNTPs, 5-10 µl of genomic DNA, 0.5 µM of each primer, and 0.5 mg/ml of non-acetylated BSA (MBI Fermentas) were added. For buffer-preserved tissue or blood samples and to each PCR reaction, 1.2x PCR Buffer (Invitrogen), 1 unit of *Taq* DNA Polymerase (Invitrogen), 2.25 mM $MgCl_2$, 0.8 mM dNTPs, 50 ng of genomic DNA, and 0.5 µM of each primer were added. Thermocycling consisted of initial denaturation at 94° C for 3 min, followed by 35 (for modern samples) up to 55 (for ancient-DNA samples) cycles in 94° C for 30s, 50° C for 45s and 72° C for 1 min. The final extension was 7 min at 72° C. Negative controls were carried for all PCR reactions. In the case of ancient-DNA samples, PCR replicates were conducted for the same and/or different extraction replicates from a set of randomly chosen samples in order to authenticate the respective sequences.

All PCR products were purified with the GeneClean Turbo Kit (Bio 101, catalogue #1101-600, Q-Biogene) following the manufacturer's instructions. Sequencing of both strands of the product was carried out using the ABI Prism Dye Terminator cycle sequencing ready reaction kit (catalogue #4314477, Perkin-Elmer) for the ABI Prism model 377 DNA automated sequencer following the recommendations of the manufacturer.

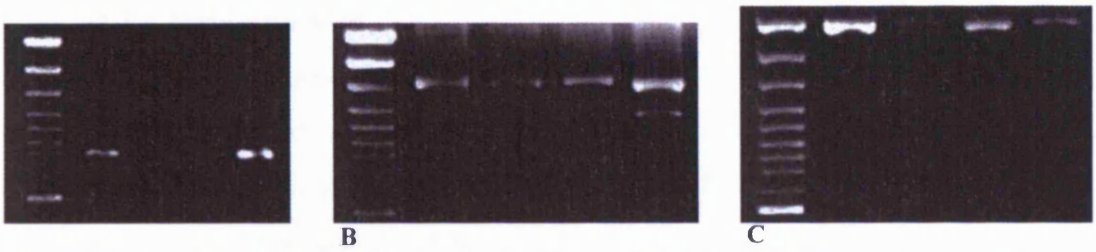

Figure 3.2 Agarose gel images showing the double banding in PCR amplification results of genet mtDNA using versatile primers. **A** - Control region left domain with primers L15774/H16498 (Shields & Kocher 1991); **B** – Complete control region with primers L15926/H00651 (Kocher *et al.* 1989); **C** – Complete cytochrome b and control region with primers L14724b/H00651 (Irwin *et al.* 1991; Kocher *et al.* 1989).

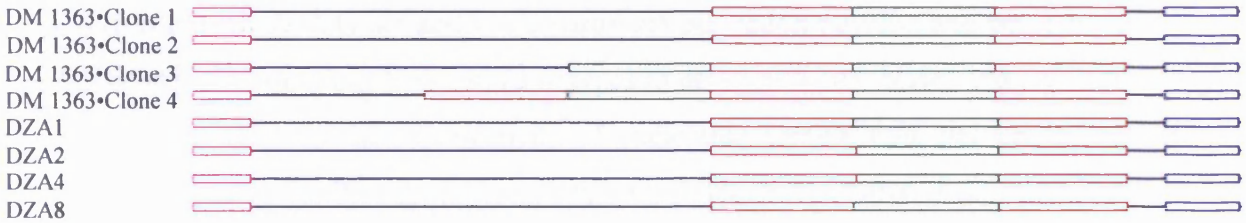

Figure 3.3 Length heteroplasmy of a VNTR in the control region left domain with a motif of 81 bp. VNTR length variation is represented by the alternating red and green blocks; conserved core of the control region is in blue and the tRNA-Pro in pink. The first four sequences are different clones of a single South African genet and the last four are of European genets derived directly from the PCR.

Table 3.1 List of primers designed for this study and respective sequences. The letters identify the primer position on the heavy (H) or light (L) strand and the number identifies the 3' end of the primer following the *Felis catus* mtDNA numbering (Lopez *et al.* 1996).

Gene	Primer identification	Nucleotide sequence (5'→3')	Observations
ND-5	L12603 Genetta	AGA AGT AAT CCA TTG GTC TTA GGA ACC	
	L13766 Genetta	GCG CCT ATT AAT TGG AAG CAT	
	H13978 Genetta	TTG GTG GAA AGC GGT GAA T	
	H13982 Genetta	TTA TTG GTG GAA RGC GGT	
Cytochrome *b*	L15467 Genetta	ATT CCT TCT GAG GRG CAA	
	L15471 Genetta	TTT CTG AGG AGC AAC CGT	only for *G. genetta*
	H15665 Genetta	TCT GAG TCA GAT AYT ACG CC	
Control Region	L16212 Genetta	GGA TAC CTT GGT CTT GTA A	
	L16290 Genetta	ATC AGC ACC CAA AGC TGA	
	H16418 Genetta	GCA CTA ATA GGT AGA TTG	
	H16421 Genetta	GCG GAG TAT TAA TAG GTA GA	only for *G. genetta*

3.2.3 Data analysis

3.2.3.1 Phylogenetic analysis

The forward and reverse sequences obtained were at first aligned and assembled, using Sequencher^{TM} 3.1.2 (Gene Codes Corporation Inc.) software, for each gene and individual, and subsequently checked by eye, as recommended when insertions and deletions are absent or straightforward to resolve (Graur & Li 2000), for manual editing.

To evaluate if phylogenetic signal is significantly stronger than random noise in the structure of the sequences, the g_1-test (Hillis & Huelsenbeck 1992) with 1,000,000 randomly generated trees and the permutation tail probability (PTP) test (Faith & Cranston 1991) with heuristic tree search and 1,000 replicates were performed. Both tests were applied to the whole data set and to its partitions per codon position and per gene. Base frequencies were compared between haplotypes to detect potential biases and a χ^2 test was carried out to measure the homogeneity of nucleotide composition, per genes and per codon position, among lineages. The incongruence length difference (ILD) test (Farris *et al.* 1994) was used to assess the congruence between the three gene fragments and, hence, their suitability to be analytically combined, and also as an indicator of the homogeneity among codon positions in the two protein-coding genes. The two homogeneity partition tests, for genes and codon positions, were carried out with 1,000 randomisations each and with invariable sites removed. The transition:transversion ratio of the sequences was estimated and, to determine if the commonest substitutions in protein-coding genes, transitions in the third position of codons, were saturated, uncorrected pairwise differences against corrected pairwise differences were plotted in a graph that reveals when observed divergence reached an asymptote. Finally, the likelihood ratio test (LRT), which compares trees with and without an ultrametric constraint (Felsenstein 1981), was used to estimate if all the lineages were evolving at a similar rate (molecular clock hypothesis).

PAUP version 4.0b10 (Swofford 2002) was used to implement those tests and also to conduct phylogenetic reconstructions of the data set using the methods of maximum parsimony (MP), minimum evolution (ME) and maximum likelihood (ML). Unweighted MP analysis was accomplished with heuristic tree search, random sequence addition, 1,000 replicates, and rearrangements by tree-bisection-reconnection (TBR) limited to 2,000,000 per replicate, a recommended approach to minimise computational time without significantly hindering the search for an optimal tree (Swofford 2002). ME reconstruction was carried out using LogDet distances between sequences (Lockhart *et al.* 1994) and with

an evolutionary model tested as the best fit to the data; both heuristic and neighbour-joining (NJ) tree searches were compared. The model of sequence evolution to correct the genetic distance matrix for metric tree reconstruction by the ME method, was selected with hierarchically nested LRTs as implemented in ModelTest version 3.06 (Posada & Crandall 1998). ML analysis was also performed using as starting tree the semi-strict consensus obtained from the MP trees to estimate sequence evolution parameters that best fit the data, which were subsequently used in tree heuristic evaluation with rearrangements by tree-bisection-reconnection (TBR) limited to 5,000. Corrected pairwise distances between sequences were calculated with both the model selected by ModelTest and with the Kimura 2-parameter model. The latter was applied in one of the most thorough attempts to calibrate genetic distances taxonomically, using the cytochrome *b* gene (Johns & Avise 1998), and this allows evaluation of the systematic value of the observed genetic distances in the data set. Bootstrapping (Felsenstein 1985), with 1,000 replicates for MP and ME analyses and 100 replicates for ML, was performed to measure the support for clades throughout all the topologies. Phylogenetic reconstruction was also implemented using Bayesian inference with Mr. Bayes version 3.0 (Huelsenbeck & Ronquist 2001) where four independent Markov chains were run (three heated and one cold) for 1,000,000 Metropolis-coupled Markov chain generations, with tree sampling each 50 generations and burn-in after 10,000 trees (Huelsenbeck *et al.* 2001). The evolutionary model used in the Bayesian analysis was the same applied for genetic distance correction and ML analysis, as estimated as best fit to the overall data set by ModelTest. Posterior probabilities were calculated to measure node reliability of the estimated tree topology.

3.2.3.2 Population genetics and phylogeographic analyses

For the population genetics and phylogeographic analyses, samples were grouped accordingly with sampling locality in defined geographic areas (Table 3.2) whose limits roughly correspond to obvious geographic isolates and/or to proposed subspecies, thereby allowing testing of taxonomic hypotheses. The fact that the limits of the areas coincide as well in several cases with biome transitions (Figure 3.4) also permitted to assess the correlation between genetic structure and contemporary vegetation belts. Each geographic area was equated in practical terms to a "population" for the population genetics analyses.

The program Arlequin version 2.000 (Schneider *et al.* 2000) was used to compute measures of intraspecific genetic diversity, tests of selective neutrality of the sequences, and for calculating the mismatch, or pairwise differences, distributions. The hypothesis of

demographic expansion for each population was assessed by estimating the relevant parameters (Θ_0, Θ_1, and τ) under this scenario using a generalised least-squares approach (Schneider & Excoffier 1999); the statistical significance of the estimated parameters was determined with 1,000 coalescent simulation replicates. The raggedness index (Harpending 1994), a measure of the smoothness of the observed mismatch distribution and hence of the stationary *versus* expanding nature of the populations, was also calculated for each population and assessed with 1,000 bootstrap replicates.

Table 3.2 Geographic ranges and centres of the areas defined for the phylogeographic analysis of *G. genetta*.

Geographic Area	Latitude/Longitude Range	Geographic Centre
Europe	[51° 49' 00"N ; 36° 00' 00"N];	40° 45' 37"N
	[09° 36' 00"W; 10° 24' 00"E]	02° 45' 23"W
North Africa	[36° 00' 00"N; 30° 00' 00"N];	35° 42' 46"N
	[10° 00' 00"W; 20° 00' 00"E]	09° 25' 51"E
Arabia	[30° 00' 00"N; 12° 50' 00"N];	20° 24' 49"N
	[35° 00' 00"E; 60° 00' 00"E]	44° 49' 41"E
West Africa	[20° 00' 00"N; 04° 50' 00"N];	15° 44' 12"N
	[17° 60' 00"W; 10° 00' 00"E]	15° 35' 36"W
Central Africa	[20° 00' 00"N; 09° 00' 00"S];	10° 54' 52"N
	[10° 00' 00"E; 33° 30' 00"E]	13° 50' 08"E
East Africa	[17° 60' 00"N; 10° 00' 00"S];	07° 20' 55"N
	[33° 30' 00"E; 52° 00' 00"E]	38° 03' 37"E
Southern Africa	[10° 00' 00"S; 28° 50' 00"S];	23° 31' 51"S
	>28° 50' 00"E when > 28° 50' 00"S	26° 27' 21"E
Cape+OFS	>28°50' 00"S	32° 33' 31"S
	<28°50' 00"E	20° 33' 47"E

The program Chiperm version 1.2 (Posada 2000) was used to implement the test for detecting significant associations between haplotypes and geographical locations presented by Hudson *et al.* (1992a). The significance of the χ^2 is approximated with Monte Carlo simulation (Roff & Bentzen 1989) by permuting the contingency tables; 100,000 permutations were performed.

Arlequin version 2.000 was also used to compute parameters of structure among populations and to carry out an analysis of the molecular variance (AMOVA), as described in Excoffier *et al.* (1992). AMOVA is a method that estimates the proportion of the genetic variation at different hierarchical levels, using information from the geographical distribution of haplotypes, their frequencies and nucleotide differences. It yields an analogous estimator to Wright's F_{ST}, Φ_{ST}, that measures the fraction of variance explained by population subdivision, thereby indicating the level of differentiation among demes. For both the AMOVA and the construction of a minimum spanning network (MSN; Rohlf

1973) connecting all *G. genetta* haplotypes found in this study, a matrix of genetic distances among haplotypes corrected under an evolution model selected as best fit to the data by ModelTest was used. The significance of the fixation index Φ_{ST} was tested with 100,000 permutations. The amount of gene flow between pairs of populations was estimated with the parameter M ($=N_f m$) and as described in the method introduced by Slatkin (1993). The test of exact population differentiation based on haplotype frequencies of Raymond & Rousset (1995) was performed and the results tested for significance at $\alpha=0.05$ through a Markov chain with 100,000 steps and burn-in after 10,000 steps. Finally, the net number of nucleotide differences between populations (Nei & Li 1979) was calculated and used in a test of the correlation between population genetic differences and geographic distances (Hutchinson & Templeton 1999).

With DNAsp version 4.0 (Rozas *et al.* 2003) different estimators of F_{ST} and M were applied to the data set for comparison with those above and to investigate the support given by different methods. The method described by Hudson *et al.* (1992b) for F_{ST} calculation was implemented and tested with 10,000 permutations; estimates of M were derived from these pairwise F_{ST} values and also from N_{ST} values (Lynch & Crease 1990). Presence of genetic differentiation was also examined with a recently proposed statistic, S_{nn}, by Hudson (2000) and results for population pairwise comparisons were compared with the ones obtained with the statistic K_s^* (Hudson *et al.* 1992a).

A Mantel test to evaluate the importance of isolation by distance in shaping the genetic structure of *G. genetta* populations was carried out using the software IBD version 1.4 (Bohonak 2003) with 100,000 randomisations. The regressed matrices were the one containing the net number of nucleotide differences between population pairs and the one with distances in Km between the geographic centres of each pair of the areas that were predefined.

The net genetic distance between populations or species (d_A; Nei 1987) was calculated for all pairwise comparisons in the multispecies data set, including both *G. genetta* units and the other genet species. These values of genetic distance were used to derive approximate timings for the splits between clades using the equality $d_A=2\mu t$ where μ is the neutral mutation rate per nucleotide and t is the population/species divergence time. For recent species or population divergences, the use of d_A allows correcting for the ancestral polymorphism present in the samples due to the fact that gene divergence often predates population divergence (Takahata & Slatkin 1990; Edwards & Beerli 2000).

However, it must be considered that this method assumes constancy of sizes between the ancestral and the two descendant clades (Arbogast *et al.* 2002). In the absence of any relevant fossil data that could allow the calibration of a genet mtDNA molecular clock, two different calibrations derived from reliable fossil record in other mammal groups, and already tested in several species, were used. Divergence times were estimated both assuming the traditional mtDNA molecular clock with a substitution rate of 0.010 substitutions per site per lineage per My (Brown *et al.* 1979, 1982) and a recently proposed new rate, accounting for rate heterogeneity among sites, for mammals and birds of 0.026 (Arbogast & Slowinski 1998).

Nested clade analysis (NCA) it is a phylogeographic method that attempts to disentangle population structure from population history by inferring the current or historical process that most parsimoniously can explain the hierarchical nesting of haplotype clades within a statistical cladogram (Templeton 1998). The method is based on the observation that different causes of geographical association (range expansion, long-distance colonization, isolation by distance and/or past fragmentation) may generate characteristic patterns on how haplotypes are located within a gene tree or network (Avise *et al.* 1987).

For the NCA, the first step, which is to infer a set of 95% plausible haplotype networks, which clades are hierarchically nested, using the algorithm given in Templeton *et al.* (1992), was carried out with the program TCS version 1.13 (Clement *et al.* 2000). The program Geodis version 2.0 (Posada *et al.* 2000) was employed to measure the geographical information of the clades and to test the significance of geographical association for each clade by 100,000 random permutations of clades against sampling locations. Inferences about evolutionary processes and events that most likely account for observed haplotype-geography associations, or possible artefacts due to sampling inadequacies, were made using the key presented in the appendix of Templeton (2004).

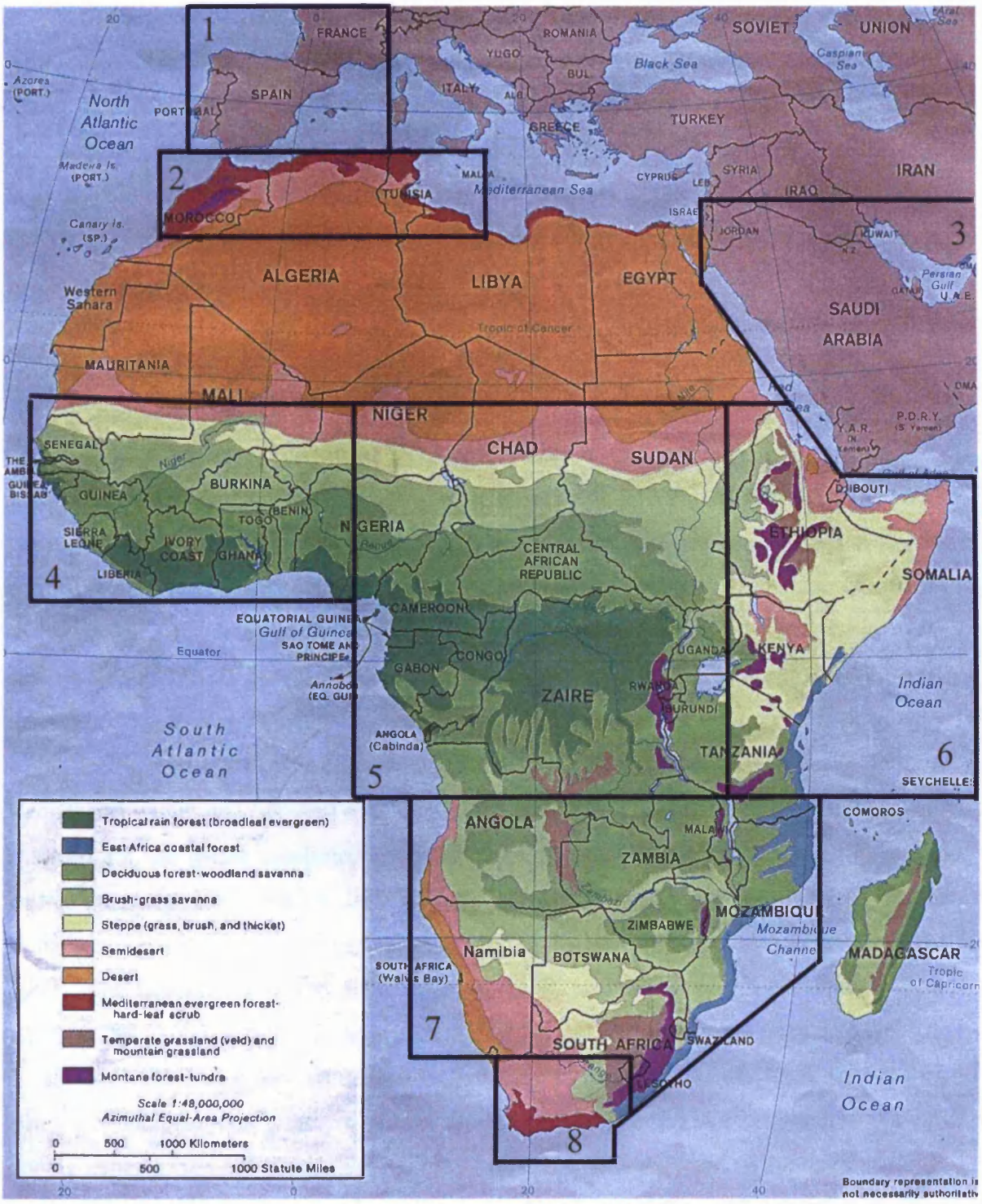

Figure 3.4 Vegetation map of Africa and representation of the geographic units in which the distribution of *G. genetta* was subdivided for the phylogeographic analysis. 1- Europe, 2- North Africa, 3- Arabia, 4- West Africa, 5- Central Africa, 6- East Africa, 7- Southern Africa, 8- Cape and Orange Free State. For convenience, the name "Southern Africa" was kept to designate the whole of Africa below the 10° S parallel excluding its extreme south-west (Cape and Orange Free State). Template map courtesy of the General Libraries, University of Texas at Austin.

3.3 Results

3.3.1 Reliability of DNA sequences from museum specimens

Sequences derived from ancient-DNA material always showed the highest similarities with sequences produced from equivalent, in taxonomic and/or geographic terms, modern samples. Results were congruent among independent extraction rounds (two per sample) and different PCR replicates (two per sample), which is regarded as authentication against contamination (Hassanin & Douzery 2000) and amplification errors induced by miscoding lesions in ancient specimens (Hansen *et al.* 2001). Finally, several instances of museum samples yielding unique haplotypes (i.e. showing singleton substitutions) within the data set were observed, suggesting absence of cross-contamination in the laboratory procedures.

3.3.2 Characterisation of DNA sequences

Sequences of 533-534 bp in length were obtained; 210 nucleotides from the ND-5 gene, 198 nucleotides from the cytochrome *b* gene, and 125-126 nucleotides from the control region. In comparison with the other genet species analysed, *G. genetta* was found to possess a 1 bp deletion in the control region fragment, which was present in every sample throughout its range. The reading frames for the two protein-coding genes were determined by alignment with the complete mtDNA sequence of the domestic cat (Lopez *et al.* 1996), and no stop codons were found in any haplotype. Homogeneity of base frequencies within and between species was revealed by the χ^2 test (P=1.000), either when assessed for the genes combined or when assessed separately according with gene and codon positions partitions. A low number of guanines (G) was encountered in the sequences for the three loci, a typical result in the mtDNA of vertebrates (Tamura & Nei 1993). The frequencies of the nucleotides A, C. G, and T for the ND-5 fragment were 0.329, 0.269, 0.090, and 0.312, respectively. For the cytochrome *b* they were 0.264, 0.305, 0.145, and 0.286, and for the control region they were 0.307, 0.308, 0.078, and 0.307. Among codon positions, a pattern of bias similar to the one found in mitochondrial protein-coding genes of several vertebrates (Irwin *et al.* 1991) was detected in the data; adenines dominating at first positions, thymidines at second positions, and adenines at third positions. The frequencies were, at first positions, 0.318, 0.239, 0.203, and 0.240. For the second positions they were 0.220, 0.303, 0.075, and 0.403, and for the third positions they were 0.348, 0.324, 0.075, and 0.253. The average apparent transition/transversion ratio was

of 10, a bias within the typical range found in the mtDNA of vertebrates (Graur & Li 2000). Saturation plots showed a slight saturation of transitions at the third positions of codons (Fig. 3.5) but since incongruence as measured by the ILD test was not detectable among codon positions (P-value=0.277) it was decided to retain all characters in the analyses. This approach has been already demonstrated as potentially advantageous (Vidal & Lecointre 1998), as well as the essential role that third position transitions may play in the recovery of accurate phylogenies (Bjorklund 1999). The ILD test for the partition genes also did not detect any significant incompatibility for these character sets (P-value=0.555).

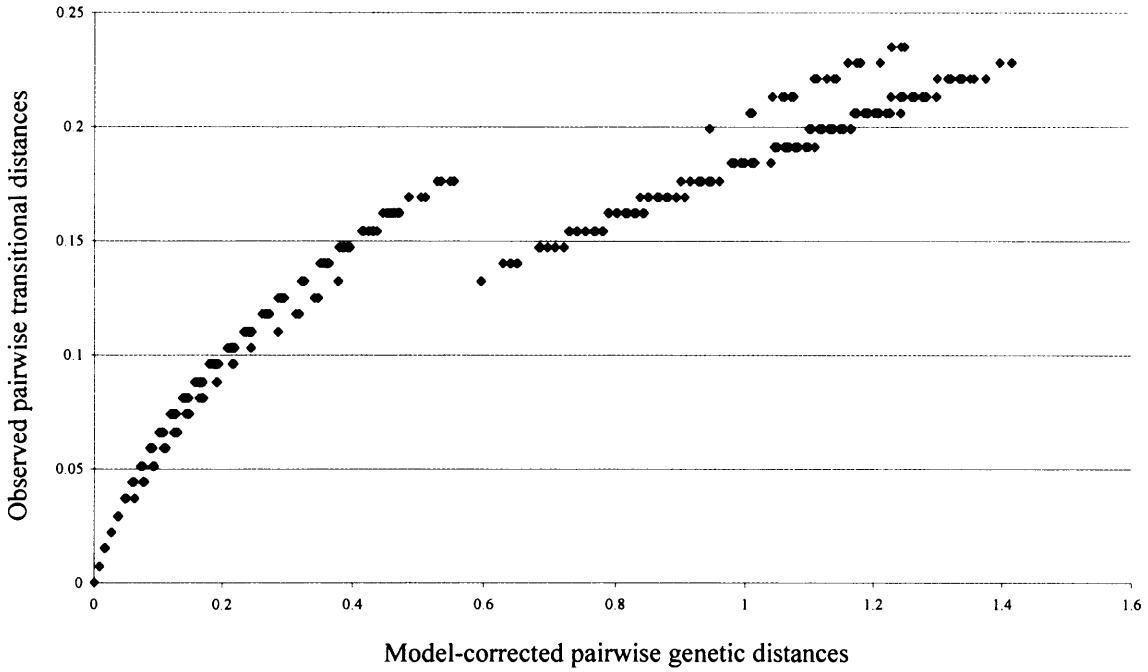

Figure 3.5 Graph plotting corrected pairwise genetic distances against observed transitional differences at third positions of ND-5 and Cyt *b* codons to assess the presence and level of saturation with increasing genetic divergence. The saturation effect is light for the cloud of distances in the left, corresponding to within species comparisons, but slightly heavier for the cloud of inter-species comparisons in the right.

3.3.3 Phylogenetic analysis

It proved impossible to amplify successfully the control region fragment for the species *G. angolensis* with any primer combination of the four designed control region primers used in this study. Consequently, the sequences combined per sample for the interspecific comparisons were only the ones from the protein-coding genes, with a total length of 408 bp, whereas for the phylogeographic analysis of *G. genetta*, partial sequences of the three loci, with a total of 533 bp, were analysed. For the two protein-coding genes, 50 haplotypes were observed among the 132 samples analysed; Table 3.3 lists the

haplotypes, together with the samples and the geographic areas defined in this study in which each haplotype was present. The haplotypes were designated accordingly with the genet species in which they were observed, but in two instances a haplotype was found to be shared between two different species. There were 95 variable characters in the 408 bp data set, of which 76 were parsimony informative and 19 were autapomorphies. Table 3.4 shows the polymorphism pattern for all the 95 variable sites among genet haplotypes. The PTP test was statistically significant (P=0.001) which suggests the presence of a strong phylogenetic signal in the distribution of character states among the taxa. For the *G. genetta*-only data set, 35 haplotypes were detected among the 96 samples; addition of the control region sequences to the protein-coding block revealed 5 additional lineages. Tables 3.5 and 3.6 provide information, in terms of samples and locations, concerning the distribution of the haplotypes found in *G. genetta* and Table 3.7 presents nucleotide variation over the 98 variable sites, of which 62 were parsimony informative, in the 533 bp sequence. A distribution of 10^6 trees generated randomly from this second data set was significantly skewed (g_1=-0,813 $P < 0.01$, mean ± SD tree length = 343.706 ± 11.453, range = 259-375) suggesting the presence of strong phylogenetic structure in the data set. The results of the LRT for a molecular clock assumption was statistically significant at α=0.05 for the null hypothesis of rate homogeneity among all lineages; LR=40.32 (df=48) in the multispecies data set and LR=23.60 (df=33) for *G. genetta*. The best-fitting model of sequence evolution determined by ModelTest was the TrN (Tamura & Nei 1993) with gamma-distributed correction for rate heterogeneity among sites. The shape parameter of the distribution was 0.365 for the phylogenies including only *G. genetta* populations and 0.223 for the phylogenies including all species. Table 3.8 shows the corrected genetic distances between *G. genetta* haplotypes in the 533 bp data set.

The different methods of phylogenetic analysis generated consistent results, in which the distinction of the major clades was clear in spite of some topological and branch-length differences in tree areas characterised by low bootstrap values. For the haplotype genealogy within *G. genetta* a MP tree and the tree obtained through Bayesian analysis are shown in Fig. 3.6 and 3.7; only bootstrap values equal or higher than 70% were considered, as this amount as been showed to be a reliability threshold (Hillis & Bull 1993). The MP tree is a 50% majority-rule consensus of 129 steps in length (CI=0.783; RI=0.898) and the Bayesian tree is the 50% majority-rule consensus of 10,000 MCMC samples retained after burn-in.

Table 3.3 List of genet haplotypes for the combined two protein-coding gene fragments included in the multispecies phylogenetic analysis, together with the samples and geographic areas where each of them was found.

Haplotype	Samples	Geographic Area
Gge1	PNPG 3 1, PNPG 12 1, Mertola1, G42, DZA1, DZA2, DZA3, Donana1, Donana2, Donana3, Donana4, Donana5, Donana7, T37, T39, T44, T46, T67, T83, T107, T87, ROM59287	Europe, North Africa
Gge2	Donana6	Europe
Gge3	PC25	North Africa
Gge4	Batna1, NMS25864, NMS25865	North Africa, Central Africa
Gge5	T69	West Africa
Gge6	T60, NMW32814	West Africa
Gge7	T61	West Africa
Gge8	T09	West Africa
Gge9	NMS25863	Central Africa
Gge10	LACM14468, LACM14469, LACM14477	Central Africa
Gge11	NMWB2145	Central Africa
Gge12	LACM56721	East Africa
Gge13	Bern1025642	East Africa
Gge14	EritreaG1, EritreaG3	East Africa
Gge15	EritreaG2	East Africa
Gge16	PC30	East Africa
Gge17	TM12831	East Africa
Gge18	T104, NWRC1, NWRC3, NWRC4, NWRC5, NMSG2, Oman1	Arabia
Gge19	NWRC2	Arabia
Gge20	NMSG1, TC31	Arabia
Gge21	Rotterdam1	Southern Africa
Gge22	Griffin1, Griffin212, TM30163, TM14840, MVZ117819, Hamburg3815, PC74, MHNG1492029, KM13857, NMB8544, KM24151, TM29543, KM31133	Southern Africa, Cape+OFS
Gge23	KM27713	Cape+OFS
Gge24	TM12054	Southern Africa
Gge25	NMS27346	Southern Africa
Gge26	TM28497	Southern Africa
Gge27	TM6540, MVZ118447, PC138, NMS27344, NMS27348, KM19288, TM40740, TM23971, TM27716, TM24611, NMB3159, TM32528, TM28218, TM16796, DM1363	Southern Africa
Gge28	NMS27341	Southern Africa
Gge29	TM23464, NMB7835	Southern Africa
Gge/Gti	NMB9279, NMB3946, KM29723, TC38, TC39, NGP2152, NGP2160, NGP2161, NGP2164, NGP2172, TM28335, TM28666, KM30813, KM30814	Southern Africa, Cape+OFS
Gti1	A274	Cape+OFS
Gti2	TM32193, KM30812	Cape+OFS
Gti3	A270, A271, A273	Cape+OFS
Gti4	A272	Cape+OFS
Gti5	KM31185, NMB4786	Cape+OFS
Gti/Gru	KM31276, TM16638, NMB7013, NMB6904	Southern Africa, Cape+OFS
Gti6	NMB4470	Cape+OFS
Gru1	TM10348, TM8886	Southern Africa
Gru2	Angola1	Southern Africa
Gru3	Angola2	Southern Africa
Gru4	PC90	Southern Africa
Gru5	DM2232	Southern Africa
Gru6	ROM35695, TM12656	Southern Africa
Gru7	TM11449, CZ242	Southern Africa
Gru8	DM1089	Southern Africa
Gru9	TM6058	Southern Africa
Gru10	TM9839	Southern Africa
Gan1	TM17713	Southern Africa
Gan2	MVZ118449, TM9847, TM9849	Southern Africa
Gan3	TM9842	Southern Africa

For convenience, the name Southern Africa was kept to designate the whole of Africa below the 10° S parallel excluding its extreme south-west (Cape and Orange Free State).

Table 3.4 Variable sites and polymorphism pattern in the combined ND-5+Cyt *b* 408 bp fragment among the haplotypes included in the multispecies analysis. The bold vertical line marks the border between the two genes.

Haplotype	3 6 9 2 8 1 3 7 0 5 1 7 0 3 4 6 9 2 5 4 7 9 1 3 9 1 5 2 4 3 6 9 3 4 4 5 1 9 8 9 0 0 3 6 0 2 5 8 5 0	3 2 8 1 7 6 7 5 7 8 9 1 4 7 0 6 9 2 5 0 4 6 2 4 0 3 4 6 2 4 5 1 2 7 6 0 2 3 4 0 3 2 5 7 8
Gge1	T G A C T T A C A T G T G T C T T A A C C T G G T C T C G A C C G C C C T T C C C T T T C A A T A C	C A C A C C G T A T C A A A G A A C T C T A A T T C T C C T C G G G T C G T C A T C C C A
Gge2	. . . . . T . . . . . T	. . . . . T . . . . .
Gge3	. . . . C . T . . . A . . . . G . . . . . T . . . . T . . C T . G . .	. . . . . A . . . . C . . . . C . . . . . C . . . T . . G
Gge4	. . . . C . T . . . . . G . . . . . T . . . . T . G . .	. . . . . A . . . . C . . . . C . . . . . C . . C . . T . . G
Gge5	. . . . C . T . . . C A . . . G . . . . . T . . C . . T . . C T . G . .	. . . . . A . . . . C . . . . C . . . . . C . . . T . . G
Gge6	. . . . C . T . . . . A . . . . G . . . . . T . . C . . T . . C T . G . .	. . . . . A . . . . C . . . . C . . . . . C . . . T . . G
Gge7	. . . . C . T . . . . A . . . . G . . . . . T . . C . . T . . C T . G . .	. . . . . A . . . . C . . . . C . . . . . C . . C T . . G
Gge8	. . . . C . T . . . . A . . . . G . . . . . T . . . . T . . C T . G . .	. . . . . A . G . . C . . . . C . . . . . C . . . T . . G
Gge9	. . . . C . T . . . . A . . . . G . . . . . T . . . . T . G . .	. . . . . A . . . . C . . . . C . . . . . C . . C . . T . . G
Gge10	. . . C C . T . . . . . G . . . . . T . . . . T . G . .	. . . . . A . . . . C . . . . C . . . . . C . . C . . T . . G
Gge11	. . . . C . T . . . . . G . . . . . T . . . . T . G . .	. . . . . A . . . T C . . . . C . . . . . C . . C . . T . . G
Gge12	. . . . C . T . . A . . . . . G . . . . . A . . T A . . . . . T . . . T . G . .	. . . . . A . . . . C . . . T C . . . . . C . G . T . .
Gge13	. . . . C . T . . A . . . . . G . . . . . A . . T A . . . . . T . . . T . G . .	. . . . . A . . . . C . . . T C . . . . . C . . . T . .
Gge14	. . . . C . T . . A . . . . . G . . . . . T T . . . T . G . .	. . . . . T G . G A . . . . C . . . T C . . . A . . . . C . . . T . . G
Gge15	. . . . C . T . . A . . . . . G . . . . . T . . . T . G . .	. . . . . T . . G A . . . . C . . . T C . . . A . . . . C . . . T . . G
Gge16	. . . . C . T T . . . A . . . . G . T C . . . . . G . . . . . T . . . T . G . .	G . . . . . A . . . . C . . . . C . . . . . C . G . T . . G
Gge17	. . . . C . T T . . . A . . . . G . T C . . . . . A G . . . . . T . . . T . G . .	G . . . . . A . . . . C . . . . C . . . . . C . G . T . . G
Gge18	. . . . . T . C . . . . . G . . C . . C . . . . . A . . C . . T . . C T	. . . . T . . C . . . . A . . . . C . . . . C . . T . . . . T A . . . . .
Gge19	. . . . . T . C . . . . . G . . C . . C . . . . . A . . C . . T . . C T	. . . . T . . C . . . . A G . . . . C . . . . C . . T . . . . T A . . . . .
Gge20	. . . . . T . C . . A . . . . G . . C . . C . . . . . A . . C . . T . . C T	. . . . T . . C . . . . A . . . . C . . . . C . . T . . . . T A . . . . .
Gge21	. . . . . T . . . . . C . A . . . . . T . . T . G . . T	. . G . . A . . . . . A . . T . . C . . . . C . . . . . A . . . . C . . . . . G
Gge22	. . . . . T . . . . . G . . C . A . . . . . T . . T . G . .	. . G . . A . . . . . A . . T . . C . . C . C . . . . . A . . . . C . . . . . G
Gge23	. . . . . T . . . . . G . . C . A . . . . . T . . T . G . .	. . G . . . . . A . . T . . C . . . . C . . . . . A . . . . C . . . . . G
Gge24	. . . . . T . . . . . G . . C . A . . . . . T . . T . G . .	. . . . . A . . T . . C . . . . C . . . . . T . . . . C . . . . . G
Gge25	. . . . . T G . . . . . G . . C . A . . . . . T . . . . . T . . T . G . .	. . . . . A . . T . . C . . . . C . . . . . T . . . . C . . . . . G
Gge26	. . . . . T . . . . . G . . C . A . . . . . T . . T . G . .	. . . . . A . G T . . C . . . . C . . . . . T . . . . C . . . . . G
Gge27	. . . . . T . . . . . G . . C . A . . . . . T . . T . G . .	. . . . . A . . T . . C . . . . C . . . . . A . . . . C . . . . . G
Gge28	. . . . . T . . . . . C . . G . . C . A . . . . . T . . T . G . .	. . . . . A . . T . . C . . . . C . . . . . A . . . . C . . . . . G
Gge29	. . . . . T . . . . . G . . C . A . . . . . T . . . . . T . . T . G . .	. . . . . A . . T . . C . . . . C . . . . . A . . . . C . . . . . G
Gge/Gti	C A G T . . G T T . . . . . C G G . . C . . . T . . . . T . . . . . T . T . C C T G . C . T	T T . . T . A . . G . . A . . . C . C . C . . C T . . T . . A . . C . . . . T . .
Gti1	C A G T . C G T T . . . . . C G G . . C . . . T . . . . T . . . . . T . T . C C T G . C . T	T T . . T . A . . G . . A . . . C . C . C . T C T . . T . . A . . C . . . . T . .
Gti2	C A G T . . G T T . . . . . C G G . . C . . . T C . . . T . . . . . T . T . C C T G . C . T	T T . . T . A . . G . . A . . . C . C . C . . C T . . T . . A . . C . . . . T . .
Gti3	C A . T . . . T C . . C . C T . C G . . . C A A C T . T . . . . . C . . T . C . T G G C . .	T T . . T . G . . G . . A . G T . . C . . C . . . . . A . . C . . C . . . .
Gti4	C A . T . . . T C . . C . C T . C G . . . C A A C T . T . . . . . C . . T . C . T G G C . .	T T . . T . G . . G . . A . G T C . C . . C . . . . . A . . C . . C . . . .
Gti5	C A . T . . . T C . . C . C T . C G . . . C . A C T . T . . . . . C . . T . C . T G G C . .	T T . . T . G . . G . . A . G T . . C . . C . . . . . A . . C . . C . . . .
Gti/Gru	C A . T . . . T C . . C . C T . C G . . . C . A . T . T . . . . . T . . C . . T . C . T G . C . .	T T . . T . G . . G . . A . G T . . C . . C . . . . . A . . C . . C . . . .
Gti6	C A . T . . . T C . . C . C T . C G . . . C . A . T . T . . . . . T . . C . . T . C . T G . C . .	T T . . T . G . . G . . A . G T . . C . . C . . C . . C . . A . . C . . C . . . .
Gru1	C A . T . . . T C . . C . C . . C . . . . C . A . T . T A G T . . . . . C . . T . C . T G G C . .	T T . . T . G . . G . . A . G T . . C . . C . . C T . . . . . A . . C . . . . .
Gru2	C A . T . . . T C . . C A . . . C . . . . C . A . T . T . G T . . . . . C . . T . C . . . G C . .	T T . T T . G . . . G G . A . G T . . C . . C . . C T . . . . . A . . C . . . . .
Gru3	C A . T . . . T C . . C . C . . C . . . . C . A . T . . . . T . . . . . C . . T . C . . . . C . .	T T . . T . G . . G . . A . G T . . C . . C . . C T . . . . . A . . C . . . . .
Gru4	C A . T . . . T C . . C . C . . C . . . . C . A . T . T . G T . . . . . C . . T . C . T G G C . .	T T . . T A G . . . . . A . G T . . C . . C . . C T . . . . . A . . C . . . . .
Gru5	C A . T . . . T C . . C . C . . C . . . . C . A . T . T A G T . . . . . C . . T . C . T G G C . .	T T . . T . G . . G . . A . G T . . C . . C . . C T . . . . . A . . C . . . . . T .
Gru6	C A . T . . . T C . . C . C T . C G . . . C . A C T . T . . T . . . . . C . . T . C . T G G C . .	T T . . T . G . . G . . A . G T . . C . . C . . C . . . . . A . . C . . . . .
Gru7	C A . T . . . T C . . C . C T . C G . . . C . A . T . T . . . . T . . C . . T . C . T G G C . .	T T . . T . G . . G . . A . G T . . C . . C . . C . . . . . A . . C . . C . . . .
Gru8	C A . T . . . T C . . C . C T . C G . . . C . A . T . T . . . . T . . C . . T . C . T G G C . .	C T . . T . G . . G . . A . G T . . C . . C . . C . . . . . A . . C T . C . . . .
Gru9	C A . T . . . T C . . C . C T . C G . . . C . A C T . T . . T . . . . T . C . . T . C . T G G C . .	T T . . T . G G . . G . . A . G T . . C G . C . . C . . . . . A . . C . . . . .
Gru10	C A . T . . . T C . . C . C . . C . . . . C . A . T . T . . T . . T . . . C . . T . C . . G G C G .	T T . . T . G . . G . . A . . T . . C . . C . . C T . . . . . A . . C . . . . .
Gan1	C A . T . . G T C . . . . . C G G T . C . . . T . . . . T . . . . . T . C . . G . . .	T T T . . T . A . C . . . . A . . . . . C . G C . . C T T . T . . A . . C . . . . T . .
Gan2	C A . T . . G T C . . . . . C G G T . C . . . T . . . . T . . . . . T . C . . G . . .	T T T . . T . A . C . . . . A . . . . . C . . C . . C T T . T . . A . . C . . . . T . .
Gan3	C A . T . . G T C . . . . . C G G T . C . . . T . . . . T . . . . . T C C . . G . . .	T T T . . T . A . C . . . . A . . . . . C . . C . . C T T . T A . A . . C . . . . T . .

Table 3.5 List of *G. genetta* haplotypes for the combined two protein-coding genes and control region fragments included in the phylogeographic analysis, together with the samples and geographic areas where each of them was found.

Haplotype	Samples	Geographic Area
Gg1	PNPG 3 1, PNPG 12 1, Mertola1, G42, DZA1, DZA2, DZA3, Donana1, Donana2, Donana3, Donana4, Donana5, Donana7, T39, T44, T107, T87, ROM59287	Europe, North Africa
Gg2	Donana6	Europe
Gg3	T37, T46, T67, T83	Europe
Gg4	PC25	North Africa
Gg5	Batna1	North Africa
Gg6	T69	West Africa
Gg7	T60, NMW32814	West Africa
Gg8	T61	West Africa
Gg9	T09	West Africa
Gg10	NMS25863	Central Africa
Gg11	NMS25864, NMS25865	Central Africa
Gg12	LACM14468, LACM14469, LACM14477	Central Africa
Gg13	NMWB2145	Central Africa
Gg14	LACM56721	East Africa
Gg15	Bern1025642	East Africa
Gg16	EritreaG1	East Africa
Gg17	EritreaG2	East Africa
Gg18	EritreaG3	East Africa
Gg19	PC30	East Africa
Gg20	TM12831	East Africa
Gg21	T104, NWRC1, NWRC4, NWRC5, NMSG2, Oman1	Arabia
Gg22	NWRC2	Arabia
Gg23	NWRC3	Arabia
Gg24	NMSG1, TC31	Arabia
Gg25	Rotterdam1	Southern Africa
Gg26	Griffin1, Griffin212, TM30163, TM14840, MVZ117819, PC74, MHNG1492029, NMB8544, KM24151, TM29543, KM31133	Southern Africa, Cape+OFS
Gg27	KM27713	Cape+OFS
Gg28	TM12054	Southern Africa
Gg29	NMS27346	Southern Africa
Gg30	TM28497	Southern Africa
Gg31	TM6540, TM28218, TM16796	Southern Africa
Gg32	MVZ118447, NMS27344, NMS27348, KM19288, TM40740, TM23971, TM27716, NMB3159, TM32528, DM1363	Southern Africa
Gg33	PC138, TM24611	Southern Africa
Gg34	TM23464, NMB7835	Southern Africa
Gg35	NMB3946, KM29723, TC38, TC39, NGP2152, NGP2160, NGP2161, NGP2164, NGP2172	Southern Africa, Cape+OFS

For convenience, the name Southern Africa was kept to designate the whole of Africa below the 10° S parallel excluding its extreme south-west (Cape and Orange Free State).

Table 3.6 Distribution of *G. genetta* haplotypes at each of the geographic areas. The number of sequences, different haplotypes and unique haplotypes are indicated for each column.

Haplotype	Europe	North Africa	West Africa	Central Africa	East Africa	Arabia	Southern Africa	Cape+OFS	Sequences
Gg1	16	2	0	0	0	0	0	0	18
Gg2	1	0	0	0	0	0	0	0	1
Gg3	4	0	0	0	0	0	0	0	4
Gg4	0	1	0	0	0	0	0	0	1
Gg5	0	1	0	0	0	0	0	0	1
Gg6	0	0	1	0	0	0	0	0	1
Gg7	0	0	2	0	0	0	0	0	2
Gg8	0	0	1	0	0	0	0	0	1
Gg9	0	0	1	0	0	0	0	0	1
Gg10	0	0	0	1	0	0	0	0	1
Gg11	0	0	0	2	0	0	0	0	2
Gg12	0	0	0	3	0	0	0	0	3
Gg13	0	0	0	1	0	0	0	0	1
Gg14	0	0	0	0	1	0	0	0	1
Gg15	0	0	0	0	1	0	0	0	1
Gg16	0	0	0	0	1	0	0	0	1
Gg17	0	0	0	0	1	0	0	0	1
Gg18	0	0	0	0	1	0	0	0	1
Gg19	0	0	0	0	1	0	0	0	1
Gg20	0	0	0	0	1	0	0	0	1
Gg21	0	0	0	0	0	6	0	0	6
Gg22	0	0	0	0	0	1	0	0	1
Gg23	0	0	0	0	0	1	0	0	1
Gg24	0	0	0	0	0	2	0	0	2
Gg25	0	0	0	0	0	0	1	0	1
Gg26	0	0	0	0	0	0	7	4	11
Gg27	0	0	0	0	0	0	0	1	1
Gg28	0	0	0	0	0	0	1	0	1
Gg29	0	0	0	0	0	0	1	0	1
Gg30	0	0	0	0	0	0	1	0	1
Gg31	0	0	0	0	0	0	3	0	3
Gg32	0	0	0	0	0	0	10	0	10
Gg33	0	0	0	0	0	0	2	0	2
Gg34	0	0	0	0	0	0	2	0	2
Gg35	0	0	0	0	0	0	1	8	9
Sequences	21	4	5	7	7	10	29	13	96
Different	3	3	4	4	7	4	10	3	
Unique	2	2	4	4	7	4	8	1	

Table 3.7 Variable sites and polymorphism pattern in the combined ND-5+Cyt *b*+CR 533 bp fragment among *G. genetta* haplotypes. The bold vertical lines mark the borders between loci.

Haplotype	1 1 1 2 2 2 3 4 5 5 6 6 7 7 8 8 9 9 0 1 2 2 2 3 4 5 5 6 6 7 8 8 9 9 9 9 1 3 6 9 2 8 1 3 7 0 5 1 7 0 9 2 5 7 9 3 9 1 4 3 6 9 3 4 1 9 8 9 0 3 6 0 2 5 8 0	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 4 4 4 2 8 1 6 7 5 8 9 1 7 0 6 9 2 5 0 4 4 0 3 4 6 5 1 2 7 6 0 2 3 0 3 2 5 8	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 2 3 3 4 4 5 5 5 5 6 8 8 8 8 9 0 0 1 1 1 2 2 2 2 3 9 2 8 4 5 5 6 8 9 3 0 5 7 7 5 9 4 6 8 3 4 6 7 0
Gg1	T G A C T T A C A T G T G T A A C T G T C G A C C G C T T C C C T T C A A T C	A C A C G T T C A A G A A C T C T T T C T C C G G G T C G T A T C C A	T T C T C A C T C G C C G C G T T C C C C T A T
Gg2	. . . . . T	. . . . . T	. . . . . T T . . . . .
Gg3	. . . . .	. . . . .	. . . . . A . . . . .
Gg4	. . . . . C . T . . . . A . . G . . . . . T . . . . T . C T . G . .	. . . . . A . . . . . C . . . C . . . . . C . . T . G .	C . . T . . . . . T . . . . . C . C
Gg5	. . . . . C . T . . . . . G . . . . . T . . T . G . .	. . . . . A . . . . . C . . . C . . . . . C . . C . . T . G .	C . . . . . T . T . . . . . C . . . C . C
Gg6	. . . . . C . T . . . C A . . G . . . . . T . C . . T . C T . G . .	. . . . . A . . . . . C . . . C . . . . . C . . T . G .	C . . T . . . . T A T . . . . . T . . . C . C
Gg7	. . . . . C . T . . . . A . . G . . . . . T . C . . T . C T . G . .	. . . . . A . . . . . C . . . C . . . . . C . . T . G .	C . . T . . . T . T . . . . . T . . . C . C
Gg8	. . . . . C . T . . . . A . . G . . . . . T . C . . T . C T . G . .	. . . . . A . . . . . C . . . C . . . . . C . C T . G .	C . . T . . . T . T . . . . . T . . . C . C
Gg9	. . . . . C . T . . . . A . . G . . . . . T . . . . T . C T . G . .	. . . . . A . G . . . C . . . C . . . . . C . . T . G .	C . . T . . . T . T . . . . . T . . . C . C
Gg10	. . . . . C . T . . . . A . . G . . . . . T . . T . G . .	. . . . . A . . . . . C . . . C . . . . . C . . C . . T . G .	C . . . . . T . T . . . . . C . C
Gg11	. . . . C . T . . . . . G . . . . . T . . T . G . .	. . . . . A . . . . . C . . . C . . . . . C . . C . . T . G .	C . . . . . T . T . . . . . C . C
Gg12	. . . . C C . T . . . . . G . . . . . T . . T . G . .	. . . . . A . . . . . C . . . C . . . . . C . . C . . T . G .	C . . . . . T . T . A . . . . . C . C
Gg13	. . . . . C . T . . . . . G . . . . . T . . T . G . .	. . . . . A . . . . T C . . . C . . . . . C . . C . . T . G .	C . . . . . T A T . . . . . C . C
Gg14	. . . . . C . T . . A . . . . G . . . . . A . . T A . . . . . T . . T . G . .	. . . . . A . . . . . C . . T C . . . . . C G . T . .	C . . . . . T . T . . . A . . . . . C . .
Gg15	. . . . . C . T . . A . . . . G . . . . . A . . T A . . . . . T . . T . G . .	. . . . . A . . . . . C . . T C . . . . . C . . T . .	C C . . . . . T . T . . . A . . . . . C . .
Gg16	. . . . . C . T . . A . . . . G . . . . . T T . . T . G . .	. . . . . T G G A . . . . . C . . T C . . A . . . . . C . . T . G .	C . . . . . T . T . . . A . . . . . C . C
Gg17	. . . . . C . T . . A . . . . G . . . . . T . . T . G . .	. . . . . T . G A . . . . . C . . T C . . A . . . . . C . . T . G .	C . . . . . T . T . . . A . . . . . C . C
Gg18	. . . . . C . T . . A . . . . G . . . . . T T . . T . G . .	. . . . . T G G A . . . . . C . . T C . . A . . . . . C . . T . G .	C . . . . . T . T . . T A . . . . . C . C
Gg19	. . . . . C . T T . . . A . . G T C . . . . G . . . . . T . . T . G . .	G . . . . . A . . . . . C . . C . . . . . C G . T . G .	C . . . . . T . T . . . A . . . . . C . .
Gg20	. . . . . C . T T . . . A . . G T C . . . A G . . . . . T . . T . G . .	G . . . . . A . . . . . C . . C . . . . . C G . T . G .	C . . . . . T . T . . . A . C . . . . C . .
Gg21	. . . . . T . C . . . . . G . C . C . . . . . A . C . . . T . C T . . .	. . . T . . C . . . A . . . . . C . . C . T . . . . T A	. . C . G . . . . T . . . . . T . . . .
Gg22	. . . . . T . C . . . . . G . C . C . . . . . A . C . . . T . C T . . .	. . . T . . C . . . A G . . . . C . . C . T . . . . T A	. . C . G . . . . T . . . . . T . . . .
Gg23	. . . . . T . C . . . . . G . C . C . . . . . A . C . . . T . C T . . .	. . . T . . C . . . A . . . . . C . . C . T . . . . T A	. . C . G A . . . T . . . . . T . . . .
Gg24	. . . . . T . C . . A . . G . C . C . . . . . A . C . . . T . C T . . .	. . . T . . C . . . A . . . . . C . . C . T . . . . T A	. . C . G . . . . T . . . . . T . . . .
Gg25	. . . . . T . . . . . C A . . . . . T . . T . G . T	. . G . A . . . . . A . . T . . C . . . C . . . A . . . . C . . . . G	C . . . . . T . T . . . A . . . T . . C . .
Gg26	. . . . . T . . . . . G . C A . . . . . T . . T . G . .	. . G . A . . . . . A . . T . . C . C . C . . . A . . . . C . . . . G	C . . . . . T . T . . . A . . . T . . C . .
Gg27	. . . . . T . . . . . G . C A . . . . . T . . T . G . .	. . G . . . . . A . . T . . C . . . C . . . A . . . . C . . . . G	C . . . . . T . T . . . A . . . T . . C . .
Gg28	. . . . . T . . . . . G . C A . . . . . T . . T . G . .	. . . . . A . . T . . C . . . C . . . T . . . . C . . . . G	C . . . . . T . T . . . . . T . . C . .
Gg29	. . . . . T G . . . . . G . C A . . . . T . . . . . T . . T . G . .	. . . . . A . . T . . C . . . C . . . T . . . . C . . . . G	C . . T . . . T . T . . . . . T . . C . .
Gg30	. . . . . T . . . . . G . C A . . . . . T . . T . G . .	. . . . . A . G T . . C . . . C . . . T . . . . C . . . . G	C . . . . . T . T . . . . . T . T C . .
Gg31	. . . . . T . . . . . G . C A . . . . . T . . T . G . .	. . . . . A . . T . . C . . . C . . . A . . . . C . . . . G	C . . T . . . T . T . . . . . T . . C . .
Gg32	. . . . . T . . . . . G . C A . . . . . T . . T . G . .	. . . . . A . . T . . C . . . C . . . A . . . . C . . . . G	C . . . . . T . T . . . . . T . . C . .
Gg33	. . . . . T . . . . . G . C A . . . . . T . . T . G . .	. . . . . A . . T . . C . . . C . . . A . . . . C . . . . G	C . . . . . T . T . . . A . . . T . . C . .
Gg34	. . . . . T . . . . . G . C A . . . . T . . . . . T . . T . G . .	. . . . . A . . T . . C . . . C . . . A . . . . C . . . . G	C . . . . . T . T . . . . . T . . C . .
Gg35	C A G T . . G T T . . . . C G G . C . . T . . T . . . . . T . T C C T G . C T	T T . T . A . . G . A . . . C . C C . . C T T . . A . . . C . . . T .	C T . T . . C T . T . . . . . C G .

Table 3.8 Genetic distances, corrected with the model of evolution suggested by ModelTest (TrN+G with gamma shape parameter = 0.365), between *G. genetta* ND-5+Cytb+CR haplotypes.

Gg1																																				
Gg2	0.008																																			
Gg3	0.002	0.010																																		
Gg4	0.043	0.043	0.046																																	
Gg5	0.041	0.041	0.043	0.014																																
Gg6	0.056	0.056	0.059	0.010	0.020																															
Gg7	0.051	0.051	0.054	0.006	0.016	0.004																														
Gg8	0.054	0.054	0.056	0.008	0.018	0.006	0.002																													
Gg9	0.051	0.051	0.053	0.006	0.016	0.008	0.004	0.006																												
Gg10	0.041	0.041	0.043	0.010	0.004	0.016	0.012	0.014	0.012																											
Gg11	0.038	0.038	0.041	0.012	0.002	0.018	0.014	0.016	0.014	0.002																										
Gg12	0.043	0.043	0.046	0.016	0.006	0.022	0.018	0.020	0.018	0.006	0.004																									
Gg13	0.043	0.043	0.046	0.016	0.006	0.018	0.018	0.020	0.018	0.006	0.004	0.008																								
Gg14	0.048	0.048	0.046	0.029	0.022	0.036	0.031	0.034	0.031	0.022	0.020	0.024	0.024																							
Gg15	0.048	0.048	0.046	0.029	0.022	0.036	0.031	0.034	0.031	0.022	0.020	0.024	0.024	0.004																						
Gg16	0.056	0.056	0.053	0.027	0.020	0.033	0.029	0.031	0.029	0.020	0.018	0.022	0.022	0.022	0.022																					
Gg17	0.051	0.051	0.048	0.022	0.016	0.029	0.024	0.027	0.024	0.016	0.014	0.018	0.018	0.018	0.018	0.004																				
Gg18	0.059	0.059	0.056	0.029	0.022	0.036	0.031	0.033	0.031	0.022	0.020	0.024	0.024	0.024	0.024	0.002	0.006																			
Gg19	0.054	0.054	0.051	0.024	0.022	0.031	0.027	0.029	0.027	0.018	0.020	0.024	0.024	0.024	0.029	0.031	0.027	0.034																		
Gg20	0.059	0.059	0.056	0.029	0.027	0.036	0.031	0.034	0.031	0.022	0.024	0.029	0.029	0.025	0.029	0.036	0.031	0.038	0.004																	
Gg21	0.048	0.043	0.051	0.054	0.056	0.067	0.062	0.065	0.062	0.056	0.054	0.059	0.059	0.059	0.059	0.073	0.067	0.076	0.065	0.070																
Gg22	0.051	0.046	0.054	0.056	0.059	0.070	0.064	0.067	0.064	0.059	0.056	0.062	0.062	0.062	0.062	0.076	0.070	0.079	0.067	0.073	0.002															
Gg23	0.052	0.046	0.054	0.057	0.060	0.071	0.065	0.068	0.065	0.060	0.057	0.062	0.062	0.062	0.063	0.077	0.071	0.080	0.068	0.074	0.002	0.004														
Gg24	0.051	0.046	0.054	0.051	0.059	0.065	0.059	0.062	0.059	0.054	0.056	0.062	0.062	0.062	0.062	0.076	0.070	0.079	0.062	0.068	0.002	0.004	0.004													
Gg25	0.048	0.048	0.046	0.038	0.031	0.046	0.041	0.043	0.041	0.031	0.029	0.034	0.034	0.038	0.038	0.041	0.036	0.043	0.034	0.038	0.064	0.067	0.068	0.067												
Gg26	0.051	0.051	0.048	0.036	0.029	0.043	0.038	0.041	0.038	0.029	0.027	0.031	0.031	0.036	0.036	0.038	0.034	0.041	0.031	0.036	0.062	0.064	0.065	0.064	0.006											
Gg27	0.046	0.046	0.043	0.031	0.024	0.038	0.034	0.036	0.033	0.024	0.022	0.027	0.027	0.031	0.031	0.033	0.029	0.036	0.027	0.031	0.056	0.059	0.059	0.059	0.006	0.004										
Gg28	0.041	0.041	0.044	0.027	0.020	0.034	0.029	0.032	0.029	0.020	0.018	0.022	0.022	0.031	0.032	0.034	0.029	0.036	0.027	0.032	0.051	0.054	0.055	0.054	0.012	0.010	0.006									
Gg29	0.049	0.049	0.051	0.029	0.027	0.036	0.032	0.034	0.031	0.027	0.025	0.029	0.029	0.039	0.039	0.041	0.036	0.043	0.032	0.036	0.059	0.062	0.063	0.062	0.018	0.016	0.012	0.006								
Gg30	0.046	0.046	0.049	0.032	0.025	0.039	0.034	0.036	0.029	0.025	0.022	0.027	0.027	0.036	0.036	0.039	0.034	0.041	0.031	0.036	0.057	0.059	0.060	0.059	0.016	0.014	0.010	0.004	0.010							
Gg31	0.043	0.043	0.046	0.024	0.022	0.031	0.027	0.029	0.027	0.022	0.020	0.024	0.024	0.034	0.034	0.036	0.031	0.038	0.029	0.034	0.054	0.056	0.057	0.056	0.012	0.010	0.006	0.004	0.006	0.008						
Gg32	0.041	0.041	0.043	0.027	0.020	0.034	0.029	0.031	0.029	0.020	0.018	0.022	0.022	0.031	0.031	0.033	0.029	0.036	0.027	0.031	0.051	0.053	0.054	0.054	0.010	0.008	0.004	0.002	0.008	0.006	0.002					
Gg33	0.043	0.043	0.041	0.029	0.022	0.036	0.031	0.034	0.031	0.022	0.020	0.024	0.024	0.029	0.029	0.031	0.027	0.034	0.024	0.029	0.054	0.056	0.057	0.056	0.008	0.006	0.002	0.004	0.010	0.008	0.004	0.002				
Gg34	0.043	0.043	0.046	0.029	0.022	0.036	0.031	0.034	0.031	0.022	0.020	0.024	0.024	0.034	0.034	0.036	0.031	0.038	0.029	0.034	0.054	0.056	0.057	0.056	0.012	0.010	0.006	0.004	0.006	0.008	0.004	0.002	0.004			
Gg35	0.118	0.111	0.121	0.096	0.100	0.107	0.100	0.103	0.099	0.100	0.096	0.103	0.103	0.110	0.111	0.113	0.113	0.117	0.098	0.105	0.111	0.114	0.115	0.114	0.103	0.106	0.100	0.094	0.088	0.101	0.090	0.093	0.096	0.090		

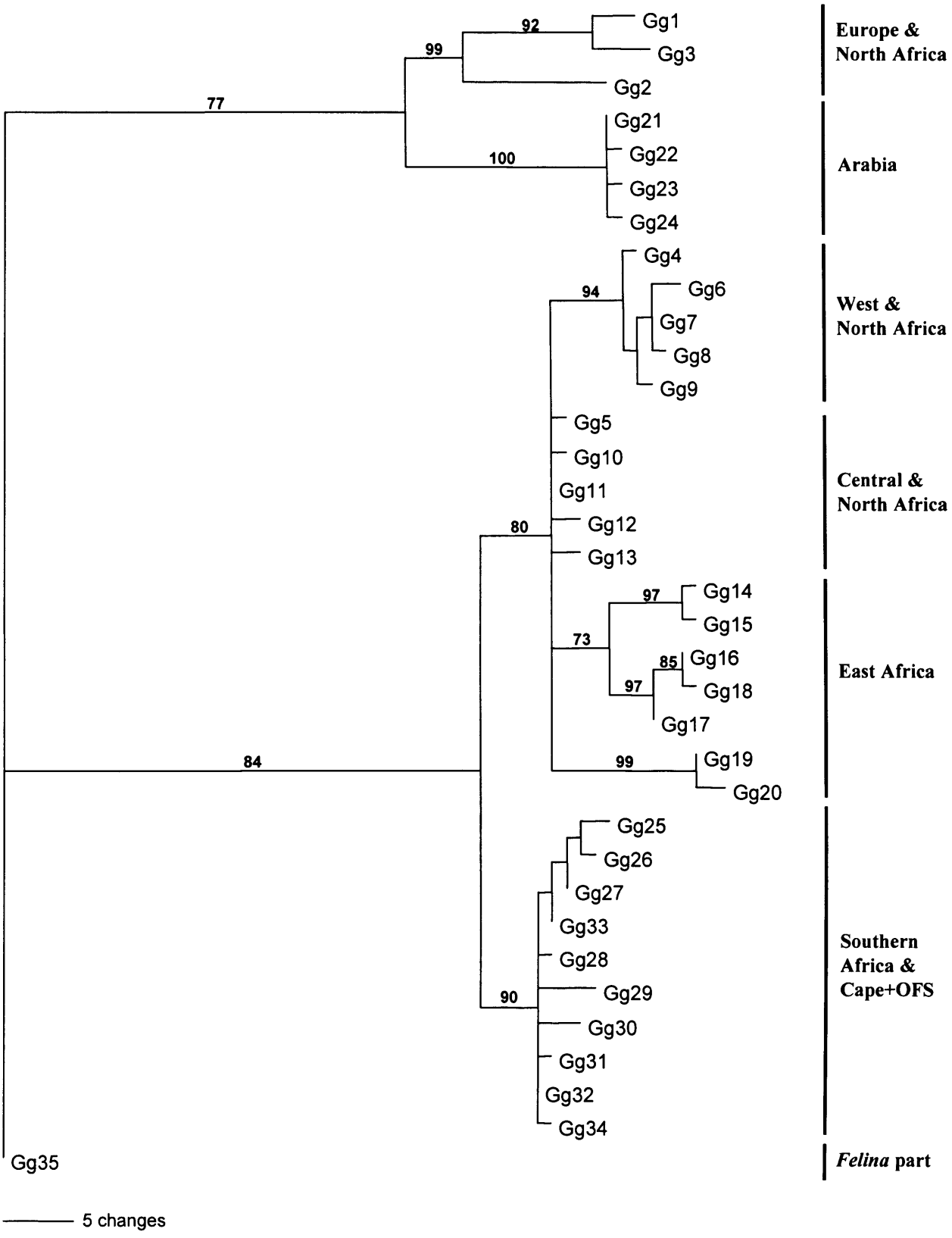

Figure 3.6 Phylogram of *G. genetta* haplotypes obtained with maximum parsimony as tree-search objective function. Haplotypes are classified in accordance with the geographic areas where they occur with the exception of a highly divergent haplotype found in part of the specimens phenotypically classified as belonging to the *felina* form of the *Genetta genetta* complex. Only bootstrap support values for each clade equal or higher than 70% are shown.

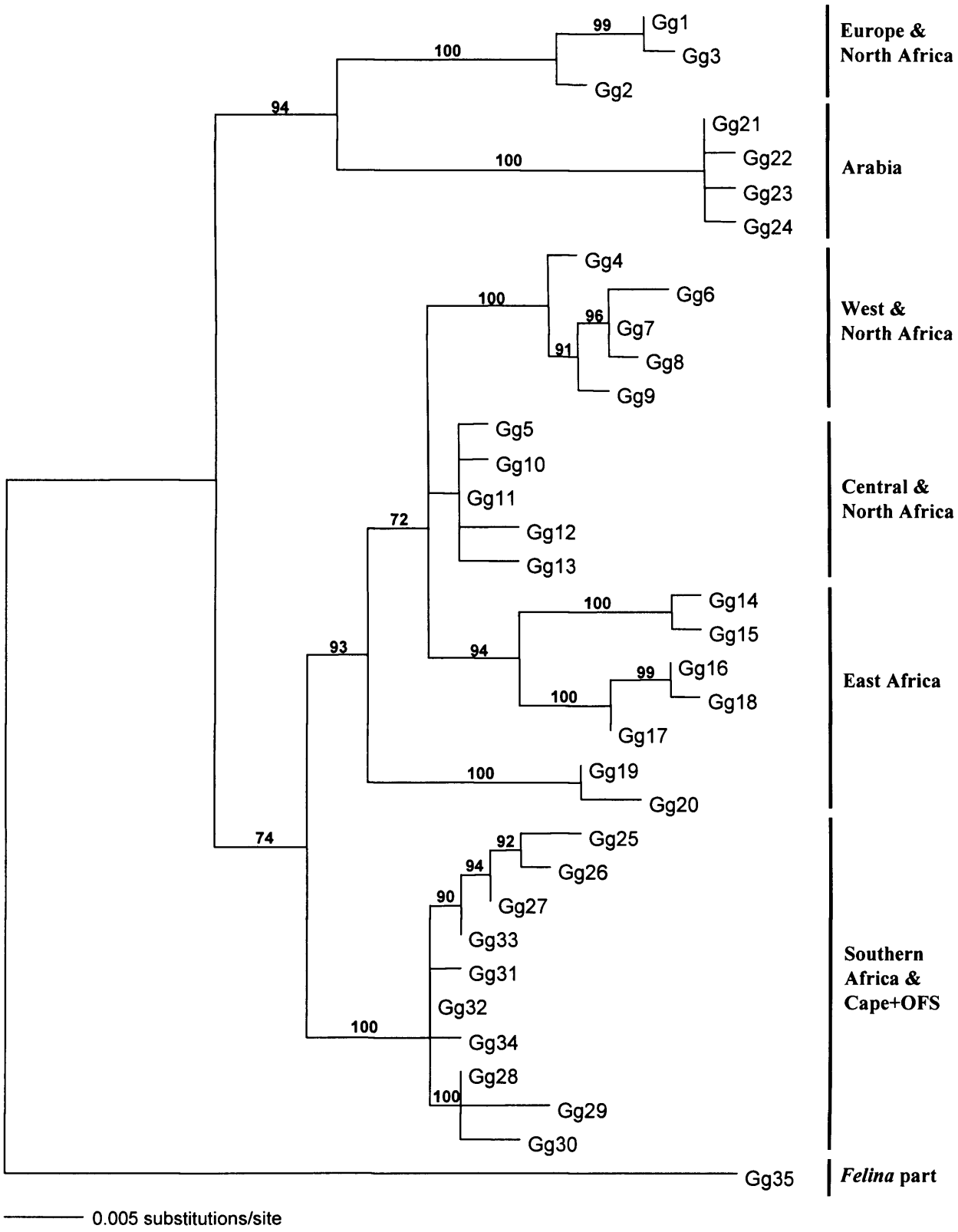

Figure 3.7 Phylogram of *G. genetta* haplotypes obtained using TrN+G (α=0.365) as maximum likelihood evolution model and Bayesian MCMC tree search. Haplotypes are classified in accordance with the geographic areas where they occur with the exception of a highly divergent haplotype found in part of the specimens phenotypically classified as belonging to the *felina* form of the *Genetta genetta* complex. Only posterior probabilities for each clade equal or higher than 70% are shown.

The most obvious features, common to both trees, are the clear separation of European and Arabian haplotypes from the majority of the African haplotypes and the presence of one highly divergent haplotype (Gg35) in some specimens (9 out of 14 sampled individuals) of *felina*, the Cape subspecies of *G. genetta*. However, the other 5 analysed *felina* specimens exhibited haplotypes (Gg26, Gg27) that fell within a clade containing all the lineages from Southern Africa, with the exception of Gg35. The Southern Africa & Cape+OFS clade formed a reciprocally monophyletic relationship with all other African lineages. Other main features are the ubiquitous associations of North Africa lineages, the apparent divergence of Somalian haplotypes (Gg19, Gg20) within the East African region, and the unresolved polytomic structure of the Central African haplotypes (Gg10-Gg13) indicating shallow genetic divergence from the MRCA of that region in the tree. The main difference between the two topologies concerns the basal clades, with the MP tree showing a polytomy joining three clades possibly the result of a long-branch attraction process (Hendy & Penny 1989) caused by the highly divergent haplotype Gg35. Maximum parsimony is usually a useful and reliable method for tree reconstruction at low levels of genetic divergence but is sensitive to the presence of odd evolutionarily distant clades within an otherwise homogeneous data set.

For the multispecies phylogenetic reconstruction, Figs. 3.8 and 3.9 present respectively a ME tree with LogDet transformed distances (matrix of distances in Table 3.9) and a ML tree with the evolutionary model TrN+G (α=0.223) (matrix of distances in Table 3.10). Once again, only bootstrap values equal or higher than 70% are presented. Comparison of phylogenies for LogDet transformed distances and ML transformed distances allows an examination of the assumptions of time reversibility and stationary substitution matrices of the ML model (Swofford 2002). An unexpected result in both trees is the existence of a mixed-species clade, including a shared haplotype (Gge/Gti) between some *felina* specimens (the same ones that possess Gg35 in the three-loci data set) and some *tigrina* specimens. Even more surprising it is the fact that this clade it is neither closely related with other *tigrina* haplotypes (Gti3-Gti6), or with other *felina* haplotypes (Gge22 and Gge23), which are within the *G. genetta* complex cluster in accordance with the traditional taxonomy, but instead groups as a reciprocally monophyletic sister group with *angolensis*. Another relevant result is the observation that *angolensis* and the *G. genetta* complex are more closely related to each other than to any other genet species in Southern Africa.

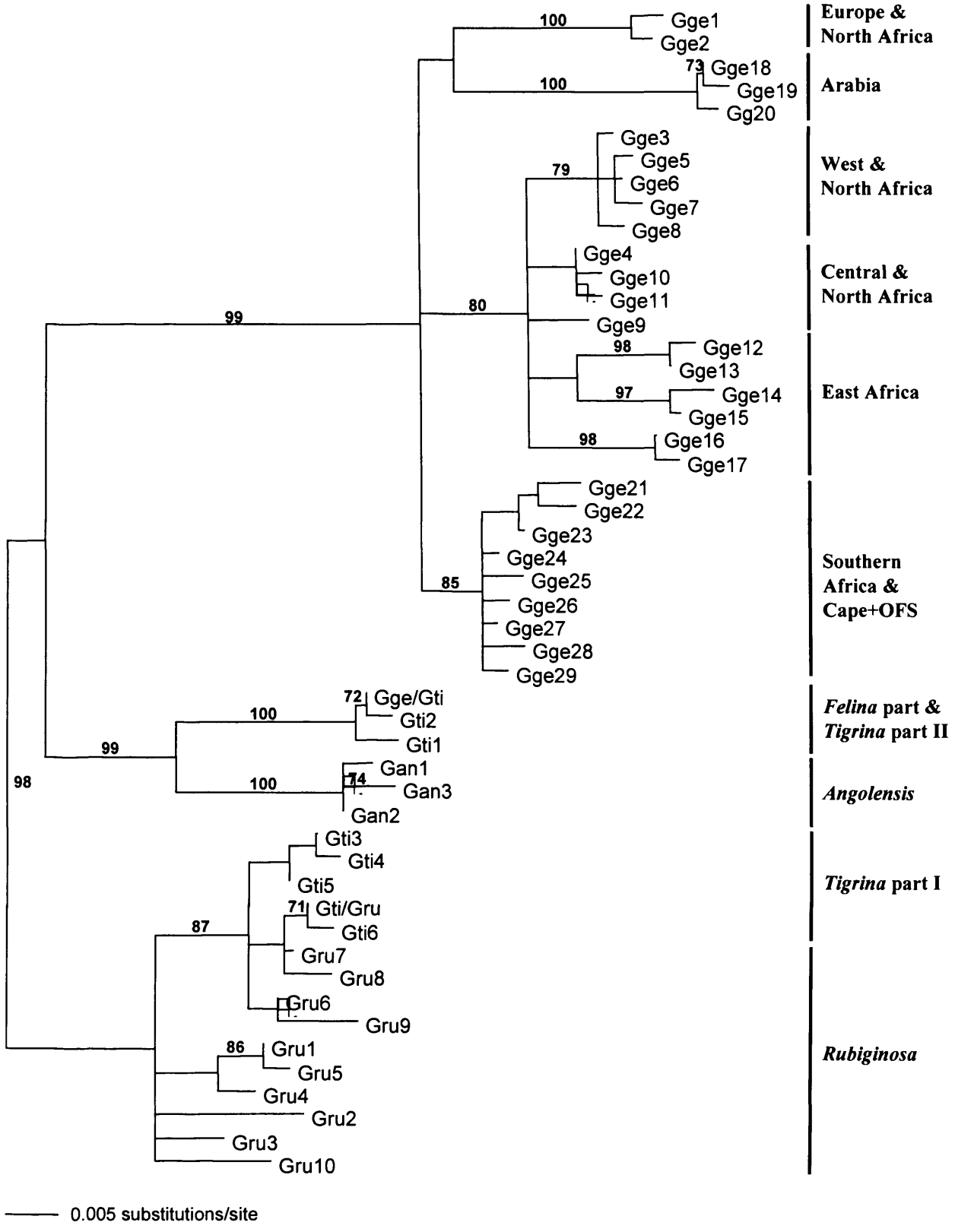

Figure 3.8 Phylogram of genet haplotypes obtained using LogDet for distance transformation and minimum evolution (ME) as optimality criterion. *G. genetta* haplotypes are classified in accordance with the geographic areas where they occur, with the exception of the highly divergent section of *felina* specimens, whereas the haplotypes of other species included in the analysis are simply designated by the species name. Only bootstrap support values for each clade equal or higher than 70% are shown.

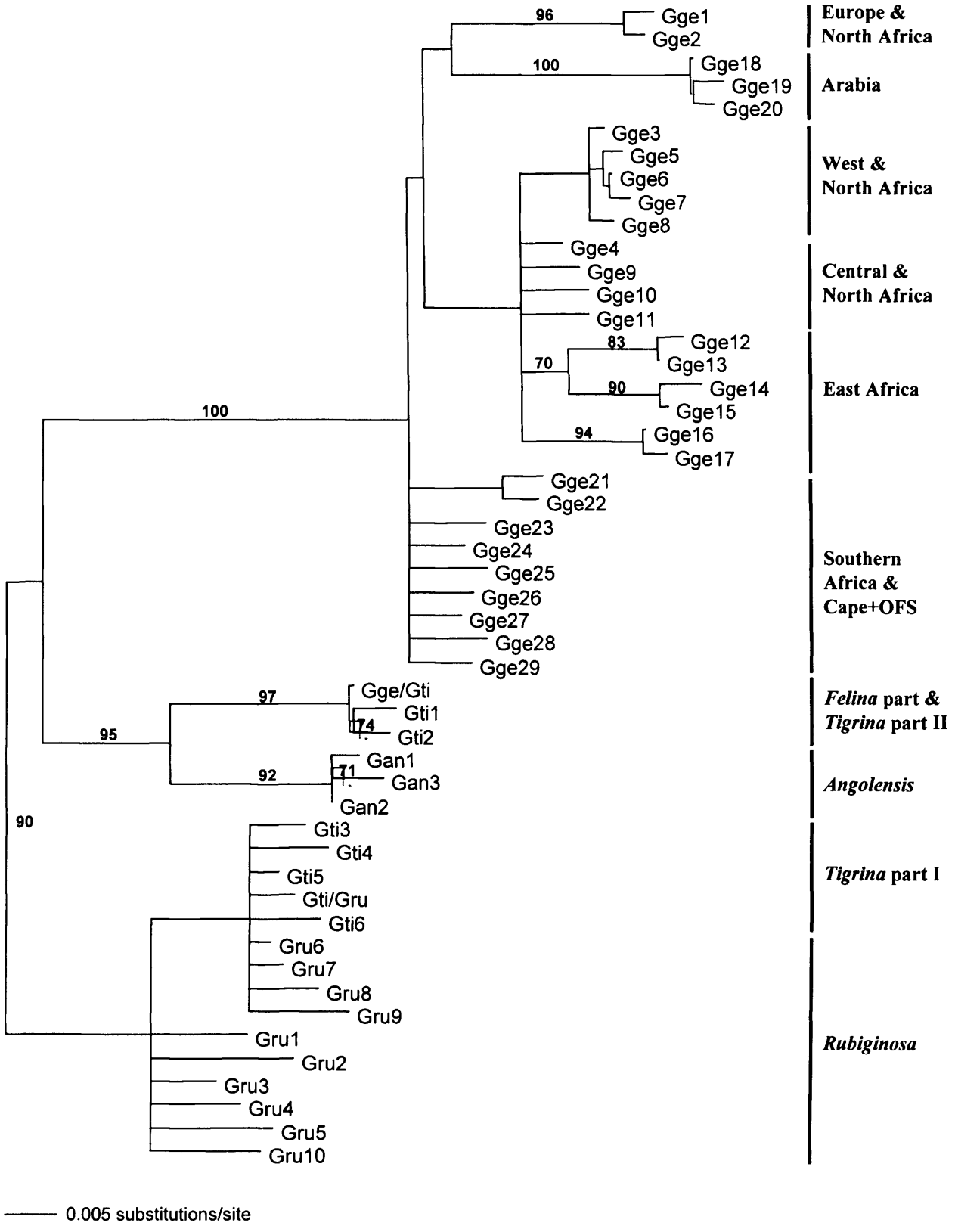

Figure 3.9 Phylogram of genet haplotypes obtained using TrN+G (α=0.223) as the maximum likelihood evolution model and heuristic tree search. *G. genetta* haplotypes are classified in accordance with the geographic areas where they occur, with the exception of the highly divergent part of *felina* specimens, whereas the haplotypes of other species included in the analysis are simply designated by the species name. Only bootstrap support values for each clade equal or higher than 70% are shown.

Table 3.9 LogDet transformed genetic distances between all ND-5+Cyt *b* haplotypes included in the multispecies phylogenetic analysis.

Gge1																																																	
Gge2	0.004																																																
Gge3	0.041	0.041																																															
Gge4	0.034	0.034	0.010																																														
Gge5	0.045	0.045	0.004	0.014																																													
Gge6	0.043	0.043	0.002	0.012	0.002																																												
Gge7	0.045	0.045	0.004	0.014	0.004	0.002																																											
Gge8	0.045	0.045	0.004	0.014	0.008	0.006	0.008																																										
Gge9	0.039	0.038	0.006	0.004	0.011	0.009	0.011	0.010																																									
Gge10	0.037	0.037	0.012	0.002	0.016	0.014	0.016	0.016	0.006																																								
Gge11	0.037	0.037	0.012	0.002	0.016	0.014	0.016	0.016	0.006	0.004																																							
Gge12	0.049	0.049	0.032	0.025	0.036	0.034	0.036	0.035	0.029	0.027	0.027																																						
Gge13	0.045	0.045	0.027	0.021	0.032	0.030	0.032	0.031	0.025	0.023	0.023	0.004																																					
Gge14	0.055	0.055	0.029	0.023	0.034	0.031	0.034	0.033	0.027	0.025	0.025	0.033	0.029																																				
Gge15	0.049	0.049	0.023	0.017	0.028	0.026	0.028	0.027	0.021	0.019	0.020	0.027	0.023	0.006																																			
Gge16	0.054	0.054	0.021	0.023	0.026	0.023	0.026	0.025	0.019	0.025	0.025	0.037	0.041	0.043	0.037																																		
Gge17	0.059	0.059	0.025	0.027	0.030	0.027	0.030	0.029	0.023	0.029	0.029	0.033	0.037	0.047	0.041	0.004																																	
Gge18	0.046	0.042	0.045	0.044	0.050	0.048	0.050	0.049	0.048	0.046	0.046	0.051	0.047	0.064	0.058	0.058	0.063																																
Gge19	0.050	0.046	0.049	0.048	0.054	0.052	0.054	0.053	0.052	0.050	0.050	0.054	0.051	0.068	0.062	0.063	0.067	0.004																															
Gge20	0.050	0.046	0.041	0.047	0.046	0.044	0.046	0.045	0.044	0.050	0.050	0.055	0.051	0.068	0.062	0.054	0.059	0.004	0.008																														
Gge21	0.047	0.047	0.038	0.032	0.043	0.040	0.043	0.042	0.036	0.034	0.034	0.056	0.051	0.052	0.046	0.047	0.052	0.060	0.065	0.065																													
Gge22	0.051	0.051	0.034	0.028	0.039	0.036	0.039	0.038	0.032	0.030	0.030	0.051	0.047	0.048	0.042	0.043	0.047	0.056	0.060	0.060	0.008																												
Gge23	0.044	0.044	0.028	0.022	0.032	0.030	0.032	0.032	0.026	0.024	0.024	0.044	0.040	0.042	0.036	0.037	0.041	0.049	0.053	0.053	0.009	0.006																											
Gge24	0.040	0.040	0.024	0.018	0.028	0.026	0.028	0.028	0.022	0.020	0.020	0.040	0.036	0.038	0.032	0.033	0.037	0.045	0.049	0.049	0.016	0.012	0.006																										
Gge25	0.046	0.046	0.030	0.024	0.034	0.032	0.034	0.034	0.028	0.026	0.026	0.046	0.042	0.044	0.038	0.036	0.041	0.051	0.055	0.055	0.021	0.017	0.012	0.006																									
Gge26	0.044	0.044	0.028	0.022	0.032	0.030	0.032	0.024	0.026	0.024	0.024	0.044	0.040	0.042	0.036	0.037	0.041	0.049	0.053	0.053	0.019	0.015	0.009	0.004	0.009																								
Gge27	0.040	0.040	0.024	0.018	0.028	0.026	0.028	0.028	0.022	0.020	0.020	0.040	0.036	0.038	0.032	0.033	0.037	0.045	0.049	0.049	0.013	0.010	0.004	0.002	0.008	0.006																							
Gge28	0.043	0.043	0.026	0.020	0.031	0.029	0.031	0.030	0.024	0.022	0.022	0.042	0.038	0.040	0.034	0.035	0.039	0.047	0.052	0.052	0.016	0.012	0.006	0.004	0.010	0.008	0.002																						
Gge29	0.043	0.043	0.026	0.020	0.031	0.029	0.031	0.030	0.024	0.022	0.022	0.042	0.038	0.040	0.034	0.035	0.039	0.047	0.051	0.052	0.016	0.012	0.006	0.004	0.006	0.008	0.002	0.004																					
Gge/Gti	0.097	0.092	0.091	0.089	0.096	0.094	0.096	0.095	0.094	0.092	0.092	0.105	0.101	0.103	0.105	0.098	0.103	0.087	0.091	0.091	0.102	0.102	0.095	0.090	0.089	0.094	0.090	0.093	0.088																				
Gti1	0.103	0.097	0.091	0.089	0.096	0.094	0.096	0.095	0.094	0.092	0.092	0.100	0.096	0.098	0.100	0.098	0.103	0.092	0.096	0.096	0.106	0.107	0.100	0.095	0.094	0.099	0.095	0.098	0.093	0.004																			
Gti2	0.100	0.095	0.094	0.092	0.099	0.096	0.099	0.098	0.096	0.094	0.095	0.108	0.103	0.106	0.107	0.101	0.106	0.089	0.094	0.094	0.104	0.104	0.097	0.093	0.092	0.097	0.093	0.095	0.091	0.002	0.006																		
Gti3	0.104	0.104	0.103	0.096	0.098	0.100	0.098	0.098	0.100	0.098	0.098	0.112	0.108	0.109	0.112	0.107	0.113	0.108	0.113	0.113	0.091	0.095	0.088	0.084	0.087	0.079	0.084	0.086	0.086	0.068	0.073	0.071																	
Gti4	0.107	0.106	0.105	0.098	0.100	0.103	0.100	0.101	0.103	0.101	0.101	0.115	0.110	0.112	0.114	0.110	0.115	0.111	0.115	0.116	0.093	0.098	0.090	0.086	0.090	0.081	0.086	0.089	0.089	0.066	0.071	0.068	0.002																
Gti5	0.099	0.099	0.098	0.091	0.093	0.096	0.093	0.094	0.096	0.094	0.094	0.107	0.103	0.105	0.107	0.103	0.108	0.103	0.108	0.108	0.086	0.090	0.083	0.079	0.083	0.075	0.079	0.082	0.082	0.064	0.069	0.067	0.004	0.006															
Gti/Gru	0.096	0.095	0.103	0.096	0.098	0.100	0.098	0.098	0.100	0.098	0.098	0.113	0.108	0.109	0.112	0.108	0.113	0.105	0.109	0.109	0.091	0.095	0.088	0.084	0.088	0.079	0.084	0.086	0.086	0.060	0.065	0.063	0.012	0.014	0.008														
Gti6	0.098	0.098	0.106	0.098	0.100	0.103	0.100	0.101	0.103	0.101	0.101	0.115	0.111	0.112	0.114	0.110	0.115	0.107	0.111	0.112	0.093	0.098	0.090	0.086	0.090	0.081	0.086	0.089	0.089	0.063	0.068	0.065	0.014	0.016	0.010	0.002													
Gru1	0.102	0.101	0.100	0.093	0.095	0.098	0.100	0.096	0.098	0.096	0.096	0.101	0.097	0.107	0.109	0.096	0.092	0.111	0.115	0.116	0.089	0.093	0.086	0.081	0.080	0.077	0.082	0.084	0.079	0.065	0.070	0.067	0.026	0.028	0.022	0.026	0.028												
Gru2	0.099	0.099	0.094	0.096	0.089	0.092	0.094	0.090	0.092	0.098	0.098	0.113	0.108	0.110	0.112	0.090	0.095	0.114	0.118	0.109	0.091	0.095	0.088	0.084	0.083	0.079	0.084	0.086	0.082	0.076	0.081	0.078	0.040	0.042	0.036	0.040	0.042	0.021											
Gru3	0.080	0.080	0.092	0.085	0.087	0.090	0.092	0.087	0.090	0.088	0.087	0.102	0.097	0.098	0.101	0.096	0.101	0.094	0.098	0.099	0.080	0.085	0.077	0.073	0.072	0.069	0.073	0.076	0.071	0.057	0.061	0.059	0.030	0.032	0.026	0.022	0.025	0.019	0.021										
Gru4	0.098	0.097	0.096	0.089	0.091	0.094	0.096	0.092	0.094	0.092	0.092	0.106	0.102	0.112	0.105	0.092	0.097	0.107	0.111	0.112	0.076	0.080	0.082	0.077	0.076	0.073	0.078	0.080	0.075	0.069	0.074	0.072	0.030	0.032	0.025	0.030	0.032	0.011	0.025	0.023									
Gru5	0.104	0.104	0.103	0.096	0.098	0.100	0.103	0.098	0.100	0.098	0.098	0.104	0.099	0.110	0.112	0.099	0.095	0.114	0.118	0.118	0.091	0.095	0.088	0.084	0.083	0.079	0.084	0.086	0.082	0.067	0.072	0.070	0.028	0.030	0.024	0.028	0.030	0.002	0.024	0.021	0.013								
Gru6	0.100	0.099	0.098	0.091	0.093	0.096	0.098	0.094	0.096	0.094	0.094	0.107	0.103	0.105	0.107	0.103	0.108	0.103	0.108	0.108	0.086	0.090	0.083	0.079	0.078	0.075	0.079	0.082	0.077	0.060	0.064	0.062	0.008	0.010	0.004	0.012	0.014	0.017	0.032	0.022	0.021	0.020							
Gru7	0.100	0.099	0.098	0.091	0.093	0.096	0.093	0.094	0.096	0.094	0.094	0.107	0.103	0.105	0.107	0.103	0.108	0.109	0.113	0.113	0.086	0.090	0.083	0.079	0.083	0.075	0.079	0.082	0.082	0.064	0.069	0.067	0.008	0.010	0.004	0.004	0.006	0.022	0.036	0.026	0.026	0.024	0.008						
Gru8	0.102	0.102	0.101	0.094	0.096	0.098	0.096	0.097	0.098	0.097	0.097	0.110	0.106	0.107	0.110	0.105	0.110	0.111	0.116	0.116	0.089	0.093	0.086	0.082	0.086	0.077	0.082	0.084	0.084	0.069	0.074	0.072	0.012	0.014	0.008	0.008	0.010	0.026	0.041	0.030	0.030	0.028	0.013	0.004					
Gru9	0.110	0.110	0.109	0.102	0.104	0.106	0.109	0.104	0.106	0.104	0.104	0.118	0.114	0.115	0.118	0.114	0.119	0.114	0.118	0.118	0.096	0.101	0.094	0.089	0.088	0.085	0.089	0.092	0.087	0.070	0.075	0.072	0.017	0.019	0.013	0.021	0.024	0.027	0.041	0.031	0.031	0.029	0.009	0.018	0.022				
Gru10	0.093	0.093	0.097	0.090	0.092	0.094	0.097	0.101	0.094	0.093	0.093	0.106	0.102	0.103	0.106	0.101	0.106	0.107	0.111	0.112	0.085	0.089	0.082	0.078	0.077	0.082	0.078	0.080	0.075	0.061	0.066	0.064	0.030	0.032	0.026	0.026	0.028	0.019	0.029	0.019	0.023	0.021	0.022	0.022	0.026	0.031			
Gan1	0.088	0.083	0.092	0.085	0.097	0.095	0.097	0.096	0.090	0.088	0.088	0.101	0.097	0.108	0.101	0.097	0.101	0.083	0.087	0.087	0.103	0.098	0.091	0.086	0.086	0.090	0.087	0.089	0.084	0.035	0.040	0.037	0.075	0.077	0.071	0.067	0.069	0.072	0.078	0.059	0.068	0.074	0.066	0.071	0.076	0.076	0.063		
Gan2	0.084	0.079	0.088	0.081	0.093	0.091	0.093	0.092	0.086	0.084	0.084	0.097	0.093	0.104	0.097	0.093	0.097	0.079	0.083	0.083	0.098	0.094	0.087	0.082	0.082	0.086	0.083	0.085	0.080	0.031	0.036	0.034	0.071	0.073	0.067	0.063	0.066	0.068	0.074	0.055	0.064	0.070	0.062	0.067	0.072	0.072	0.059	0.004	
Gan3	0.091	0.086	0.095	0.088	0.100	0.098	0.100	0.099	0.093	0.091	0.091	0.105	0.100	0.102	0.095	0.100	0.105	0.086	0.091	0.091	0.106	0.101	0.094	0.089	0.089	0.093	0.090	0.092	0.087	0.037	0.042	0.040	0.078	0.080	0.074	0.070	0.072	0.075	0.082	0.062	0.071	0.077	0.069	0.074	0.079	0.079	0.066	0.009	0.006

Table 3.10 Corrected genetic distances, using the model of evolution suggested by ModelTest (TrN+G with gamma shape parameter = 0.223), between all ND-5+Cyt *b* haplotypes included in the multispecies phylogenetic analysis.

Gge1
Gge2	0.005
Gge3	0.046	0.046
Gge4	0.038	0.038	0.010
Gge5	0.054	0.054	0.005	0.016
Gge6	0.050	0.050	0.002	0.013	0.002
Gge7	0.054	0.054	0.005	0.016	0.005	0.002
Gge8	0.049	0.049	0.002	0.013	0.008	0.005	0.008
Gge9	0.042	0.042	0.008	0.002	0.013	0.010	0.013	0.010
Gge10	0.042	0.042	0.013	0.002	0.019	0.016	0.019	0.016	0.005
Gge11	0.042	0.042	0.013	0.002	0.019	0.016	0.019	0.016	0.005	0.005
Gge12	0.053	0.053	0.028	0.022	0.035	0.031	0.035	0.031	0.025	0.025	0.025
Gge13	0.050	0.050	0.025	0.019	0.031	0.028	0.031	0.028	0.022	0.022	0.022	0.002
Gge14	0.061	0.061	0.028	0.022	0.035	0.031	0.035	0.031	0.025	0.025	0.025	0.028	0.025
Gge15	0.053	0.053	0.022	0.016	0.028	0.025	0.028	0.025	0.019	0.019	0.019	0.022	0.019	0.005
Gge16	0.062	0.062	0.022	0.022	0.029	0.025	0.029	0.025	0.019	0.025	0.025	0.035	0.038	0.042	0.035
Gge17	0.066	0.066	0.025	0.025	0.032	0.028	0.032	0.028	0.022	0.028	0.028	0.032	0.035	0.045	0.038	0.002
Gge18	0.058	0.050	0.054	0.054	0.062	0.058	0.062	0.058	0.058	0.058	0.058	0.062	0.058	0.079	0.070	0.071	0.075
Gge19	0.062	0.054	0.058	0.058	0.066	0.062	0.066	0.061	0.062	0.062	0.062	0.066	0.062	0.084	0.075	0.075	0.080	0.002
Gge20	0.062	0.054	0.050	0.058	0.058	0.054	0.058	0.054	0.054	0.062	0.062	0.067	0.063	0.084	0.075	0.067	0.071	0.002	0.005
Gge21	0.049	0.049	0.038	0.031	0.046	0.042	0.046	0.042	0.035	0.035	0.035	0.053	0.049	0.053	0.045	0.046	0.049	0.066	0.070	0.070
Gge22	0.053	0.053	0.035	0.028	0.042	0.038	0.042	0.038	0.031	0.031	0.031	0.049	0.045	0.049	0.042	0.042	0.046	0.061	0.065	0.066	0.008
Gge23	0.045	0.045	0.028	0.022	0.035	0.031	0.035	0.031	0.025	0.025	0.025	0.042	0.038	0.042	0.035	0.035	0.038	0.053	0.057	0.057	0.008	0.005
Gge24	0.043	0.043	0.026	0.019	0.032	0.029	0.032	0.029	0.022	0.022	0.022	0.039	0.036	0.039	0.032	0.032	0.036	0.051	0.055	0.055	0.013	0.010	0.005
Gge25	0.050	0.050	0.032	0.025	0.039	0.036	0.039	0.035	0.029	0.029	0.029	0.046	0.043	0.046	0.039	0.036	0.039	0.059	0.063	0.063	0.019	0.016	0.010	0.005
Gge26	0.046	0.046	0.029	0.022	0.036	0.032	0.036	0.025	0.025	0.025	0.025	0.042	0.039	0.042	0.035	0.036	0.039	0.055	0.058	0.059	0.016	0.013	0.008	0.002	0.008
Gge27	0.042	0.042	0.025	0.019	0.032	0.028	0.032	0.028	0.022	0.022	0.022	0.038	0.035	0.038	0.031	0.032	0.035	0.050	0.053	0.053	0.010	0.008	0.002	0.003	0.008	0.005
Gge28	0.046	0.046	0.028	0.022	0.035	0.032	0.035	0.031	0.025	0.025	0.025	0.042	0.038	0.042	0.035	0.035	0.038	0.054	0.057	0.058	0.013	0.010	0.005	0.005	0.010	0.008	0.002
Gge29	0.046	0.046	0.028	0.022	0.035	0.032	0.035	0.031	0.025	0.025	0.025	0.042	0.038	0.042	0.035	0.035	0.038	0.054	0.057	0.058	0.013	0.010	0.005	0.005	0.005	0.008	0.002	0.005
Gge/Gti	0.164	0.150	0.142	0.142	0.156	0.149	0.156	0.148	0.149	0.149	0.149	0.170	0.163	0.169	0.169	0.143	0.150	0.136	0.142	0.143	0.147	0.154	0.141	0.139	0.132	0.145	0.135	0.141	0.128
Gti1	0.179	0.164	0.142	0.142	0.156	0.149	0.156	0.148	0.149	0.149	0.149	0.155	0.149	0.155	0.154	0.143	0.150	0.150	0.156	0.157	0.161	0.169	0.154	0.153	0.146	0.159	0.148	0.155	0.141	0.005
Gti2	0.172	0.157	0.149	0.149	0.163	0.156	0.163	0.155	0.156	0.156	0.156	0.178	0.171	0.177	0.177	0.150	0.157	0.143	0.149	0.150	0.154	0.161	0.147	0.146	0.139	0.152	0.141	0.148	0.135	0.002	0.008
Gti3	0.172	0.172	0.163	0.149	0.149	0.156	0.149	0.156	0.156	0.156	0.156	0.178	0.171	0.177	0.177	0.157	0.164	0.171	0.178	0.179	0.129	0.135	0.122	0.120	0.127	0.114	0.117	0.123	0.123	0.088	0.099	0.093
Gti4	0.180	0.180	0.171	0.156	0.156	0.163	0.156	0.164	0.163	0.164	0.164	0.186	0.179	0.185	0.185	0.164	0.172	0.179	0.186	0.187	0.136	0.142	0.129	0.127	0.133	0.120	0.123	0.129	0.129	0.083	0.093	0.088	0.002
Gti5	0.165	0.165	0.156	0.142	0.142	0.149	0.142	0.149	0.149	0.149	0.149	0.171	0.164	0.170	0.170	0.151	0.157	0.164	0.170	0.171	0.123	0.129	0.116	0.114	0.120	0.108	0.111	0.117	0.117	0.084	0.094	0.089	0.002	0.005
Gti/Gru	0.158	0.158	0.163	0.149	0.149	0.156	0.149	0.156	0.156	0.156	0.156	0.178	0.171	0.178	0.177	0.157	0.164	0.173	0.179	0.180	0.129	0.135	0.122	0.120	0.127	0.114	0.117	0.123	0.123	0.079	0.089	0.084	0.010	0.013	0.008
Gti6	0.166	0.166	0.171	0.156	0.156	0.163	0.156	0.164	0.163	0.164	0.164	0.186	0.179	0.185	0.185	0.164	0.172	0.180	0.187	0.188	0.136	0.142	0.129	0.127	0.133	0.120	0.123	0.129	0.129	0.084	0.094	0.089	0.013	0.016	0.010	0.002
Gru1	0.164	0.164	0.156	0.142	0.142	0.149	0.156	0.149	0.149	0.149	0.149	0.157	0.150	0.170	0.170	0.138	0.132	0.179	0.186	0.187	0.123	0.128	0.116	0.114	0.108	0.108	0.111	0.117	0.105	0.073	0.083	0.078	0.025	0.028	0.022	0.025	0.028
Gru2	0.157	0.157	0.150	0.149	0.136	0.143	0.150	0.143	0.143	0.156	0.156	0.178	0.171	0.178	0.177	0.133	0.139	0.187	0.194	0.180	0.129	0.135	0.122	0.120	0.114	0.114	0.117	0.123	0.111	0.087	0.097	0.092	0.042	0.045	0.038	0.042	0.045	0.019
Gru3	0.126	0.126	0.144	0.131	0.130	0.137	0.144	0.137	0.137	0.137	0.137	0.157	0.151	0.157	0.157	0.139	0.145	0.152	0.159	0.159	0.112	0.117	0.106	0.103	0.098	0.098	0.101	0.106	0.095	0.065	0.074	0.070	0.032	0.035	0.028	0.025	0.028	0.016	0.019
Gru4	0.158	0.158	0.150	0.136	0.136	0.143	0.150	0.143	0.143	0.143	0.143	0.164	0.157	0.177	0.163	0.132	0.139	0.172	0.179	0.180	0.106	0.112	0.111	0.109	0.103	0.103	0.106	0.112	0.100	0.078	0.087	0.083	0.028	0.031	0.025	0.028	0.031	0.008	0.022	0.019
Gru5	0.172	0.172	0.163	0.149	0.149	0.156	0.163	0.156	0.156	0.156	0.156	0.164	0.158	0.178	0.177	0.145	0.139	0.187	0.194	0.195	0.129	0.135	0.122	0.120	0.114	0.114	0.117	0.123	0.111	0.078	0.088	0.083	0.028	0.031	0.025	0.028	0.031	0.002	0.022	0.019	0.010
Gru6	0.165	0.165	0.156	0.143	0.142	0.149	0.156	0.149	0.149	0.149	0.149	0.171	0.164	0.170	0.170	0.151	0.157	0.164	0.170	0.171	0.123	0.129	0.116	0.114	0.108	0.108	0.111	0.117	0.105	0.074	0.084	0.079	0.008	0.010	0.005	0.013	0.016	0.016	0.031	0.022	0.019	0.019
Gru7	0.165	0.165	0.156	0.143	0.142	0.149	0.142	0.149	0.149	0.149	0.149	0.171	0.164	0.170	0.170	0.151	0.157	0.179	0.186	0.187	0.123	0.129	0.116	0.114	0.121	0.108	0.111	0.117	0.117	0.084	0.094	0.089	0.008	0.010	0.005	0.002	0.005	0.022	0.038	0.028	0.025	0.025	0.010
Gru8	0.172	0.172	0.164	0.149	0.149	0.156	0.149	0.157	0.156	0.157	0.157	0.178	0.172	0.178	0.178	0.158	0.164	0.187	0.194	0.195	0.130	0.135	0.123	0.121	0.127	0.114	0.117	0.123	0.123	0.094	0.104	0.099	0.013	0.016	0.010	0.008	0.010	0.028	0.046	0.035	0.031	0.032	0.016	0.005
Gru9	0.186	0.186	0.177	0.162	0.162	0.169	0.177	0.169	0.169	0.169	0.169	0.192	0.185	0.191	0.191	0.170	0.177	0.185	0.192	0.193	0.141	0.147	0.134	0.132	0.125	0.126	0.128	0.135	0.122	0.088	0.098	0.093	0.016	0.019	0.013	0.022	0.025	0.025	0.041	0.031	0.028	0.028	0.008	0.019	0.025
Gru10	0.151	0.151	0.157	0.143	0.143	0.150	0.157	0.164	0.150	0.150	0.150	0.172	0.165	0.171	0.171	0.152	0.158	0.180	0.187	0.188	0.124	0.129	0.117	0.115	0.109	0.121	0.112	0.118	0.106	0.074	0.083	0.079	0.031	0.035	0.028	0.025	0.028	0.016	0.025	0.016	0.019	0.019	0.022	0.022	0.028	0.031
Gan1	0.143	0.130	0.150	0.136	0.164	0.157	0.164	0.156	0.143	0.143	0.143	0.163	0.157	0.177	0.163	0.144	0.151	0.131	0.136	0.137	0.155	0.148	0.135	0.133	0.126	0.139	0.129	0.136	0.123	0.042	0.050	0.046	0.098	0.104	0.093	0.089	0.094	0.083	0.087	0.065	0.078	0.087	0.083	0.093	0.104	0.098	0.074
Gan2	0.138	0.124	0.143	0.130	0.158	0.150	0.158	0.150	0.137	0.137	0.137	0.157	0.151	0.170	0.156	0.138	0.145	0.125	0.131	0.131	0.149	0.142	0.129	0.127	0.121	0.133	0.123	0.130	0.117	0.039	0.046	0.042	0.094	0.099	0.089	0.084	0.089	0.078	0.082	0.061	0.074	0.083	0.079	0.089	0.099	0.093	0.070	0.002
Gan3	0.151	0.137	0.157	0.143	0.172	0.165	0.172	0.164	0.150	0.150	0.150	0.172	0.165	0.171	0.157	0.152	0.158	0.138	0.144	0.144	0.163	0.156	0.142	0.140	0.133	0.146	0.136	0.143	0.130	0.046	0.054	0.050	0.104	0.110	0.099	0.094	0.100	0.088	0.092	0.070	0.083	0.093	0.089	0.099	0.110	0.103	0.079	0.008	0.005

Table 3.11 shows minimum and maximum genetic divergences for all inter- and intraclade comparisons in the multispecies data set. Intervals of intraclade distances, using the evolution model TrN+G with α=0.223, are on the diagonal of the matrix, and intervals of interclade distances using the same model are below the diagonal. Intervals of interclade distances as estimated with the Kimura-2P model are above the diagonal to allow comparison with the results in Johns & Avise (1998). As discussed above, part of the *felina* specimens presented a very divergent haplotype (Gge/Gti) from any other *G. genetta* haplotype, and related with haplotypes found in *G. tigrina* and *G.* angolensis. Consequently, the clade with the Gge/Gti haplotype or its equivalent in the within *G. genetta* analysis (Gg35) was treated as a distinct unit, labelled "*Felina* part", in both the multispecies and single-species analyses. The fact that all interclade distances are higher than all intraclade distances, with the exception of cases where haplotypes are shared between clades, together with the results from the phylogenetic reconstructions, indicates that some of the units in *G. genetta* apparently represent independent evolutionary lineages (Avise 1994). It is important to note that in the two genes data set, North and Central Africa share one haplotype but the same specimens are separated when their control region sequences are also considered.

Table 3.11 Minimum and maximum genetic divergence percentages for all inter- and intraclade comparisons for the clades considered in the multispecies phylogenetic analysis. Below and on the diagonal: distances using the model and parameters suggested by ModelTest as best fitting the data (TrN+G with gamma shape parameter = 0.223). Above diagonal: distances using the Kimura-2P model as in Johns & Avise (1998). Underlined values indicate pairwise comparisons between clades that share haplotypes.

	Europe	North Africa	West Africa	Central Africa	East Africa	Arabia	Southern Africa	Cape+OFS	*Felina* part	*Tigrina* part I	*Tigrina* part II	*Rubiginosa*	*Angolensis*
Europe	0.0-0.5%	0.0-4.6%	4.9-5.3%	3.8-4.2%	4.9-6.5%	4.9-6.1%	4.1-5.3%	4.6-5.3%	13.5-14.8%	14.2-16.2%	13.5-16.2%	11.1-17.0%	11.1-13.5%
North Africa	0.0-4.6%	0.0-4.6%	0.2-5.3%	0.0-4.2%	1.6-6.5%	4.9-6.1%	1.9-5.3%	2.2-5.3%	12.9-14.8%	12.9-16.2%	12.9-16.2%	11.1-17.0%	11.7-14.2%
West Africa	4.9-5.4%	0.2-5.4%	0.0-0.8%	1.0-1.9%	2.5-3.5%	5.3-6.6%	2.5-4.6%	3.1-4.2%	13.5-14.2%	12.9-14.8%	13.5-14.8%	11.7-16.2%	13.5-15.5%
Central Africa	3.8-4.2%	0.0-4.2%	1.0-1.9%	0.0-0.5%	1.6-2.8%	5.3-6.1%	1.9-3.5%	2.2-3.1%	12.9-13.5%	12.9-14.8%	12.9-14.2%	11.7-15.5%	11.7-13.5%
East Africa	5.0-6.6%	1.6-6.6%	2.5-3.5%	1.6-2.8%	0.0-4.5%	5.7-8.4%	3.1-5.3%	3.4-4.9%	13.7-15.5%	14.4-17.0%	13.5-16.2%	12.5-17.7%	13.1-16.2%
Arabia	5.0-6.2%	5.0-6.2%	5.4-6.6%	5.4-6.2%	5.8-8.4%	0.0-0.5%	4.9-7.0%	5.3-6.6%	12.3-12.9%	14.8-17.0%	12.3-14.2%	13.5-17.7%	11.1-12.9%
Southern Africa	4.2-5.3%	1.9-5.3%	2.5-4.6%	1.9-3.5%	3.1-5.3%	5.0-7.0%	0.0-1.9%	0.0-1.6%	11.5-14.2%	9.4-12.9%	11.5-15.5%	8.4-13.5%	10.4-14.8%
Cape+OFS	4.5-5.3%	2.2-5.3%	3.1-4.2%	2.2-3.1%	3.5-4.9%	5.3-6.6%	0.0-1.6%	0.0-0.5%	12.9-14.2%	10.6-12.9%	12.9-15.5%	9.5-13.5%	11.7-14.2%
Felina part	15.0-16.4%	14.2-16.4%	14.8-15.6%	14.2-14.9%	14.3-17.0%	13.6-14.3%	12.8-15.4%	14.1-15.4%	0.0%	7.9-8.4%	0.0-0.5%	6.6-9.4%	3.8-4.6%
Tigrina part I	15.8-18.0%	14.2-18.0%	14.2-16.4%	14.2-16.4%	15.1-18.6%	16.4-18.8%	11.4-13.6%	11.6-14.2%	7.9-8.8%	0.0-1.6%	7.9-9.9%	0.0-4.6%	8.4-10.5%
Tigrina part II	15.0-17.9%	14.2-17.9%	14.8-16.3%	14.2-15.6%	14.3-17.8%	13.6-15.7%	12.8-16.9%	14.1-16.9%	0.0-0.5%	7.9-9.9%	0.0-0.8%	7.0-10.5%	3.8-5.3%
Rubiginosa	12.6-18.6%	12.6-18.6%	13.0-17.7%	13.1-16.9%	13.2-19.2%	15.2-19.5%	9.8-14.7%	10.6-14.7%	6.5-9.4%	0.0-4.5%	6.5-10.4%	0.0-4.6%	6.1-11.0%
Angolensis	12.4-15.1%	13.0-15.7%	15.0-17.2%	13.0-15.0%	13.8-17.7%	12.5-14.4%	11.7-16.3%	12.9-15.6%	3.9-4.6%	8.4-11.0%	3.9-5.4%	6.1-11.0%	0.0-0.8%

3.3.4 Population genetics and phylogeographic analyses

Estimated values for parameters of genetic diversity and the results of neutrality tests, which support the assumption of neutral evolution, are summarised per geographic area in Table 3.12. The low values of both haplotype and nucleotide diversity in Europe, Arabia, and the Cape+OFS region may be indicators of recent bottlenecks or founder events driven by a small number of lineages (Grant & Bowen 1998). Similarly, the larger values of gene diversity together with low values of nucleotide diversity observed for West, Central and Southern Africa may suggest that these populations passed though a more ancient bottleneck. Finally, the large values of both gene diversity and nucleotide diversity present in North and East Africa seems to suggest that these populations maintain large effective population sizes, either kept due to internal subdivision or secondary contact with other demes. Several authors have noted that the D value of the Tajima's test (Tajima 1989a) is sensitive to population demography as well as to non-neutral molecular evolution (Fu & Li 1993; Rand 1996). Population growth or a recent bottleneck will be reflected in more negative values of D, whereas population structure will drive D towards more positive values. The estimated D values are therefore also in agreement with a large N_e in East and North Africa and a small N_e in Europe and Arabia. The mismatch distributions were unimodal for all geographic areas with the exception of North Africa and East Africa and Table 3.13 shows the estimated parameters and the raggedness index for each area. However, although these results are concordant with the predictions from the estimated measures of genetic diversity, none of the mismatch distributions under the sudden expansion model was statistically significant at the confidence level of 95% and the hypothesis of no population expansion could not be rejected. This probably indicates that larger sample sizes would be required to achieve the necessary statistical power to determine with high confidence the demographic history of each area.

Genetic differentiation between the geographic areas was strongly and consistently supported by different statistics (χ^2=584.476 P=0.000; K_S*=1.142 P=0.000; S_{nn}=0.845 P=0.000) and the analysis of the molecular variance showed that 90% of the total variance comes from the variance between the predefined geographic areas. Tables 3.14 and 3.15 show pairwise values of fixation indexes and estimates of gene flow obtained with different methods and Table 3.16 presents the results of an exact test of population differentiation based on haplotype frequencies. Pairwise fixation indexes and estimates of gene flow given by the different methods were broadly similar, and congruent with the

pattern of differentiation suggested by both phylogenetic trees and genetic divergence estimates. Results of the exact test in general were concordant with the pattern revealed by the fixation indexes and gene flow estimates, although the non-differentiation between West and East Africa and between the Cape+OFS and East Africa were not. However, since this test is based on haplotype frequencies only, and therefore sensitive to small sample sizes, incongruent results may be expected. The result of the Mantel test indicated a moderate correlation between genetic distance and geographic distance (Z=2419045.448, r=0.368, P=0.030). Table 3.17 presents the matrices that were regressed: a matrix of net nucleotide differences between populations and a matrix of the geographic distances between the centres of the areas. This result may be consequence of a globally moderate correlation throughout the species range or of the existence in the whole distribution area of a mosaic of regions presently with and without restricted gene flow.

The minimum spanning network of *G. genetta* haplotypes is presented in Fig. 3.10. Both the topology and spanning lengths of the network were very informative and when applied to the *G. genetta* data set the MSN did not produce a star-like genealogy stemming from a central most frequent haplotype, a criticism made at this method by Cassens *et al.* (2003). The Arabian clade, although significantly divergent (24 mutational steps), it is much closer to European and North African haplotypes than to East African ones (52 mutational steps) in spite of its adjacent position with the latter region. The haplotypes from Central Africa (Gg10 and Gg11) have a core position in the network. The graph also shows very clearly the existence of two distinct lineages in East Africa and of a wide genetic divergence between part of the *felina* specimens (Gg35) and all other lineages in Southern Africa, including haplotypes found in other *felina* specimens (Gg26 and Gg27).

Although the MSN it is a useful diagrammatic representation of the phylogeographic partition of the genetic variation, it is also a result that cannot be interpreted in a statistically objective way. This is in contrast with the idea behind Templeton's NCA (1998, 2004). Figure 3.11 shows the nested design for the *G. genetta* complex haplotypes as defined by a 95% plausible set of cladograms (Templeton *et al.* 1992) and subsequent treatment of topologically ambiguous connections (Templeton & Sing 1993). Table 3.18 presents the results of the nested contingency analysis for testing the geographical association of clades with both genetic and geographic variation. The null hypothesis of no association was rejected at the confidence level of 95% for only three nested clades, all of them of high hierarchical level. This illustrates that unless sampling is thorough and intense across a species range NCA will not provide detailed inference on

small-scale patterns but, on the other hand, it may provide corroboration to inferred associations and processes that pass its strict filtering procedure. Table 3.19 shows the results of the NCA for clades 3-1, 3-2 and 3-4 and the inferred biogeographic/demographic process putatively responsible for the observed haplotypic composition of clades in their area of occurrence. In two of the cases, the inference was that of geographical associations caused by contiguous range expansion. The two areas involved are the ones connecting the Cape and Orange Free State with the rest of Southern Africa and connecting East Africa with Central and North Africa. Isolation by distance was inferred to explain the genetic relationship between West, Central and North Africa.

Table 3.20 presents estimates of divergence times among all the clades in the multispecies data set using d_A as measure of the genetic distance between clades and two different calibrations of the mtDNA molecular clock. Significant results are the estimated contemporary splits between Arabia and North Africa and between *felina* and *angolensis*, either in the Middle Pleistocene, if we assume the faster rate as more accurate, or in the Early Pleistocene, if we assume the rate of 1% as more accurate (Pesole *et al.* 1999).

Table 3.12 Values of genetic diversity and results of neutrality tests for the samples from each of the geographic regions. # indicates parameters that cannot be estimated when the number of haplotypes equals the number of samples; underlined quantities indicate significant deviations from the null hypothesis at the confidence level of 95%.

	Sequences	Haplotypes	S (polymorphic sites)	H (gene diversity)
Europe	21	3	5	0.400 ± 0.114
North Africa	4	3	23	0.833 ± 0.222
West Africa	5	4	5	0.900 ± 0.161
Central Africa	7	4	5	0.810 ± 0.130
East Africa	7	7	22	1.000 ± 0.076
Arabia	10	4	3	0.644 ± 0.152
Southern Africa	28	9	12	0.812 ± 0.052
Cape+OFS	5	2	2	0.400 ± 0.237

	π (mean number of pairwise differences)	π/nucleotide (nucleotide diversity)	Ewens' Θ(k)	Watterson's Θ(S)
Europe	0.731 ± 0.566	0.001 ± 0.001	0.704 [0.203; 2.202]	1.390 ± 0.746
North Africa	16.657 ± 9.453	0.031 ± 0.021	3.766 [0.775; 18.233]	12.546 ± 7.088
West Africa	2.044 ± 1.367	0.004 ± 0.003	7.106 [1.543; 33.076]	2.400 ± 1.513
Central Africa	2.055 ± 1.304	0.004 ± 0.003	3.029 [0.842; 10.887]	2.041 ± 1.223
East Africa	11.858 ± 6.121	0.022 ± 0.013	#	8.980 ± 4.357
Arabia	0.772 ± 0.613	0.001 ± 0.001	1.956 [0.598; 6.128]	1.061 ± 0.704
Southern Africa	2.851 ± 1.548	0.005 ± 0.003	4.192 [1.878; 9.016]	3.084 ± 1.276
Cape+OFS	0.814 ± 0.690	0.002 ± 0.002	0.691 [0.149; 3.179]	0.960 ± 0.758

	Tajima's $\Theta(\pi)$	Tajima's D	Watterson's F P-value	Fu's Fs P-value
Europe	0.731 ± 0.632	-1.486	0.700	0.577
North Africa	16.657 ± 11.288	1.329	1.000	0.882
West Africa	2.044 ± 1.598	-1.124	1.000	0.118
Central Africa	2.055 ± 1.494	-0.099	0.712	0.385
East Africa	11.876 ± 7.012	0.94	#	0.124
Arabia	0.772 ± 0.694	-1.035	0.880	<u>0.034</u>
Southern Africa	2.851 ± 1.723	-0.391	0.695	0.293
Cape+OFS	0.814 ± 0.806	-0.973	1.000	0.624

Table 3.13 Mean, variance, and estimated parameters of the mismatch distributions for each geographic unit of *G. genetta*. Parameters were estimated with a generalized non-linear least-square method and their P-values derived through parametric bootstrap (Schneider & Excoffier 1999).

	m (mean)	v (variance)	τ (tau)	Θ_0	Θ_1	Sum of square deviations (P-value)	Raggedness Index (P-value)
Europe	0.705	1.511	6.000	0.000	0.629	0.019 (P=0.559)	0.189 (P=0.722)
North Africa	14.167	73.367	20.948	0.007	1993.750	0.195 (P=0.079)	0.306 (P=0.751)
West Africa	2.000	1.333	2.265	0.000	4730.000	0.019 (P=0.734)	0.110 (P=0.824)
Central Africa	2.000	1.700	2.647	0.000	13.674	0.038 (P=0.348)	0.150 (P=0.470)
East Africa	10.476	28.862	13.867	0.011	41.029	0.041 (P=0.545)	0.086 (P=0.599)
Arabia	0.756	0.416	0.967	0.000	2382.500	0.033 (P=0.154)	0.222 (P=0.213)
Southern Africa	2.720	4.415	4.605	0.001	4.735	0.030 (P=0.362)	0.068 (P=0.437)
Cape+OFS	0.800	1.067	3.039	0.001	0.870	0.147 (P=0.111)	0.680 (P=0.454)

Table 3.14 Above diagonal: population pairwise Fst values (10000 permutations) accordingly with the method described in Hudson *et al.* 1992b. Below diagonal: population pairwise Φst values (100000 permutations) following the AMOVA procedure introduced by Excoffier *et al.* 1992 (corrected distances with the model TrN and gamma shape = 0.365 were used for the AMOVA). All values were significant at α=0.050.

	Europe	North Africa	West Africa	Central Africa	East Africa	Arabia	Southern Africa	Cape+OFS
Europe	*	0.259	0.943	0.931	0.765	0.968	0.863	0.698
North Africa	0.634	*	0.456	0.324	0.314	0.683	0.451	0.462
West Africa	0.967	0.517	*	0.766	0.577	0.950	0.786	0.635
Central Africa	0.954	0.436	0.777	*	0.429	0.946	0.72	0.612
East Africa	0.876	0.350	0.542	0.425	*	0.807	0.534	0.513
Arabia	0.973	0.820	0.967	0.960	0.857	*	0.885	0.703
Southern Africa	0.850	0.593	0.725	0.651	0.603	0.852	*	0.463
Cape+OFS	0.758	0.435	0.521	0.531	0.494	0.672	0.541	*

Table 3.15 Above diagonal: M values (M=Nm for haploid data) obtained using Slatkin's (1995) method. Below diagonal: population pairwise Nm values averaged from estimates derived of Fst (Hudson *et al.* 1992b) and Nst (Lynch & Crease 1990) values tested with 10000 permutations.

	Europe	North Africa	West Africa	Central Africa	East Africa	Arabia	Southern Africa	Cape+OFS
Europe	*	0.29	0.02	0.02	0.07	0.01	0.09	0.16
North Africa	1.43	*	0.47	0.65	0.93	0.11	0.34	0.65
West Africa	0.03	0.60	*	0.14	0.42	0.02	0.19	0.46
Central Africa	0.04	1.04	0.15	*	0.68	0.02	0.27	0.44
East Africa	0.15	1.08	0.37	0.67	*	0.08	0.33	0.51
Arabia	0.02	0.23	0.03	0.03	0.12	*	0.09	0.24
Southern Africa	0.08	0.61	0.14	0.19	0.44	0.07	*	0.42
Cape+OFS	0.22	0.58	0.29	0.32	0.47	0.21	0.58	*

Table 3.16 Exact test of population differentiation based on haplotype frequencies (Raymond & Rousset 1995) with a Markov chain length of 100000 steps. An * indicates value non-significant at α=0.050.

	Europe	North Africa	West Africa	Central Africa	East Africa	Arabia	Southern Africa	Cape+OFS
Europe	0							
North Africa	0.089+-0.005*	0						
West Africa	0.000+-0.000	0.212+-0.003*	0					
Central Africa	0.000+-0.000	0.058+-0.002*	0.046+-0.002	0				
East Africa	0.000+-0.000	0.495+-0.008*	0.487+-0.005*	0.091+-0.003*	0			
Arabia	0.000+-0.000	0.013+-0.001	0.009+-0.001	0.001+-0.000	0.008+-0.001	0		
Southern Africa	0.000+-0.000	0.002+-0.001	0.001+-0.000	0.000+-0.000	0.001+-0.000	0.000+-0.000	0	
Cape+OFS	0.000+-0.000	0.001+-0.000	0.001+-0.000	0.000+-0.000	0.000+-0.000	0.000+-0.000	0.000+-0.000	0

Table 3.17 Above diagonal: distances in Km between the centres of the geographic areas. Below diagonal: net number of nucleotide differences between populations (D_A; Nei & Li 1979).

	Europe	North Africa	West Africa	Central Africa	East Africa	Arabia	Southern Africa	Cape+OFS
Europe	*	1201	3043	3693	5479	5005	7755	8491
North Africa	3.182	*	3328	2791	4290	3836	6822	7674
West Africa	27.968	8.467	*	3224	5900	6370	6316	6600
Central Africa	21.617	4.473	7.176	*	2686	3473	4066	4883
East Africa	22.661	6.960	9.692	5.142	*	1624	3655	4806
Arabia	26.574	20.282	33.948	30.744	30.947	*	5273	6426
Southern Africa	22.309	9.325	15.338	10.421	10.627	28.315	*	1157
Cape+OFS	35.129	21.600	26.048	23.610	23.343	35.045	14.909	*

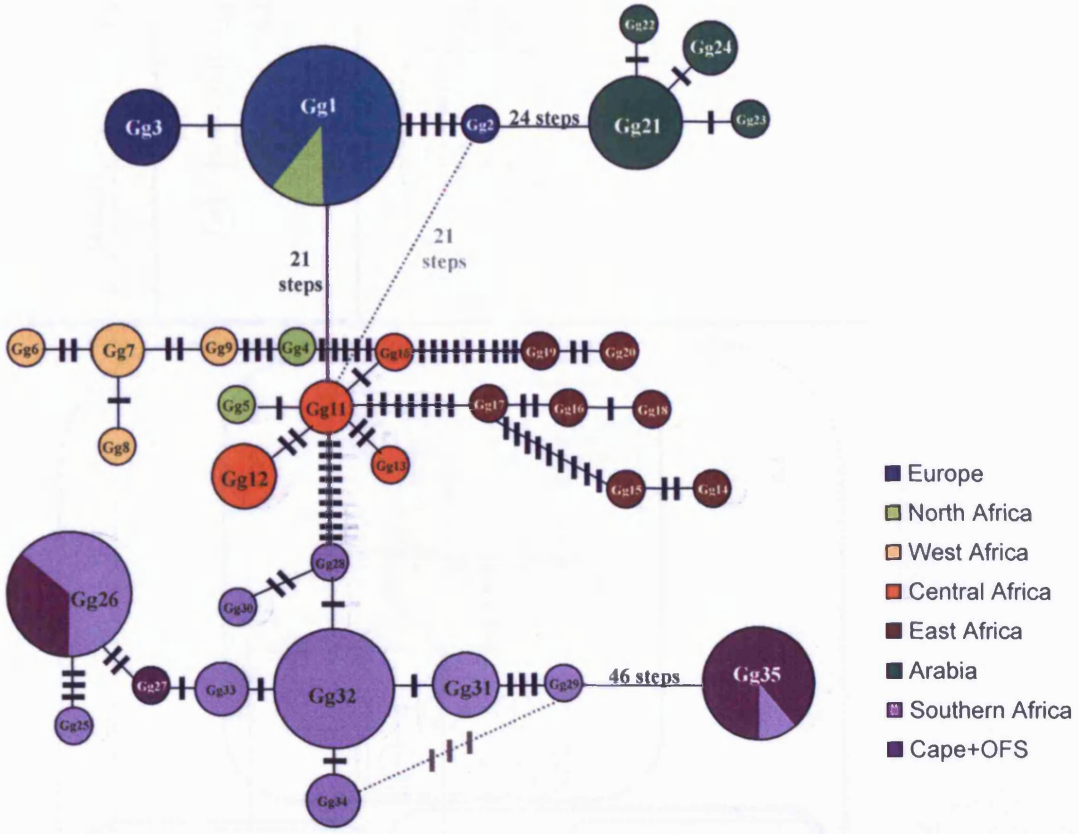

Figure 3.10 *G. genetta* haplotype minimum spanning network. Codes inside circles are haplotype names as given in Table 3.5 and the size of each circle is proportional to the haplotype frequency, with the smallest size equal to one. Haplotype geographical occurrence is portrayed by the different colours and the frequency of occurrence among areas it is proportional to slice size in the pie. Mutational steps are represented by black bars on lines connecting haplotypes when steps are less than 10. Grey lines represent alternative links between haplotypes.

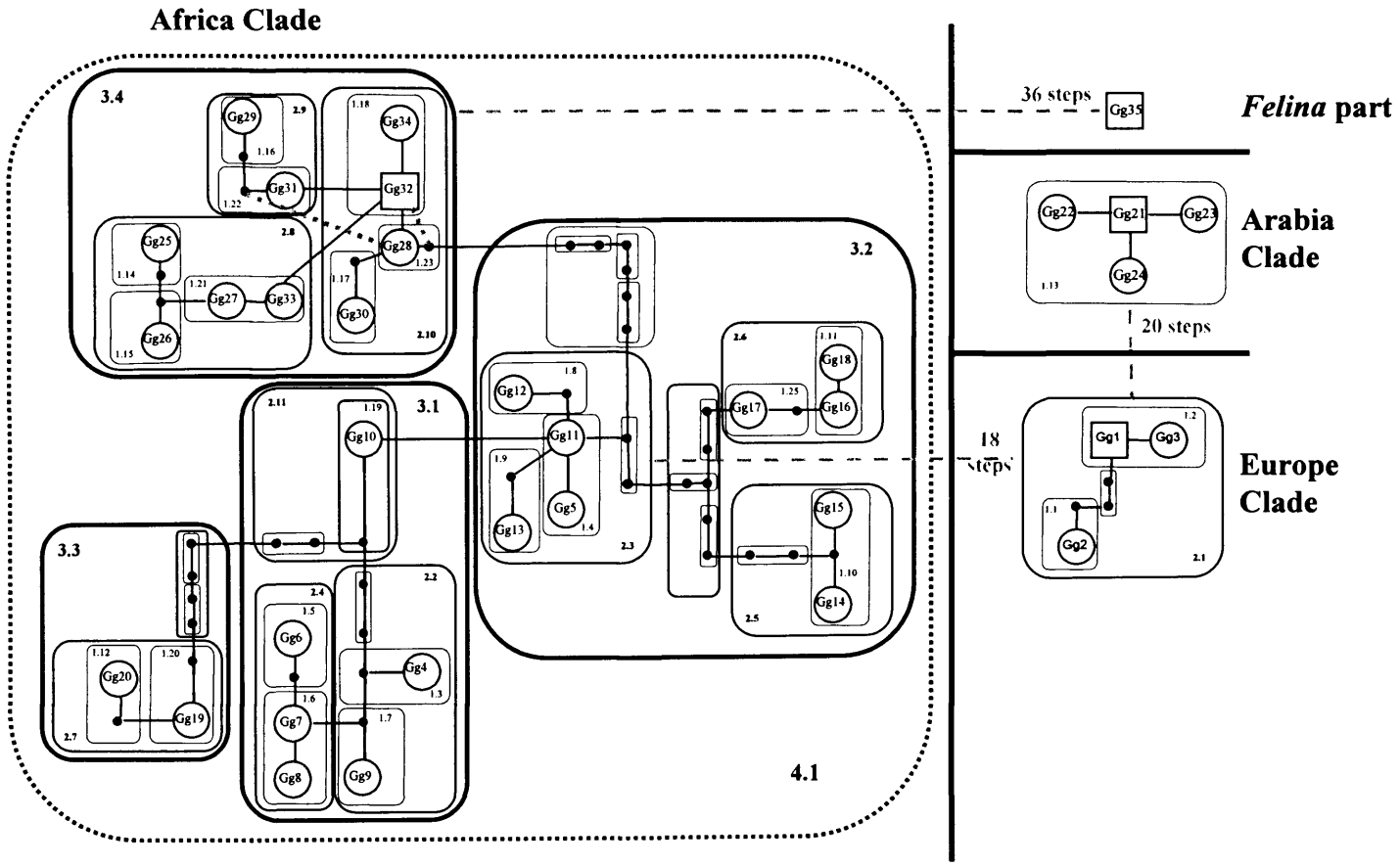

Figure 3.11 *G. genetta* statistical parsimony haplotype cladogram following the procedures of Templeton *et al.* (1995). Connections ≤ nine substitutions have a 0.95 probability of being parsimonious. Haplotype designations correspond to those in Table 3.5. Substitutions are represented by dashes and unsampled haplotypes are represented by black dots. Bold lines indicate the partitioning of the network into four parsimonious clades, three of them with a geographic basis of allopatry. Broken lines between these clades represent the connections implying the minimum number of observed mutations. Broken lines connecting haplotypes within the Africa clade represent alternative links. Hierarchical nesting design specified is by boxes and numbered clade designations.

Table 3.18 Nested contingency analysis of geographical associations for clades with geographical and genetic variation in the statistical parsimony network.

Clade	Permutational chi-square statistic	Probability
1-2	0.489	1.000
1-4	3.000	0.332
1-21	3.000	0.334
2-1	0.100	1.000
2-2	2.000	1.000
2-3	1.556	1.000
2-8	0.546	1.000
3-1	9.800	0.049*
3-2	12.000	0.004*
3-4	7.071	0.027*
Total cladogram	100.424	0.000*

* Significant at the 0.05 level.

Table 3.19 Results of the nested clade analysis for the Africa clade of *G. genetta*. D_c and D_n are, respectively, the clade and nested distance in kilometers of each included clade in the nested clades being examined. Values in bold mean that they are statistically significant, either significantly large (L) or small (S).

Nested clades	Inclusive clades	D_C	D_N	Inference chain; Final inference	Corresponding geographic area
3-1	2-2 (Interior)	**1643^L**	01798^L	1-2-3-4-no; Restricted gene flow with isolation by distance	Between West, Central and North Africa
	2-4 (Tip)	0	867		
	2-11 (Interior)	0	2509		
	I-T	**1095^L**	**1168^L**		
3-2	2-3 (Interior)	**976^S**	1414	1-2-11-12-no; Contiguous range expansion	Between East, Central and North Africa
	2-5 (Tip)	0	1786		
	2-6 (Tip)	0	1786		
	I-T	976	-372		
3-4	2-8 (Tip)	**577^L**	**592^L**	1-2-11-12-no; Contiguous range expansion	Between Southern Africa and the Cape+OFS
	2-9 (Tip)	0	**332^S**		
	2-10 (Interior)	0	**332^S**		
	I-T	**-445^S**	**-205^S**		

Table 3.20 Divergence time estimates derived from the equation $d=2\mu t$ using net nucleotide differences as distance and two different calibrations of the mtDNA neutral molecular clock. Below diagonal: net number of nucleotide differences per site between populations or species (Nei 1987). Above diagonal: divergence time estimates in My; left value derived from the application of the fastest substitution rate and right value derived from the application of the slowest substitution rate. Underlined values indicate pairwise comparisons between clades that share haplotypes.

	Europe	North Africa	West Africa	Central Africa	East Africa	Arabia	Southern Africa	Cape+OFS	*Felina* part	*Tigrina* part I	*Tigrina* part II	*Rubiginosa*	*Angolensis*
Europe	-	0.096; 0.250	0.731; 1.900	0.615; 1.600	0.635; 1.650	0.846; 2.200	0.654; 1.700	0.750; 1.950	1.692; 4.400	1.654; 4.300	1.692; 4.400	1.500; 3.900	1.500; 3.900
North Africa	0.005	-	0.192; 0.500	0.115; 0.300	0.173; 0.450	0.577; 1.500	0.288; 0.750	0.385; 1.000	1.385; 3.600	1.385; 3.600	1.385; 3.600	1.250; 3.250	1.269; 3.300
West Africa	0.038	0.010	-	0.212; 0.550	0.250; 0.650	0.808; 2.100	0.462; 1.200	0.558; 1.450	1.577; 4.100	1.538; 4.000	1.577; 4.100	1.404; 3.650	1.577; 4.100
Central Africa	0.032	0.006	0.011	-	0.154; 0.400	0.808; 2.100	0.346; 0.900	0.442; 1.150	1.557; 4.050	1.557; 4.050	1.557; 4.050	1.404; 3.650	1.462; 3.800
East Africa	0.033	0.009	0.013	0.008	-	0.769; 2.000	0.365; 0.950	0.462; 1.200	1.500; 3.900	1.500; 3.900	1.462; 3.800	1.346; 3.500	1.423; 3.700
Arabia	0.044	0.030	0.042	0.042	0.040	-	0.769; 2.000	0.865; 2.250	1.500; 3.900	1.692; 4.400	1.519; 3.950	1.596; 4.150	1.423; 3.700
Southern Africa	0.034	0.015	0.024	0.018	0.019	0.040	-	0.038; 0.100	1.500; 3.900	1.308; 3.400	1.500; 3.900	1.173; 3.050	1.404; 3.650
Cape+OFS	0.039	0.020	0.029	0.023	0.024	0.045	0.002	-	1.615; 4.200	1.423; 3.700	1.615; 4.200	1.269; 3.300	1.519; 3.950
Felina part	0.088	0.072	0.082	0.081	0.078	0.078	0.078	0.084	-	1.096; 2.850	0.000; 0.000	0.923; 2.400	0.615; 1.600
Tigrina part I	0.086	0.072	0.080	0.081	0.078	0.088	0.068	0.074	0.057	-	1.096; 2.850	0.096; 0.250	1.154; 3.000
Tigrina part II	0.088	0.072	0.082	0.081	0.076	0.079	0.078	0.084	0.000	0.057	-	0.923; 2.400	0.615; 1.600
Rubiginosa	0.078	0.065	0.073	0.073	0.070	0.083	0.061	0.066	0.048	0.005	0.048	-	0.942; 2.450
Angolensis	0.078	0.066	0.082	0.076	0.074	0.074	0.073	0.079	0.032	0.060	0.032	0.049	-

3.4 Discussion

3.4.1 Genetic structure and phylogeographic patterns

The results of this study suggest, as illustrated by a global F_{ST} value of 90%, that the presently observed geographic distribution has correspondence with how genetic variation is structured and that the majority of the proposed evolutionary units in *G. genetta* have indeed a genetic basis. However, the phylogeographic architecture connecting the geographic populations, as inferred from several different analytical tools, cannot be explained only by the present distribution and indicates the contribution of past processes in its shaping. The observed weak to moderate correlation between geographic and genetic distances indicates that the current geographic arrangement, although reflecting the main genetic partitions, is recent enough or has passed through cyclic changes to prevent its impact to be overwhelming on the genetic structure of the species complex (Bohonak 1999). Indeed, absence of genetic equilibrium is usually argued as evidence for historical events, such as climatic cycles promoting the expansion and contraction of suitable habitat or geological processes conducive to barrier formation and vicariance, on the basis of observed genetic partitions (Avise 2000a). Furthermore, in the case of species with strong dispersal abilities, patterns incompatible with isolation by distance may also be explained by leptokurtic colonisation (Ibrahim *et al.* 1996) and this has been shown to occur in several carnivores (Vila *et al.* 1999; Davison *et al.* 2001).

The topologies for the haplotype relationships within *G. genetta* obtained using either tree methods or network methods were essentially the same. This provides strong support to the inferred genealogy and enables discussion of relevant hypotheses. The clustering of the Central African haplotypes together with their core position in the minimum spanning network (MSN), bridging all the other African populations, suggests that a substantial part of the diversification presently observed may be associated with vicariant or dispersal events stemming from this geographic area (Castelloe & Templeton 1994). The topology of the phylogenetic trees, the levels of sequence divergence, and the estimates of divergence times all indicate that the earliest diversification originating from Central Africa is the one at the origin of the Southern African clade. Its monophyly suggests that the south was successfully colonised only once and the Middle Pleistocene estimate for the timing of its divergence it is compatible with a vicariant event associated with an increased central African rainforest belt in a period of global warming (Flagstad *et al.* 2001). Increased water levels, with the formation of extensive lakes and swamps in

Central Africa, are also reported for the interglacial periods of the Middle Pleistocene (Dupont *et al.* 2001). The results of the nested clade analysis depict the evolution of the Southern African clade as a contiguous range expansion, leading to the colonisation of a large area. Although presently occupying a very extensive area, the genetic variation in the clade is compatible with an ancient bottlenecked origin. A similar pattern within a Southern African lineage was encountered by Flagstad *et al.* (2001) in the hartebeest and was attributed to the relative constancy of the Pleistocene climate in the region.

A second diversification episode might have been promoted by the allopatric fragmentation of East Africa, which is a frequently inferred biogeographic process in the evolutionary history of African taxa (Freitag & Robinson 1993; Arctander *et al.* 1999; Gagneux *et al.* 1999). Around the estimated divergence time between East and Central Africa (200,000-400,000 yr BP), the combination of a warm and humid period with volcanic or tectonic activity along the Rift Valley would be a scenario likely to isolate populations between the two sides of the Rift (Colinvaux 1997; Jensen-Seaman & Kidd 2001). In fact, from 0.6 million yr BP wooded savannah and grassland were already dominant in the eastern side of the Valley (Cerling 1992). In contrast with the process that originated the southern lineage, the East African clade apparently retained a much higher proportion of the genetic variability present in the ancestral population after splitting from Central Africa. The high levels of both haplotype and nucleotide diversity in the East African clade are also a likely consequence of the differentiation of allopatric lineages between Somalia and Kenya within the region. Considering the pattern in the MSN of the lineages in East Africa, and also the topologies of the phylogenetic trees, it seems that the East African lineages started to diverge at the same time or even before the divergence of the region with Central Africa. In contrast with Southern Africa, where the Pleistocene climate was more stable as compared to the rest of the continent (Lancaster 1979), East Africa has a more variable climatic history and it is richer in physiographic barriers and habitat discontinuities likely to conduce to interdemic divergences (Faulkes *et al.* 1998).

The close association of North Africa (NA) with both Central Africa (CA) and West Africa (WA) are revealed by the topology in the MSN, the estimates of sequence divergence, and the paraphyly of NA haplotypes in relation to the other two geographic areas in the phylogenetic trees. Additionally, the inference of restricted gene flow between these three regions by the nested clade analysis (NCA) seems to indicate that, although now fragmented, the period of time since they are separated is too short to allow detectable independent evolution. Indeed, the potential for gene flow between refuge populations is

not surprising if the frequency of glacial cycles and the relative short-term interval separating shifts in population distributions is taken into account (Bartlein & Prentice 1989). A closer scrutiny of the F_{ST}/Φ_{ST} results shows, however, that this inference of the NCA does not apply to the relation between CA and WA, but only to the relationship of these two areas with NA. A possible scenario compatible with these observations for this part of Africa is one where the independent connection of NA to both CA and WA, through two separate savannah corridors, might have been established and then interrupted by the contraction and expansion of the Sahara desert (deMenocal 1995). In several wet and warm periods of the Pleistocene the Sahara desert is likely to have diminished considerably, and savannah and grassland corridors may have occurred (Sarnthein 1978). A "green" Sahara it is well documented for the last climatic optimum, 6,000 yr BP (Jolly *et al.* 1998), and similar instances are likely to have occurred before, even if they are obviously more and more difficult to trace as we go backwards in time.

The findings observed for the European and Arabian regions have further implications in the way we may reconstruct the evolutionary history of common genets west and north of the Abyssinian plateau. The European clade it is clearly the outcome of a colonisation from NA and the sharing of a haplotype (Gg1) between the two areas, which are geographically isolated, suggests a recent colonisation. The European haplotypes are therefore almost certainly a subset of the genetic variation found in NA and the other two haplotypes found in Europe (Gg2 and Gg3) are likely to represent two additional founding lineages. Although Gg3 is only one nucleotide different from Gg1 (corrected d = 0.002) it would take, even accepting a fast divergence rate of 5.2% per million years, around 38,000 years for Gg1 to mutate into Gg3, a time interval incompatible with an anthropogenic introduction of genets into Europe. For the same reason, unless the occurrence of natural dispersal events in the last 100,000 years is accepted (but see below), the estimated divergence time between Europe and NA it is also almost certainly an overestimation. Departures from expectations of $d=2\mu t$ are a normal effect when the assumption of equal size between the two daughter populations is violated (Arbogast *et al.* 2002). The Arabian clade is related by all the results with NA, more precisely with haplotypes Gg1 and Gg2 as illustrated by the MSN. Haplotype Gg2 was only recovered in Europe but, as discussed above, it is almost certainly, unless now extinct, a haplotype that was not sampled from NA. The ancestor of the Arabian clade it is then most likely a lineage from North Africa, which was not sampled or it is now extinct in the area, different from the colonisers of Europe but even more so from any other African haplotype recovered in this study. A

similar case where the origin of a clade, detected to result from long-distance colonisation and which haplotypes were not found in any candidate founding area, was assigned with basis on genetic divergence and phylogenetic clustering is described by Pitra *et al.* (2002) for the sable antelope.

The significant divergence of Gg1 and Gg2 from all other haplotypes present in the triangle NA-CA-WA may be seen as evidence for their antiquity and imply that in the latter region haplotypes with more than 4% of sequence divergence coexist in sympatry. The most likely explanation for this is the secondary admixture of lineages that experienced extended periods in allopatry (Vila *et al.* 1999; Avise 2000a). The high levels of both haplotype and nucleotide diversity observed in NA could therefore be the result of a cyclic admixture of resident lineages diverging by genetic drift with lineages arriving from the south through temporary savannah corridors. The haplotypes Gg1 and Gg2, which are more divergent from the WA and CA clades, would be more ancient residents in NA whereas Gg4 and Gg5 would be more recent arrivals. Considering the highest value of genetic divergence among NA haplotypes and the fastest rate of sequence divergence of 5.2% per million years, the presence of common genets in NA possibly goes back to the Middle Pleistocene. It is important to stress how critical additional samples from NA, but also from WA and CA, are to assess this hypothesis, especially when taking into account that geographic areas with high haplotypic diversity are more sensitive to poor sampling in terms of not detecting additional variants (Sjogren & Wyoni 1994). The hypothesis of cyclic connections, through savannah corridors, would be substantiated by the finding of additional lineages in NA, also present in WA or CA, which could be attributed to migration events in different interglacial periods.

The genealogical affinity of NA with both Europe and Arabia together with the high genetic diversity present in NA has two important implications. First, common genets are likely to have been present in NA since well before the observed genetic differences with both WA and CA would suggest and, consequently, the inferred paraphyly of NA haplotypes cannot be explained just by a simple colonisation process (Thorpe *et al.* 1994; Austerlitz *et al.* 1997). Although levels of divergence are shallow, it seems more plausible that the NA population has, along with WA and CA, a history of episodic fragmentation in which periods of interrupted gene flow are relatively short. This would have the effect of retarding population differentiation, increasing overall effective population size, and it is a type of demographic structure that has been theoretically explored in the context of metapopulation dynamics and source-sink systems (Slatkin 1985a; McCauley 1991; Nei &

Takahata 1993). Second, the colonisation of the Arabian Peninsula is likely to have occurred during a warm and humid interglacial period in the Middle Pleistocene in which areas of savannah or grassland linked NA with the Levant. The Sinai region has been proposed as a land bridge during a climatic optimum also for the invasion of Asia by the leopard (Uphyrkina *et al.* 2001). In a period of re-establishment of dry and cool conditions, and the return of the desert to most of the area of this NA-Arabia corridor, genets in the latter region would have become an isolated deme and with a distribution restricted to the non-desert areas of the Arabian Peninsula. Long distance colonisation and range expansion followed by fragmentation through extinction of geographically intermediate populations may produce similar outcomes (an isolate) and be genetically indistinguishable (Templeton 2004). Although this might be true, it seems nevertheless that the former process is a better explanation for the observed low genetic diversity and increased divergence observed in the Arabian deme. The foundation of the Arabian population, according to the divergence time estimates, occurred before the diversification of the Southern and East African lineages. Consequently, it seems that common genets were already distributed over a range stretching from East to North Africa before the vicariance of Southern and East Africa. This may explain the outcome of contiguous range expansion for the clade 3-2 in the NCA, referring to a process prior to the allopatric fragmentation of East Africa. The indication that genets may have been present in NA since the Middle Pleistocene together with the estimated recent date for the Europe-NA split seems to support the scenario of introduction by man to Europe, as natural dispersal apparently did not occur in the previous 500,000 years. However, it is important to emphasise that the divergence time estimate between NA and Arabia may be biased upward due to an expected increased lineage sorting rate in a population resulting from a founder effect (Nei *et al.* 1975; Hoelzer *et al.* 1998).

3.4.2 The *felina* case

One of the most striking findings of this study was the recovery of a highly divergent haplotype (Gg35) in two specimens out of nine positively identified as members of *felina* (Fig. 3.XIII), the form of the *G. genetta* complex from the Cape and Orange Free State. This haplotype showed a minimum of 9% sequence divergence in relation to any other *G. genetta* haplotype and of 10% in relation to other haplotypes from the Cape in specimens also identified as *felina* (Fig. 3.IX). High levels of intraspecific diversity in sympatry have already been found in gazelles (Arctander *et al.* 1996), antelopes (Pitra *et*

al. 2001) and jackals (Wayne *et al.* 1990). Among the explanations for this phenomenon are lineage variability in the rate of evolution, the effect of natural selection, and secondary contact of populations previously allopatric for long periods. However, the fact that in the multispecies data set the haplotype Gge/Gti is placed by all analyses as more closely related to the haplotypes of a different species, *G. angolensis*, than to any other *G. genetta* haplotype asks for a different interpretation. Furthermore, the haplotype Gge/Gti it is also found in individuals of the species *G. tigrina* (Fig. 3.XIV) morphologically indistinguishable of other *tigrina* specimens (Fig. 3.X) with haplotypes that cluster with "*rubiginosa*", as expected from the current taxonomy. Still in specimens of *G. tigrina* related haplotypes with Gge/Gti were found, Gti1 and Gti2, showing a maximum of 0.5% sequence divergence to Gge/Gti.

One hypothesis to explain these results is one where *felina* is a distinct species, possibly sister clade to *G. angolensis*, that would had became restricted to the Cape during a cool and dry period in which the Kalahari and the Namib would had acted as barriers (Lancaster 1979; Stokes *et al.* 1998). It would have been the only genet species in the Cape region until the recent arrival of *tigrina*, estimated from its divergence time with "*rubiginosa*" to have occurred sometime between 100,000-200,000 years BP. Introgressive hybridisation between the two species may have resulted and this could explain the presence in some *tigrina* specimens of *angolensis*-like haplotypes (Gge/Gti, Gti1, and Gti2). More recently, as estimated from the divergence between the Southern African clade and the Cape+OFS clade, the southern expansion of *G. genetta* would have reached the Cape and OFS, and extensive hybridisation with *felina* would have occurred, explaining the several *felina* specimens detected with a *genetta*-like mitochondrial genome. However, this hypothesis does not conform well to the fact that *felina* is morphologically more similar to *pulchra* than to *angolensis*, an observation that has led all genet classifications to place *felina* either as a subspecies or as a related species to *G. genetta* and never particularly close to *angolensis*. Moreover, the finding in some *felina* specimens of an *angolensis*-like haplotype, in comparison with a larger fraction carrying *genetta*-like haplotypes, may be simply consequence of past hybridisation between *felina* and *angolensis* with genome introgression of the latter into the former, followed by divergence through genetic drift. Range shifts and genetic drift have also been invoked in previous studies to explain similar cases in which a population is partly introgressed by allopatric foreign mtDNA (Patton & Smith 1994).

Therefore, an alternative hypothesis is to accept that *felina* is indeed a member of the *G. genetta* complex, as suggested by its morphology, and that the highly divergent haplotype found in some of its specimens is the result of ancient introgression from *G. angolensis*. It is possible that *felina*, by being the southernmost geographic population of the species complex, descends from the leading edge of the wave expanding southwards that followed fragmentation, as described above, from the equatorial populations. *G. angolensis* and *G. genetta* are ecologically separated (Kingdon 1997; Crawford-Cabral in press), which is a central mechanism in the reduction of interspecific gene flow among related species with areas of sympatry (Templeton 1989). However, if the encounter between the *G. genetta* wave invading Southern Africa and the resident *G. angolensis* occurred during a period of environmental instability in which the two taxa survived and met in pockets of suitable habitat and ecological segregation was removed or minimised, then spatially and temporally restricted hybridisation could have ensued. Furthermore, if such contact involved only a fraction of the leading edge pioneers of *G. genetta* and was associated with demographic crisis this could be compatible with the detected specimens presenting the *angolensis*-like haplotype being confined to a subset within the *felina* subspecies.

One expectation from the first hypothesis would be to find individuals belonging to the *pulchra* subspecies of *G. genetta*, particularly from areas adjacent to the Cape and OFS, containing the divergent haplotype found in *felina*, whereas an expectation in accordance with the second hypothesis would be to recover *angolensis* specimens with a *genetta*-like haplotype suggesting past and localised hybridisation. Neither of these expectations was detected here but their absence, even if confirmed over an extended sample set, may not allow the extraction of any definitive conclusion, as bidirectional introgression seems to be rare (Martinsen *et al.* 2001; Rohwer *et al.* 2001), a fact that may also explain why no *felina* samples were retrieved with *tigrina*-like haplotypes. In contrast, the finding that all the *tigrina* individuals, found in this study to be introgressed, only presented the *angolensis*-like haplotype and not the *genetta*-like haplotype, which is nevertheless more frequent within the sympatric *felina*, is unexpected and of difficult interpretation. It may possibly be an indication that, if the second hypothesis is correct, hybridisation between *tigrina* and *felina* has been exclusively a past event and limited to the time of arrival of the southern wave of *G. genetta* to the Cape and OFS areas. Interspecific gene flow would be prevented afterwards when the ecological segregation that is observed today between *tigrina* and *felina* (Stuart 1991) was established. The

importance of stochastic processes of colonisation and range shifts in the initiation and disappearance of hybridisation processes has been recently underlined (Babik *et al.* 2003).

It is evident that more samples from all the species involved and results using nuclear DNA markers are required for assessing these and other competing hypotheses, evaluating the importance of past and present hybridisation, and determining if introgression is unidirectional or bidirectional. Hybridisation among close congeneric species has been reported for several carnivore families (Gottelli *et al.* 1994; Davison *et al.* 2000; Beaumont *et al.* 2001) and both its evolutionary importance and usefulness as a system to analyse the process of speciation is now well documented (Dowling & Secor 1997; Hewitt 2001).

G. genetta and *G. tigrina* have been reported to hybridise in captivity (Gray 1971), although no information about the fertility of the F1 generation was recorded, and a *tigrina-felina* morphological hybrid was identified in the skins collection of the British Museum (Gaubert *et al.* in press). It is clear that the available information is too fragmentary in order to be helpful to the interpretation of the findings of the present study, and the only certainty at the moment is the existence of introgressive hybridisation in the mtDNA of different genet species from Southern Africa.

3.4.3 The origins of *G. genetta*

For the origin of the small-spotted genets, the area Central-East Africa and the date Middle Pliocene have been proposed (Crawford-Cabral 1981b; Gaubert *et al.* 2004). The fossil information is scarce, and frequently genet fossils cannot be assigned to the species level, thereby not contributing to support or contradict these suggestions, drawn from other sources of evidence, for the appearance of *G. genetta*. Considering the habitat preferences of *G. genetta* throughout its range for steppe, grassland and dry woodlands, an origin associated with the onset of cooler and drier climatic conditions in Africa (deMenocal 1995) it is a likely scenario. Fossils identified with certainty exist from the Early Pleistocene of East Africa and Late Pleistocene of North Africa, but only for the last 2,000 years in Europe (Morales 1994; Lariviere & Calzada 2001). Genet fossils not identified beyond the genus level are also known from the Late Pliocene onwards in North Africa (Geraads 1997) and it is a feasible scenario, considering the location, that they indeed represent common genets.

The results of the present study are compatible with this available information but it seems clear that the recovered data set it is not genealogically deep enough to allow the drawing of conclusions about a place and time for the origin of *G. genetta*. Instead, the inferred phylogeographic pattern seems to be the product of the interaction of several layers of Pleistocenic events, starting in the Middle or Early Pleistocene, depending on the accepted neutral mutation rate, until very recent times (colonisation of Europe in the Holocene).

3.4.4 Taxonomic findings

The genetic structure of *G. genetta* revealed in this study may partially be related with the forms of the complex referred above as potentially relevant on taxonomic grounds on the basis of morphological and geographical criteria. No support for previously described groups of subspecies was found, although the affinities between Central, West, North Africa, and Europe reported by Crawford-Cabral (1981b) were confirmed.

The results suggest a long and independent evolutionary history of the Arabian clade, and its level of genetic divergence is higher than 5% with any other *G. genetta* population. Johns & Avise (1998) have showed that 5% is the typical level of genetic distance revealed by mtDNA protein-coding genes between sister species in mammals. Taking all this into account, plus recognised morphological differentiation (Crawford-Cabral 1981b), the raising of *granti* to species level would appear to be justifiable.

The data revealed the existence of a highly divergent haplotype in some *felina* specimens, also found in some individuals of *G. tigrina*, which is phylogenetically close to the haplotypes of a different species, *G. angolensis*, and suggests the occurrence of introgressive hybridisation among genet species in Southern Africa. However, due to the need of confirmation of these results with both more samples from the region and the use of nuclear DNA markers, the potential taxonomic significance of these evolutionary processes cannot be properly addressed with the available evidence. Furthermore, the proposed extended range of *felina*, as a distinct species, to other countries in Southern Africa (Gaubert 2003b) is not supported by this study, as only *pulchra* haplotypes were found in Namibia and Angola. The proposed redefinition for the area of distribution of *felina* was exclusively based on the morphological similarity of scattered individuals in other regions of Southern Africa with *felina* specimens from the Cape, which may just be the result of convergence in the coat pattern (Crawford-Cabral 1981b). Still in Southern

Africa, the monophyly and the genetic divergence higher than 2%, which is a value commonly found among mammalian subspecies (Gravlund *et al.* 1998), with any other *G. genetta* unit supports the validity of a distinct subspecies for the region.

In East Africa, the topologies of the phylogenetic trees and the spanning pattern of the MSN suggest subdivision in the region, but more samples are needed, particularly from Ethiopia, to allow assessing the taxonomic significance of this finding for a region otherwise likely to contain a single subspecies, *hararensis*. Also, the absence of samples from Tanzania in this study prevents the evaluation of the taxonomic value of the subspecies *neumanni* and how it relates with *hararensis*.

The situation in Central, West, and North Africa, is more complicated since these areas are apparently connected as a metapopulation in terms of their mtDNA evolution but each of them possesses some degree of morphological and genetic differentiation; the latter it is more evident between West and Central Africa. Again, more samples and analyses using nuclear DNA markers are expected to help uncover the evolutionary structure for this region of Africa. Finally, Europe comprises a subset of the genetic variation of North Africa and, although now essentially isolated and therefore constituting a relevant evolutionary process on itself (Erwin 1991), it seems not to represent a unique parcel of the gene diversity within the species (Patton & Smith 1994).

3.4.5 Final remarks

This study shows that the phylogeographic analysis of species, which besides possessing a very extensive distribution also have high vagility can yield interesting results. Some of these can be striking as they are the product of a combination of the current range of the species, past events, and high dispersal ability, but likely to be relevant for a better understanding of general patterns across whole continents. The importance of savannah/grassland refuges in the Sahel, East and Southern Africa, a conclusion also reached in a recent phylogeographic study of the common warthog (Muwanika *et al.* 2003), for the maintenance and diversification of pan-African taxa during the Pleistocene climatic cycles is supported. Furthermore, additional scenarios that may be more specific of mammals able to colonise semi-desertic habitats were inferred from the common genet phylogeographic data. To apply a comprehensive set of analytical tools, coming from different perspectives and disciplines of evolutionary research, can only be advantageous and may allow us to extract the maximum of information from a data set with deficiencies

both in sampling and number of screened independent markers. Finally, it seems clear that to revisit the phylogeography of *G. genetta* with critical samples, nuclear markers adequate for phylogeographic and hybridisation analysis (Hare 2001; Zhang & Hewitt 2003), and revision of morphological traits with more robust techniques (Rohlf 2001), it is both a necessary and exciting prospect.

Appendix to Chapter 3

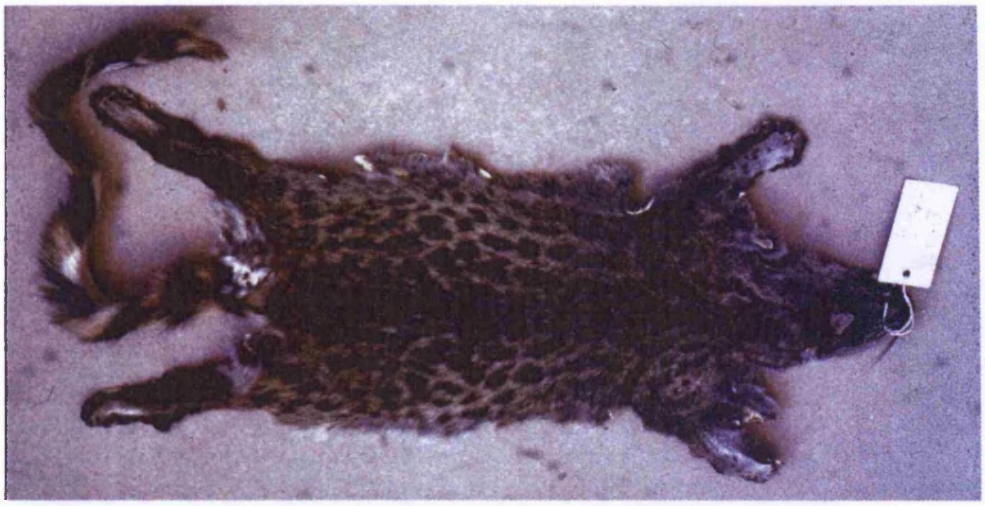

Figure 3.I *genetta*; Bocage Museum "12-3-67 Arraiolos".

Figure 3.II *afra*; Vienna Natural History Museum "NMW B2463".

Figure 3.III *senegalensis*; Vienna Natural History Museum "NMW 32814".

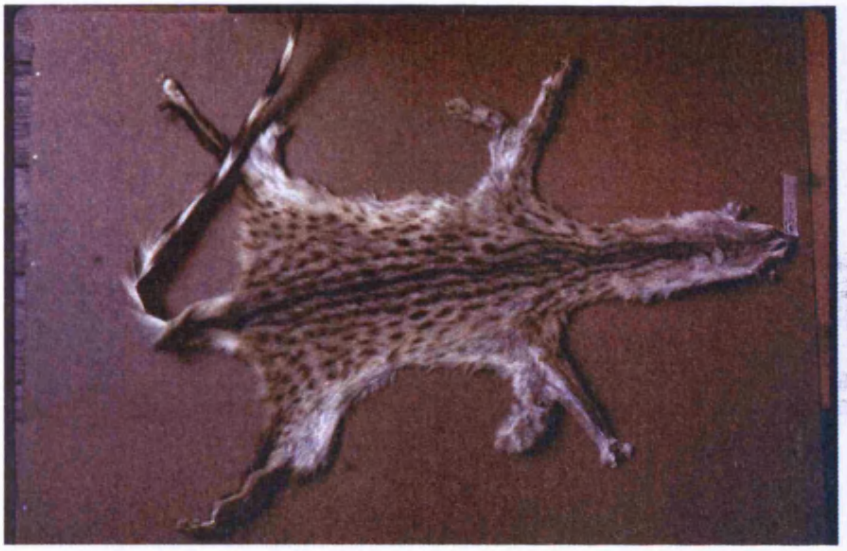

Figure 3.IV *dongolana*; Paris Natural History Museum "3308".

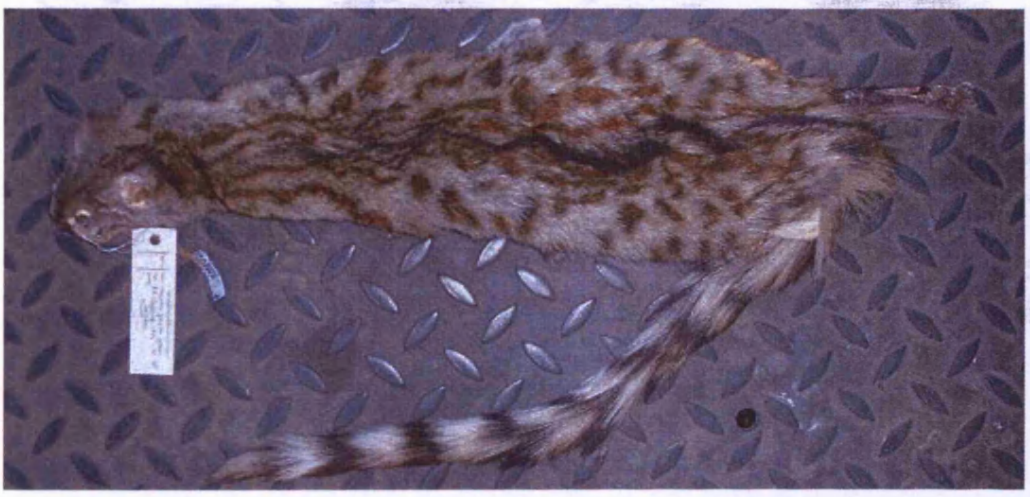

Figure 3.V *granti*; Museum of Scotland "G2".

Figure 3.VI *hararensis*; Los Angeles County Natural History Museum "LACM 56721".

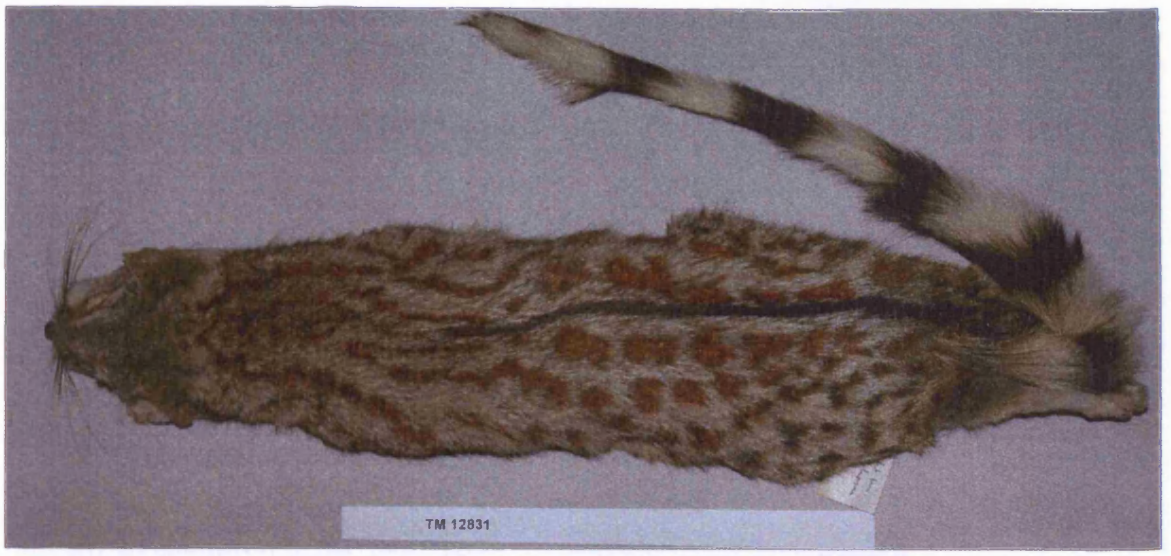

Figure 3.VII *guardafuensis*; Transvaal Museum "TM12831".

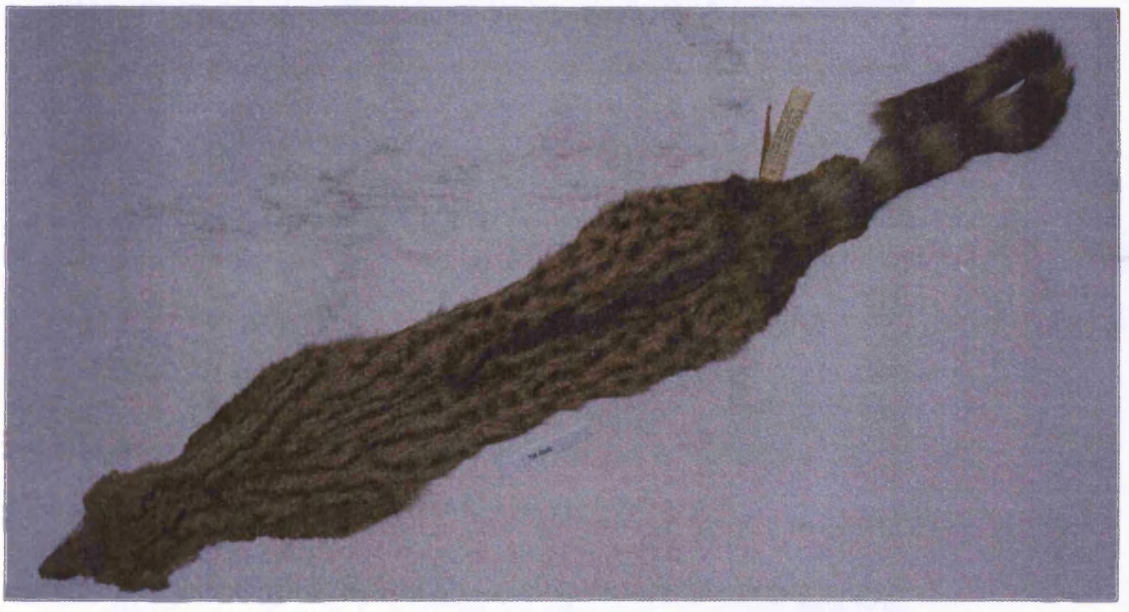

Figure 3.VIII *pulchra*; Transvaal Museum "TM6540".

Figure 3. IX *felina*; Bloemfontein National Museum "NMB7835".

Figure 3.X *Genetta tigrina*; Amathole Museum "KM31185".

Figure 3.XI *Genetta "rubiginosa"*; Transvaal Museum "TM10056".

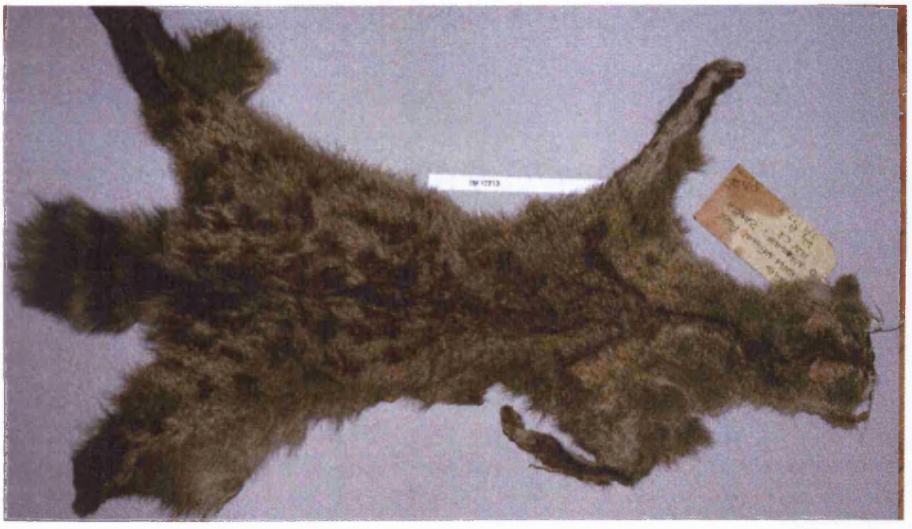

Figure 3. XII *Genetta angolensis*; Transvaal Museum "TM17713".

Figure 3.XIII *felina* with *angolensis*-like haplotype; Amathole Museum "KM29723".

Figure 3.XIV *Genetta tigrina* with *angolensis*-like haplotype; Amathole Museum "KM30812".

Table 3.I Description of the samples included in the study by species name, biological type, sampling locality, geographic coordinates and source of loan or donation.

Species Name	Sample Reference	Biological Type	Sampling Locality	Geographic Coordinates	Source	Curator / Collector
G. genetta	PNPG 3 1	Tissue	Gerês Mountain, Portugal	41° 47' 60N 08° 00' 00W	PNPG Tissue Bank – ICN Portugal	Henrique Carvalho, Joel Ferraz
G. genetta	PNPG 12 1	Tissue	Gerês Mountain, Portugal	41° 47' 60N 08° 00' 00W	PNPG Tissue Bank – ICN Portugal	Henrique Carvalho, Joel Ferraz
G. genetta	Mértola1	Tissue	Mértola, Portugal	37° 37' 59N 07° 40' 00W	PNVG – ICN Portugal	Carlos Carrapato
G. genetta	G42	Tissue	Grândola, Portugal	38° 10' 00N 08° 33' 59W	DZA – Lisbon University	Iris Pereira, Margarida Santos-Reis
G. genetta	DZA1	Tissue	Barrancos, Portugal	38° 07' 60N 06° 58' 60W	DZA – Lisbon University	Iris Pereira, Margarida Santos-Reis
G. genetta	DZA2	Tissue	Alvito, Portugal	38° 15' 00N 07° 58' 60W	DZA – Lisbon University	Iris Pereira, Margarida Santos-Reis
G. genetta	DZA3	Tissue	Portugal	39° 33' 00N 07° 38' 00W	DZA – Lisbon University	Iris Pereira, Margarida Santos-Reis
G. genetta	Donana1	Blood	Madrid, Spain	40° 23' 60N 03° 40' 60W	CSIC – Donana, Spain	Ana Piriz, Francisco Palomares
G. genetta	Donana2	Blood	Madrid, Spain	40° 23' 60N 03° 40' 60W	CSIC – Donana, Spain	Ana Piriz, Francisco Palomares
G. genetta	Donana3	Blood	Donana, Spain	37° 00' 00N 06° 19' 59W	CSIC – Donana, Spain	Ana Piriz, Francisco Palomares
G. genetta	Donana4	Blood	Donana, Spain	37° 00' 00N 06° 19' 59W	CSIC – Donana, Spain	Ana Piriz, Francisco Palomares
G. genetta	Donana5	Blood	Huelva, Spain	37° 15' 30N 06° 57' 03W	CSIC – Donana, Spain	Ana Piriz, Francisco Palomares
G. genetta	Donana6	Blood	Spain	39° 60' 00N 02° 60' 00W	CSIC – Donana, Spain	Ana Piriz, Francisco Palomares
G. genetta	Donana7	Hair	Donana, Spain	37° 00' 00N 06° 19' 59W	CSIC – Donana, Spain	Ana Piriz, Francisco Palomares
G. genetta	T37	Tissue	Gironde, France	44° 55' 00N 00° 30' 00W	Paris NHM	Philippe Gaubert
G. genetta	T39	Tissue	Languedoc, France	44° 00' 00N 04° 00' 00E	Paris NHM	Philippe Gaubert
G. genetta	T44	Tissue	Lot et Garonne, France	44° 19' 59N 00° 30' 00E	Paris NHM	Philippe Gaubert
G. genetta	T46	Tissue	Gironde, France	44° 55' 00N 00° 30' 00W	Paris NHM	Philippe Gaubert
G. genetta	T67	Tissue	Charente-Maritime, France	45° 30' 00N 00° 45' 00W	Paris NHM	Philippe Gaubert
G. genetta	T83	Tissue	Gironde, France	44° 55' 00N 00° 30' 00W	Paris NHM	Philippe Gaubert
G. genetta	T107	Hair	Pyrenees, France	42° 40' 00N 01° 00' 00E	Paris NHM	Philippe Gaubert
G. genetta	T87	Hair	Jijel, Algeria	36° 47' 59N 05° 46' 00E	Paris NHM	Philippe Gaubert

G. genetta	ROM59287ᵎ	Museum skin	Tunisia	33° 45' 00N 09° 60' 00E	Royal Ontario Museum	Judith Eger
G. genetta	NMWB2463*ᴟ	Museum skin	Meknes, Morocco	33° 55' 00N 05° 30' 00W	Wien NHM	Barbara Herzig, Friederike Spitzenberger
G. genetta	PC25	Museum skin	El Fedja, Tunisia	36° 40' 47N 09° 58' 53E	Powell-Cotton Museum	Malcolm Harman
G. genetta	Batna1	Hair	Batna, Algeria	35° 32' 48N 06° 00' 28E	Belezma National Park	Aissa Laabed
G. genetta	T69	Tissue	Tentane, Mauritania	20° 12' 00N 13° 22' 00W	Paris NHM	Philippe Gaubert
G. genetta	T60	Tissue	Dakar, Senegal	14° 40' 00N 17° 25' 60W	Paris NHM	Philippe Gaubert
G. genetta	T61	Tissue	Dakar, Senegal	14° 40' 00N 17° 25' 60W	Paris NHM	Philippe Gaubert
G. genetta	NMW32814*	Museum skin	Richard Toll, Senegal	16° 28' 00N 15° 41' 00W	Wien NHM	Barbara Herzig, Friederike Spitzenberger
G. genetta	T09	Tissue	Emnal'here, Mali	12° 38' 59N 08° 00' 00W	Paris NHM	Philippe Gaubert
G. genetta	NMS25863*	Museum skin	Farniso, Nigeria	12° 00' 00N 08° 31' 00E	National Museum of Scotland	Jerry Herman, Ruth Pollitt, Andrew Kitchener
G. genetta	NMS25864*	Museum skin	Farniso, Nigeria	12° 00' 00N 08° 31' 00E	National Museum of Scotland	Jerry Herman, Ruth Pollitt, Andrew Kitchener
G. genetta	NMS25865*	Museum skin	Farniso, Nigeria	12° 00' 00N 08° 31' 00E	National Museum of Scotland	Jerry Herman, Ruth Pollitt, Andrew Kitchener
G. genetta	LACM14468*	Museum skin	Ubangi, Chad	09° 00' 00N 18° 00' 00E	Los Angeles County NHM	Jim Dines, Ines Horovitz
G. genetta	LACM14469*	Museum skin	Ubangi, Chad	09° 00' 00N 18° 00' 00E	Los Angeles County NHM	Jim Dines, Ines Horovitz
G. genetta	LACM14477*	Museum skin	Fada, Chad	17° 11' 05N 21° 35' 25E	Los Angeles County NHM	Jim Dines, Ines Horovitz
G. genetta	NMWB2145*	Museum skin	Mongalla, Sudan	05° 12' 08N 31° 46' 22E	Wien NHM	Barbara Herzig, Friederike Spitzenberger
G. genetta	LACM56721*	Museum skin	Isiolo, Kenya	00° 21' 00N 37° 34' 60E	Los Angeles County NHM	Jim Dines, Ines Horovitz
G. genetta	Bern1025642ᵛ	Museum skin	Athi River, Kenya	01° 26' 60S 36° 58' 60E	Bern NHM	Pieter Lueps, Beatrice Blochlinger
G. genetta	MHNG1119085*ᴟᵎ	Museum skin	Kenya	00° 07' 00S 37° 60' 00E	Geneve NHM	Manuel Ruedi
G. genetta	Hamburg64ᴟ	Museum skin	Nairobi, Kenya	01° 16' 60S 36° 49' 00E	Hamburg Zoological Museum	Harald Schliemann, Nelson Mascarenhas
G. genetta	EritreaG1	Tissue	Hagas, Eritrea	15° 41' 60N 38° 16' 00E	University of Asmara	Hezy Shoshany, Rebekka Deleu
G. genetta	EritreaG2	Tissue	Hagas, Eritrea	15° 41' 60N 38° 16' 00E	University of Asmara	Hezy Shoshany, Rebekka Deleu
G. genetta	EritreaG3	Tissue	Mehdaf, Eritrea	15° 01' 00N 38° 15' 00E	University of Asmara	Hezy Shoshany, Rebekka Deleu

G. genetta	PC30	Museum skin	Jubba, Somalia	01° 25' 59N 42° 26' 00E	Powell-Cotton Museum	Malcolm Harman
G. genetta	TM12831*	Museum skin	El Bur Rungno, Somalia	04° 40' 60N 46° 37' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	T104	Hair	Taiz, Yemen	14° 46' 60N 44° 01' 60E	Paris NHM	Philippe Gaubert
G. genetta	NWRC1	Blood	Taif, Saudi Arabia	21° 16' 00N 40° 25' 00E	NWRC Taif, Saudi Arabia	Stephane Ostrowski
G. genetta	NWRC2	Tissue	Taif, Saudi Arabia	21° 16' 00N 40° 25' 00E	NWRC Taif, Saudi Arabia	Stephane Ostrowski
G. genetta	NWRC3	Tissue	Bani Sa'ad, Saudi Arabia	20° 49' 00N 40° 45' 00E	NWRC Taif, Saudi Arabia	Stephane Ostrowski
G. genetta	NWRC4	Tissue	Raydah, Saudi Arabia	18° 12' 00N 42° 24' 00E	NWRC Taif, Saudi Arabia	Stephane Ostrowski
G. genetta	NWRC5	Tissue	Taif, Saudi Arabia	21° 16' 00N 40° 25' 00E	NWRC Taif, Saudi Arabia	Stephane Ostrowski
G. genetta	NMSG1*!	Tissue	Oman	21° 27' 00N 55° 60' 00E	National Museum of Scotland	Jerry Herman, Ruth Pollitt, Andrew Kitchener
G. genetta	NMSG2*!	Tissue	Oman	21° 27' 00N 55° 60' 00E	National Museum of Scotland	Jerry Herman, Ruth Pollitt, Andrew Kitchener
G. genetta	TC31	Tissue	Salallah, Oman	26° 03' 58N 56° 23' 13E	Paris NHM	Philippe Gaubert
G. genetta	Oman1	Tissue	Dhofar, Oman	20° 30' 30N 55° 31' 40E	Oman NHM	Andrew Spalton
G. genetta	Rotterdam1	Hair	Okahandja, Namibia	22° 01' 00S 16° 52' 60E	Rotterdam Zoo	Simone de Vries
G. genetta	Griffin1!	Tissue	Namibia	22° 60' 00S 18° 35' 00E	Namibia Ministry of Environment	Mike Griffin
G. genetta	Griffin212!	Tissue	Namibia	22° 60' 00S 18° 35' 00E	Namibia Ministry of Environment	Mike Griffin
G. genetta	TM30163*v	Museum skin	Outjo , Namibia	20° 07' 00S 16° 08' 59E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	TM14840	Museum skin	Gobabeb, Namibia	23° 32' 59S 15° 01' 59E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	MVZ117819*	Museum skin	Ovamboland, Namibia	18° 00' 00S 16° 00' 00E	Berkeley Museum of Vertebrate Zoology	James Patton, Yuri Leite
G. genetta	Hamburg3815	Museum skin	Gambos, Angola	15° 45' 00S 14° 04' 60E	Hamburg Zoological Museum	Harald Schliemann, Nelson Mascarenhas
G. genetta	PC74!	Museum skin	Angola	10° 60' 00S 17° 60' 00E	Powell-Cotton Museum	Malcolm Harman
G. genetta	MHNG1492029*	Museum skin	Mupa, Angola	16° 10' 00S 15° 45' 00E	Geneve NHM	Manuel Ruedi
G. genetta	KM13857	Museum skin	Ngamiland, Botswana	20° 30' 00S 22° 40' 00E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. genetta	NMB8544*	Museum skin	Prieska, South Africa	29° 40' 59S 22° 45' 59E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. genetta	KM24151*	Museum skin	Steytlerville, South Africa	33° 14' 52S 24° 22' 50E	Amathole Museum	Fred Kigozi, Lloyd Wingate

G. genetta	TM29543	Museum skin	Beaufort West, South Africa	32° 21' 00S, 22° 34' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	KM31133*▽	Museum skin	Montagu, South Africa	33° 42' 52S 20° 06' 57E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. genetta	KM27713*	Museum skin	Robertson, South Africa	33° 50' 25S 19° 55' 00E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. genetta	TM12054*	Museum skin	Bulawayo, Zimbabwe	20° 08' 59S 28° 34' 59E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	NMS27346*	Museum skin	Sabie River, South Africa	23° 30' 00S 31° 06' 00E	National Museum of Scotland	Jerry Herman, Ruth Pollitt, Andrew Kitchener
G. genetta	TM28497	Museum skin	Warmbat, South Africa	24° 52' 60S 28° 16' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	TM6540*	Museum skin	Tsotsoroga Pan, Botswana	18° 44' 00S 24° 21' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	MVZ118447*	Museum skin	Kweneng, Botswana	24° 00' 00S 25° 00' 00E	Berkeley Museum of Vertebrate Zoology	James Patton, Yuri Leite
G. genetta	PC138	Museum skin	M'Kuzi, South Africa	27° 40' 00S 32° 01' 60E	Powell-Cotton Museum	Malcolm Harman
G. genetta	NMS27341*	Museum skin	Sabie River, South Africa	23° 30' 00S 31° 06' 00E	National Museum of Scotland	Jerry Herman, Ruth Pollitt, Andrew Kitchener
G. genetta	NMS27344*	Museum skin	Sabie River, South Africa	23° 30' 00S 31° 06' 00E	National Museum of Scotland	Jerry Herman, Ruth Pollitt, Andrew Kitchener
G. genetta	NMS27348*	Museum skin	Sabie River, South Africa	23° 30' 00S 31° 06' 00E	National Museum of Scotland	Jerry Herman, Ruth Pollitt, Andrew Kitchener
G. genetta	KM19288	Museum skin	Nyamandhlovu, Zimbabwe	19° 50' 00S 28° 15' 00E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. genetta	TM40740*▽	Museum skin	Standerton, South Africa	26° 56' 60S 29° 15' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	TM23464	Museum skin	Delarey, South Africa	26° 40' 60S 25° 28' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	TM23971	Museum skin	Pretoria, South Africa	25° 45' 00S 28° 10' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	TM27716	Museum skin	Lichtenburg, South Africa	26° 08' 60S 26° 10' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	TM24611	Museum skin	Letaba, South Africa	23° 49' 00S 30° 19' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney

G. genetta	NMB7835*	Museum skin	Vryburg, South Africa	26° 56' 60S 24° 43' 60E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. genetta	NMB3159*	Museum skin	Vryburg, South Africa	26° 56' 60S 24° 43' 60E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. genetta	TM32528*	Museum skin	Hay, South Africa	28° 00' 00S 22° 00' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	TM28218*	Museum skin	Vryburg, South Africa	26° 56' 60S 24° 43' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	TM16796*	Museum skin	Gordonia Mata, South Africa	28° 12' 00S 24° 37' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. genetta	DM1363	Tissue	Chelmsford, South Africa	24° 49' 00S 29° 55' 00E	Durban Museum	Peter Taylor
G. genetta	NMB9279*	Museum skin	Lindley, South Africa	27° 52' 00S 27° 55' 00E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. genetta	NMB3946*	Museum skin	Bethlehem, South Africa	28° 13' 60S 28° 18' 00E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. genetta	KM29723*	Museum skin	Beaufort West, South Africa	32° 21' 00S, 22° 34' 60E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. genetta	TC38	Hair	Victoria West, South Africa	31° 23' 59S 23° 07' 00E	Paris NHM	Philippe Gaubert
G. genetta	TC39	Hair	Victoria West, South Africa	31° 23' 59S 23° 07' 00E	Paris NHM	Philippe Gaubert
G. genetta	NGP2152	Tissue	Hopefield, South Africa	33° 02' 04S 18° 21' 08E	Cape Nature Conservancy	Guy Palmer
G. genetta	NGP2160	Tissue	Porterville, South Africa	33° 01' 00S 18° 58' 60E	Cape Nature Conservancy	Guy Palmer
G. genetta	NGP2161	Tissue	Piketberg, South Africa	32° 58' 33S 18° 60' 18E	Cape Nature Conservancy	Guy Palmer
G. genetta	NGP2164	Tissue	Lambert's Bay, South Africa	32° 06' 00S 18° 19' 60E	Cape Nature Conservancy	Guy Palmer
G. genetta	NGP2172	Tissue	Stellenbosch, South Africa	33° 55' 60S 18° 51' 00E	Cape Nature Conservancy	Guy Palmer
G. tigrina	A274	DNA Extract	East London, South Africa	33° 01' 59S 27° 55' 00E	Cape Town University	Eric Harley
G. tigrina	TM32193*	Museum skin	Swellendam, South Africa	34° 01' 60S 20° 25' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. tigrina	TM28335*	Museum skin	Clanwilliam, South Africa	32° 10' 60S 18° 52' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. tigrina	TM28666*	Museum skin	Knysna, South Africa	34° 01' 60S 23° 01' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. tigrina	KM30812*	Museum skin	Bredasdorp, South Africa	34° 31' 60S 20° 01' 60E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. tigrina	KM30813*	Museum skin	Betty's Bay, South Africa	34° 22' 00S 18° 55' 60E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. tigrina	KM30814*	Museum skin	Wellington, South Africa	33° 37' 60S 19° 00' 00E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. tigrina	TM29786*∃	Museum skin	Humansdorp, South Africa	34° 01' 60S 24° 46' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney

G. tigrina	A270	DNA Extract	East London, South Africa	33° 01' 59S 27° 55' 00E	Cape Town University	Eric Harley
G. tigrina	A271	DNA Extract	East London, South Africa	33° 01' 59S 27° 55' 00E	Cape Town University	Eric Harley
G. tigrina	A272	DNA Extract	East London, South Africa	33° 01' 59S 27° 55' 00E	Cape Town University	Eric Harley
G. tigrina	A273	DNA Extract	East London, South Africa	33° 01' 59S 27° 55' 00E	Cape Town University	Eric Harley
G. tigrina	KM31185*	Museum skin	Bathurst, South Africa	33° 28' 60S 26° 49' 60E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. tigrina	KM31276*	Museum skin	Msikaba River, South Africa	31° 10' 60S 29° 36' 00E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. tigrina	NMB4786*	Museum skin	Barkly East, South Africa	30° 58' 00S 27° 36' 00E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. tigrina	NMB4470*	Museum skin	Reddersburg, South Africa	29° 38' 60S 26° 10' 00E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. "rubiginosa"	TM10348	Museum skin	Waterberg, Namibia	20° 31' 00S 17° 13' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	Angola1	Tissue	Quissama, Angola	09° 58' 60S 14° 28' 60E	Quissama National Park	Pedro Vaz Pinto
G. "rubiginosa"	Angola2	Tissue	Quissama, Angola	09° 58' 60S 14° 28' 60E	Quissama National Park	Pedro Vaz Pinto
G. "rubiginosa"	TM8886	Museum skin	Secheli, Botswana	24° 53' 00S 25° 58' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	PC90	Museum skin	Bambi, Botswana	18° 00' 00S 21° 10' 00E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	DM2232	Tissue	Umhloti, South Africa	29° 39' 00S 31° 06' 00E	Durban Museum	Peter Taylor
G. "rubiginosa"	ROM35695	Museum skin	Zimbabwe	-	Royal Ontario Museum	Judith Eger
G. "rubiginosa"	TM11449*	Museum skin	Inyanga, Zimbabwe	18° 13' 00S 32° 45' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM16638*	Museum skin	Bethlehem, South Africa	28° 13' 60S 28° 18' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	NMB7013*	Museum skin	Moletsanes, Lesotho	29° 10' 00S 28° 03' 00E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. "rubiginosa"	NMB6904*	Museum skin	Mokhotlong, Lesotho	29° 16' 60S 29° 04' 60E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. "rubiginosa"	DM1089	Tissue	St. Lucia, South Africa	28° 21' 00S 32° 25' 00E	Durban Museum	Peter Taylor
G. "rubiginosa"	CZ242	Museum skin	Mambone, Mozambique	20° 58' 60S 33° 38' 60E	Centro de Zoologia, IICT, Portugal	João Crawford-Cabral
G. "rubiginosa"	TM12656*	Museum skin	Kayombo, Zambia	13° 03' 00S 23° 51' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM6058*	Museum skin	Mofu, Zambia	11° 05' 60S 30° 21' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM9839*	Museum skin	Chisasa, Zambia	12° 05' 60S 25° 30' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney

G. angolensis	TM17713*	Museum skin	Kafue, Zambia	15° 46' 00S 28° 10' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. angolensis	MVZ118449*	Museum skin	Luanshya, Zambia	13° 07' 60S 28° 23' 60E	Berkeley Museum of Vertebrate Zoology	James Patton, Yuri Leite
G. angolensis	TM9842*	Museum skin	Kasempa, Zambia	13° 26' 60S 25° 49' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. angolensis	TM9847*	Museum skin	Kasempa, Zambia	13° 26' 60S 25° 49' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. angolensis	TM9849*	Museum skin	Kasempa, Zambia	13° 26' 60S 25° 49' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney

* - photograph of the specimen available; ∃ - sample not included in the analyses due to inability of obtaining complete sequence for both the cytochrome *b* and the ND-5 gene fragments; ∀ - specimen apparently misidentified by the source; ! - sampling locality unknown beyond country level: indicated coordinates are of the country's geographic centre.

Chapter 4. Mitochondrial DNA phylogeography of the large-spotted genets (Viverridae: *Genetta*)

4.1 Introduction

The way in which genetic variation is structured among populations from different geographical areas is a product of the history, evolution and current distribution of a species (Hewitt 2001). Phylogeography is a subdiscipline of biogeography concerned with the past and present processes that govern the geographical patterns of genealogical lineages, mainly within species but also among closely related species (Avise 2000a). The main staple of molecular phylogeographic studies is still largely mitochondrial DNA (mtDNA) data, which allows reconstruction of the intra- and interpopulation relationships of haplotypes through time and space by using several complementary analytical methods (e.g. Matocq 2002; Starkey *et al.* 2003).

The climatic history of Africa during the Late Pliocene and Pleistocene has been one that followed a long and stable warm and humid period, which characterised most of Early and Middle Pliocene, and was mainly composed of regular alternations between warm/wet and cool/dry conditions (Livingstone 1975). These cyclic fluctuations certainly had a major role in shaping the recent evolution of most African taxa (Hamilton 1976) and the quantity of broad distributional DNA data that can be interpreted within the Pleistocene history of Africa has notably grown in recent years (e.g. Birungi & Arctander 2000; Van Hooft *et al.* 2002). So far, the vast majority of phylogeographic studies in pan-African mammals have been concerned with savannah-dwelling herbivores (e.g. Matthee & Robinson 1999; Muwanika *et al.* 2003). Although the number of studies now available dealing with forest species is also increasing (Jensen-Seaman & Kidd 2001; Telfer *et al.* 2003), analyses of taxa that occur simultaneously over both types of dominant habitat in Africa, and the evolutionary consequences of this niche broadness, are still rare (Eggert *et al.* 2002). For African taxa inhabiting partly or exclusively rainforest habitat, refuge theory (Diamond & Hamilton 1980; Crowe & Crowe 1982; Mayr & O'Hara 1986) is often invoked to explain differentiation of populations or species as a result of allopatric fragmentation during the cool and dry periods of the Pleistocene. However, the generality of the role of putative rainforest refuges as diversification centres has been questioned recently (Moritz *et al.* 2000), and instead the importance of marginal areas and particularly of montane habitats has been underlined (e.g. Fjeldsa & Lovett 1997). Mountain locations

can provide, through orographic moderation, buffering capacity against changes in climatic conditions and allow persistence of forest cover that represented small refuges (Brown & Ab'Saber 1979), which by becoming isolated during vicariant events (e.g. volcanism, tectonism), acted as generators of diversity (Roy 1997). Also, the possibility offered by ecotones or habitat mosaics in areas of transition between biomes for peripatric diversification, where differential selective pressures may overcome the homogenising action of gene flow (Smith *et al.* 1997), is now clearly demonstrated (Schneider *et al.* 1999; Schilthuizen 2000).

Genetta rubiginosa nec Pucheran, 1855 is the name of a very polymorphic complex of forms, distributed from the Dahomey Gap eastwards to the Abyssinian plateau and southwards down to the Natal Province of South Africa, colloquially called rusty-spotted genets (Roberts 1951; Crawford-Cabral 1981a,b; Ansell & Dowsett 1988). It is the most ubiquitous genet, occurring in a diversity of habitats ranging from tropical rainforest and woodland savannahs to steppe and montane biotopes (Crawford-Cabral 1981b). It is closely related to *Genetta tigrina* (Schreber, 1776), the blotched genet, which occurs in the coastal regions of the Western and Eastern Cape Provinces of South Africa, and to *Genetta pardina* I. Geoffroy Saint-Hillaire, 1832, the pardine genet, which occurs west of the Dahomey Gap up to Senegal. In appendix, at the end of the chapter, Figs. 4.I to 4.III show pictures of these three taxa and the map in Fig. 4.1 presents their distribution areas. The range limits of both *G. pardina* and the rusty-spotted genets at the region where they meet, the Dahomey Gap, are not precisely established yet (Grubb *et al.* 1998). The difficulty in defining interspecific boundaries between these three taxa, collectively designated as large-spotted genets (Gaubert *et al.* 2004), on the basis of only morphological characters is clearly illustrated by their taxonomic history (Schwarz 1930; Wenzel & Haltenorth 1972; Coetzee 1977; Meester *et al.* 1986; Wozencraft 1993). Crawford-Cabral (1981b) was the first author to present enough morphological evidence suggesting a trifurcate separation that has been supported by more recent studies (Schlawe 1981; Powell & Van Rompaey 1998; Crawford-Cabral & Fernandes 2001; Gaubert *et al.* 2004). Hybridisation between *G. tigrina* and the rusty-spotted genets at their contact zone in the Natal Province of South Africa has been proposed (Pringle 1977) and apparently confirmed to occur over a narrow area at the south-west of the province (Gaubert *et al.* in press). Although now accepted to be morphologically distinct, genetic distance between the two taxa is low and their divergence is estimated to have occurred during the Late Pleistocene (Gaubert *et al.* 2004).

Figure 4.1 Map showing the approximate ranges of the three taxa of large-spotted genets. 1- *Genetta pardina*, 2- *Genetta "rubiginosa"*, 3- *Genetta tigrina*.

The evolutionary affinities of the rusty-spotted genets, and also of *G. pardina*, with proposed additional genet species (Rosevear 1974; Powell & Van Rompaey 1998) living in the tropical rainforest belts of, respectively, central and western Africa, are still not clear in spite of a recent detailed contribution (Gaubert 2003a). The split between the lineages leading to these rainforest forms, to the rusty-spotted genets, and to *G. pardina*, has been estimated to be contemporary with the onset of the Pleistocene (Gaubert *et al.* 2004). Although there is now apparently some evidence indicating the existence of more than one large-spotted genet species in the area (Gaubert 2003a; Gaubert *et al.* 2004), in the present study the name *pardina* was employed in its broad sense (sensu lato) to designate all large-spotted genet populations west of the Dahomey Gap, which is the traditional treatment. Reasons for this option were the facts that the specimens' phenotype was unknown for the majority of the samples gathered from the area, which could allow species identification, and that the analysis of taxonomic diversification within the area was not one of the aims of this study.

Among the rainforest forms for which Gaubert (2003a,b) discussed a hypothetical species status, the more controversial and with highest impact for how we traditionally see the biogeography and taxonomy of the rusty-spotted genets (Crawford-Cabral 1981a,b) is *schoutedeni*. This form was originally described by Crawford-Cabral (1970) as a subspecies of the rusty-spotted genets restricted to the central area of the Democratic Republic of the Congo (DRC). However, Gaubert (2003b) ascribes to it a much wider range, from Angola and Mozambique northwards up to Ethiopia and Ghana, which implies broad sympatry with other rusty-spotted genets and raises both biogeographic and evolutionary questions amenable to investigation with molecular markers. Overall, both in terms of coat pattern and skull measures, it falls within the range of morphological variability exhibited by the rusty-spotted genets, with the exception of an apparently diagnostic cranial feature (Gaubert 2003a).

Although the status of the rusty-spotted genets as a monophyletic clade within the large-spotted genets seems supported by recent studies, the possibility of the rusty-spotted genets themselves consisting of more than one species has been often acknowledged (Kingdon 1997; Crawford-Cabral & Fernandes 2001; Gaubert *et al.* in press). They display levels of morphological variation, likely to be the result of the superimposed contribution from different causes of diversity, without parallel in any other genet species. Consequently, it becomes difficult to discriminate between individual and adaptive variation and true phylogenetic divergence among lineages, across the vast range of heterogeneous habitats separated by several types of physical barriers in their distribution area (Crawford-Cabral & Fernandes 2001). Crawford-Cabral (1981a,b) discussed all the previously described forms, regarded by different authors and in different times, as species, subspecies, races/ecotypes, or just synonyms, that can be related with the rusty-spotted genets and indicated the ones that may have evolutionary significance. In a study essentially of craniometry, but where coat patterns were also considered even if only qualitatively, the author interpreted the results as evidence to consider the rusty-spotted genets a single species but structured in subspecies across their range. The populations occurring between the Rivers Niger and Congo were included in the subspecies *fieldiana* whereas the ones from the River Niger to the Dahomey Gap were not classified, as no specimens from this area were examined, although the hypothesis of a different subspecies was suggested. Also uncertain is the classification of the specimens from southern Chad, Central African Republic, and south-western Sudan, which may belong to *fieldiana* or to a different subspecies, *aequatorialis*. In the region encompassing eastern Sudan, Eritrea and

Ethiopia, populations were grouped under the subspecies *matschiei*, but for northern and central Somalia the presence of a different subspecies, *deorum*, has been proposed (Azzaroli & Simonetta 1966). For the central lowland rainforest of the Democratic Republic of Congo, between the Congo River and the southern Katanga Province, the author described the subspecies *schoutedeni*. The populations from the central and northern Albertine Rift, throughout Uganda, and up to the Rift Valley in Kenya were considered to represent the subspecies *stuhlmanni*, and the ones occurring east of the Rift Valley, in Kenya and southern Somalia, were included in *erlangeri*. For the region stretching from the Serengeti plains to Lake Nyasa and northern Zambia the subspecies *suahelica* was proposed. Finally, in Southern Africa, two subspecies are recognized: *zambesiana* in an area from Mozambique and Zimbabwe, through Malawi and the centre and north of Zambia, to northern Angola, and *letabae* from the Transvaal and Natal regions in South Africa, through Botswana, to Namibia. Although precise geographic ranges and limits of these putative subspecies are uncertain, Fig. 4.2 attempts to represent their approximate distribution areas. In appendix, at the end of the chapter, Figs. 4.IV to 4.XI show photographs of specimens representing all but one (*deorum*) of these morphological and geographical units.

An additional issue, originally noticed by Schlawe (1980), in relation to the rusty-spotted genets is the fact that they need a new scientific name since the type specimen of *G. rubiginosa* Pucheran, 1855, is an individual of a completely different species, *Genetta thierryi* Matschie, 1902. In attention to this, the names *G. "rubiginosa"* or *G. rubiginosa* nec Pucheran, 1855 were used in this study to designate the rusty-spotted genets. There is, at the moment, a rising controversy about the scientific name that should be given to this taxon (Gaubert *et al.* 2003a,b; Grubb in press; Fernandes & Crawford-Cabral in press). To decide over this issue it is obviously critical to determine first if the rusty-spotted genets are a single species or a set of sibling cryptic species. Crawford-Cabral & Fernandes (1999) proposed that if the rusty-spotted genets are a single entity, then the valid name should be the oldest available synonym of *rubiginosa*, *Genetta fieldiana* Du Chaillu, 1860.

Finally, a set of specimens ascribed by Roberts (1951) to *Genetta mossambica* Matschie, 1902, a taxon usually regarded as either a synonymous or a subspecies of *G. angolensis* Bocage, 1882 (Crawford-Cabral 1981b; Schlawe 1981), has been suggested, on the basis of a craniometrical study, to probably represent instead an unsuspected distinct subspecies of *G. "rubiginosa"* (Crawford-Cabral & Fernandes 2001), a hypothesis open to assessment with genetic data. In the appendix at the end of the chapter Fig. 4.XII shows a

photograph of one of the *mossambica* specimens *sensu* Roberts (1951), from which a sample was analysed in this study.

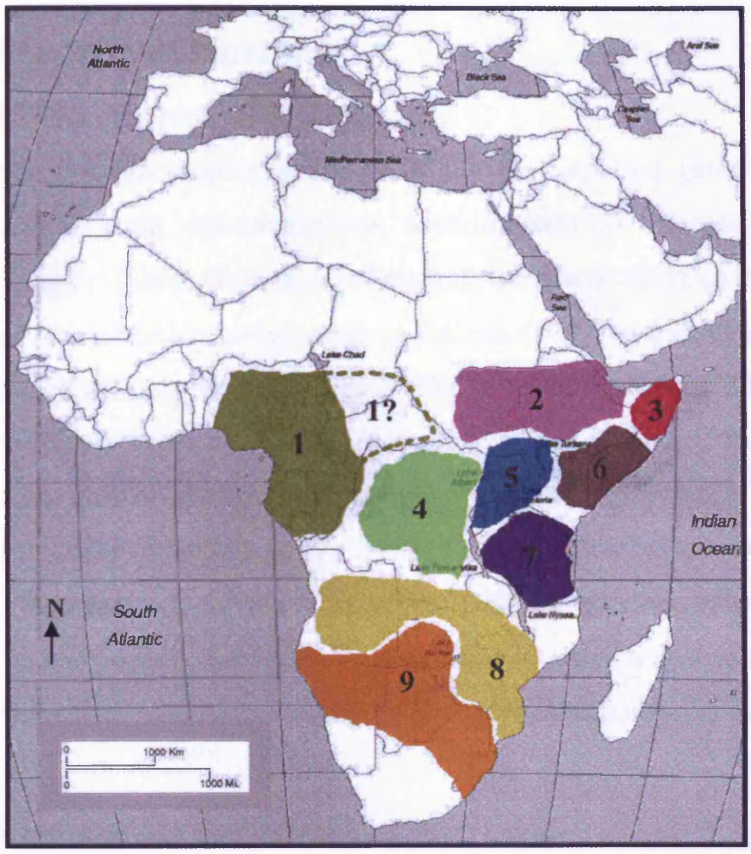

Figure 4.2 Map showing the approximate ranges of *G. "rubiginosa"* forms which, among the several others described up to date, may represent distinct evolutionary units of the *G. "rubiginosa"* complex worth formal taxonomic recognition (Crawford-Cabral *pers. comm.*). 1- *fieldiana*, 2- *matschiei*, 3- *deorum*, 4- *schoutedeni*, 5- *stuhlmanni*, 6- *erlangeri*, 7- *suahelica*, 8- *zambesiana*, 9- *letabae*.

Considering the accumulated information about the large-spotted genets, the uncertainty surrounding them, and using mitochondrial DNA (mtDNA) data of specimens from almost their whole range, several issues were addressed using the phylogeographic approach and the analytical tools now available to it. The main evolutionary questions are: (i) is the separation of the large-spotted genets in three different taxa supported by an extensive genetic survey of the group; (ii) are the rusty-spotted genets a single polytypic species or the genetic results point for the existence of multiple morphologically cryptic species; (iii) does the observed genetic structure correspond to previously proposed subspecies within the rusty-spotted genets or does it indicate different arrangements; (iv) does the phylogeographic pattern of haplotypes reflect a high influence of the current distribution of large-spotted genets or, instead, does it suggest different historic ranges and a recent establishment of the present distribution areas. If the latter is true, which past

events and biogeographic processes are likely to be involved in the evolution of this group of carnivores?

4.2 Material and methods

4.2.1 DNA samples

For the phylogeographic analyses of the large-spotted genets 118 samples were used, of several different biological types, covering with few exceptions (Somalia, Sudan, Republic of Congo, Ghana, Guinea, and Senegal) the whole range of the group. To address the affinities of *mossambica* in the sense of Roberts (1951) and its hypothesised status as a new rusty-spotted genet subspecies (Crawford-Cabral & Fernandes 2001), five samples of *G. angolensis* from Zambia were also included in the phylogenetic analyses. Table 4.I (in appendix at the end of the chapter) provides detailed information about the samples. Species or subspecies identification of samples by collectors or museum curators was assessed by comparison of the respective DNA sequences with a comprehensive, taxonomic and geographic, database of genet sequences that had been previously generated exclusively from specimens where both the phenotype and sampling locality were known.

4.2.2 Laboratory techniques

4.2.2.1 DNA extraction

Genomic DNA was isolated from the samples using different protocols in accordance with their biological type. For tissue biopsies, originated either from live-trapped or road-killed specimens, which were stored in suitable solutions [e.g. 95% ethanol with 0.01 μM EDTA or NaCl-saturated 25% DMSO (Dimethylsulphoxide)] immediately upon collection, both a "salting-out" protocol (Bruford *et al.* 1992) and a standard phenol-chloroform method (Sambrook *et al.* 1989) were successfully applied. The method described by Walsh (1991), which takes advantage of the nuclease-inhibiting chelating properties of the Chelex 100 resin, was used for DNA extraction from plucked or shed hair samples, whereas blood samples were extracted using a commercial kit (DNeasy Tissue Kit, catalogue #69506, Qiagen) and following the manufacturer's recommendations. Finally, the main source of DNA, samples from museum skins, were extracted with the same kit, but this time following the modifications described by Mundy *et al.* (1997) and using separate facilities and lab materials in an ancient-DNA-dedicated room isolated from the main lab. DNA extractions from ancient-DNA samples (e.g. museum skins and hair)

were carried out in rounds, in such a way that the next round always included a set of randomly chosen samples from the previous round in order to authenticate ancient DNA results through independent events (Hummel & Herrmann 1994).

4.2.2.2 Preliminary study of large-spotted genet mtDNA

The dominant type of sample (museum skin; N=84) determined the size of the mitochondrial fragments surveyed for all samples, as it is unlikely to regularly obtain contiguous nucleotide information for more than 200 base pairs (bp) from typical museum skins (Hofreiter *et al.* 2001). Since this was the first population-level analysis of a genet species with molecular markers, no previous information about genetic variation patterns in the genet mtDNA that could be helpful for selecting suitable 200 bp blocks for this study was available. Using DNA extracts with high molecular weight from large-spotted genet specimens sampled in different geographic regions of the distribution area, levels of genetic variation were assessed for the two mitochondrial regions most widely used in phylogeographic studies of vertebrates, the control region and the cytochrome *b* gene (reviewed in Avise 2000a). Complete DNA sequences for the cytochrome *b* gene (1140 bp) and the control region (≈ 1430 bp) were produced via polymerase chain reaction (PCR) with versatile primers, and respective PCR conditions, as described by Kocher *et al.* (1989), Shields & Kocher (1991), Irwin *et al.* (1991) and Palumbi (1996). In addition to those, Carnivora-specific primers, provided by Stephan Funk (*pers. comm.*), were also used and allowed a more straightforward sequencing of internal regions of the two genes for which no general primers are described.

The presence of more than one type of fragment in the PCR products yielded by the versatile primers, which was resistant to exhaustive PCR optimisation attempts, was detected both by multiple bands in 1.5% agarose tris-borate-EDTA (TBE) gels and by ambiguities and high background in sequence reads. This was interpreted as indicating the presence of mtDNA-like nuclear copies of the targeted fragment (*Numts*; Lopez *et al.* 1997) and/or extensive VNTR heteroplasmy in the left and right domains of the control region (described as widespread in Carnivores by Hoelzel *et al.* 1994). Both instances were confirmed through sequencing of cloned PCR products, cloned with the CopyControl™ cloning kit (Epicentre) following the manufacturer's protocol, from individuals from different geographic populations; five clones per individual were sequenced.

Figure 4.3 shows a schematic representation of the detected VNTR with an 81 bp motif in the left domain of the control region, and also of a sequence without the repetitive motif and highly divergent, that was assumed to be a *Numt* (see Cracraft *et al.* 1998), found in a fraction of the clones derived from PCR samples of a rusty-spotted genet (DM1632). To avoid the effect of heteroplasmy in the sequence data the heteroplasmic region was excluded from the fragment targeted by the PCR primers, avoiding the costly alternative of only sequencing cloned PCR products for each sample. In contrast, for eliminating co-amplification of *Numts*, to redesign PCR primers, increasing their likelihood of being mtDNA-specific by using sequences known with certainty to be of mitochondrial origin, it is one of the best approaches (Bensasson et al. 2002). In order to obtain a reliable mtDNA genet sequence, a fresh extract of total genomic DNA was used and the whole mtDNA (≈ 17,000 bp) was purified through Long PCR technology using the Expand™ Long Template PCR system (Roche) and a single set of primers suggested by Stephan Funk (*pers. comm.*). This enzymatically-driven mtDNA isolation strategy is a valid alternative to the traditional physical separation of genomes using ultra-centrifugation gradients as described by Dowling *et al.* (1996). A similar procedure has been applied successfully before to isolate mitochondrial genomes (*Cymts*; Lopez *et al.* 1997) in arthropods (Hwang *et al.* 2001) and edentates (Nelson *et al.* 1996). Long PCR was conducted in 50-µl reaction volumes with 1x Buffer 3 [10x stock contains 22.5 mM $MgCl_2$, 500 mM Tris-HCl pH 9.2, 160 mM $(NH_4)_2SO_4$, 20% v/v DMSO, 1% v/v Tween 20], 2.5 units of enzyme mix, 250 ng of genomic DNA, 1.4 mM dNTPs, and 0.5 µM of each long-PCR primer. Cycling parameters consisted of initial denaturation at 94° C for 2 min; 10 cycles at 92° C for 10s, annealing at 63° C for 30 s, and elongation at 68° C for 13 min; another 15 cycles with 20 additional seconds for the elongation step per cycle; and 7 min at 68° C for final extension.

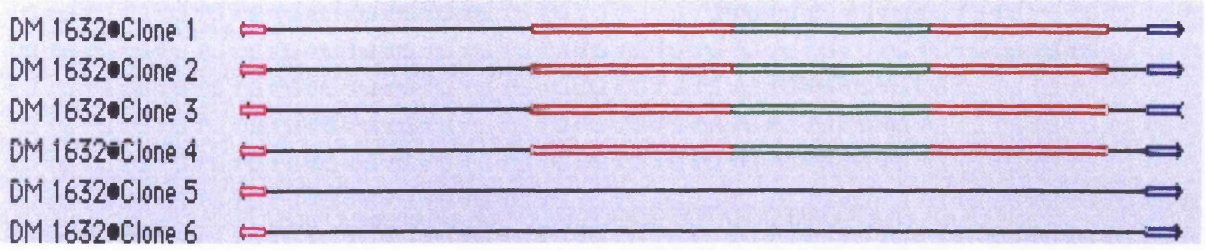

Figure 4.3 Diagrammatic representation of sequences derived from clones of a rusty-spotted genet (DM1632) PCR sample. Clones 1 to 4 contained sequences with a VNTR in the control region left domain with a motif of 81 bp, which is repeated three times in this case. Clones 5 to 6 did not show any repetitive region for the same fragment and the sequence was rich in substititutions, including several transversions, and indels. Conserved core of the control region is in blue and the tRNA-Pro in pink.

Besides isolation of the entire mtDNA molecule from a single sample, around 5 Kb of mtDNA encompassing the genes ND-5, ND-6, cytochrome *b*, and the control region was amplified, using a single primer pair, for two samples of some of the postulated taxonomic units within the large-spotted genets. Cracraft *et al.* (1998) were able to separate *Cymts* from *Numts* in tigers by resorting also to amplification of longer fragments than the ones initially elected as targets. For this PCR, the forward primer L12603 Genetta [the number identifies the 3' end of the primer following the *Felis catus* mtDNA numbering (Lopez *et al.* 1996)] was designed, based on an alignment of whole-mtDNA Carnivore sequences, and the reverse primer 12SAR-3' is listed in Palumbi (1996). Amplification was performed in 50-µl reactions with 0.8x PCR Buffer (Invitrogen; whose 10x stock contains 200 mM Tris-HCl pH 8.4 and 500 mM KCl), 2 units of *Taq* DNA Polymerase (Invitrogen), 1.75 mM $MgCl_2$, 1 mM dNTPs, 100-150 ng of genomic DNA, and 0.5 µM of each primer. The thermocycling profile consisted of initial denaturation at 94° C for 2 min, followed by 40 cycles in 94° C for 10s, 58° C for 30s and 72° C for 4 min. The final extension was 7 min at 72° C. Negative controls were carried out alongside all PCR reactions.

All the genet sequences, either derived directly from the cytochrome *b*-control region, 5 Kb, and whole-mtDNA amplifications, or from cloned PCR products, were aligned with available Carnivore sequences in the GenBank database. From this alignment, genet mtDNA specific primer pairs were designed for three substitution-rich fragments, smaller than 200 bp and each belonging to a different gene, to be screened in all samples. The sequences of these primers for the genes ND-5, cytochrome *b*, and the control region, are listed in Table 4.1.

Table 4.1 List of primers designed for this study and respective sequences. The letters identify the primer position on the heavy (H) or light (L) strand and the number identifies the 3' end of the primer following the *Felis catus* mtDNA numbering (Lopez *et al.* 1996).

Gene	Primer identification	Nucleotide sequence (5'→3')
ND-5	L12603 Genetta	AGA AGT AAT CCA TTG GTC TTA GGA ACC
	L13766 Genetta	GCG CCT ATT AAT TGG AAG CAT
	H13978 Genetta	TTG GTG GAA AGC GGT GAA T
	H13982 Genetta	TTA TTG GTG GAA RGC GGT
Cytochrome *b*	L15467 Genetta	ATT CCT TCT GAG GRG CAA
	H15665 Genetta	TCT GAG TCA GAT AYT ACG CC
Control Region	L16212 Genetta	GGA TAC CTT GGT CTT GTA A
	L16290 Genetta	ATC AGC ACC CAA AGC TGA
	H16418 Genetta	GCA CTA ATA GGT AGA TTG

4.2.2.3 PCR and sequencing

PCRs of all samples for the three gene fragments were carried out in 25-µl reaction volumes. For museum skin or hair samples and to each PCR reaction, 1.2x PCR Buffer (Invitrogen), 1.25 units of *Taq* DNA Polymerase (Invitrogen), 3 mM $MgCl_2$, 0.8 mM dNTPs, 5-10 µl of genomic DNA, 0.5 µM of each primer, and 0.5 mg/ml of non-acetylated BSA (MBI Fermentas) were added. For buffer-preserved tissue or blood samples and to each PCR reaction, 1.2x PCR Buffer (Invitrogen), 1 unit of *Taq* DNA Polymerase (Invitrogen), 2.25 mM $MgCl_2$, 0.8 mM dNTPs, 50 ng of genomic DNA, and 0.5 µM of each primer were added. Thermocycling consisted of initial denaturation at 94° C for 3 min, followed by 35 (for modern samples) up to 55 (for ancient-DNA samples) cycles in 94° C for 30s, 50° C for 45s and 72° C for 1 min. The final extension was 7 min at 72° C. Negative controls were carried for all PCR reactions. In the case of ancient-DNA samples, PCR replicates were conducted for the same or different extraction replicates from a set of randomly chosen samples in order to authenticate the respective sequences.

All PCR products were purified with the GeneClean Turbo Kit (Bio 101, catalogue #1101-600, Q-Biogene), following the manufacturer's instructions. Sequencing of both strands of the product was carried out using the ABI Prism Dye Terminator cycle sequencing ready reaction kit (catalogue #4314477, Perkin-Elmer) for the ABI Prism model 377 DNA automated sequencer following the recommendations of the manufacturer.

4.2.3 Data analysis

4.2.3.1 Phylogenetic analysis

The forward and reverse sequences obtained were at first aligned and assembled, using Sequencher^{TM} 3.1.2 (Gene Codes Corporation Inc.) software, for each gene and individual, and subsequently checked by eye, as recommended when indels are absent or not difficult to resolve (Graur & Li 2000), for manual editing.

To evaluate if phylogenetic signal is significantly stronger than random noise in the structure of the sequences, the g_1-test (Hillis & Huelsenbeck 1992) with 1,000,000 randomly generated trees and the permutation tail probability (PTP) test (Faith & Cranston 1991) with heuristic tree search and 1,000 replicates were performed. Both tests were applied to the whole data set and to its partitions per codon position and per gene. Base frequencies were compared between haplotypes to detect potential biases and a χ^2 test was

carried out to measure the homogeneity of nucleotide composition, per gene and per codon position, among lineages. The incongruence length difference (ILD) test (Farris *et al.* 1994) was used to assess the congruence between the three gene fragments and, hence, their suitability to be analytically combined, and also as an indicator of the homogeneity among codon positions in the two protein-coding genes. The two homogeneity partition tests, for genes and codon positions, were carried out with 1,000 randomisations each and with invariable sites removed. Finally, the likelihood ratio test (LRT), which compares trees with and without an ultrametric constraint (Felsenstein 1981), was used to estimate if all the lineages were evolving at the same rate (molecular clock hypothesis). PAUP version 4.0b10 (Swofford 2002) was used to implement those tests and also to conduct phylogenetic reconstructions of the data set using the methods of maximum parsimony (MP), minimum evolution (ME) and maximum likelihood (ML).

Unweighted MP analysis was accomplished with heuristic tree search, random sequence addition, 1,000 replicates, and rearrangements by tree-bisection-reconnection (TBR) limited to 2,000,000 per replicate, a recommended approach to minimise computational time without significantly hindering the search for an optimal tree (Swofford 2002). ME reconstruction was carried out with an evolutionary model tested as the best fit to the data; both heuristic and neighbour-joining (NJ) tree searches were compared. The model of sequence evolution to correct the genetic distance matrix for metric tree reconstruction by the ME method, was selected with hierarchically nested LRTs as implemented in ModelTest version 3.06 (Posada & Crandall 1998). ML analysis was also performed using, as a starting tree, the semistrict consensus obtained from the MP trees to estimate sequence evolution parameters that best fit the data, which were subsequently used in tree heuristic evaluation with rearrangements by tree-bisection-reconnection (TBR) limited to 5,000. Corrected pairwise distances between sequences were calculated with both the model selected by ModelTest and with the Kimura 2-parameter model. The latter was applied in one of the most recent attempts to calibrate genetic distances taxonomically, using the cytochrome *b* gene (Johns & Avise 1998), and may allow assessment of the systematic value of the observed genetic distances in the present data set. Bootstrapping (Felsenstein 1985), with 1,000 replicates for MP and ME analyses and 100 replicates for ML, was performed to measure the support for clades throughout all the topologies. Phylogenetic reconstruction was also implemented using Bayesian inference with Mr. Bayes version 3.0 (Huelsenbeck & Ronquist 2001) where four independent Markov chains were run (three heated and one cold) for 1,000,000

Metropolis-coupled Markov chain generations, with tree sampling each 50 generations and burn-in after 10,000 trees (Huelsenbeck *et al.* 2001). The evolutionary model used in the Bayesian analysis was the same applied for genetic distance correction and ML analysis, as estimated as best fit to the overall data set by ModelTest. Posterior probabilities were calculated to measure node reliability of the estimated tree topology.

4.2.3.2 Population genetics and phylogeographic analyses

For the population genetics and phylogeographic analyses, samples were grouped according to sampling locality in predefined geographic areas (Table 4.2) in which limits correspond in several instances to proposed species or subspecies within the large-spotted genets, thereby allowing testing of taxonomic hypotheses. The few exceptions correspond to cases where the ranges of more than one subspecies are included within a single biogeographic or climatic region (Figure 4.4), as one of the aims of this study was also to assess the correlation between genetic structure and contemporary vegetation belts. Each geographic area was equated in practical terms to a "population" for the population genetics analyses.

Table 4.2 Geographic ranges and centres of the areas defined for the phylogeographic analysis of large-spotted genets.

Geographic Area	Latitude/Longitude Range	Geographic Centre
Senegal-Dahomey Gap	[13° 40' 00"N; 04° 50' 00"N]	07° 41' 41"N
	[17° 60' 00"W; 00° 00' 00"]	10° 19' 01"W
Dahomey Gap-DRC	[13° 40' 00"N; 09° 00' 00"S]	06° 52' 05"N
	[00° 00' 00"; 20° 00' 00"E]	14° 15' 54"E
DRC-Abyssinia	[13° 40' 00"N; 09° 00' 00"S]	00° 55' 46"S
	[20° 00' 00"E; 33° 30' 00"E]	32° 24' 21"E
East Africa	[17° 60' 00"N; 09° 00' 00"S]	01° 57' 36"N
	[33° 30' 00"E; 52° 00' 00"E]	38° 32' 26"E
Southern Africa "North"	[09° 00' 00"S; 20° 00' 00"S]	14° 42' 30"S
		27° 30' 26"E
Southern Africa "South"	[20° 00' 00"S; 28° 50' 00"S];	29° 24' 16"S
	>28° 50' 00"E when > 28° 50' 00"S	32° 23' 26"E
Cape+OFS	>28°50' 00"S	31° 55' 43"S
	<28°50' 00"E	27° 32' 11"E

The program Arlequin version 2.000 (Schneider *et al.* 2000) was used to compute measures of intraspecific genetic diversity, tests of selective neutrality of the sequences, and for calculating the mismatch, or pairwise difference, distributions. The hypothesis of demographic expansion for each population was assessed by estimating the relevant parameters (Θ_0, Θ_1, and τ) under this scenario using a generalised least-squares approach

(Schneider & Excoffier 1999); the statistical significance of the estimated parameters was determined with 1,000 coalescent simulation replicates. The raggedness index (Harpending 1994), a measure of the smoothness of the observed mismatch distribution and hence of the stationary *versus* expanding nature of the populations, was also calculated for each population and assessed with 1,000 bootstrap replicates.

The program Chiperm version 1.2 (Posada 2000) was used to implement the test for detecting significant associations between haplotypes and geographical locations presented by Hudson *et al.* (1992a). The significance of the χ^2 is aproximated with Monte Carlo (Roff & Bentzen 1989) by permuting the contingency tables; 100,000 permutations were performed.

Arlequin version 2.000 was also used to compute parameters of structure among populations and to carry out an analysis of the molecular variance (AMOVA), as described in Excoffier *et al.* (1992). AMOVA is a method that estimates the proportion of the genetic variation at different hierarchical levels, using information from the geographical distribution of haplotypes, their frequencies and nucleotide differences. It yields an analogous estimator to Wright's F_{ST}, Φ_{ST}, that measures the fraction of variance explained by population subdivision, thereby indicating the level of differentiation among demes. For both the AMOVA and the construction of a minimum spanning network (MSN; Rohlf 1973) connecting all large-spotted genet haplotypes found in this study, a matrix of genetic distances among haplotypes corrected under an evolutionary model selected as best fit to the data by ModelTest was used. The significance of the fixation index Φ_{ST} was tested with 100,000 permutations. The amount of gene flow between pairs of populations was estimated with the parameter M ($=N_f m$) and as described in the method introduced by Slatkin (1993). The test of exact population differentiation based on haplotype frequencies of Raymond & Rousset (1995) was performed and the results tested for significance at $\alpha=0.05$ through a Markov chain with 100,000 steps and burn-in after 10,000 steps. Finally, the net number of nucleotide differences between populations (Nei & Li 1979) was calculated and used in a test of the correlation between population genetic differences and geographic distances (Hutchinson & Templeton 1999).

With DNAsp version 4.0 (Rozas *et al.* 2003) different estimators of F_{ST} and M were applied to the data set for comparison with the ones above and to investigate the support given by different methods to inferred quantities. The method described by Hudson *et al.* (1992b) for F_{ST} calculation was implemented and tested with 10,000 permutations;

estimates of M were derived from these pairwise F_{ST} values and also from N_{ST} values (Lynch & Crease 1990). Presence of genetic differentiation was also examined with a recently proposed statistic, S_{nn}, by Hudson (2000) and results for population pairwise comparisons were crossed with the ones obtained with the statistic K_s^* (Hudson *et al.* 1992a).

A Mantel test to evaluate the importance of isolation by distance in shaping the genetic structure of large-spotted genet populations was carried out using the software IBD version 1.4 (Bohonak 2003) with 100,000 randomisations. The regressed matrices were the one containing the net number of nucleotide differences between population pairs and the one with distances in Km between the geographic centres of each pair of areas that were predefined.

The net genetic distance between populations or species (d_A; Nei 1987) was calculated for all pairwise comparisons, including both *mossambica* and *G. angolensis* in addition to the large-spotted genet populations. These values of genetic distance were used to derive approximate timings for the splits between clades using the equality $d_A=2\mu t$ where μ is the neutral mutation rate per nucleotide and t is the population/species divergence time. For recent species or population divergences, the use of d_A allows correction of the ancestral polymorphism present in the samples due to the fact that gene divergence often predates population divergence (Takahata & Slatkin 1990; Edwards & Beerli 2000). However, it must be considered that this method assumes constancy of sizes between the ancestral and the two descendant clades (Arbogast *et al.* 2002). In the absence of any relevant fossil data that could allow the calibration of a genet mtDNA molecular clock, two different calibrations derived from reliable fossil record in other mammal groups, and already tested in several species, were used. Divergence times were estimated both assuming the traditional mtDNA molecular clock with a substitution rate of 0.010 substitutions per site per lineage per My (Brown *et al.* 1979, 1982) and a recently proposed rate, accounting for rate heterogeneity among sites, for mammals and birds of 0.026 (Arbogast & Slowinski 1998).

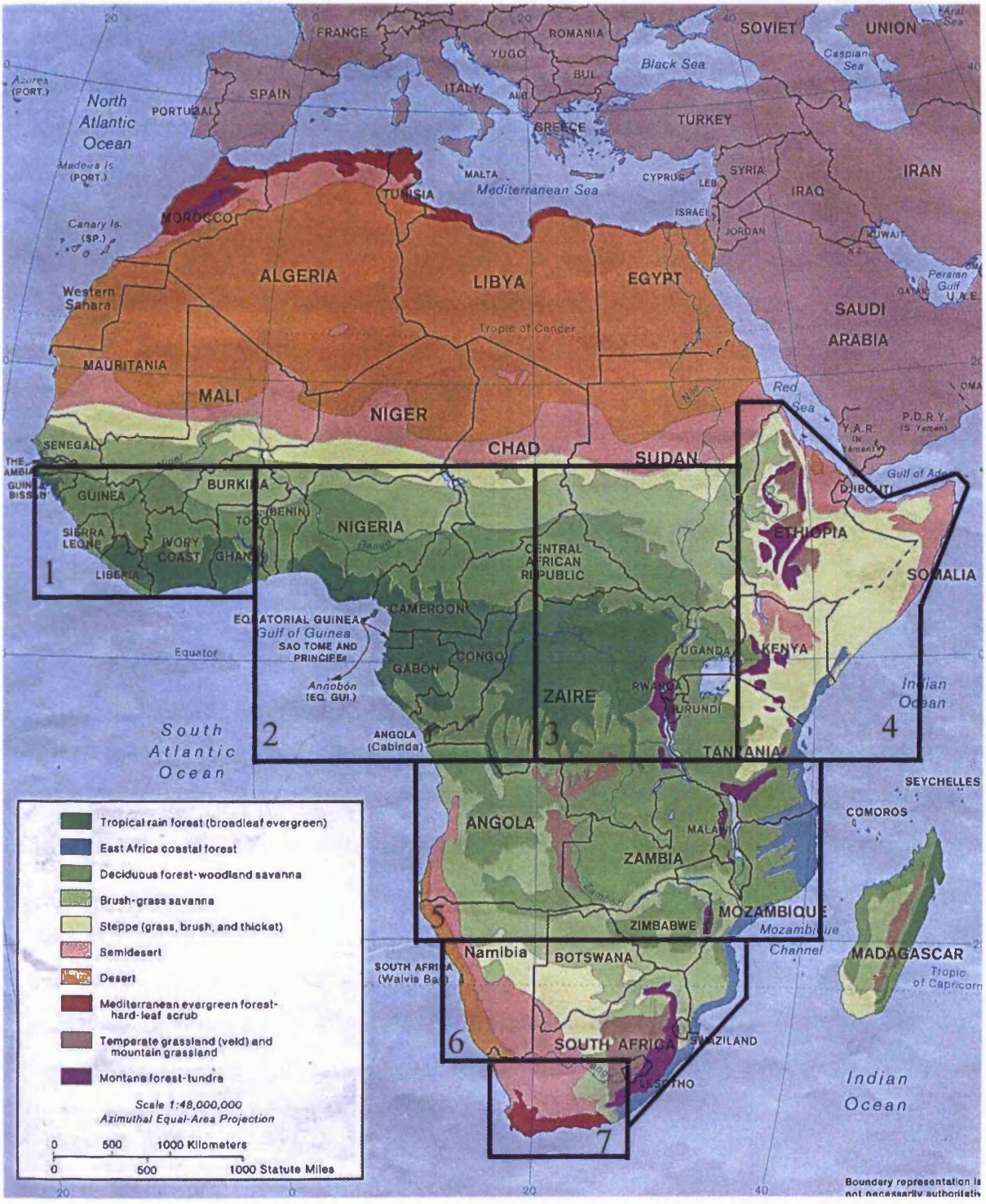

Figure 4.4 Vegetation map of Africa and representation of the geographic units in which the distribution of the large-spotted genets was subdivided for the phylogeographic analysis. 1- Senegal-Dahomey Gap, 2- Dahomey Gap-DRC, 3- DRC-Abyssinia, 4- East Africa, 5- Southern Africa "North", 6- Southern Africa "South", 7- Cape and Orange Free State. For convenience, the name "Southern Africa South" was kept to designate the whole of Africa below the 10° S parallel excluding its extreme south-west (Cape and Orange Free State).

Nested clade analysis (NCA) is a phylogeographic method that attempts to disentangle population structure from population history by inferring the current or historical process that most parsimoniously explain the hierarchical nesting of haplotype clades within a statistical cladogram (Templeton 1998). The method is based on the observation that different causes of geographical association (range expansion, long-distance colonization, isolation by distance and/or past fragmentation) usually yield specific patterns of how haplotypes are located within a gene tree or network (Avise *et al.* 1987).

For NCA, the first step, which is to infer a set of 95% plausible haplotype networks, in which clades are hierarchically nested, using the algorithm given in Templeton *et al.* (1992), was carried out with the program TCS version 1.13 (Clement *et al.* 2000). The program Geodis version 2.0 (Posada *et al.* 2000) was employed to measure the geographical information of the clades and to test the significance of geographical association for each clade by 100,000 random permutations of clades against sampling locations. Inferences about evolutionary processes and events that most likely account for observed haplotype-geography associations, or possible artefacts due to sampling inadequacies, were made using the key presented in the appendix of Templeton (2004).

4.3 Results

4.3.1 Reliability of DNA sequences from museum specimens

Sequences derived from ancient-DNA material always showed the highest similarities with sequences produced from equivalent, in taxonomic and/or geographic terms, modern samples. Results were congruent among independent extraction rounds (two per sample) and different PCR replicates (two per sample), which is regarded as authentication against contamination (Hassanin & Douzery 2000) and amplification errors induced by miscoding lesions in ancient specimens (Hansen *et al.* 2001). Finally, several instances of museum samples yielding unique haplotypes (i.e. showing singleton substitutions) within the data set were observed, suggesting absence of cross-contamination in the laboratory procedures.

4.3.2 Characterisation of DNA sequences

It proved impossible in the time available to amplify successfully the control region fragment for the species *G. angolensis* and for some large-spotted genet samples with any

primer combination of the three control region primers designed for this study. Consequently, the data set (408 bp in length) was composed of only the two concatenated protein-coding gene fragments: 210 nucleotides from the ND-5 gene and 198 nucleotides from the cytochrome *b* gene. The reading frames for the two protein-coding genes were determined by alignment with the complete mtDNA sequence of the domestic cat (Lopez *et al.* 1996), and no stop codons were found in any haplotype. Homogeneity of base frequencies within and between taxa was revealed by the χ^2 test (P=1.000), either when assessed for the genes combined or when assessed separately according with gene and codon position partitions. A low number of guanines (G) was encountered in the sequences of the two genes, a typical result in the mtDNA of vertebrates (Tamura & Nei 1993). The frequencies of the nucleotides A, C. G, and T for the concatenated fragment were 0.294, 0.293, 0.118, and 0.295, respectively. Among codon positions, a pattern of bias similar to the one found in mitochondrial protein-coding genes of several vertebrates (Irwin *et al.* 1991) was detected in the data; A's dominating at first positions, T's at second positions, and A's at third positions. The frequencies were, at first positions, 0.319, 0.238, 0.203, and 0.240. For the second positions they were 0.220, 0.299, 0.074, and 0.407, and for the third positions they were 0.344, 0.343, 0.076, and 0.237. The average apparent transition/transversion ratio was 11, a bias within the typical range found in the mtDNA of vertebrates (Graur & Li 2000). The ILD test for both the partition genes (P-value=0.791) and the partition codons (P-value=0.857) did not detect any significant incompatibility for these character sets.

4.3.3 Phylogenetic analyses

Fifty-five haplotypes were observed among the 125 samples analysed; Tables 4.3 and 4.4 provide information, in terms of samples and locations, concerning the distribution of the haplotypes found in this study. The haplotypes were designated according to the genet taxon in which they were observed, but in one case a haplotype was found to be shared between two different species. There were 94 variable characters in the 408 bp data set, of which 59 were parsimony informative and 35 were autapomorphies. Table 4.5 shows the polymorphism pattern for all the 94 variable sites among genet haplotypes. The PTP test was statistically significant (P=0.001) which suggests the presence of a strong phylogenetic signal in the distribution of character states among the taxa. A distribution of 10^6 trees generated randomly from the data set was significantly skewed (g_1=-0,298 P <

0.01, mean ± SD tree length = 392.975 ± 15.226, range = 308-443) suggesting the presence of strong phylogenetic structure in the data set. The results of the LRT for a molecular clock assumption was statistically significant at α=0.05 for the null hypothesis of rate homogeneity among all lineages; LR=52.26 (df=50) in the large-spotted genets data set and LR=65.38 (df=53) with the further inclusion of *G. angolensis*. The best-fitting model of sequence evolution determined by ModelTest was the TrN (Tamura & Nei 1993) with gamma distributed correction for rate heterogeneity among sites. The shape parameter of the distribution was 0.270 for the phylogenies including only large-spotted genet populations and 0.313 for the phylogenies including also *G. angolensis*. Table 4.6 shows the corrected genetic distances between all 55 haplotypes uncovered in the present study.

For the haplotype genealogy within large-spotted genets a ML tree and a MP tree are respectively shown in Fig. 4.5 and 4.6; only bootstrap values equal or higher than 70% were considered, as this amount as been showed to be a reliability threshold (Hillis & Bull 1993). The MP tree it is a 50% majority-rule consensus of 128 steps in length (CI=0.672; RI=0.884) and the ML tree with the evolutionary model TrN+G (α=0.270) had a score of (-ln L) = 1384.612. Two obvious features can be deducted from the branching pattern, which is essentially the same in both trees. First, the populations west of the Dahomey Gap and in the Cape plus Orange Free State, usually considered as separate species from the rusty-spotted genets, were not reciprocally monophyletic with them. Second, there is a significant paraphyly of rusty-spotted genet lineages among demes, particularly between Southern Africa and Tanzania/Kenya, and no simple correspondence between geographic distribution and genetic structure. A more detailed scrutiny of the trees reveals affinities between *tigrina* haplotypes and the "*rubiginosa*" haplotypes from south-eastern Southern Africa, between *pardina* haplotypes and the "*rubiginosa*" haplotypes from the Dahomey Gap-DRC area, and the fact that all but one (Gr28) haplotypes from Ethiopia form a completely separate clade from all other haplotypes in East Africa. A major split among the lineages stretching from the Albertine Rift to the Guinea-Liberian forest block in West Africa and those from East and Southern Africa, with intergradation in the area between the Albertine Rift and the Rift Valley (haplotypes Gr20 and Gr24), is also noticeable. Finally, the set of specimens from northern Mozambique, more precisely from Boror, a region in the coastal forest between Quelimane and Pemba, and identified as *mossambica* by Roberts (1951) groups with rusty-spotted genet samples (Gr23) from the Mombassa coast in Kenya (Malindi-Lamu districts) in a distinct clade.

Table 4.3 List of haplotypes, for the concatenated two protein-coding gene fragments, uncovered by the present study together with the samples and geographic areas where each of them was found.

Haplotype	Samples	Geographic Area
Gr1	TM10348, TM8886, TM45132, DM3335, DM3499, DM4475, DM1632, DM2178, DM2190, DM1636, PC125, TM40333, TPNR1, TM8689	Southern Africa "South", Southern Africa "North"
Gr2	Angola1	Southern Africa "North"
Gr3	Angola2	Southern Africa "North"
Gr4	CZ27, TM9839	Southern Africa "North"
Gr5	PC50, PC60	Southern Africa "North"
Gr6	PC90, TM12468	Southern Africa "North"
Gr7	DM2232	Southern Africa "South"
Gr8	ROM35695, TM12656, AMNH161763	Southern Africa "North"
Gr9	ROM35438, TM11449, TM8691, DM1618, DM5611, TM38712, CZ242	Southern Africa "South", Southern Africa "North"
Gr10	DM1089	Southern Africa "South"
Gr11	DM1617, DM2189, TM39551, TM39726, PT82, TM39910	Southern Africa "South"
Gr12	TM44830	Southern Africa "South"
Gr13	NMS27342	Southern Africa "South"
Gr14	TM6058, TM9188, TM9898, TM9899, TM9904, Tanzania 4 3, IoZ5056, IoZ2275, TC80, TM17698	Southern Africa "North", East Africa, DRC-Abyssinia
Gr15	TM9190	Southern Africa "North"
Gr16	TM9191	Southern Africa "North"
Gr17	AMNH161757	Southern Africa "North"
Gr18	Tanzania 12 2	East Africa
Gr19	Tanzania 3 4	East Africa
Gr20	Tanzania 96, TC60, AMNH187744, LACM53729, LACM53731	East Africa, DRC-Abyssinia
Gr21	T64, LACM53728	East Africa
Gr22	LACM56723	East Africa
Gr23	LACM42933, LACM42935	East Africa
Gr24	AMNH187743	East Africa
Gr25	NMWB2143	East Africa
Gr26	G1, G2, G3, G6, G7	East Africa
Gr27	G4	East Africa
Gr28	G5	East Africa
Gr29	G8	East Africa
Gr30	G9	East Africa
Gr31	FMNH2814	DRC-Abyssinia
Gr32	LACM56724	DRC-Abyssinia
Gr33	LACM36392, PCCx	DRC-Abyssinia
Gr34	PCAx, PC164, PC145	DRC-Abyssinia, Dahomey Gap-DRC
Gr35	PC160, PC143	Dahomey Gap-DRC
Gr36	SMF5831, Wanzie1, Terv7315M303, AMNH241389, AMNH236485, PC99, Terv9647M3	Dahomey Gap-DRC
Gr37	Terv9391M60, Terv9553M1, Terv9849M3, Terv9849M4	Dahomey Gap-DRC
Gr38	Terv7315M301	Dahomey Gap-DRC
Gr39	AMNH241390, PC394	Dahomey Gap-DRC
Gr40	Terv9849M2	Dahomey Gap-DRC
Gr41	Shamie4, Shamie5	Dahomey Gap-DRC
Gp1	MHNG173626, LACM33808	Senegal-Dahomey Gap
Gp2	AMNH241388	Senegal-Dahomey Gap
Gp3	ROMFN28730	Senegal-Dahomey Gap
Gp4	Bissau2	Senegal-Dahomey Gap
Gt1	A270, A271, A273	Cape+OFS
Gt2	A272	Cape+OFS
Gt3	KM31185, NMB4786	Cape+OFS
Gt/Gr	KM31276, TM16638, NMB7013, NMB6904	Southern Africa "South", Cape+OFS
Gt4	NMB4470	Cape+OFS
Gm1	TM970	Southern Africa "North"
Gm2	TM1357	Southern Africa "North"
Ga1	TM17713	Southern Africa "North"
Ga2	MVZ118449, TM9847, TM9849	Southern Africa "North"
Ga3	TM9842	Southern Africa "North"

For convenience, the name Southern Africa "South" was kept to designate the whole of Africa below the 20° S parallel excluding its extreme south-west (Cape and Orange Free State).

Table 4.4 Distribution of the genet haplotypes uncovered in the present study at each of the geographic areas. The number of sequences, different haplotypes and unique haplotypes are indicated for each column (*continues in next page*).

Haplotype	Senegal-Dahomey Gap	Dahomey Gap-DRC	DRC-Abyssinia	East Africa	Southern Africa "North"	Southern Africa "South"	Cape+OFS	Sequences
Gr1	0	0	0	0	1	13	0	14
Gr2	0	0	0	0	1	0	0	1
Gr3	0	0	0	0	1	0	0	1
Gr4	0	0	0	0	2	0	0	2
Gr5	0	0	0	0	2	0	0	2
Gr6	0	0	0	0	2	0	0	2
Gr7	0	0	0	0	0	1	0	1
Gr8	0	0	0	0	3	0	0	3
Gr9	0	0	0	0	3	4	0	7
Gr10	0	0	0	0	0	1	0	1
Gr11	0	0	0	0	0	6	0	6
Gr12	0	0	0	0	0	1	0	1
Gr13	0	0	0	0	0	1	0	1
Gr14	0	0	1	2	5	0	0	8
Gr15	0	0	0	0	1	0	0	1
Gr16	0	0	0	0	1	0	0	1
Gr17	0	0	0	0	1	0	0	1
Gr18	0	0	0	1	0	0	0	1
Gr19	0	0	0	1	0	0	0	1
Gr20	0	0	2	3	0	0	0	5
Gr21	0	0	0	2	0	0	0	2
Gr22	0	0	0	1	0	0	0	1
Gr23	0	0	0	2	0	0	0	2
Gr24	0	0	0	1	0	0	0	1
Gr25	0	0	0	1	0	0	0	1
Gr26	0	0	0	5	0	0	0	5
Gr27	0	0	0	1	0	0	0	1
Gr28	0	0	0	1	0	0	0	1
Gr29	0	0	0	1	0	0	0	1
Gr30	0	0	0	1	0	0	0	1
Gr31	0	0	1	0	0	0	0	1
Gr32	0	0	1	0	0	0	0	1
Gr33	0	0	2	0	0	0	0	2
Gr34	0	2	1	0	0	0	0	3
Gr35	0	2	0	0	0	0	0	2
Gr36	0	7	0	0	0	0	0	7
Gr37	0	4	0	0	0	0	0	4
Gr38	0	1	0	0	0	0	0	1
Gr39	0	2	0	0	0	0	0	2
Gr40	0	1	0	0	0	0	0	1
Gr41	0	2	0	0	0	0	0	2
Gp1	2	0	0	0	0	0	0	2
Gp2	1	0	0	0	0	0	0	1
Gp3	1	0	0	0	0	0	0	1
Gp4	1	0	0	0	0	0	0	1

Table 4.4 Distribution of the genet haplotypes uncovered in the present study at each of the geographic areas. The number of sequences, different haplotypes and unique haplotypes are indicated for each column (*continued*).

Haplotype	Senegal-Dahomey Gap	Dahomey Gap-DRC	DRC-Abyssinia	East Africa	Southern Africa "North"	Southern Africa "South"	Cape+OFS	Sequences
Gt1	0	0	0	0	0	0	3	3
Gt2	0	0	0	0	0	0	1	1
Gt3	0	0	0	0	0	0	2	2
Gt/Gr	0	0	0	0	0	3	1	4
Gt4	0	0	0	0	0	0	1	1
Gm1	0	0	0	0	1	0	0	1
Gm2	0	0	0	0	1	0	0	1
Ga1	0	0	0	0	1	0	0	1
Ga2	0	0	0	0	3	0	0	3
Ga3	0	0	0	0	1	0	0	1
Sequences	5	21	8	23	30	30	8	125
Different	4	8	6	14	17	8	5	
Unique	4	7	3	12	14	5	4	

Table 4.5 Variable sites and polymorphism pattern in the combined ND-5+Cyt b 408 bp fragment among the haplotypes uncovered in the present study. The bold vertical line marks the border between the two genes.

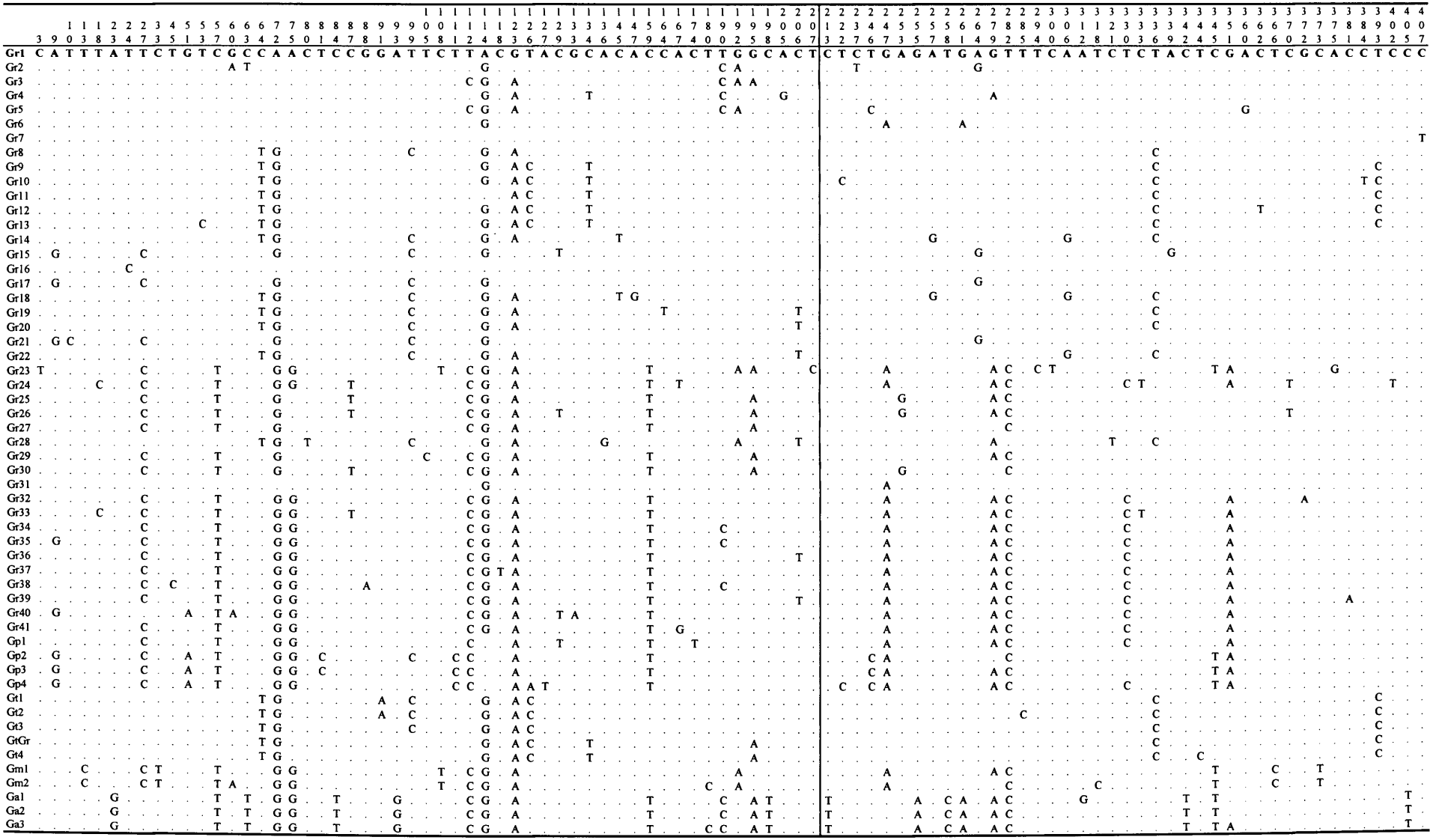

Table 4.6 Corrected genetic distances, using the model of evolution suggested by ModelTest (TrN+G with gamma shape parameter = 0.313), between all the ND-5+Cyt *b* haplotypes uncovered in the present study.

Gt1
Gt2	0.002
Gt3	0.002	0.005
Gt/Gr	0.010	0.013	0.007
Gt4	0.013	0.015	0.010	0.002
Gr1	0.024	0.027	0.021	0.024	0.027
Gr2	0.040	0.043	0.036	0.040	0.043	0.018
Gr3	0.030	0.033	0.027	0.024	0.027	0.015	0.018
Gr4	0.030	0.033	0.027	0.024	0.027	0.015	0.024	0.015
Gr5	0.033	0.037	0.030	0.033	0.037	0.018	0.021	0.007	0.018
Gr6	0.027	0.030	0.024	0.027	0.030	0.007	0.021	0.018	0.018	0.021
Gr7	0.027	0.030	0.024	0.027	0.030	0.002	0.021	0.018	0.018	0.021	0.010
Gr8	0.007	0.010	0.005	0.013	0.016	0.015	0.030	0.021	0.021	0.024	0.018	0.018
Gr9	0.007	0.010	0.005	0.002	0.005	0.021	0.036	0.027	0.021	0.030	0.024	0.024	0.010
Gr10	0.013	0.016	0.010	0.007	0.010	0.027	0.043	0.033	0.027	0.037	0.030	0.030	0.016	0.005
Gr11	0.010	0.013	0.007	0.005	0.007	0.018	0.040	0.030	0.024	0.033	0.027	0.021	0.013	0.002	0.007
Gr12	0.010	0.013	0.007	0.005	0.007	0.024	0.040	0.030	0.024	0.033	0.027	0.027	0.013	0.002	0.007	0.005
Gr13	0.010	0.013	0.007	0.005	0.007	0.024	0.040	0.030	0.024	0.033	0.027	0.027	0.013	0.002	0.007	0.005	0.005
Gr14	0.015	0.018	0.013	0.021	0.024	0.024	0.039	0.030	0.030	0.033	0.027	0.027	0.007	0.018	0.024	0.021	0.021	0.021
Gr15	0.030	0.033	0.027	0.036	0.039	0.021	0.030	0.033	0.033	0.036	0.024	0.024	0.021	0.033	0.039	0.036	0.036	0.036	0.029
Gr16	0.027	0.030	0.024	0.027	0.030	0.002	0.021	0.018	0.018	0.021	0.010	0.005	0.018	0.024	0.030	0.021	0.027	0.027	0.027	0.024
Gr17	0.024	0.027	0.021	0.030	0.033	0.015	0.024	0.027	0.027	0.030	0.018	0.018	0.015	0.027	0.033	0.030	0.030	0.030	0.024	0.005	0.018
Gr18	0.018	0.021	0.015	0.024	0.027	0.027	0.042	0.033	0.033	0.036	0.030	0.030	0.010	0.021	0.027	0.024	0.024	0.024	0.002	0.032	0.030	0.026
Gr19	0.013	0.016	0.010	0.018	0.021	0.021	0.036	0.027	0.027	0.030	0.024	0.024	0.005	0.016	0.021	0.018	0.018	0.018	0.013	0.027	0.024	0.021	0.015
Gr20	0.010	0.013	0.007	0.016	0.018	0.018	0.033	0.024	0.024	0.027	0.021	0.021	0.002	0.013	0.018	0.016	0.016	0.016	0.010	0.024	0.021	0.018	0.013	0.002
Gr21	0.027	0.030	0.024	0.033	0.036	0.018	0.027	0.030	0.030	0.033	0.021	0.021	0.018	0.030	0.036	0.033	0.033	0.033	0.027	0.007	0.021	0.002	0.029	0.024	0.021
Gr22	0.013	0.015	0.010	0.018	0.021	0.021	0.036	0.027	0.027	0.030	0.024	0.024	0.005	0.015	0.021	0.018	0.018	0.018	0.007	0.027	0.024	0.021	0.010	0.005	0.002	0.024
Gr23	0.077	0.082	0.073	0.070	0.074	0.065	0.077	0.050	0.065	0.061	0.061	0.069	0.065	0.073	0.082	0.077	0.078	0.078	0.077	0.072	0.069	0.064	0.081	0.073	0.069	0.068	0.073
Gr24	0.071	0.075	0.067	0.071	0.075	0.058	0.078	0.058	0.059	0.062	0.055	0.062	0.059	0.067	0.075	0.071	0.071	0.071	0.070	0.065	0.062	0.058	0.074	0.067	0.063	0.061	0.066	0.048
Gr25	0.043	0.047	0.040	0.037	0.040	0.033	0.050	0.027	0.033	0.036	0.036	0.036	0.033	0.040	0.047	0.043	0.043	0.043	0.043	0.039	0.036	0.033	0.046	0.040	0.037	0.036	0.040	0.036	0.030
Gr26	0.050	0.054	0.047	0.044	0.047	0.040	0.057	0.033	0.040	0.043	0.043	0.043	0.040	0.047	0.054	0.050	0.051	0.051	0.050	0.039	0.043	0.039	0.053	0.047	0.043	0.043	0.047	0.043	0.030	0.005
Gr27	0.034	0.037	0.030	0.027	0.031	0.024	0.040	0.018	0.030	0.027	0.027	0.027	0.024	0.030	0.037	0.034	0.034	0.034	0.033	0.030	0.027	0.024	0.036	0.030	0.027	0.027	0.030	0.033	0.034	0.007	0.013
Gr28	0.024	0.027	0.021	0.030	0.033	0.033	0.043	0.033	0.033	0.037	0.036	0.036	0.015	0.027	0.033	0.030	0.030	0.030	0.024	0.039	0.036	0.033	0.027	0.015	0.013	0.036	0.015	0.074	0.075	0.047	0.054	0.043
Gr29	0.040	0.044	0.037	0.034	0.037	0.030	0.046	0.024	0.030	0.033	0.033	0.033	0.030	0.037	0.044	0.040	0.040	0.040	0.040	0.036	0.033	0.030	0.043	0.037	0.034	0.033	0.037	0.033	0.034	0.007	0.013	0.005	0.044
Gr30	0.040	0.043	0.037	0.034	0.037	0.030	0.046	0.024	0.036	0.033	0.033	0.033	0.030	0.037	0.043	0.040	0.040	0.040	0.040	0.036	0.033	0.030	0.043	0.037	0.033	0.033	0.036	0.040	0.033	0.002	0.007	0.005	0.050	0.010
Gr31	0.024	0.027	0.021	0.024	0.027	0.005	0.018	0.015	0.015	0.018	0.002	0.007	0.015	0.021	0.027	0.024	0.024	0.024	0.024	0.021	0.007	0.015	0.027	0.021	0.018	0.018	0.021	0.057	0.051	0.033	0.040	0.024	0.033	0.030	0.030
Gr32	0.050	0.054	0.047	0.050	0.054	0.039	0.057	0.040	0.040	0.043	0.036	0.043	0.040	0.047	0.054	0.050	0.050	0.050	0.050	0.046	0.043	0.039	0.053	0.047	0.043	0.043	0.046	0.030	0.019	0.021	0.027	0.018	0.054	0.018	0.024	0.033
Gr33	0.058	0.062	0.054	0.058	0.062	0.046	0.065	0.047	0.047	0.050	0.043	0.050	0.047	0.054	0.062	0.058	0.058	0.058	0.057	0.053	0.050	0.046	0.061	0.054	0.050	0.050	0.054	0.037	0.008	0.021	0.027	0.024	0.062	0.024	0.024	0.040	0.010
Gr34	0.050	0.054	0.047	0.050	0.054	0.040	0.050	0.033	0.033	0.036	0.036	0.043	0.040	0.047	0.054	0.050	0.050	0.050	0.050	0.046	0.043	0.039	0.053	0.047	0.043	0.043	0.047	0.030	0.019	0.021	0.027	0.018	0.054	0.018	0.024	0.033	0.005	0.010
Gr35	0.054	0.057	0.050	0.054	0.057	0.043	0.053	0.036	0.036	0.039	0.040	0.046	0.043	0.050	0.058	0.054	0.054	0.054	0.053	0.042	0.046	0.036	0.057	0.050	0.047	0.039	0.050	0.033	0.022	0.024	0.030	0.021	0.057	0.021	0.027	0.036	0.007	0.013	0.002
Gr36	0.050	0.054	0.047	0.050	0.054	0.040	0.057	0.040	0.040	0.043	0.036	0.043	0.040	0.047	0.054	0.050	0.050	0.050	0.050	0.046	0.043	0.039	0.053	0.040	0.037	0.043	0.040	0.030	0.019	0.021	0.027	0.018	0.047	0.018	0.024	0.033	0.005	0.010	0.005	0.007
Gr37	0.050	0.054	0.047	0.050	0.054	0.040	0.057	0.040	0.040	0.043	0.036	0.043	0.040	0.047	0.054	0.050	0.050	0.050	0.050	0.046	0.043	0.039	0.053	0.047	0.043	0.043	0.047	0.030	0.019	0.021	0.027	0.018	0.054	0.018	0.024	0.033	0.005	0.010	0.005	0.007	0.005
Gr38	0.058	0.062	0.054	0.058	0.062	0.046	0.057	0.040	0.040	0.043	0.043	0.050	0.047	0.054	0.062	0.058	0.058	0.058	0.057	0.053	0.050	0.046	0.061	0.054	0.050	0.049	0.054	0.037	0.025	0.027	0.033	0.024	0.062	0.024	0.030	0.040	0.010	0.016	0.005	0.007	0.010	0.010
Gr39	0.055	0.059	0.051	0.055	0.059	0.043	0.062	0.044	0.044	0.047	0.040	0.047	0.044	0.051	0.059	0.055	0.055	0.055	0.054	0.050	0.047	0.043	0.058	0.044	0.040	0.047	0.044	0.034	0.022	0.024	0.030	0.021	0.051	0.021	0.027	0.037	0.008	0.013	0.008	0.010	0.002	0.008	0.013
Gr40	0.061	0.065	0.057	0.061	0.065	0.049	0.061	0.050	0.050	0.053	0.046	0.053	0.050	0.057	0.065	0.061	0.061	0.061	0.060	0.049	0.053	0.049	0.064	0.057	0.054	0.053	0.057	0.047	0.034	0.036	0.036	0.033	0.065	0.033	0.039	0.043	0.018	0.024	0.018	0.016	0.018	0.018	0.024	0.021
Gr41	0.050	0.054	0.047	0.050	0.054	0.039	0.057	0.039	0.040	0.043	0.036	0.043	0.040	0.047	0.054	0.050	0.050	0.050	0.050	0.046	0.043	0.039	0.053	0.047	0.043	0.042	0.046	0.030	0.016	0.021	0.027	0.018	0.054	0.018	0.024	0.033	0.005	0.010	0.005	0.007	0.005	0.005	0.010	0.008	0.018
Gp1	0.058	0.062	0.054	0.058	0.062	0.040	0.065	0.046	0.047	0.050	0.043	0.043	0.047	0.054	0.062	0.051	0.058	0.058	0.057	0.046	0.043	0.046	0.061	0.054	0.050	0.049	0.054	0.037	0.025	0.027	0.027	0.024	0.062	0.024	0.030	0.040	0.010	0.016	0.010	0.013	0.010	0.010	0.016	0.013	0.018	0.010
Gp2	0.061	0.065	0.057	0.069	0.073	0.050	0.076	0.057	0.065	0.053	0.054	0.054	0.050	0.065	0.073	0.062	0.069	0.069	0.061	0.049	0.054	0.043	0.064	0.057	0.054	0.046	0.057	0.047	0.048	0.043	0.050	0.033	0.073	0.039	0.039	0.050	0.030	0.037	0.030	0.027	0.030	0.030	0.037	0.034	0.034	0.030	0.030
Gp3	0.069	0.073	0.065	0.069	0.073	0.050	0.076	0.057	0.057	0.053	0.053	0.054	0.057	0.065	0.073	0.062	0.069	0.069	0.068	0.057	0.054	0.049	0.072	0.065	0.061	0.053	0.065	0.040	0.041	0.036	0.043	0.033	0.073	0.033	0.039	0.050	0.024	0.030	0.024	0.021	0.024	0.024	0.030	0.028	0.027	0.024	0.024	0.005
Gp4	0.080	0.084	0.075	0.080	0.084	0.063	0.092	0.071	0.071	0.066	0.067	0.067	0.071	0.075	0.075	0.071	0.080	0.080	0.083	0.070	0.067	0.062	0.087	0.080	0.075	0.066	0.079	0.052	0.045	0.047	0.055	0.044	0.089	0.044	0.051	0.063	0.028	0.034	0.028	0.025	0.028	0.028	0.034	0.031	0.031	0.028	0.028	0.019	0.013
Gm1	0.062	0.066	0.058	0.062	0.066	0.050	0.061	0.043	0.050	0.047	0.047	0.054	0.050	0.058	0.066	0.062	0.062	0.062	0.061	0.057	0.054	0.050	0.065	0.058	0.054	0.053	0.058	0.034	0.048	0.036	0.043	0.033	0.058	0.033	0.040	0.043	0.030	0.037	0.030	0.033	0.030	0.030	0.037	0.034	0.047	0.030	0.037	0.047	0.040	0.052
Gm2	0.070	0.074	0.066	0.070	0.074	0.057	0.062	0.051	0.065	0.054	0.054	0.061	0.058	0.066	0.074	0.070	0.070	0.070	0.069	0.064	0.061	0.057	0.073	0.066	0.062	0.061	0.065	0.047	0.063	0.050	0.057	0.040	0.074	0.047	0.046	0.050	0.043	0.051	0.043	0.047	0.043	0.043	0.051	0.048	0.054	0.043	0.051	0.054	0.054	0.067	0.010
Ga1	0.090	0.095	0.086	0.082	0.086	0.076	0.080	0.061	0.068	0.072	0.072	0.080	0.077	0.086	0.095	0.090	0.090	0.090	0.089	0.093	0.080	0.084	0.093	0.086	0.081	0.089	0.085	0.073	0.083	0.053	0.061	0.050	0.095	0.050	0.057	0.076	0.061	0.069	0.053	0.057	0.061	0.061	0.061	0.066	0.072	0.061	0.069	0.080	0.072	0.088	0.073	0.090
Ga2	0.086	0.091	0.082	0.078	0.082	0.072	0.076	0.057	0.065	0.068	0.069	0.077	0.073	0.082	0.091	0.086	0.086	0.086	0.085	0.089	0.077	0.080	0.089	0.082	0.077	0.085	0.081	0.069	0.079	0.050	0.058	0.047	0.091	0.047	0.053	0.072	0.057	0.065	0.050	0.053	0.057	0.057	0.057	0.062	0.069	0.057	0.065	0.076	0.069	0.084	0.069	0.086	0.002
Ga3	0.095	0.100	0.091	0.086	0.091	0.081	0.085	0.065	0.073	0.076	0.077	0.085	0.082	0.091	0.100	0.095	0.095	0.095	0.094	0.098	0.085	0.089	0.098	0.091	0.086	0.094	0.090	0.070	0.079	0.057	0.065	0.054	0.100	0.054	0.061	0.081	0.058	0.066	0.050	0.054	0.058	0.058	0.058	0.062	0.069	0.057	0.065	0.077	0.069	0.084	0.078	0.086	0.007	0.005

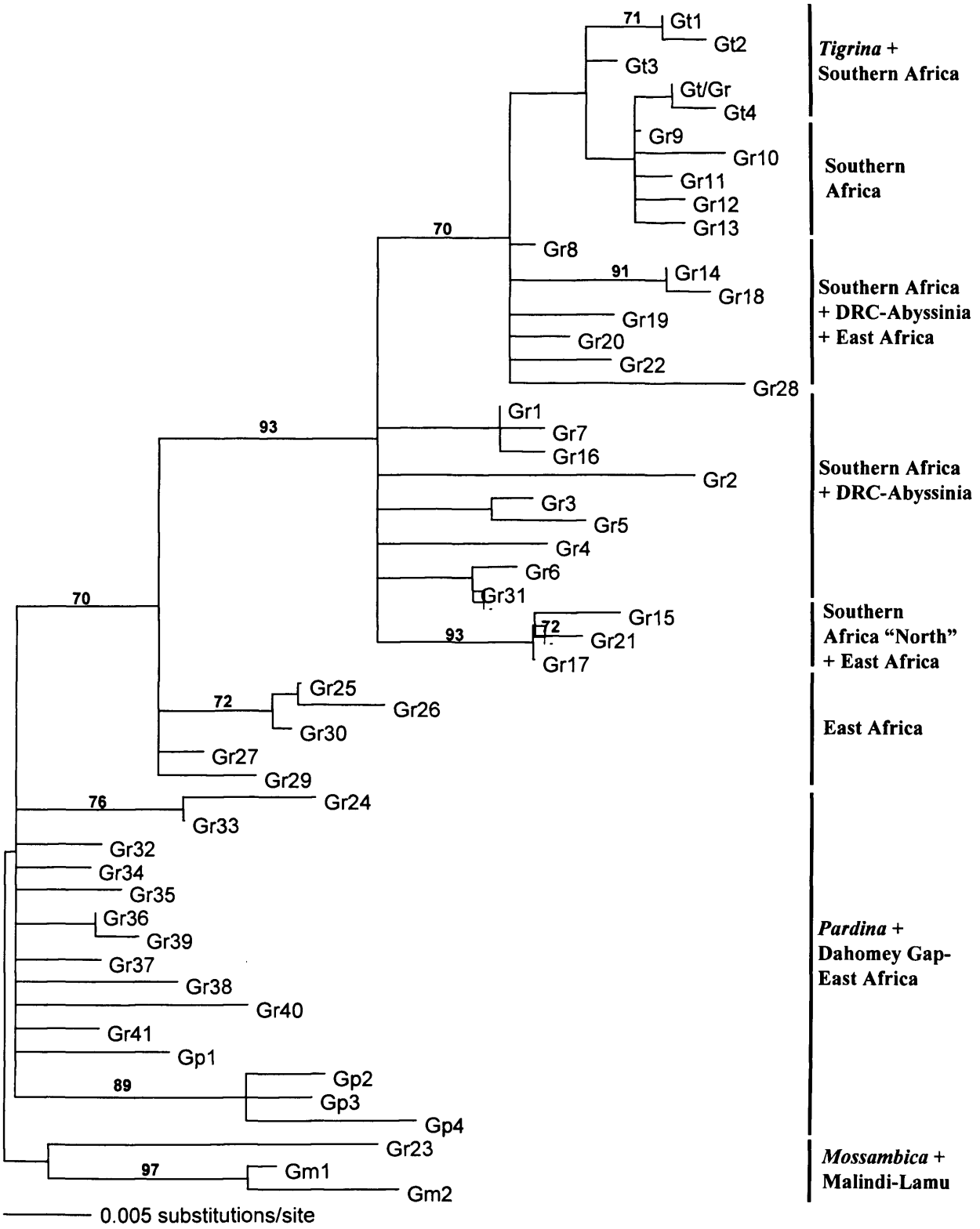

Figure 4.5 Phylogram of large-spotted genet haplotypes obtained using TrN+G (α=0.270) as the maximum likelihood evolution model and heuristic tree search. *G. "rubiginosa"* haplotypes are classified in accordance with the geographic areas where they occur whereas the haplotypes of the other taxa included in the analysis are simply designated by their name. Only bootstrap support values for each clade equal or higher than 70% are shown.

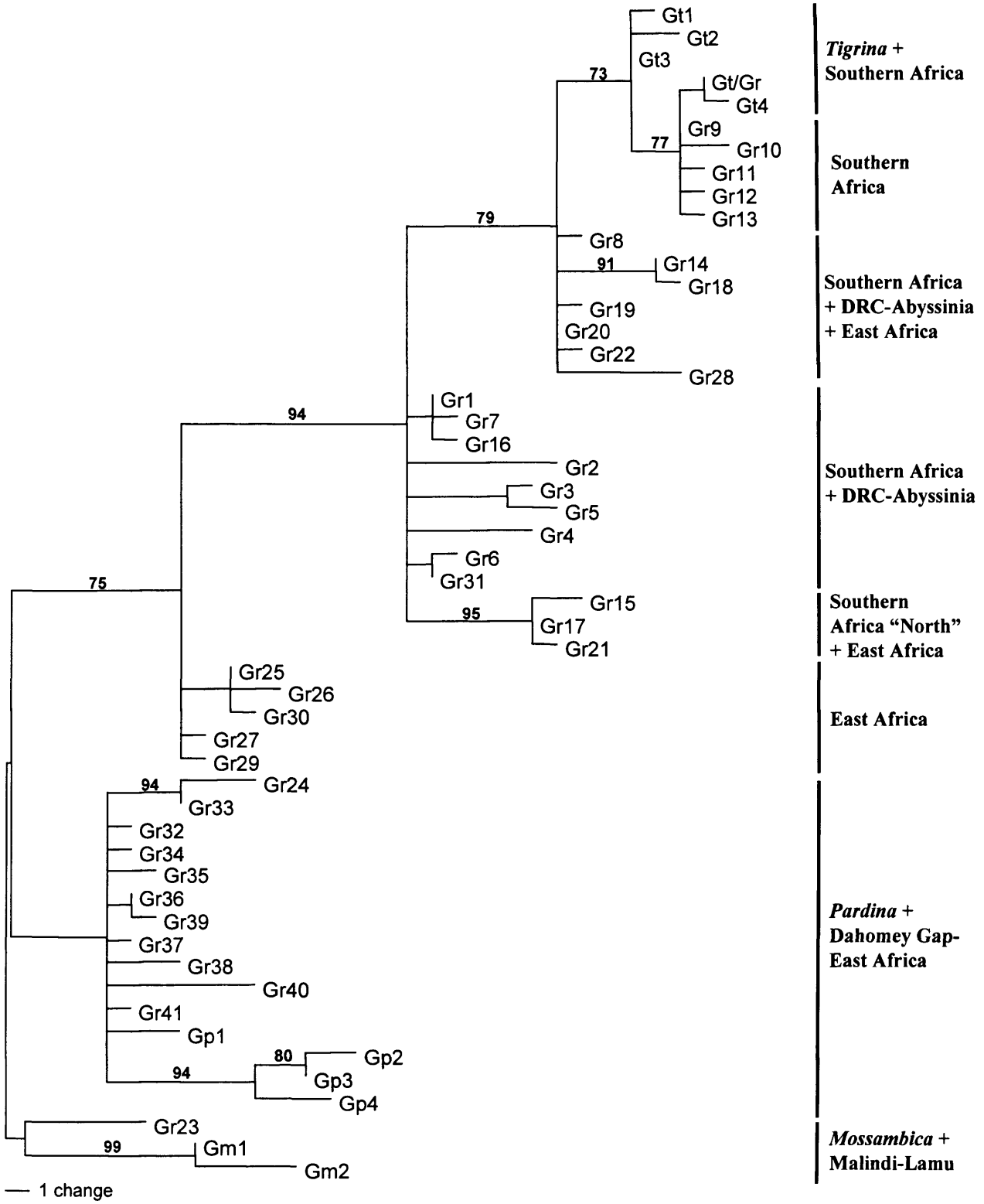

Figure 4.6 Phylogram of large-spotted genet haplotypes obtained with maximum parsimony as tree-search objective function. *G. "rubiginosa"* haplotypes are classified in accordance with the geographic areas where they occur whereas the haplotypes of the other taxa included in the analysis are simply designated by their name. Only bootstrap support values for each clade equal or higher than 70% are shown.

Haplotypes of *G. angolensis* were included as outgroup in additional phylogenetic reconstructions, aiming both to assess the affinities of *mossambica* and to gain information on the evolutionary sequence of divergence within the large-spotted genets. The results are presented in Figs. 4.7 and 4.8, which refer respectively to a 50% majority-rule consensus Bayesian tree of 10,000 MCMC samples retained after burn-in and to a ML tree with a score of (-ln L) = 1477.018. Once again, only bootstrap values equal or higher than 70% were considered. Besides confirming most of the clusters observed in the two previous topologies, the addition of *angolensis* haplotypes suggests that the *mossambica* specimens are in fact rusty-spotted genets belonging to an ancient clade in the East and Southern African context. A striking result is the seemingly closer affinity of this clade, which contains the *mossambica* haplotypes and the ones from the Kenyan coast, with samples west of the Rift Valley than with other clades in East and/or Southern Africa.

Table 4.7 shows minimum and maximum genetic divergences for all inter- and intraclade comparisons in the data set. Intervals of intraclade distances, using the evolution model TrN+G with α=0.270, are on the diagonal of the matrix, and intervals of interclade distances using the same model are below the diagonal. Intervals of interclade distances as estimated with the Kimura-2P model are above the diagonal to allow comparison with the results in Johns & Avise (1998). East Africa shows a remarkable degree of intraclade genetic variance that suggests a large effective population size, internal subdivision in separate demes, and/or secondary contact of formerly allopatric lineages. Also conspicuous is the fact that *mossambica* is at least 3% divergent apart from any other rusty-spotted genet clade.

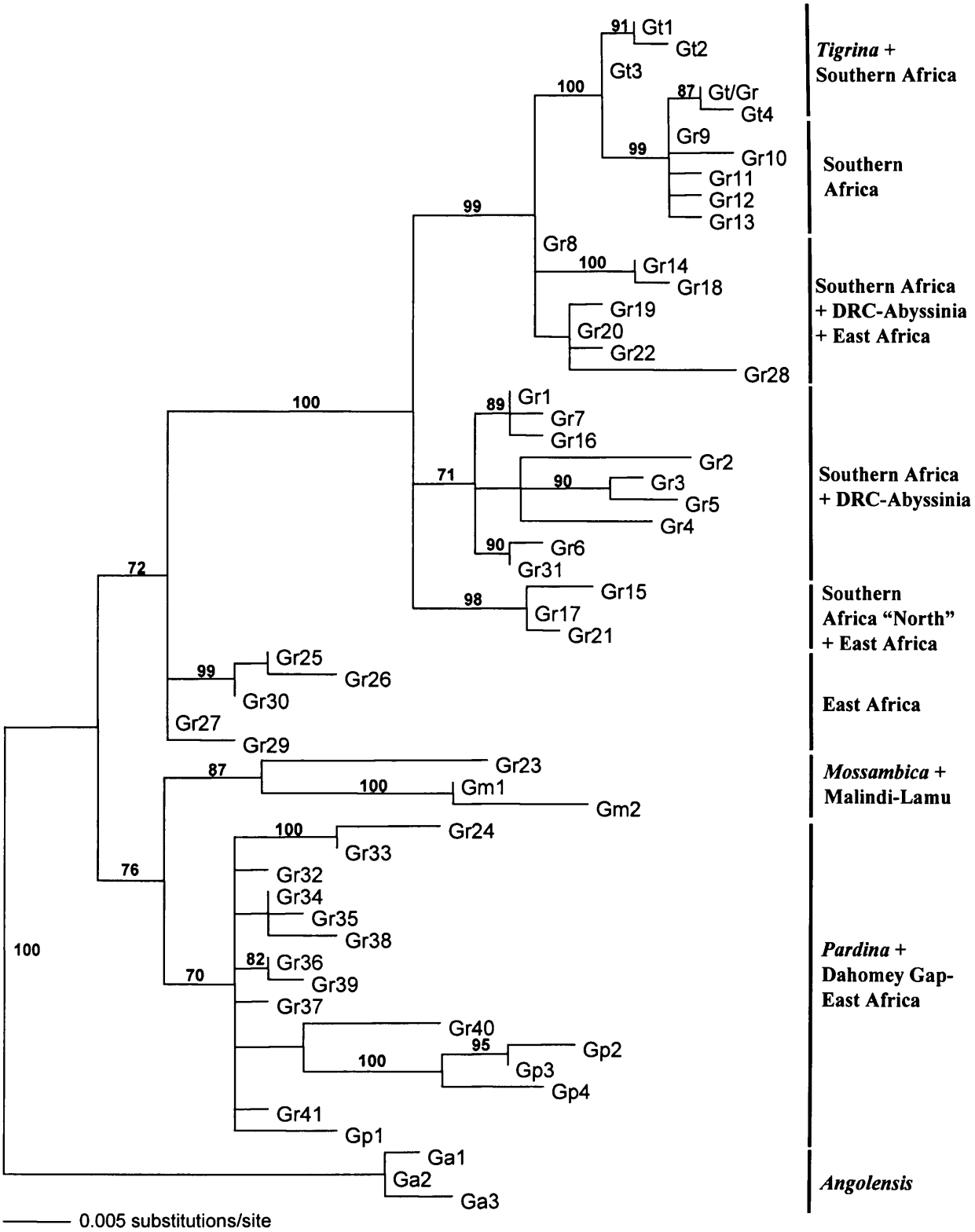

Figure 4.7 Phylogram of genet haplotypes obtained using TrN+G (α=0.313) as maximum likelihood evolution model and Bayesian MCMC tree search. *G. "rubiginosa"* haplotypes are classified in accordance with the geographic areas where they occur whereas the haplotypes of the other taxa included in the analysis are simply designated by their name. Only posterior probabilities for each clade equal or higher than 70% are shown.

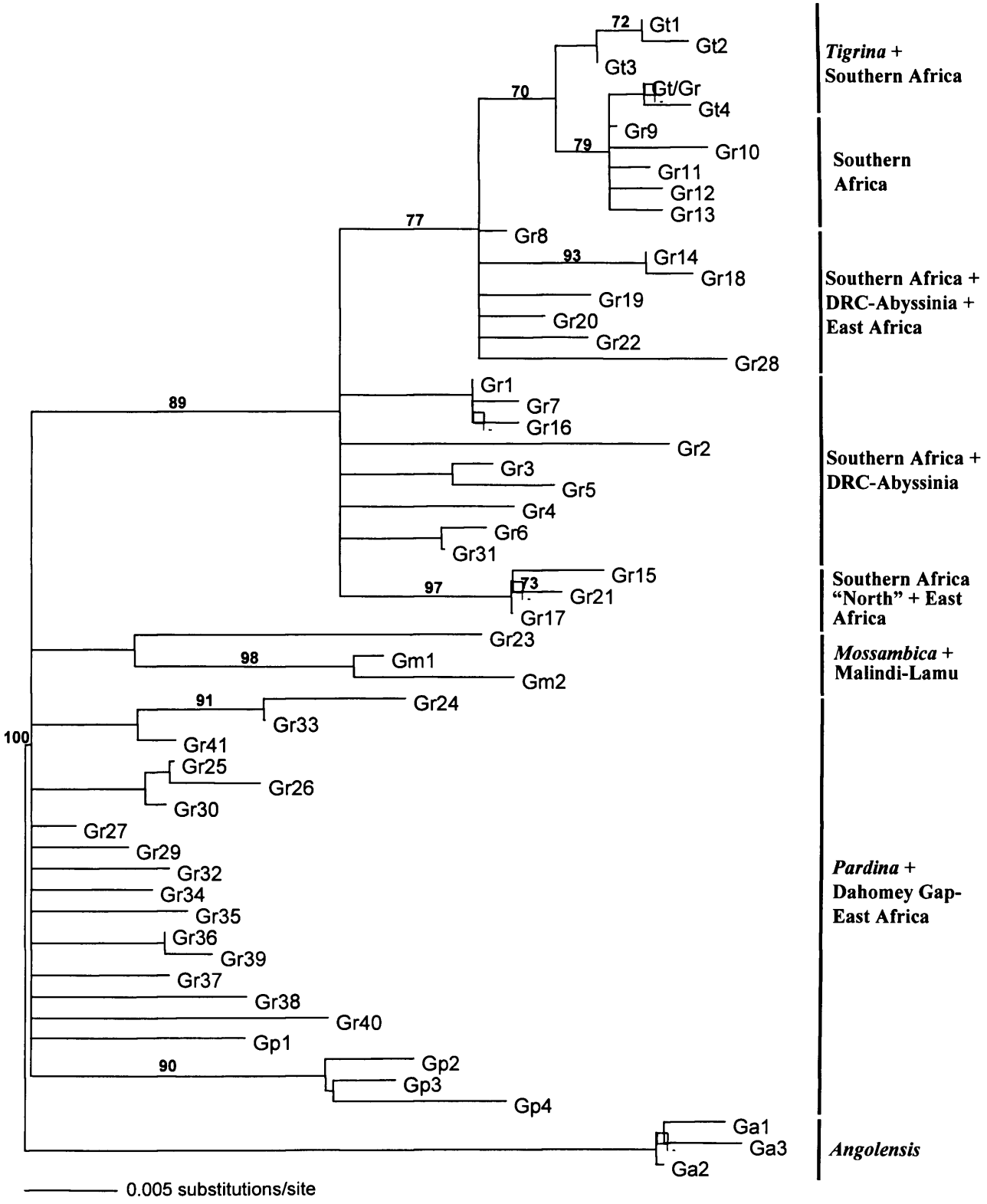

Figure 4.8 Phylogram of genet haplotypes obtained using TrN+G (α=0.313) as the maximum likelihood evolution model and NJ tree search. *G. "rubiginosa"* haplotypes are classified in accordance with the geographic areas where they occur whereas the haplotypes of the other taxa included in the analysis are simply designated by their name. Only bootstrap support values for each clade equal or higher than 70% are shown.

Table 4.7 Minimum and maximum genetic divergence percentages for all inter- and intraclade comparisons among all the clades defined in this study. Below and on the diagonal: distances using the model and parameters suggested by ModelTest as best fitting the data (TrN+G with gamma shape parameter = 0.270). Above diagonal: distances using the Kimura-2P model as in Johns & Avise (1998). Underlined values indicate pairwise comparisons between clades that share haplotypes.

	Senegal-Dahomey Gap	Dahomey Gap-DRC	DRC-Abyssinia	East Africa	Southern Africa "North"	Southern Africa "South"	Cape+OFS	*Mossambica*	*Angolensis*
Senegal-Dahomey Gap	0.0-3.0%	1.0-3.7%	1.0-7.9%	2.4-8.4%	4.0-8.8%	4.0-7.5%	5.4-7.9%	3.7-6.4%	6.5-8.8%
Dahomey Gap-DRC	1.0-3.7%	0.0-2.4%	0.0-6.1%	1.8-6.5%	3.3-6.1%	4.0-6.5%	4.7-6.5%	3.0-5.4%	5.0-7.3%
DRC-Abyssinia	1.0-8.3%	0.0-6.0%	0.0-5.7%	0.0-6.1%	0.0-6.5%	0.5-6.1%	0.8-6.1%	3.0-6.9%	5.0-9.4%
East Africa	2.4-8.9%	1.6-6.5%	0.0-6.2%	0.0-8.1%	0.0-7.7%	1.3-8.1%	0.8-8.1%	3.3-7.3%	4.7-9.9%
Southern Africa "North"	4.0-9.2%	3.3-6.2%	0.0-6.5%	0.0-7.8%	0.0-3.6%	0.0-4.0%	0.2-4.3%	4.3-6.9%	5.7-9.9%
Southern Africa "South"	4.0-8.0%	3.9-6.5%	0.5-6.2%	1.3-8.2%	0.0-4.3%	0.0-3.0%	0.0-3.0%	5.0-7.3%	7.3-9.9%
Cape+OFS	5.4-8.4%	4.7-6.5%	0.7-6.2%	0.7-8.2%	0.2-4.3%	0.0-3.0%	0.0-1.5%	5.7-7.3%	7.7-9.9%
Mossambica	3.7-6.7%	3.0-5.4%	3.0-6.9%	3.3-7.4%	4.3-6.9%	5.0-7.4%	5.8-7.4%	0.0-1.0%	6.9-9.0%
Angolensis	6.5-8.8%	5.0-7.2%	5.0-9.4%	4.7-10.0%	5.7-9.8%	7.2-10.0%	7.8-10.0%	6.9-9.0%	0.0-0.7%

4.3.4 Population genetics and phylogeographic analysis

Estimated values for parameters of genetic diversity and the results of neutrality tests, which support the assumption of neutral evolution, are summarised per geographic area in Table 4.8. The high values of both haplotype and nucleotide diversity in East Africa, the area between the Albertine Rift and the Rift Valley, and the northern part of Southern Africa, are compatible with large effective population sizes. On the other hand, the large values of gene diversity together with low values of nucleotide diversity observed for the region between the Dahomey Gap and the Congolese forest, and also for the Cape+OFS area suggest the influence of a bottleneck in the pattern of lineage sorting (Grant & Bowen 1998). The mismatch distributions were unimodal for the two geographic areas for which a bottleneck was inferred and Table 4.9 shows the estimated parameters and the raggedness index for each area. However, the results of the mismatch distribution analysis, under the sudden expansion model, were statistically significant at the confidence level of 95% only for the area Dahomey Gap-DRC.

The presence of genetic differentiation between the geographic areas was supported by different statistics (χ^2=602.376 P=0.000; K_S*=1.683 P=0.000; S_{nn}=0.767 P=0.000) but the analysis of the molecular variance showed that just 50% of the total variance is explained by that found between predefined geographic areas. Tables 4.10 and 4.11 show pairwise values of fixation indices and estimates of gene flow obtained with different methods and Table 4.12 presents the results of an exact test of population differentiation based on haplotype frequencies. Pairwise fixation indices and estimates of gene flow given by the different methods were broadly similar, and the most relevant result is the indication of significant migration connecting the Albertine Rift and East Africa. This finding had been already detected from the pattern in the phylogenetic trees of haplotypes where an intergradation between lineages from East Africa and the Albertine Rift was observed. Results of the exact test were in general concordant with the pattern revealed by the fixation indexes and gene flow estimates, although the non-differentiation between the Cape+OFS and Senegal-Dahomey Gap areas was an unexpected result. However, since this test is based on haplotype frequencies only, and is therefore over-sensitive to small sample sizes, a few incongruent outcomes are not unexpected. The result of the Mantel test indicated a marked degree of correlation between genetic distance and geographic distance (Z=742318.869, r=0.804, P=0.000). Table 4.13 presents the matrices that were regressed: a matrix of net nucleotide differences between populations and a matrix of the geographic

distances between the centres of the areas. This result suggests that geographic profiles of genetic variation are, at least in large patches of the distribution area, under the effect of isolation by distance. Large-spotted genets are then possibly genetically connected, even if weakly, across several of the recognised physical and ecological barriers within their range, which would produce a pattern of allopatric fragmentation in a species with weaker dispersal abilities or less ecological plasticity. Alternatively, isolation by distance may be consequence of historical association recent enough, so that insufficient time has passed for drift to obscure the previous equilibrium, and/or of population sizes sufficiently large to minimise the effects of drift (Barber 1999).

The minimum spanning network of large-spotted genet haplotypes is presented in Figure 4.9. The topology and spanning lengths are mostly concordant with the hierarchies observed in the phylogenetic trees and the same biogeographic affinities are apparent among clades. As suggested by the significant paraphyly observed in the phylogenetic trees, a complex history of migration and genetic drift between Southern Africa, the southern and central parts of the Albertine Rift, and Kenya/Tanzania, is confirmed in the MSN. However, an important result is the observation that haplotypes (e.g. Gr3 and Gr8) both seemingly central in the context of this radiation and with the closest affinities within it to haplotypes from the Congolese forest block occur in northern areas of Southern Africa and not in Kenya/Tanzania. The fact that the connection between Southern African haplotypes, through Gr3, and Congolese haplotypes (Gr34) is established by intermediate Ethiopian haplotypes (Gr27 and Gr29) might be interpreted as an instance of homoplasy due to shared ancestral polymorphism. Both the haplotypes from the Kenyan coast and from the *mossambica* specimens are linked, although with a marked degree of genetic divergence, to a haplotype from Uganda (Gr32), which is very similar to several haplotypes from the Nigeria-DRC forest block. An independent divergence, within the East Africa context, of Ethiopian haplotypes (Gr25-Gr27 and Gr29-Gr30) stemming from the haplotypes west of the Rift Valley it is also recorded in the MSN by the connection between Gr29 and Gr34. Finally, the high diversity of lineages present in the region between the Albertine Rift and the Rift Valley (Gr20, Gr24, Gr32, and Gr33) is clear from their disparate positions in the network.

Table 4.8 Values of genetic diversity and results of neutrality tests for the samples from each of the geographic regions.

	Sequences	Haplotypes	S (polymorphic sites)	H (gene diversity)
Senegal-Dahomey Gap	5	4	14	0.900 ± 0.161
Dahomey Gap-DRC	21	8	13	0.852 ± 0.054
DRC-Abyssinia	8	6	24	0.929 ± 0.084
East Africa	23	14	44	0.937 ± 0.033
Southern Africa "North"	23	12	31	0.925 ± 0.032
Southern Africa "South"	30	8	14	0.766 ± 0.061
Cape+OFS	8	5	6	0.857 ± 0.108

	π (mean number of pairwise differences)	π/nucleotide (nucleotide diversity)	Ewens' Θ(k)	Watterson's Θ(S)
Senegal-Dahomey Gap	8.321 ± 4.650	0.020 ± 0.013	7.106 [1.543; 33.076]	6.720 ± 3.685
Dahomey Gap-DRC	2.658 ± 1.476	0.007 ± 0.004	4.242 [1.776; 9.810]	3.613 ± 1.526
DRC-Abyssinia	13.136 ± 6.628	0.032 ± 0.019	9.231 [2.613; 34.341]	9.256 ± 4.318
East Africa	14.630 ± 6.799	0.036 ± 0.019	14.231 [6.501; 31.554]	11.922 ± 4.223
Southern Africa "North"	8.796 ± 4.211	0.022 ± 0.012	9.410 [4.287; 20.576]	8.399 ± 3.075
Southern Africa "South"	5.080 ± 2.345	0.013 ± 0.007	3.226 [1.409; 7.041]	3.534 ± 1.404
Cape+OFS	2.484 ± 1.495	0.006 ± 0.004	4.694 [1.394; 16.076]	2.314 ± 1.308

	Tajima's $\Theta(\pi)$	Tajima's D	Watterson's F P-value	Fu's Fs P-value
Senegal-Dahomey Gap	8.321 ± 5.436	0.738	1.000	0.643
Dahomey Gap-DRC	2.658 ± 1.647	-1.099	0.387	0.237
DRC-Abyssinia	13.139 ± 7.550	0.848	0.652	0.661
East Africa	14.630 ± 7.582	-0.062	0.733	0.467
Southern Africa "North"	8.796 ± 4.696	-0.321	0.380	0.460
Southern Africa "South"	5.080 ± 2.821	0.992	0.691	0.785
Cape+OFS	2.484 ± 1.703	0.087	0.786	0.234

Table 4.9 Mean, variance, and estimated parameters of the mismatch distributions for each geographic area. Parameters were estimated with a generalized non-linear least-square method and their P-values derived through parametric bootstrap (Schneider & Excoffier 1999).

	m (mean)	v (variance)	τ (tau)	Θ_0	Θ_1	Sum of square deviations (P-value)	Raggedness Index (P-value)
Senegal-Dahomey Gap	7.400	14.933	10.638	0.000	111.406	0.057 (P=0.432)	0.150 (P=0.743)
Dahomey Gap-DRC	2.505	3.572	2.467	0.000	39.541	0.041 (P=0.019)	0.141 (P=0.042)
DRC-Abyssinia	10.750	38.972	17.438	0.000	28.984	0.086 (P=0.102)	0.255 (P=0.076)
East Africa	11.731	45.412	15.609	0.023	25.118	0.019 (P=0.311)	0.042 (P=0.104)
Southern Africa "North"	7.692	11.889	9.202	0.006	47.656	0.018 (P=0.086)	0.041 (P=0.072)
Southern Africa "South"	4.584	14.340	9.377	0.000	6.462	0.061 (P=0.109)	0.068 (P=0.305)
Cape+OFS	2.357	3.571	4.768	0.012	3.905	0.056 (P=0.407)	0.153 (P=0.536)

Table 4.10.Above diagonal: population pairwise Fst values (10000 permutations) accordingly with the method described in Hudson *et al.* 1992b. Below diagonal: population pairwise Φst values (100000 permutations) following the AMOVA procedure introduced by Excoffier *et al.* 1992 (corrected distances with the model TrN and gamma shape = 0.270 were used for the AMOVA). An * indicates value non-significant at α=0.050.

	Senegal-Dahomey Gap	Dahomey Gap-DRC	DRC-Abyssinia	East Africa	Southern Africa "North"	Southern Africa "South"	Cape+OFS
Senegal-Dahomey Gap	-	0.370	0.339	0.421	0.604	0.676	0.767
Dahomey Gap-DRC	0.510	-	0.270	0.439	0.672	0.778	0.861
DRC-Abyssinia	0.350	0.387	-	0.044	0.184	0.340	0.457
East Africa	0.401	0.431	0.035*	-	0.180	0.332	0.421
Southern Africa "North"	0.651	0.699	0.233	0.193	-	0.151	0.358
Southern Africa "South"	0.762	0.798	0.453	0.363	0.158	-	0.417
Cape+OFS	0.831	0.882	0.479	0.346	0.290	0.369	-

Table 4.11.Above diagonal: M values (M=Nm for haploid data) obtained using Slatkin's (1991) method. Below diagonal: population pairwise Nm values averaged from estimates derived of Fst (Hudson *et al.* 1992b) and Nst (Lynch & Crease 1990) values tested with 10000 permutations.

	Senegal-Dahomey Gap	Dahomey Gap-DRC	DRC-Abyssinia	East Africa	Southern Africa "North"	Southern Africa "South"	Cape+OFS
Senegal-Dahomey Gap	-	0.48	0.93	0.75	0.27	0.16	0.10
Dahomey Gap-DRC	0.85	-	0.79	0.66	0.22	0.13	0.07
DRC-Abyssinia	0.97	1.35	-	13.85	1.64	0.60	0.54
East Africa	0.68	0.64	11.03	-	2.09	0.88	0.94
Southern Africa "North"	0.33	0.24	2.20	2.27	-	2.66	1.22
Southern Africa "South"	0.24	0.14	0.97	1.00	2.81	-	0.85
Cape+OFS	0.15	0.08	0.59	0.69	0.90	0.70	-

Table 4.12 Exact test of population differentiation based on haplotype frequencies (Raymond & Rousset 1995) with a Markov chain length of 100000 steps. An * indicates value non-significant at α=0.050.

	Senegal-Dahomey Gap	Dahomey Gap-DRC	DRC-Abyssinia	East Africa	Southern Africa "North"	Southern Africa "South"	Cape+OFS
Senegal-Dahomey Gap	0						
Dahomey Gap-DRC	0.001+-0.000	0					
DRC-Abyssinia	0.118+-0.002*	0.001+-0.000	0				
East Africa	0.017+-0.002	0.000+-0.000	0.205+-0.011*	0			
Southern Africa "North"	0.006+-0.002	0.000+-0.000	0.025+-0.002	0.000+-0.000	0		
Southern Africa "South"	0.000+-0.000	0.000+-0.000	0.000+-0.000	0.000+-0.000	0.000+-0.000	0	
Cape+OFS	0.054+-0.003*	0.000+-0.000	0.023+-0.002	0.002+-0.001	0.001+-0.000	0.000+-0.000	0

Table 4.13.Above diagonal: distances in Km between the centres of the geographic areas. Below diagonal: net number of nucleotide differences between populations (DA; Nei & Li 1979).

	Senegal-Dahomey Gap	Dahomey Gap-DRC	DRC-Abyssinia	East Africa	Southern Africa "North"	Southern Africa "South"	Cape+OFS
Senegal-Dahomey Gap	-	2711	4830	5444	4854	6149	5957
Dahomey Gap-DRC	3.296	-	2190	2743	2807	4472	4535
DRC-Abyssinia	6.339	2.969	-	753	1623	3164	3482
East Africa	9.663	6.778	0.579	-	2215	3546	3940
Southern Africa "North"	16.356	13.648	2.818	2.810	-	1708	1914
Southern Africa "South"	17.206	16.218	5.124	5.186	1.239	-	542
Cape+OFS	22.346	19.588	7.170	6.712	3.246	2.800	-

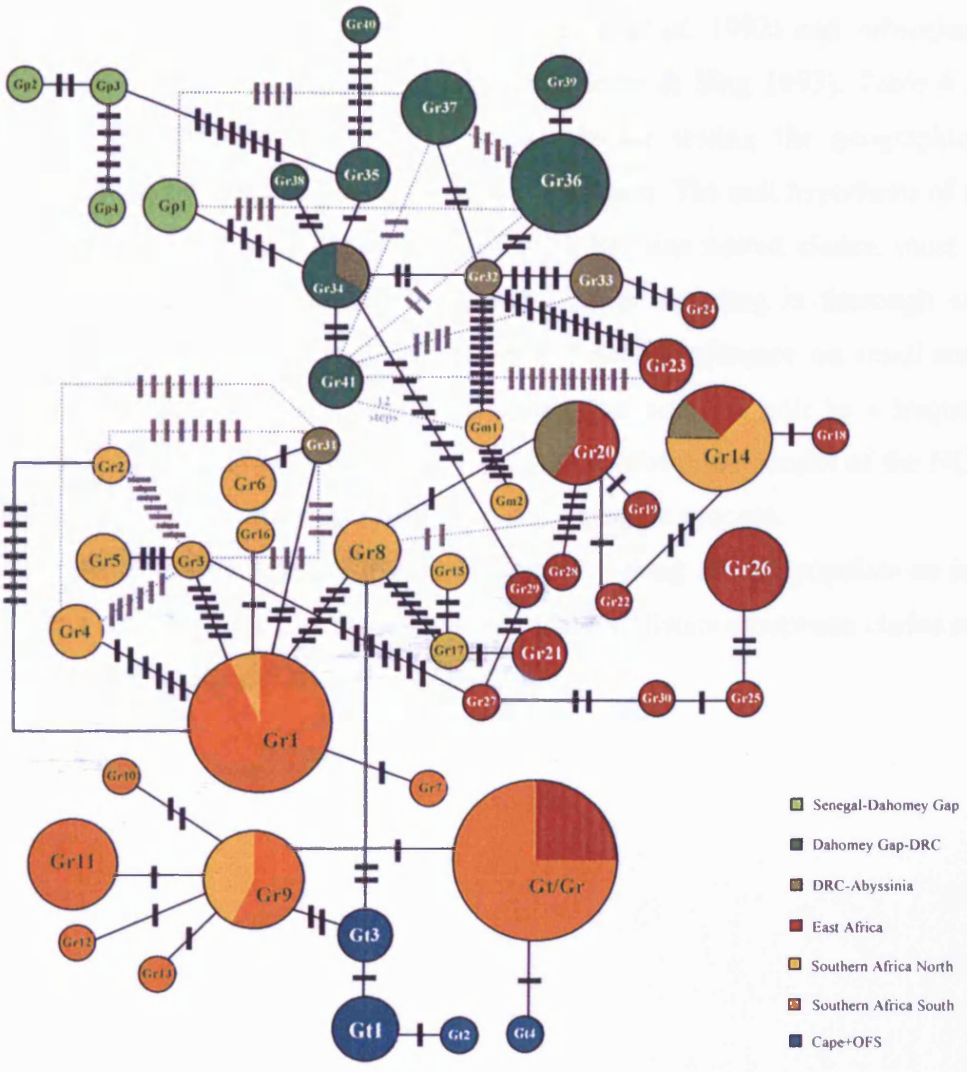

Figure 4.9 Large-spotted genets haplotype minimum spanning network. Codes inside circles are haplotype names as given in Table 4.3 and the size of each circle is proportional to the haplotype frequency, with the smallest size equal to one. Haplotype geographical occurrence is portrayed by the different colours and the frequency of occurrence among areas it is proportional to slice size in the pie. Mutational steps are represented by black bars on lines connecting haplotypes when steps are less than 10. Grey lines represent alternative links between haplotypes.

Figure 4.10 shows the nested design for the large-spotted genet haplotypes as defined by a 95% plausible set of cladograms (Templeton *et al.* 1992) and subsequent treatment of topologically ambiguous connections (Templeton & Sing 1993). Table 4.14 presents the results of the nested contingency analysis for testing the geographical association of clades with both genetic and geographic variation. The null hypothesis of no association was rejected at the confidence level of 95% for nine nested clades, most of them of high hierarchical level. This illustrates that unless sampling is thorough and intense across a species range, NCA will not provide detailed inference on small-scale processes and for large-scale interpretations an inconclusive outcome will be a frequent result. This is dramatically illustrated by Table 4.15, which shows the results of the NCA for clades with significant association and the inferred geographic process.

Table 4.16 presents estimates of divergence times among all the populations and taxa analysed in this study using d_A as measure of the genetic distance between clades and two different calibrations of the mtDNA molecular clock.

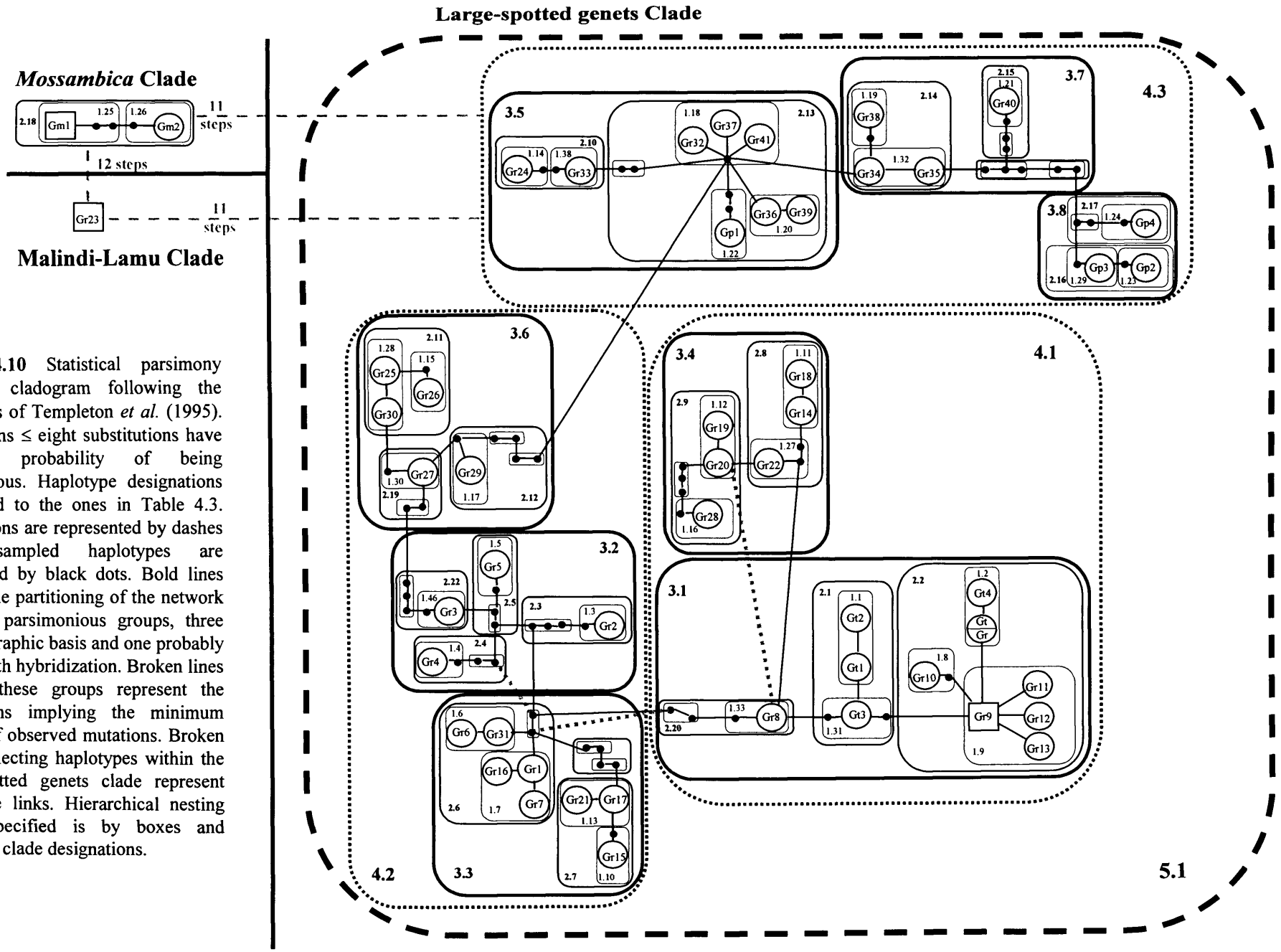

Figure 4.10 Statistical parsimony haplotype cladogram following the procedures of Templeton *et al.* (1995). Connections ≤ eight substitutions have a 0.95 probability of being parsimonious. Haplotype designations correspond to the ones in Table 4.3. Substitutions are represented by dashes and unsampled haplotypes are represented by black dots. Bold lines indicate the partitioning of the network into four parsimonious groups, three with geographic basis and one probably related with hybridization. Broken lines between these groups represent the connections implying the minimum number of observed mutations. Broken lines connecting haplotypes within the Large-spotted genets clade represent alternative links. Hierarchical nesting design specified is by boxes and numbered clade designations.

Table 4.14 Nested contingency analysis of geographical associations for clades with geographical and genetic variation in the statistical parsimony network.

Clade	Permutational chi-square statistic	Probability
1-2	1.875	0.400
1-6	3.000	0.334
1-7	7.510	0.243
1-9	4.286	0.494
1-11	2.250	0.443
1-12	0.600	1.000
1-13	3.000	0.334
1-18	7.000	0.144
1-28	2.000	1.000
1-32	0.833	1.000
2-2	7.875	0.140
2-6	11.479	0.011*
2-7	1.333	1.000
2-8	0.741	1.000
2-9	0.240	1.000
2-10	3.000	0.336
2-11	2.917	0.286
2-13	19.543	0.003*
2-14	0.240	1.000
3-1	31.900	0.000*
3-3	13.606	0.003*
3-4	5.073	0.139
3-5	15.556	0.006*
3-6	0.321	1.000
3-7	0.194	1.000
4-1	37.023	0.000*
4-2	39.707	0.000*
4-3	22.240	0.003*
Total cladogram	108.669	0.000*

* Significant at the 0.05 level

Table 4.15 Results of the nested clade analysis for the large-spotted genets clade. D_c and D_n are, respectively, the clade and nested distance in kilometers of each included clade in the nested clades being examined. Values in bold mean that they are statistically significant, either significantly large (L) or small (S).

Nested clades	Inclusive clades	D_C	D_N	Inference chain; Final inference	Corresponding geographic area
2-6	1-6 (Tip) 1-7 (Tip) I-T	772 **426^S** -	1734 877 -	1-2-Tip/Interior status cannot be determined; Inconclusive outcome	Between Southern Africa and DRC
2-13	1-18 (Interior) 1-20 (Tip) 1-22 (Tip) I-T	927 0 0 927	1325 676 2043 400	1-2-Tip/Interior status cannot be determined; Inconclusive outcome	Between DRC and Dahomey Gap
3-1	2-1 (Interior) 2-20 (Interior) 2-2 (Tip) I-T	**0^S** 0 496 **-495^S**	369 **1575^L** 520 251	1-2-3-4-no; Restricted gene flow with isolation by distance	Between Southern Africa and the Cape+OFS
3-3	2-6 (Interior) 2-7 (Tip) I-T	1111 1078 32	1205 1361 -156	1-2-Tip/Interior status cannot be determined; Inconclusive outcome	Between Southern Africa, DRC and East Africa
3-5	2-10 (Tip) 2-13 (Interior) I-T	190 1332 1142	**2344^L** 1206 **-1138^S**	1-2-Tip/Interior status cannot be determined; Inconclusive outcome	Between East Africa and the Dahomey Gap
4-1	3-1 (Interior) 3-4 (Tip) I-T	**391^S** **703^S** -312	**1220^{SL}** **2004^L** **-784^S**	1-2-3-5-6-Too few clades (≤2) to determine concordance; Insufficient genetic resolution to discriminate between range expansion/colonization and restricted gene flow	Between Southern Africa, DRC and East Africa
4-2	3-2 (Interior) 3-6 (Interior) 3-3 (Interior) I-T	**0^S** **293^S** 1267 -	**608^S** 1601 1457 -	1-2-Tip/Interior status cannot be determined; Inconclusive outcome	Between Southern Africa, DRC and East Africa
4-3	3-5 (Interior) 3-7 (Interior) 3-8 (Tip) I-T	1241 927 0 1159	1423 1113 **2278^L** **-935^S**	1-2-Tip/Interior status cannot be determined; Inconclusive outcome	Between East Africa and Senegal

Table 4.16 Divergence time estimates derived from the equation d=2μt using net nucleotide differences as distance and two different calibrations of the mtDNA neutral molecular clock. Below diagonal: net number of nucleotide differences per site between populations or species (Nei 1987). Above diagonal: divergence time estimates in My; left value derived from the application of the fastest substitution rate and right value derived from the application of the slowest substitution rate.

	Senegal-Dahomey Gap	Dahomey Gap-DRC	DRC-Abyssinia	East Africa	Southern Africa "North"	Southern Africa "South"	Cape+OFS	*Mossambica*	*Angolensis*
Senegal-Dahomey Gap	-	0.135; 0.350	0.212; 0.550	0.327; 0.850	0.538; 1.400	0.596; 1.550	0.750; 1.950	0.500; 1.300	0.865; 2.250
Dahomey Gap-DRC	0.007	-	0.115; 0.300	0.269; 0.700	0.500; 1.300	0.577; 1.500	0.712; 1.850	0.481; 1.250	0.808; 2.100
DRC-Abyssinia	0.011	0.006	-	0.019; 0.050	0.096; 0.250	0.192; 0.500	0.269; 0.700	0.423; 1.100	0.769; 2.000
East Africa	0.017	0.014	0.001	-	0.096; 0.250	0.192; 0.500	0.250; 0.650	0.462; 1.200	0.769; 2.000
Southern Africa "North"	0.028	0.026	0.005	0.005	-	0.058; 0.150	0.135; 0.350	0.615; 1.600	0.942; 2.450
Southern Africa "South"	0.031	0.030	0.010	0.010	0.003	-	0.115; 0.300	0. 712; 1.850	1.038; 2.700
Cape+OFS	0.039	0.037	0.014	0.013	0.007	0.006	-	0.846; 2.200	1.154; 3.000
Mossambica	0.026	0.025	0.022	0.024	0.032	0.037	0.044	-	1.019; 2.650
Angolensis	0.045	0.042	0.040	0.040	0.049	0.054	0.060	0.053	-

4.4 Discussion

4.4.1 Haplotype genealogies, gene flow and habitat dynamics

Rusty-spotted genets are presently adapted to a wide diversity of habitats ranging from tropical rainforest and woodland savannahs to steppe and montane biotopes. Like several other carnivore species, they are highly mobile and thus able to disperse across physical barriers that may be unsurpassable to other types of terrestrial vertebrates. Indeed, the results of this study indicate that isolation by distance plays an important role in the shaping of their genetic structure, at least in substantial parts of the range. However, reconstruction of haplotype genealogies using either phylogenetic trees or networks showed some patterns that might be better accounted for if we consider the effect of past geological events, climatic cycles and vegetation changes.

Crawford-Cabral (1981b) suggested rainforest as the primary habitat in the evolution of the rusty-spotted genets, with secondary invasion of open landscapes such as grassland and woodland savannah. The branching pattern in the phylogenetic trees rooted with *angolensis* haplotypes, where all the rusty-spotted genet rainforest clades are basal, support this hypothesis. The divergence time estimates also indicate, at least considering the set of sampled haplotypes, the Congolese forest block as the likely ancestral area of the group. The tropical rainforest belt in Africa is known to have passed through cyclic contractions and expansions of its range during, respectively, glacial and interglacial periods that started in the Late Pliocene (Maley 1996). During glacial maxima rainforests were frequently reduced to only 5-25% of their present extension, whereas in times of climatic optima they apparently connected West and East Africa through a large corridor (Livingstone 1975). Periods of forest expansion were likely to promote differentiation through colonisation of new available areas and switches to vacant niches (Grubb 1978). On the other hand, forest contraction, by leaving behind demes in islands of suitable habitat, was certainly also a possible mechanism for diversification through allopatric fragmentation (Diamond & Hamilton 1980). This latter scenario is compatible with the finding in this study of lineages in forest areas of the African east coast that are genetically more similar to the ones in the Albertine Rift and westwards than to any other in East Africa. Contraction of an extended African forest during a cool and dry period, estimated from the genetic divergence between demes to have occurred in the Early or Middle Pleistocene depending on the molecular clock accepted as more accurate, would have left isolated pockets of forest in specific regions. This estimate should be regarded with the

uncertainty conveyed by the effects of stochastic lineage extinction and sampling variance on single-locus genealogies. Although they may be inaccurate in absolute terms, they are used in this study as an attempt to reconstruct a relative sequential order of events and to circumstantially correlate these with historical processes externally dated. The level of genetic divergence between haplotypes (Table 4.6) points to a slightly earlier split of *mossambica* than of the deme in the Kenyan coast, in accordance with the observation that reduction of the forest block was retarded along the equator (Nichol 1999). The role of the forests along the coastline between Kenya and Mozambique as refuges for forest species during periods of climatic stress and the number of biogeographic relicts and endemisms contained in this type of habitat has been reported before (Dinesen *et al.* 1994; Roy *et al.* 2001). Their relative climatic and ecological stability (Prell *et al.* 1980) allowed survival and diversification of old lineages in a wide array of animal and plant taxa (Clausnitzer 2003). The hypothesis raised by the results produced here for the samples from the coastal forests of Kenya and Mozambique would be further supported if samples from Tanzania, from the coast and also from the adjacent Eastern Arc Mountains (e.g. East Usambaras), that were not available for this study showed a similar pattern. Also interesting would be to see if any trace of restricted gene flow could be detected between the Tanzanian populations and the one in the Mombassa coast or the one in the Quelimane-Pemba coast. At least judging by their genetic divergence of around 4%, the latter two seemingly remained isolated since they became fragmented from a broader forest block.

An additional remnant in East Africa, apparently more recent than the previous one as suggested by the degree of genetic distance to the haplotypes in the Congolese forest, was detected in the data set for Ethiopia, particularly the Bale Mountains. Indeed, Ethiopian haplotypes (Gr25-Gr27 and Gr29-Gr30) showed closer affinities with the ones westwards of the Albertine Rift than with any other East African lineage. This observation is compatible with the persistence of a deme in the Abyssinian plateau that once could have been the easternmost part of a range of rusty-spotted genets dwelling in an expanded forest block. A montane diversification model in which mountain biotopes acted as refuges and promoters of differentiation due to their distinct environmental characteristics has been proposed and confirmed by patterns of endemism (Fjeldsa & Lovett 1997; Roy 1997). Another hypothesis is that these Ethiopian haplotypes are the extreme of a cline connected to the Congolese forest block through southern Sudan and Central African Republic but samples from Sudan were not available to test it. However, the fact that a related haplotype

to Gr25-Gr27 and Gr29-Gr30 was not recovered from any of the samples from Uganda removes some circumstantial support for this hypothesis.

The effects of alternating cycles of contraction and expansion of the rainforest, in the evolution of the fauna and flora within the Guinean-Congolese range, has been considered as possibly mediated by refuges in the Guinea/Liberia uplands, Cameroon-Gabon and Ituri forest (Hamilton 1976; Maley 1991). However, a dominant role for these refuges as centres of persistence of Pliocene lineages instead of as centres of diversification of new lineages during the Pleistocene has been underlined recently (Fjeldsa & Lovett 1997). Rainfall and habitat heterogeneity would have allowed the survival of diversity-maintaining processes and gene flow through gallery forest could prevent divergence among forest patches (Kellnan *et al.* 1994). From this debate it is emerging that lowland rainforest refuges probably acted as survival centres for species, without actually promoting speciation, but may be associated with several intraspecific radiations (Roy *et al.* 2001). Furthermore, the importance of these refuges as a mechanism for lineage differentiation within species seems dependent on both the ecological plasticity and colonisation abilities of any given species (Querouil *et al.* 2003). No evidence of differentiation was observed among the rusty-spotted genets occurring in the area between the Dahomey Gap and eastern DRC, as portrayed by the lack of clade structure of their haplotypes in the phylogenetic trees and the shallow genetic distances among them. Moreover, the pattern of substitution differences that can be inferred from the MSN, with a star-phylogeny stemming from a central haplotype (Gr34), was congruent with an ancient bottleneck followed by rapid expansion. The latter can be associated with a historical contraction of the rainforest range and population survival in a small refuge, but a substantially larger number of samples would be necessary to determine its location. Also, since genets have strong dispersal abilities and riparian forest connections between refuges in the Cameroon and eastern DRC are hypothesised (Moreau 1963; but see Colyn *et al.* 1991), additional samples could allow one to test the hypothesis of a single *versus* multiple refuges. In contrast, differentiation among postulated Pleistocene rainforest refuges was indeed found between Cameroon/Gabon and Liberia/Ivory Coast. West Africa was characterised by anomalous events and highly unstable life zone boundaries in the Pleistocene (Runge 1996) and the opening and closing of the savannah-like Dahomey Gap was a regular consequence of stochastic oceanic changes (Dupont *et al.* 2000). The estimated divergence time for the evolutionary split between populations in opposite flanks of the River Volta was sometime between 135,000-350,000 years BP, which coincides

with a known arid period for the region around 250,000 years BP (Jahns 1996). A recent study in the genetic structure of forest elephant populations (Debruyne *et al.* 2003) found haplotype genealogies compatible with vicariant evolution on both sides of the Dahomey Gap, but also evidence for restricted gene flow in the form of reciprocal introgression. Although expected, in another highly mobile species, to find similar clear traces of migration across a historically variable barrier, such as the Dahomey Gap, no sharing of lineages was detected between the Guinea/Liberia and Nigeria/Cameroon blocks. This may be viewed as supporting the distinct specific status of the populations west of the River Volta, but since a relation of reciprocal monophyly was not observed in the phylogenetic reconstructions and the level of genetic divergence was not deep, this can be also the result of insufficient sampling.

East Africa shows a very high level of intraclade diversity, a consequence of the presence in the area of three divergent haplotype groups, which certainly contributed to an artificial lowering of the global inter-areas fixation index estimated with the AMOVA procedure. Besides the two previously discussed biogeographic relicts in the Mombassa coast and in Ethiopia, haplotypes (Gr18-Gr22) that are close to the ones in Southern Africa were found from Kenya, west of the Rift Valley, to northern Tanzania. The fact that this third cluster of lineages in East Africa, in contrast with the other two in the region, is distantly related from the one in the Congolese forest block, as portrayed by the topologies of both phylogenetic trees and MSN, begs the question as to its origins. The spanning pattern in the MSN and the values of genetic distances between the different regional haplotypes in this cluster and the ones west of the Albertine Rift support a scenario in which a large-scale diversification of rusty-spotted genets into the woodland savannah habitat occurred to the south of the Congo rainforest. The estimated high levels of genetic diversity for the north of Southern Africa are compatible with the role of this area as the origin of a lineage radiation. The estimated divergence times suggest that this event occurred before the retreat of an expanded forest that led to the allopatric fragmentation of the *mossambica* clade. In a dry and cool period, in which the effect of an extended Kalahari northwards could have changed the climate and vegetation of the southern Congo basin (Sarnthein 1978), opportunities for colonisation attempts of a new niche in newly formed savannah-forest ecotones in marginal areas were likely to have happened (Roy 1997). It is important to note that a similar process is likely to have occurred, possibly even earlier, into the woodland savannah stretching from north of the Congo rainforest to Senegal. However, samples from this belt did not show a pattern of divergence from the

rainforest belt immediately at its south. Possibly, the northern limit to dispersal imposed by the steppe and semi-desert band below the Sahara had a role in preventing the spatial separation that could enable the generation of detectable divergence using neutral markers. However, this does not mean that differentiation in selected genes did not occur between rainforest and savannah populations of this area. It is important to underline that although large-spotted genets can be found today in a wide array of African biomes, they are not present, in contrast with the other highly successful genet taxon (*G. genetta*), in arid or semi-arid regions and in areas of open habitat, such as woodland savannah, they tend to be associated with their mesic sites (Skinner & Smithers 1990; Kingdon 1997).

From the southern Congo basin, invasion of empty and available habitat southwards, through Angola and Zambia, and eastwards, through the corridor between Lakes Tanganyika and Nyasa would have ensued. Nersting & Arctander (2001) also uncovered phylogeographic architectures in impala and kudu likely to be consequence of the colonisation of East Africa from Southern Africa through the same woodland savannah passage in the Pleistocene. However, the observed paraphyly in the trees of haplotypes and the lack of geographic continuity in the MSN branching pattern indicates that a process of contiguous range expansion was overlaid by other factors. Cyclic expansions and contractions of arid and semi-arid habitats, mostly regulated in Southern Africa by the influence of the Namib and Kalahari deserts (Nichol 1999), may have contributed to a stochastic distribution of lineages. A similar lack of regional structure in Southern Africa was revealed in another species that depends on the availability of standing water in woodland savannah, the sable antelope (Matthee & Robinson 1999). Cycles of environmental heterogeneity coupled with strong dispersal abilities could explain why related haplotypes are found in distant locations, such as the eastern side of the central Albertine Rift (Gr31) and Botswana (Gr6) or in the case of Gr14, which was recovered both in Kenya and Katanga. A mixture of historical environmental heterogeneity and high levels of migration superimposed over range expansion has been also invoked to explain low population differentiation in the buffalo within and between Southern and East Africa (Simonsen *et al.* 1999; Van Hooft *et* al. 2001).

The arrival of a wave of rusty-spotted genets into the Cape and into East Africa is estimated from the calibrations for the regional splits to have occurred, in both cases, sometime between 100,000-300,000 years BP. The population in the Cape is recognised as being morphologically distinct, but genetic divergence with neighbouring populations is low, as confirmed by the results of this study, and hybridisation over a narrow contact zone

with rusty-spotted genets from the Natal Province has been proposed (Gaubert *et al.* in press). Also, as referred above, the populations in Tanzania and central Kenya are not, as suggested by haplotypes Gr14 and Gr18-Gr22, genetically differentiated from the ones in Southern Africa. An interesting biogeographic result involving Kenyan haplotypes was the detection of a region of admixture, located between the Albertine Rift and the Rift Valley, in which lineages from the Congolese haplogroup (Gr24, Gr32, and Gr33) meet with haplotypes from this postulated more recent invasion of Kenya (Gr20). The estimated high gene and nucleotide diversities for the area DRC-Abyssinia are a consequence of this phenomenon of confluence of very distinct lineages. Locations with high genetic diversity resulting from the secondary contact of units that diverged in allopatry have been described before (Tiedeman *et al.* 1998). In particular, the importance of this region for hybridisation has been exemplified with the study of a kob intergradation area in Uganda (Birungi & Arctander 2000). The whole Albertine Rift is a recognised biogeographic boundary and biodiversity hotspot (Kasangaki *et al.* 2003) and the finding in this study of a haplotype from Burundi that clusters with haplotypes from South Africa, Zimbabwe and Botswana, further documents this.

Evidence of secondary contact was also detected for Ethiopia, to where apparently the expanding wave coming from south has arrived (haplotype Gr28). In contrast, it was not found in the coastal forest areas of Southern and East Africa included in this study. However, in both cases, more samples would be needed to evaluate, respectively, the extension and age of the intergradation in Ethiopia and the real allopatry between the coastal relicts and the more recent colonisers of the central areas in East Africa.

4.4.2 Taxonomy and conservation

Large-spotted genets are regarded, since the landmark work of Crawford-Cabral (1981a,b), as a group composed by at least three species: *G. pardina* occurring west of the River Volta, *G. tigrina* in the Cape and Orange Free State provinces of South Africa, and *G. "rubiginosa"* in a vast area between these two. Recently, the first reconstruction of the genus *Genetta* phylogeny using both morphology and molecular markers lent additional support to such partition of this section of the genus tree (Gaubert *et al.* 2004). Within this context, from the results of the present study, the first genetic survey of an extensive set of large-spotted genets, two findings should be underlined. They are that between the rusty-spotted genets and any of the two marginal taxa neutral molecular divergence is low and

reciprocal monophyly was not recovered in the phylogenetic trees of haplotypes. The interpretation of these observations is not necessarily the questioning of the trifurcation but also the realisation for the need of more samples from the marginal areas, to assess them with both a longer mitochondrial DNA fragment and nuclear markers, and also the importance of morphological analysis with sophisticated techniques. Although their level of neutral genetic divergence is low when comparing with typical values among sister species in mammals (Johns & Avise 1998), morphological and behavioural differentiation can be accelerated, even in the presence of gene flow, by selection across steep ecological gradients (Schneider *et al.* 1999). This might apply particularly to the case of *tigrina*, which showed a lower genetic divergence to "*rubiginosa*" and where an obvious physical barrier to gene flow is absent at their distribution boundary. The Cape region is known by the presence of abrupt climatic gradients and a highly diverse vegetation cover, generating a complex mosaic of habitats that contrasts with the dominant woodland savannah, grassland and semi-desert conditions of the surrounding areas (Roberts *et al.* 2001). This, together with a relatively stable climatic and geological history since the establishment of the Mediterranean climate in the Middle Pliocene, certainly provides opportunities for ecological differentiation of populations that colonise the Cape (Goldblatt 1997). Similarly, although West Africa has a history of fragmentation from the area eastwards of the River Volta, processes of ecological and parapatric divergence may be at the centre of *pardina* evolution and of other proposed genet forms in the Guinean/Liberian forest block (Gaubert 2003a), which are not necessarily recorded in the patterns of genetic variation of neutral markers.

An objective of this study was to relate the evolutionary structure of the rusty-spotted genet populations, estimated with genetic markers, with previously described forms that were included with uncertain taxonomic status within the *G.* "*rubiginosa*" complex. The present results suggest that from the Dahomey Gap eastwards to the western side of the Albertine Rift, in a band including both rainforest and deciduous forest/woodland savannah, a single unit exists that taxonomically may be identified with *fieldiana*, although this requires confirmation through comparison with samples from the type material of this form. This conclusion is based upon surveying samples from Benin, Nigeria, Cameroon, Gabon, southern Chad, Central African Republic, and northern DRC. The biological reality of a distinct rusty-spotted genet subspecies, *schoutedeni*, in the core of DRC, as proposed by Crawford-Cabral (1970), could not be assessed, since no relevant samples were available. The presence of a distinct clade of unique haplotypes in Ethiopia, with a level of

genetic divergence from all other rusty-spotted genet demes attributable to subspecific differentiation (Gravlund *et al.* 1998), seems to support the subspecies *matschiei* for the Abyssinian plateau. Additional samples from Ethiopia, and also from Somalia, Eritrea, and Sudan, are needed to establish the extension and limits of the range of this subspecies. The unavailability of samples from Somalia also prevented the evaluation of the hypothesis of a unique element of the evolutionary diversity within the rusty-spotted genets, classified as *deorum*, for this territory. An interesting finding was the absence of genetic structure for an extensive area encompassing the whole of Southern Africa up to Tanzania and central Kenya, suggesting the presence of a single unit. As in the cases above of *fieldiana* and *matschiei*, screening of samples from the type material of *letabae*, *zambesiana*, *suahelica* and *erlangeri* would be required in order to validate any taxonomic designation proposed for this widespread unit detected here.

This study showed that a population from the northern coast of Mozambique, previously considered as belonging to *G. mossambica*, is a divergent rusty-spotted genet deme. Together with a population from the Kenyan coast, although seemingly having an independent evolutionary history from each other, they seem to constitute biogeographic relicts from an ancient connection with the forest populations westwards of the Albertine Rift. The genetic survey of the geographically intermediate population of the Tanzanian coastal forest and of samples from the type material of *mossambica*, *erlangeri*, and *suahelica* (in the collection of the Berlin Natural History Museum) are the logical steps to clarify the taxonomy and nomenclature of these two demes. Also, additional samples of the inland areas in Kenya, Tanzania, and Mozambique, surrounding the coastal forest habitat in each of these three countries would be important to determine precise ranges and boundaries of each form and to identify potential zones of hybridisation. Nevertheless, although conditional upon the results of these further analyses, the present study clearly indicates that the eastern coastal forest biome in Africa harbours genet relicts, maybe good species, with a long and independent evolutionary trajectory and probably adapted to a singular biotope. They belong to a major centre of endemism in Africa, today with an area of around only 7% of its original extent (Myers *et al.* 2000), in which research on the ecological requirements of their members and implementation of protective measures against human destruction are conservation priorities (Mittermeier *et al.* 1998; Burgess *et al.* 1998).

The possible species status of these relict demes provides the answer to a question that this study attempted to address, if the rusty-spotted genet complex is a single polytypic

species or is composed of more than one morphologically cryptic species. Besides these cases, the results point to the supposition that the remaining rusty-spotted genet populations may indeed constitute a single species to which the name *G. fieldiana*, replacing the invalid name *G. rubiginosa*, should be assigned. However, more samples, mainly from demes that were not sampled but also from the ones for which the sampling was weak, and analyses using nuclear markers are needed before drawing a definitive conclusion concerning the taxonomic composition of the *G. "rubiginosa"* complex. Also in consequence of the absence of data from the nuclear genome, the use of the widely applied terminology of "management unit" and "evolutionary significant unit" (Moritz 1994) was deliberately avoided in this study. Conversely, the sample set was apparently robust enough to circumstantially contradict the supposed existence of two morphologically cryptic species under the colloquial name rusty-spotted genets. Gaubert (2003a) hypothesised based on a single diagnostic cranial feature that *schoutedeni* is a different species from *"rubiginosa"* but, by being sympatric and seemingly indistinguishable by their external morphology, they would have been both included in the same taxon. None of the results obtained here are compatible with the existence of two broadly sympatric species, an instance that would be reflected in a phylogeographic pattern of category II (Avise *et al.* 1987) which was clearly not observed in the present study. Nevertheless, this finding does not imply that analysis of samples from specimens positively identified as *schoutedeni*, originally described only for the DRC, which were not available for this study, would not be an important next step in the understanding of the rusty-spotted genets evolution.

4.4.3 Final remarks

This study shows how the phylogeographic approach can be helpful in uncovering unsuspected evolutionary relationships among geographic populations that are spatially assorted by an intricate history of range changes controlled by palaeo-environmental fluctuations. Consequently, with the caution implied by a non-ideal sample set and the use of a single locus, past biogeographic processes could be hypothesised that explain affinities between fragmented sets of similar habitat, establishment of regions where divergent lineages mix, and the occurrence of vast areas where genetic structure is absent. These hypotheses were related with proposed biogeographic models and previously described patterns for other African taxa, in order to evaluate how likely they are of being correct.

However, interpretation of the results and drawing of conclusions in terms of speciation and taxonomy can be difficult when a complex spatial history across time it is associated with evolution across ecological gradients, as neutral markers may not tell the whole tale.

This study also illustrates the potential of an almost infinite sample source, in practical terms, which are the museum collections. For many taxa it is very difficult to obtain a good sampling, or it may even prove impossible to implement a phylogeographic analysis over a large geographic area, by relying only in modern samples. However, the use of museum material in large scale for phylogeographic studies it is very rarely an option attempted by researchers, although it may provide most relevant results when it is indeed pursued (Prager *et al.* 1998). This study would be simply impossible without resorting to a large number of museum samples and probably all the sampling weaknesses detected after the present analysis may only be addressed with additional material of this type.

Appendix to Chapter 4

Figure 4.I *Genetta "rubiginosa"*; Transvaal Museum "TM10056".

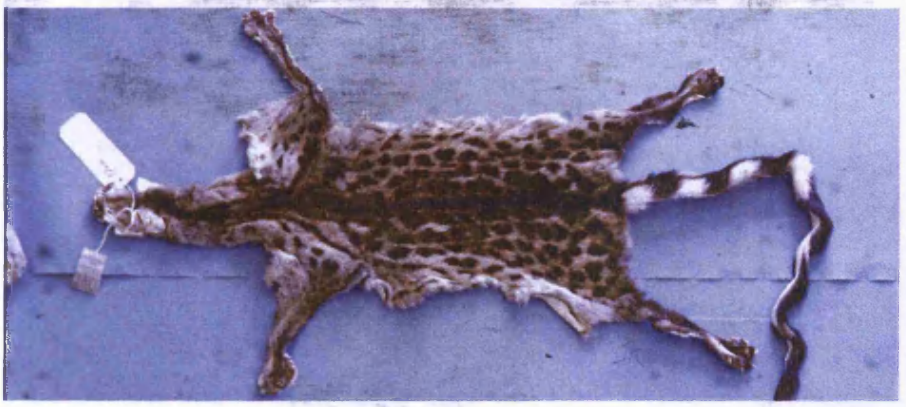

Figure 4.II *Genetta pardina*; Lisbon IICT "CZ340".

Figure 4.III *Genetta tigrina*; Amathole Museum "KM31185".

Figure 4.IV *fieldiana*; Paris Natural History Museum "1965-357".

Figure 4.V *matschiei*; British Museum "6628".

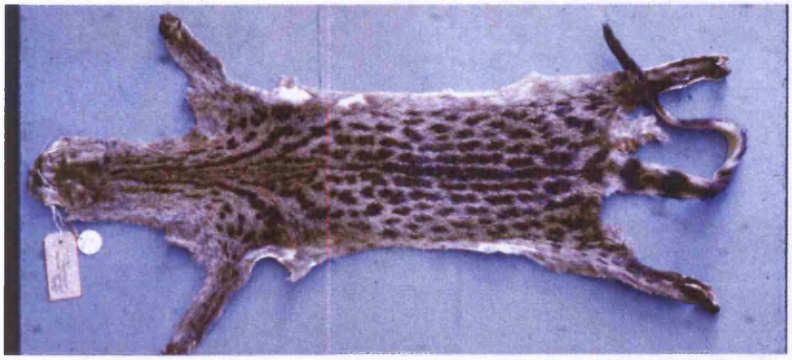

Figure 4.VI *schoutedeni*; Royal Museum for Central Africa"18949".

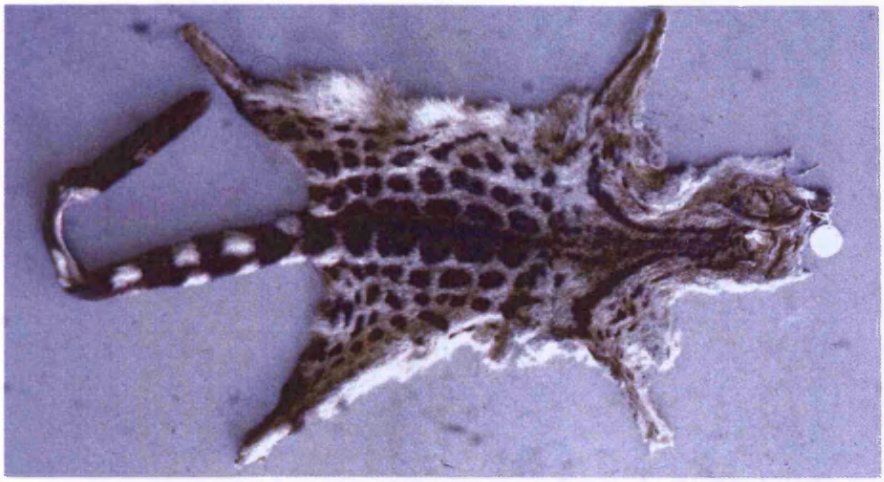

Figure 4.VII *stuhlmanni*; Royal Museum for Central Africa "23052".

Figure 4.VIII *erlangeri*; Los Angeles County Natural History Museum "LACM42933".

Figure 4. IX *suahelica*; Arusha National Park "DSCN0466".

Figure 4.X *zambesiana*; Transvaal Museum "TM9904".

Figure 4.XI *letabae*; Transvaal Museum "TM45132".

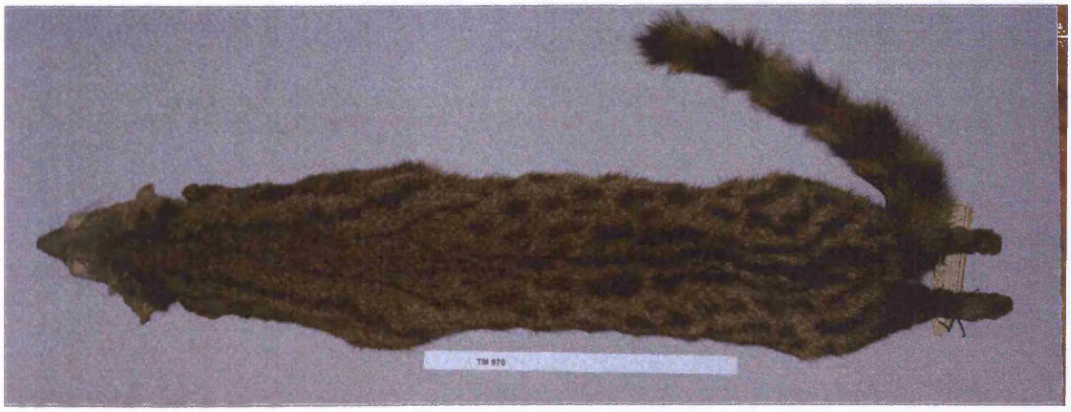

Figure 4. XII *Genetta mossambica*; Transvaal Museum "TM970".

Table 4.I Description of the samples included in the study by taxon name, biological type, sampling locality, geographic coordinates and source of loan or donation.

Species Name	Sample Reference	Biological Type	Sampling Locality	Geographic Coordinates	Source	Curator / Collector
G. "rubiginosa"	TM10348	Museum skin	Waterberg, Namibia	20° 31' 00S 17° 13' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	Angola1	Tissue	Quissama, Angola	09° 58' 60S 14° 28' 60E	Quissama National Park	Pedro Vaz Pinto
G. "rubiginosa"	Angola2	Tissue	Quissama, Angola	09° 58' 60S 14° 28' 60E	Quissama National Park	Pedro Vaz Pinto
G. "rubiginosa"	CZ27	Museum skin	Lumbala, Angola	14° 06' 00S 21° 25' 59E	Centro de Zoologia, IICT, Portugal	João Crawford-Cabral
G. "rubiginosa"	PC50ǃ	Museum skin	Angola	10° 60' 00S 17° 60' 00E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	PC60ǃ	Museum skin	Angola	10° 60' 00S 17° 60' 00E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	TM8886	Museum skin	Secheli, Botswana	24° 53' 00S 25° 58' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	PC90	Museum skin	Bambi, Botswana	18° 00' 00S 21° 10' 00E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	TM12468*	Museum skin	Maun, Botswana	19° 58' 60S 23° 25' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM45132*ᵛ	Museum skin	Ubombo, South Africa	27° 34' 00S 32° 04' 59E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	DM3335	Tissue	New Germany, South Africa	29° 48' 00S 30° 52' 60E	Durban Museum	Peter Taylor
G. "rubiginosa"	DM3499	Tissue	Kloof, South Africa	29° 46' 60S 30° 49' 60E	Durban Museum	Peter Taylor
G. "rubiginosa"	DM4475	Tissue	Umgeni River, South Africa	29° 50' 00S 31° 00' 00E	Durban Museum	Peter Taylor
G. "rubiginosa"	DM1632	Tissue	Hillary, South Africa	29° 52' 60S 30° 56' 60E	Durban Museum	Peter Taylor
G. "rubiginosa"	DM2178	Tissue	Waterfall, South Africa	29° 23' 60S 31° 03' 00E	Durban Museum	Peter Taylor
G. "rubiginosa"	DM2190	Tissue	Gillitts, South Africa	29° 48' 00S 30° 48' 00E	Durban Museum	Peter Taylor
G. "rubiginosa"	DM1636	Tissue	Westville, South Africa	29° 49' 60S 30° 55' 60E	Durban Museum	Peter Taylor
G. "rubiginosa"	DM2232	Tissue	Umhloti, South Africa	29° 39' 00S 31° 06' 00E	Durban Museum	Peter Taylor
G. "rubiginosa"	PC125	Museum skin	M'Kuzi, South Africa	27° 40' 00S 32° 01' 60E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	TM40333	Museum skin	Mbuzane, South Africa	28° 00' 00S 32° 15' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TPNR1	Museum skin	Timbavati, South Africa	24° 25' 00S 31° 19' 59E	Timbavati Private Reserve	William Briedenhann
G. "rubiginosa"	TM8689	Museum skin	Inyanga, Zimbabwe	18° 13' 00S 32° 45' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	KM19287*∃	Museum skin	Salisbury, Zimbabwe	17° 49' 60S 31° 03' 00E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. "rubiginosa"	ROM35695ǃ	Museum skin	Zimbabwe	18° 60' 00S 29° 50' 00E	Royal Ontario Museum	Judith Eger
G. "rubiginosa"	ROM65103∃ǃ	Museum skin	Zimbabwe	18° 60' 00S 29° 50' 00E	Royal Ontario Museum	Judith Eger
G. "rubiginosa"	ROM35696∃ǃ	Museum skin	Zimbabwe	18° 60' 00S 29° 50' 00E	Royal Ontario Museum	Judith Eger
G. "rubiginosa"	ROM35348ǃ	Museum skin	Zimbabwe	18° 60' 00S 29° 50' 00E	Royal Ontario Museum	Judith Eger

G. "rubiginosa"	TM11449*	Museum skin	Inyanga, Zimbabwe	18° 13' 00S 32° 45' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM8691	Museum skin	Birchenough Bridge, Zimbabwe	19° 58' 00S 32° 19' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM16638*	Museum skin	Bethlehem, South Africa	28° 13' 60S 28° 18' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	NMB7013*	Museum skin	Moletsanes, Lesotho	29° 10' 00S 28° 03' 00E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. "rubiginosa"	NMB6904*	Museum skin	Mokhotlong, Lesotho	29° 16' 60S 29° 04' 60E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. "rubiginosa"	NMB3947*∃	Museum skin	Bethlehem, South Africa	28° 13' 60S 28° 18' 00E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. "rubiginosa"	NMB6885*∃	Museum skin	Ndedema, Lesotho	29° 04' 00S 29° 13' 00E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. "rubiginosa"	DM1089	Tissue	St. Lucia, South Africa	28° 21' 00S 32° 25' 00E	Durban Museum	Peter Taylor
G. "rubiginosa"	DM1618	Tissue	Kloof, South Africa	29° 46' 60S 30° 49' 60E	Durban Museum	Peter Taylor
G. "rubiginosa"	DM1617	Tissue	Albert Falls, South Africa	29° 27' 00S 30° 23' 59E	Durban Museum	Peter Taylor
G. "rubiginosa"	DM2189	Tissue	Paradise Valley, South Africa	29° 50' 00S 30° 52' 00E	Durban Museum	Peter Taylor
G. "rubiginosa"	DM5611	Tissue	Dlinza Forest, South Africa	28° 54' 00S 31° 27' 00E	Durban Museum	Peter Taylor
G. "rubiginosa"	TM39551	Tissue	Blydschap, South Africa	23° 15' 00S 29° 46' 00E	Durban Museum	Peter Taylor
G. "rubiginosa"	TM39276	Tissue	Skewbridge, South Africa	29° 37' 00S 30° 22' 59E	Durban Museum	Peter Taylor
G. "rubiginosa"	PT82	Tissue	Blydschap, South Africa	23° 15' 00S 29° 46' 00E	Durban Museum	Peter Taylor
G. "rubiginosa"	TM39910*	Museum skin	Soutpan, South Africa	28° 43' 00S 26° 04' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM38712	Museum skin	Potgietersrus, South Africa	24° 10' 60S 28° 58' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM44830	Museum skin	Pretoria, South Africa	25° 45' 00S 28° 10' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	NMS27342*	Museum skin	Sabie River, South Africa	23° 30' 00S 31° 06' 00E	National Museum of Scotland	Jerry Herman, Ruth Pollitt, Andrew Kitchener
G. "rubiginosa"	NMS27349*∃	Museum skin	Sabie River, South Africa	23° 30' 00S 31° 06' 00E	National Museum of Scotland	Jerry Herman, Ruth Pollitt, Andrew Kitchener
G. "rubiginosa"	CZ242	Museum skin	Mambone, Mozambique	20° 58' 60S 33° 38' 60E	Centro de Zoologia, IICT, Portugal	João Crawford-Cabral
G. "rubiginosa"	PC136∃!	Museum skin	Mozambique	18° 43' 00S 37° 47' 00E	Powell-Cotton Museum	Malcolm Harman

G. "rubiginosa"	TM12656*	Museum skin	Kayombo, Zambia	13° 03' 00S 23° 51' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM6058*	Museum skin	Mofu, Zambia	11° 05' 60S 30° 21' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM9839*	Museum skin	Chisasa, Zambia	12° 05' 60S 25° 30' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM9190*	Museum skin	Ncheu, Malawi	14° 49' 00S 34° 37' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM9191*	Museum skin	Ncheu, Malawi	14° 49' 00S 34° 37' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	AMNH161757*	Museum skin	Likabula, Malawi	15° 58' 00S 35° 29' 00E	AMNH	Bob Randall, Chris Norris
G. "rubiginosa"	AMNH161758*∃	Museum skin	Nchisi, Malawi	13° 22' 00S 34° 00' 00E	AMNH	Bob Randall, Chris Norris
G. "rubiginosa"	AMNH161763*	Museum skin	Cholo, Malawi	16° 04' 00S 35° 07' 60E	AMNH	Bob Randall, Chris Norris
G. "rubiginosa"	TM9188*	Museum skin	Ncheu, Malawi	14° 49' 00S 34° 37' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM9898*	Museum skin	Salima, Malawi	13° 46' 60S 34° 25' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM9899*	Museum skin	Salima, Malawi	13° 46' 60S 34° 25' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	TM9904*	Museum skin	Salima, Malawi	13° 46' 60S 34° 25' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	Tanzania 4 3*	Tissue	Arusha, Tanzania	03° 15' 00S 36° 45' 00E	Varese University	Adriano Martinoli
G. "rubiginosa"	Tanzania 12 2*	Tissue	Arusha, Tanzania	03° 15' 00S 36° 45' 00E	Varese University	Adriano Martinoli
G. "rubiginosa"	Tanzania 3 4 *	Tissue	Arusha, Tanzania	03° 15' 00S 36° 45' 00E	Varese University	Adriano Martinoli
G. "rubiginosa"	Tanzania 96*	Tissue	Arusha, Tanzania	03° 15' 00S 36° 45' 00E	Varese University	Adriano Martinoli
G. "rubiginosa"	IoZ5056	Blood	/	/	London Zoo	Dada Gotelli
G. "rubiginosa"	IoZ2275	Blood	/	/	London Zoo	Dada Gotelli
G. "rubiginosa"	TC80	Tissue	Nairobi, Kenya	01° 16' 60S 36° 49' 00E	Paris NHM	Philippe Gaubert
G. "rubiginosa"	TC60	Tissue	Thika, Kenya	01° 03' 00S 37° 04' 60E	Paris NHM	Philippe Gaubert
G. "rubiginosa"	AMNH187744*	Museum skin	Belle Vue, Kenya	00° 13' 60S 36° 43' 00E	AMNH	Bob Randall, Chris Norris
G. "rubiginosa"	T64	Tissue	Kibwezi, Kenya	02° 25' 00S 37° 58' 00E	Paris NHM	Philippe Gaubert
G. "rubiginosa"	LACM53728*	Museum skin	Samburu, Kenya	03° 46' 00S 39° 16' 60E	Los Angeles County NHM	Jim Dines, Ines Horovitz
G. "rubiginosa"	LACM56723*	Museum skin	Isiolo, Kenya	00° 21' 00N 37° 34' 60E	Los Angeles County NHM	Jim Dines, Ines Horovitz

G. "rubiginosa"	LACM42933*	Museum skin	Bodhei, Kenya	01° 51' 00S 40° 43' 00E	Los Angeles County NHM	Jim Dines, Ines Horovitz
G. "rubiginosa"	LACM42935*	Museum skin	Kipini, Kenya	02° 31' 60S 40° 31' 00E	Los Angeles County NHM	Jim Dines, Ines Horovitz
G. "rubiginosa"	AMNH187743*	Museum skin	Belle Vue, Kenya	00° 13' 60S 36° 43' 00E	AMNH	Bob Randall, Chris Norris
G. "rubiginosa"	NMWB2143*	Museum skin	Addis Abeba, Ethiopia	09° 01' 60N 38° 42' 00E	Wien NHM	Barbara Herzig, Friederike Spitzenberger
G. "rubiginosa"	G1	Tissue	Angesu, Ethiopia	06° 45' 00N 39° 45' 00E	Edinburgh University, University of Stirling	Karen Laurenson, Simon Thirgood
G. "rubiginosa"	G2	Tissue	Angesu, Ethiopia	06° 45' 00N 39° 45' 00E	Edinburgh University, University of Stirling	Karen Laurenson, Simon Thirgood
G. "rubiginosa"	G3	Tissue	Angesu, Ethiopia	06° 45' 00N 39° 45' 00E	Edinburgh University, University of Stirling	Karen Laurenson, Simon Thirgood
G. "rubiginosa"	G4	Tissue	Sura, Ethiopia	06° 55' 60N 39° 58' 60E	Edinburgh University, University of Stirling	Karen Laurenson, Simon Thirgood
G. "rubiginosa"	G5	Tissue	Bale, Ethiopia	06° 57' 00N 40° 07' 60E	Edinburgh University, University of Stirling	Karen Laurenson, Simon Thirgood
G. "rubiginosa"	G6	Tissue	Angesu, Ethiopia	06° 45' 00N 39° 45' 00E	Edinburgh University, University of Stirling	Karen Laurenson, Simon Thirgood
G. "rubiginosa"	G7	Tissue	Angesu, Ethiopia	06° 45' 00N 39° 45' 00E	Edinburgh University, University of Stirling	Karen Laurenson, Simon Thirgood
G. "rubiginosa"	G8	Tissue	Angesu, Ethiopia	06° 45' 00N 39° 45' 00E	Edinburgh University, University of Stirling	Karen Laurenson, Simon Thirgood
G. "rubiginosa"	G9	Tissue	Angesu, Ethiopia	06° 45' 00N 39° 45' 00E	Edinburgh University, University of Stirling	Karen Laurenson, Simon Thirgood
G. "rubiginosa"	Terv77-53-M-4*∃	Museum skin	Muramvya, Burundi	03° 15' 53S 29° 36' 49E	Royal Museum for Central Africa	Wim Van Neer, Wim Wendelen
G. "rubiginosa"	Terv78-26-M-20*∃	Museum skin	Ruzizi, Burundi	03° 20' 17S 29° 13' 08E	Royal Museum for Central Africa	Wim Van Neer, Wim Wendelen
G. "rubiginosa"	FMNH2814	Tissue	Bukinayana, Burundi	02° 52' 00S 29° 16' 60E	Paris NHM	Philippe Gaubert
G. "rubiginosa"	LACM56724*	Museum skin	Ntandi, Uganda	00° 48' 00N 30° 08' 60E	Los Angeles County NHM	Jim Dines, Ines Horovitz
G. "rubiginosa"	LACM36392*	Museum skin	Budongo, Uganda	01° 38' 60N 31° 34' 60E	Los Angeles County NHM	Jim Dines, Ines Horovitz
G. "rubiginosa"	LACM53729*	Museum skin	Katera, Uganda	00° 34' 00S 31° 22' 00E	Los Angeles County NHM	Jim Dines, Ines Horovitz
G. "rubiginosa"	LACM53731*	Museum skin	Malabigambo, Uganda	00° 57' 00S 31° 34' 59E	Los Angeles County NHM	Jim Dines, Ines Horovitz

G. "rubiginosa"	Zurich13245∃	Museum skin	Kivu, Democratic Republic of Congo	02° 30' 00S 28° 00' 00E	Zurich Zoological Museum	Cesar Claude
G. "rubiginosa"	TM17698*	Museum skin	Katanga, Democratic Republic of Congo	09° 37' 59S 25° 45' 59E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. "rubiginosa"	Terv18949*∃	Museum skin	Bokungu, Democratic Republic of Congo	01° 34' 00S 23° 49' 59E	Royal Museum for Central Africa	Wim Van Neer, Wim Wendelen
G. "rubiginosa"	PCAX	Museum skin	Makala, Democratic Republic of Congo	00° 33' 00N 27° 45' 00E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	PCCX	Museum skin	Makala, Democratic Republic of Congo	00° 33' 00N 27° 45' 00E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	PC164∀	Museum skin	Maio, Chad	09° 35' 00N 19° 08' 00E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	PC145∀	Museum skin	Maio, Chad	09° 35' 00N 19° 08' 00E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	PC160∀	Museum skin	Maio, Chad	09° 35' 00N 19° 08' 00E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	PC143∀	Museum skin	Maio, Chad	09° 35' 00N 19° 08' 00E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	SMF5831	Museum skin	Pama River, Central African Republic	07° 00' 00N 16° 37' 59E	Frankfurt NHM	Dieter Kock
G. "rubiginosa"	Terv93-91-M-60*¡	Museum skin	Central African Republic	6° 35' 00N 20° 60' 00E	Royal Museum for Central Africa	Wim Van Neer, Wim Wendelen
G. "rubiginosa"	Lope1*∃	Native skin	Lope, Gabon	00° 30' 00S 11° 40' 00E	Lope Reserve	Mike Bruford, Jean Wickings
G. "rubiginosa"	Wanzie1*	Museum skin	Yaounde, Cameroon	03° 52' 00N 11° 31' 00E	Cameroon IRAD	Chris Wanzie
G. "rubiginosa"	Terv73-15-M-301*	Museum skin	Yagoua, Cameroon	11° 07' 59N 14° 03' 59E	Royal Museum for Central Africa	Wim Van Neer, Wim Wendelen
G. "rubiginosa"	Terv73-15-M-303*	Museum skin	Mokolo, Cameroon	10° 45' 00N 13° 48' 00E	Royal Museum for Central Africa	Wim Van Neer, Wim Wendelen
G. "rubiginosa"	AMNH241389*	Museum skin	Bertoua, Cameroon	04° 34' 60N 13° 40' 60E	AMNH	Bob Randall, Chris Norris
G. "rubiginosa"	AMNH241390*	Museum skin	Bertoua, Cameroon	04° 34' 60N 13° 40' 60E	AMNH	Bob Randall, Chris Norris
G. "rubiginosa"	AMNH236485*	Museum skin	Yaounde, Cameroon	03° 52' 00N 11° 31' 00E	AMNH	Bob Randall, Chris Norris
G. "rubiginosa"	PC99	Museum skin	Yabassi, Cameroon	04° 28' 00N 09° 58' 00E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	PC394	Museum skin	Batouri, Cameroon	04° 25' 60N 14° 23' 07E	Powell-Cotton Museum	Malcolm Harman
G. "rubiginosa"	Terv95-53-M-1*	Museum skin	Diebu, Nigeria	04° 37' 00N 06° 07' 60E	Royal Museum for Central Africa	Wim Van Neer, Wim Wendelen
G. "rubiginosa"	Terv98-49-M-2*	Museum skin	Okpoama, Nigeria	04° 17' 60N 06° 17' 60E	Royal Museum for Central Africa	Wim Van Neer, Wim Wendelen
G. "rubiginosa"	Terv98-49-M-3*	Museum skin	Orashi, Nigeria	04° 45' 00N 06° 46' 00E	Royal Museum for Central Africa	Wim Van Neer, Wim Wendelen

G. "rubiginosa"	Terv98-49-M-4*	Museum skin	Num, Nigeria	07° 05' 60N 08° 43' 00E	Royal Museum for Central Africa	Wim Van Neer, Wim Wendelen
G. "rubiginosa"	Terv96-47-M-3*	Museum skin	Taba, Nigeria	11° 07' 00N 07° 50' 60E	Royal Museum for Central Africa	Wim Van Neer, Wim Wendelen
G. "rubiginosa"	Taiwo*∃	Native skin	Ile-Ife, Nigeria	07° 28' 00N 04° 34' 00E	Obafemi Awolowo University	Yetunde Taiwo
G. "rubiginosa"?	Shamie1∃	Native skin	Cotounou, Benin	06° 20' 60N 02° 25' 60E	Benin IITA	Ibrahim Shamie
G. "rubiginosa"?	Shamie2∃	Native skin	Cotounou, Benin	06° 20' 60N 02° 25' 60E	Benin IITA	Ibrahim Shamie
G. "rubiginosa"?	Shamie3∃	Native skin	Cotounou, Benin	06° 20' 60N 02° 25' 60E	Benin IITA	Ibrahim Shamie
G. "rubiginosa"?	Shamie4	Native skin	Cotounou, Benin	06° 20' 60N 02° 25' 60E	Benin IITA	Ibrahim Shamie
G. "rubiginosa"?	Shamie5	Native skin	Cotounou, Benin	06° 20' 60N 02° 25' 60E	Benin IITA	Ibrahim Shamie
G. pardina ?	MHNG1736026*	Museum skin	Adiapodoume, Ivory Coast	05° 19' 60N 04° 07' 00W	Geneve NHM	Manuel Ruedi
G. pardina ?	LACM33808*	Museum skin	Wolo, Ivory Coast	09° 31' 60N 05° 25' 00W	Los Angeles County NHM	Jim Dines, Ines Horovitz
G. pardina ?	AMNH241388*	Museum skin	Lakota, Ivory Coast	05° 50' 60N 05° 40' 60W	AMNH	Bob Randall, Chris Norris
G. pardina ?	AMNH167497*∃!	Museum skin	Liberia	06° 24' 00N 08° 60' 00W	AMNH	Bob Randall, Chris Norris
G. pardina ?	ROMFN28730	Tissue	Liberia	06° 24' 00N 08° 60' 00W	Royal Ontario Museum	Judith Eger
G. pardina ?	MVZ1331309*∃!	Museum skin	Firestone Plantation, Liberia	06° 24' 00N 10° 22' 00W	Berkeley Museum of Vertebrate Zoology	James Patton, Yuri Leite
G. pardina ?	Bissau2*	Blood	Orango, Guinea-Bissau	11° 20' 60N 15° 22' 00W	Algarve University	Luis Palma
G. tigrina	A270	DNA Extract	East London, South Africa	33° 01' 59S 27° 55' 00E	Cape Town University	Eric Harley
G. tigrina	A271	DNA Extract	East London, South Africa	33° 01' 59S 27° 55' 00E	Cape Town University	Eric Harley
G. tigrina	A272	DNA Extract	East London, South Africa	33° 01' 59S 27° 55' 00E	Cape Town University	Eric Harley
G. tigrina	A273	DNA Extract	East London, South Africa	33° 01' 59S 27° 55' 00E	Cape Town University	Eric Harley
G. tigrina	KM31185*	Museum skin	Bathurst, South Africa	33° 28' 60S 26° 49' 60E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. tigrina	KM31276*	Museum skin	Msikaba River, South Africa	31° 10' 60S 29° 36' 00E	Amathole Museum	Fred Kigozi, Lloyd Wingate
G. tigrina	NMB4786*	Museum skin	Barkly East, South Africa	30° 58' 00S 27° 36' 00E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen
G. tigrina	NMB4470*	Museum skin	Reddersburg, South Africa	29° 38' 60S 26° 10' 00E	Bloemfontein National Museum	Nico Avenant, Johan Eksteen

G. mossambica?	TM970	Museum skin	Boror, Mozambique	12° 30' 00S 39° 00' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. mossambica?	TM1357	Museum skin	Boror, Mozambique	12° 30' 00S 39° 00' 00E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. angolensis	TM17713*	Museum skin	Kafue, Zambia	15° 46' 00S 28° 10' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. angolensis	MVZ118449*	Museum skin	Luanshya, Zambia	13° 07' 60S 28° 23' 60E	Berkeley Museum of Vertebrate Zoology	James Patton, Yuri Leite
G. angolensis	TM9842*	Museum skin	Kasempa, Zambia	13° 26' 60S 25° 49' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. angolensis	TM9847*	Museum skin	Kasempa, Zambia	13° 26' 60S 25° 49' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney
G. angolensis	TM9849*	Museum skin	Kasempa, Zambia	13° 26' 60S 25° 49' 60E	Transvaal Museum	Duncan McFadyen, Teresa Kearney

* - photograph of the specimen available; ∃ - sample not included in the analyses due to inability of obtaining complete sequence for both the cytochrome *b* and the ND-5 gene fragments; ∀ - specimen apparently misidentified by the source; / - sample from captive specimen without capture data records; ! - sampling locality unknown beyond country level: indicated coordinates are of the country's geographic centre.

Chapter 5. General Discussion

The main aim of this study was to attempt the reconstruction of the phylogeographic histories of two species complexes of genets, widely distributed in Africa, which have cryptic morphological variation. The information derived from haplotype genealogies and measures of genetic diversity and structure, when overlaid over the geographic distribution and evidence from the fields of palaeoclimatology and palaeoecology, is likely to convey evolutionary and taxonomic insight upon these taxa. Furthermore, it may also have larger relevance by contributing to general scenarios of historical biogeography. Two additional objectives were to extend the phylogenetic breadth of the available phylogeographic studies on the Feloidea branch of Carnivores, essentially limited to the family Felidae (e.g Eizirick *et al.* 2001) with hyenas, mongooses, civets and genets largely ignored so far, and to demonstrate the usefulness of museum samples to otherwise unfeasible large-scale population studies of cryptic taxa. In the following two sections, the evolutionary findings for each of the species complexes are summarised under the light of the initial questions and hypotheses. Then, the results are addressed in the context of the study of speciation and merits/limitations of mtDNA for its pursuit, and in terms of how comparative data can be helpful in determining critical factors responsible for departures shown by some species from dominant biogeographic patterns. Finally, recommended developments from this work that may constitute future avenues of research are outlined, some of them hopefully to be undertaken in the near future.

5.1 Small-spotted genets

Phylogenetic reconstruction including the genet species generally regarded as the most closely related to the *G. genetta* species complex suggests that the small-spotted genets apparently constitute a monophyletic clade. Nevertheless, the haplotype genealogies reconstructed with both hierarchical and network methods indicate strong population structure within *G. genetta*, as suspected in face of the highly fragmented range presently observed. The branching patterns connecting the different regional haplogroups, and the estimated genetic divergences between them, depart from what would be expected considering only the current spatial structure and imply the effects of past range shifts or dispersal, which genetic signature still remains. This is particularly true for the inferred paraphyly among lineages from the Maghreb and the Sahel, two regions presently

separated by an unsurpassable physical barrier to genets, and the link between the Arabian isolate and haplotypes exclusively present in the Maghreb and southwestern Europe.

The observed genetic affinity between North Africa and the Sahel is likely to be the result of the cyclic contractions of the Sahara during the wet and warm interglacial periods of the Quaternary in Africa, bringing both regions into genetic contact in the form of migration across two separate corridors linking North with West Africa and North with Central Africa. The colonisation of the Arabian Peninsula is probably the consequence of a long-distance dispersal from individuals of North African origin in a phase of climatic mildness in which the coastal regions of Libya and Egypt were populated. From the estimates of divergence time between pairs of geographic populations, the invasion of the Arabian Peninsula is dated as one of the oldest biogeographic events within *G. genetta*. However, this may simply be due to the acceleration of genetic divergence associated with the lineage bottlenecking implied in founder effect processes. In any case, an important consequence of this finding is the support for the presence of genets in the Maghreb at least since the initial stages of the Middle Pleistocene, or even since the Early Pleistocene if the classical mtDNA clock is used, and the antiquity of this deme is also incidentally supported by the coexistence in the region of highly divergent lineages. In spite of the ancient occurrence of genets in North Africa, populations in Europe showed a very shallow genetic divergence from the ones in the Maghreb, compatible with the palaeontological data that suggests an anthropogenic introduction within the last 2,000 years (Morales 1994). Two lineages were found in Iberia and two lineages were found in France, with one lineage common to both regions and also found in North African samples. Overall low levels of genetic differentiation between the two sides of the Mediterranean also prevented credible dating of colonisation and, taken together with the available fossil record, were interpreted as sign of anthropogenic introduction in recent studies on chameleons and woodmice (Paulo *et al.* 2002a; Michaux *et al.* 2003).

The haplotype trees, rooted with large-spotted genet sequences, indicated a pattern of allopatric fragmentation, and posterior differentiation within savannah refuges (Muwanika *et al.* 2003), of a connected range stretching from East to West Africa and up to North Africa, the latter being subsequently cyclically (re)connected to the Sahel. Whereas the Maghreb region appears to be pivotal in the origin of the non-African demes and, due to its cyclic connections with the Sahel, seems to harbour an important admixture of old resident and recently arrived lineages, Central Africa is the core area involved in all vicariant events. An early diversification into Southern Africa was possibly the

consequence of the split of a Central African population driven by rainforest expansion and formation of extensive lakes (Flagstad *et al.* 2001), followed by range expansion southwards. Isolation in East Africa is likely to be associated with geological activity along the Rift Valley (Arctander *et al.* 1999; Jensen-Seaman & Kidd 2001) and, if contemporary with a wet and warm interglacial period, this process was probably accompanied by additional subdivision within the region in scattered grassland refuges. East Africa has a notable diversity of interspersed environments that provide further opportunities for genetic divergence in pockets of suitable habitat following population reshuffling resulting of climatic-induced range shifts, and this may also explain the differentiation found between Eritrea, Kenya and Somalia. Samples from Zambia, Tanzania, and Ethiopia, which were not available for this study, would be most relevant to assess the interpretation here derived of genetic independence between Southern and East Africa, as well as its geographic boundary, and to determine the extent of population structure within East Africa. The segregation between West and Central African lineages is certainly associated with the alternated effects of an extended rainforest during warm and wet periods and a descending Sahara during the glacial phases (Runge 1996; Dupont *et al.* 2000), both contributing to reduced ranges and movements of savannah-dwelling species. It is important to note that since it is not possible to calibrate a molecular clock for the mtDNA evolution in genets based on non-genetic information about their likely diversification rate, population divergence time estimates are tentative. They are based on two significantly different rates, one traditional and widely applied (Brown *et al.* 1982) and one homeotherm-specific that accounts for rate heterogeneity among sites and is faster (Arbogast & Slowinski 1998). The latter has the practical advantage of bringing evolutionary processes closer to the present and thereby permitting their association with better documented past geological and climatological events, but unfortunately it is not possible to guarantee that is the most adequate for genets.

As mentioned above, the present study apparently supports the status of monophyletic clade for the small-spotted genets. However, a striking result, which may seem in conflict with this assertion, was the detection in some individuals of the *felina* subspecies, from the Cape and Orange Free State provinces in South Africa, of a highly divergent haplotype that clusters with, although significantly divergent from, *G. angolensis* haplotypes, a species mainly distributed in Angola and Zambia. The putative relevance of this finding is increased by a recent suggestion (Gaubert 2003b) that *felina* specimens are significantly different in their coat pattern from the other Southern African subspecies,

pulchra, supporting a specific status for *felina* in both morphological and genetic grounds. The real evolutionary and taxonomic importance of both the presence of an unexpected haplotype in some *felina* individuals and distinctive coat pattern can nevertheless be questioned. Among the nine specimens positively identified as *felina* included in this study, only two presented the haplotype in question. Furthermore, due to its adaptive value, coat pattern is known to be useful in the distinction of ecotypes and other within-species units but not to separate some closely related species in genets (Crawford-Cabral 1981b). The occurrence in *felina* of a haplotype related with the ones recovered in *G. angolensis* can also be explained by an ancient mtDNA introgression from *angolensis* into *felina*, during a period in which both forms were in contact, followed by divergence in the introgressed *felina* lineages. Introgression of mtDNA between related species with different morphologies and independent nuclear phylogenies has been reported before several times (e.g. Degnan 1993) and range shifts have been invoked in cases where the taxa are currently allopatric (Jones *et al.* 1995).

The haplotype was also found in still another different species, *G. tigrina*, which is broadly sympatric with *felina*, but the two taxa are different in their morphology and ecology (Gaubert *et al.* 2004). Occasional, or even extensive, hybridisation may occur between sympatric species, which remain distinct if ecologically segregated and/or genetically divergent enough, in spite of abundant introgression of neutral alleles (Harrison 1993; Goodman *et al.* 1999; Jiggins & Mallet 2000). Similarly to the instance here described, introgression is known to be usually unidirectional in many other hybridising organisms (e.g. Martinsen *et al.* 2001; Rohwer *et al.* 2001).

Finally, in terms of systematics and based in two independent attempts to relate genetic distance estimated from mtDNA with taxonomic rank (Johns & Avise 1998; Bradley & Baker 2001), the results of this study strongly support raising the status of one of the subspecies. Indeed, the degree of genetic divergence of the Arabian lineages, together with their detached placement in all haplotype genealogies, is indicative of a long independent evolutionary history of the Arabian isolate and, therefore, a specific status for *granti* may be warranted. The existence of two distinct subspecies of *G. genetta* in Southern Africa, as proposed in morphological and geographical grounds, is not supported by both estimates of neutral genetic distance and the absence of recognised phylogenetic partitioning, although this should be also assessed with rapidly-evolving nuclear markers (O'Brien & Mayr 1991). On the other hand, even if the whole of Southern Africa consists only of a single evolutionary unit, its geographical, ecological and genetic separation

justifies its treatment as distinct subspecies (Wilke & Pfenninger 2002), which should then be named *felina* as this name has priority over *pulchra*. The existence of at least one distinct subspecies in East Africa, *hararensis*, is another proposal derived from the findings presented here, but more samples throughout the region are needed to determine if the detected additional subdivision within East Africa is worth formal taxonomic recognition. The populations in Europe are clearly composed of a small fraction of the genetic diversity present in North Africa, corroborating the inclusion of both sides of the Mediterranean in the subspecies *genetta*. In contrast, the taxonomic relationships between *genetta*, *senegalensis* in West Africa, and *dongolana* in Central Africa, are difficult to interpret. Current geographical and ecological segregation is not coupled with significant genetic distance and severance of private portions of genetic diversity within the species, and the true meaning of this discrepancy can only be resolved with more samples and complementary genetic information (Bradley & Baker 2001).

5.2 Large-spotted genets

Phylogenetic reconstruction rooted with one related genet species, *G. angolensis*, recently identified as an older lineage within the same branch of the tree for the whole genus (Gaubert *et al.* 2004), suggested several patterns in the evolution of the large-spotted genets that could be linked with available biogeographical and palaeoecological information. Importantly, large-spotted genet haplotype genealogies support the proposal by Crawford-Cabral (1981b) of rainforest as the ancestral habitat for the group. Current genetic partitions often do not accurately reflect contemporary geographic structure, unless the latter has been stable for a reasonable amount of time to allow their coupling and blurring of previous genetic structures. This was evident in the finding that demes in the coastal forests of eastern Africa are genetically more similar to populations in the Congolese rainforest than to any other in the inland areas of eastern Africa. Such an unexpected result seems compatible with a scenario in which the contraction of an expanded rainforest, during a period of significant climatic deterioration, left isolates in islands of suitable habitat that diverged in allopatry and now constitute phylogenetic relicts (Fjeldsa & Lovett 1997; Grubb *et al.* 2003). Possibly, the lineages uncovered in specimens from the Bale Mountains in Ethiopia also constitute a biogeographic relict of a past extended rainforest block but in this case samples from throughout Ethiopia and southern Sudan are needed to assess this hypothesis. There is an ongoing discussion about the role

of rainforest refuges in processes of Pleistocene speciation, suggesting instead their relevance as survival centres for older lineages (Roy *et al.* 2001). Nevertheless, biogeographic hypotheses, involving refuges, developed to explain speciation might also (or instead) be relevant in understanding how within-species diversity may be generated, particularly if vicariance formation is cyclically recapitulated (Milot *et al.* 2000). In the case of the rusty-spotted genets, *G. "rubiginosa"*, no differentiation was detected within the area between the Albertine Rift and Nigeria and the pattern of genetic diversity is congruent with an ancient bottleneck followed by rapid demographic growth. This is compatible with a scenario of survival in a significantly reduced rainforest belt, during a glacial period, and population growth after environmental amelioration. The absence of differentiation among the haplotypes from the area indicates persistence in a single rainforest refuge, but the possibility for gene flow provided by gallery forest connections between refuges (Kellnan *et al.* 1994), allied with the high mobility of genets, does not rule out the hypothesis of multiple refuges. Shallow genetic differentiation and absence of reciprocal monophyly were revealed between the two sides of the Dahomey Gap, i.e. between *G. pardina* sensu lato and *G. "rubiginosa"*. This result is probably related with the fact that the Dahomey Gap has been an unstable biogeographical barrier and is permeable to animals with strong dispersal abilities and tolerant to the savannah habitat (e.g. Debruyne *et al.* 2003). A large-scale invasion of open habitats, such as woodland-savannah and grassland, associated with genetic diversification was inferred to have occurred south of the Congolese forest block with subsequent expansion to the whole of Southern Africa and into East Africa. The derived position of the savannah clade in the rooted phylogenetic trees suggests that the successful establishment of this radiation across a vast range was the most recent evolutionary event within the rusty-spotted genets. However, according with the divergence time estimates between haplotypes from the Congolese forest and the northern part of Southern Africa, the onset of this process apparently traces back to a date around the contraction of the forest belt that gave rise to the uncovered relicts in eastern Africa. The observed lack of geographic structure in the genetic variation of the savannah clade may be possibly due to a combination of stochasticity, introduced by the Namib and Kalahari expansions in glacial phases, in the dispersal and extinction of lineages in Southern Africa, colonisation constrained to mesic areas, and long-distance migration (Matthee & Robinson 1999; Simonsen *et al.* 1999). Recent or contemporary crossing of the Albertine Rift by migrants originated in the Congolese rainforest block was unveiled by the detection of lineage admixture with haplotypes of the savannah clade in an area

between the Albertine Rift and the Rift Valley. This area is likely to constitute a hotspot of genetic diversity due to secondary contact processes (Birungi & Arctander 2000). The results indicate that the expanding savannah clade has reached, probably in recent times, the Abyssinian region, as lineage mixing was also found among Ethiopian samples. The arrival to the Cape region of the savannah-adapted clade produced *G. tigrina*, the youngest taxon within the large-spotted genets with an origin estimated around the Middle-Late Pleistocene boundary. In spite of its low genetic divergence from *G. "rubiginosa"*, morphological differentiation is clear and ecological shift is probable as the habitats in the Cape coast are not savannah-like.

Within a taxonomic perspective, the findings of this study do not add additional support, in the form of reciprocal monophyly between clades or high levels of genetic distance, to previous studies (Crawford-Cabral 1981a,b; Gaubert *et al.* 2004) that proposed the subdivision of the large-spotted genets into three species. However, these results are not surprising if the suggested processes of speciation are viewed as ecologically-driven between parapatric units in which limited gene flow at the contact zones does not prevent adaptive divergence. The resolution of the postulated trichotomy is expected to require a large number of samples, particularly from the ecotonal contact zones, and analyses with multiple morphological and molecular markers. In contrast, the degree of genetic distance and the inferred long history of evolutionary independence of two isolates in the eastern African coast, one of them previously included within a different genet species (*G. mossambica*), from the remaining rusty-spotted genet populations, are compatible with a scenario of allopatric speciation. The relationship of the two demes needs nevertheless to be investigated with more samples from both populations and also from the geographic range connecting them. Regardless of their taxonomic rank, their relevance in terms of conservation biology is warranted considering that they represent relicts of a vanishing ecosystem (Lesica & Allendorf 1995). The savannah clade, a unit uncovered by the present study and which range encompasses the distribution area of several previously described subspecies of *G. "rubiginosa"*, constitutes a recent and derived evolutionary entity. The fact that this clade shows detectable phylogenetic partitioning and significant average genetic distance from any other rusty-spotted genet haplogroup suggests at least subspecific rank (O'Brien & Mayr 1991) and the need for additional investigation on its precise taxonomic status (Bradley & Baker 2001). The lack of differentiation within the whole region ranging from Nigeria to the western side of the Albertine Rift conforms to the existence of a single subspecies in the area, *fieldiana*, which should be also the name

used to designate from now on the rusty-spotted genets, in replacement of "*rubiginosa*". No support was found to the recently proposed expansion of geographical range and elevation of taxonomic status for *schoutedeni*, based on a single anatomical trait (Gaubert 2003a). This is hardly surprising, as it is becoming evident that if morphology and anatomy are to be relevant in taxonomic and phylogenetic studies they must consider character sets, thereby accounting for pleiotropy and developmental modularity, instead of analyses of atomised features (Leamy *et al.* 1999; Hlusko 2004).

5.3 Phylogeography, evolutionary divergence and speciation

Knowledge of genetic variation is critical to our understanding of population genetics, speciation and historical biogeography. One common approach to the study of genetic variation involves analysis of mitochondrial DNA collected at the level of geographic populations and interpreted in a genealogical or phylogenetic context. The resulting phylogeographies are the likely result of the superimposed effects on the evolution of species contributed by past and present biological and physical processes that established vicariance and promoted migration, two of the main biogeographic phenomena responsible for demographic fluctuations and population subdivision (Flagstad & Roed 2003). Molecular assessments of phylogeographic structure, in concert with information from the fields of palaeogeography and palaeoclimatology, are highly valuable for the reconstruction of evolutionary scenarios, demographic events and biogeographic processes (e.g. Fleischer *et al.* 2001; Feldman & Spicer 2002; Zheng *et al.* 2003).

Although difficult to accomplish, detailed examination of molecular variation within taxa presenting continental distributions offers opportunities to address the reality of polytypic species and gain insights into the geographical and/or ecological processes driving divergence when morphological differentiation is cryptic (Rodriguez-Robles & de Jesus-Escobar 2000; Omland *et al.* 2000; Leache & Reeder 2002). Obvious phenotypic diversification, particularly in morphological traits traditionally measured in taxonomic studies, may be absent when divergent radiation of allopatric populations is non-adaptive (i.e. not accompanied by relevant niche diversification) (Gittenberger 1991; Lessios *et al.* 2001). Even when morphological differentiation does indeed occur between demes it may de-coupled from genealogical divergence as measured by neutral markers such as mtDNA, as there are no biological reasons for a correspondence of evolutionary rates between the two processes (Fry & Zink 1998; Bromham *et al.* 2002). An increasing number of studies

seem to unequivocally demonstrate that mtDNA may be more powerful than morphology in revealing independent evolutionary clades within species complexes with uncertain and controversial partitions (e.g. Serb *et al.* 2001; Wiens & Penkrot 2002; Burbrink 2002). However, to equate such units with species it is a step further in the cataloguing of biodiversity that may not be warranted before independent sources of evidence converge to the same result (Templeton 1989; Moritz 1994; Sites & Crandall 1997). Since the evolutionary divergence resulting in the genetic isolation associated with speciation may be initially reflected upon a single type of character, the one directly involved in the segregation, the screening of several types of traits, such as genic, chromosomal, morphological, ecological, or behavioural, can only improve inference. The genetic basis of cladogenesis in terms of number of genes involved in genetic isolation, the distribution of their phenotypic effects, and their location in the genome, is still poorly understood (Coyne & Orr 1998). Additionally illustrative of how challenging it may prove to determine that speciation has occurred when differentiation is cryptic, is the difficulty in distinguishing between potential, ongoing and established genetic isolation and the use of concepts such as "incipient speciation" and "semispecies" (Liebers & Helbig 2002; Jockusch & Wake 2002). These same questions, and the subtle distinction between reproductive isolation and genetic cohesion (Templeton 1989) provided by internally coadapted genomes that remain independent even in the presence of limited gene flow (Hewitt 1993), are also central in the interpretation of the results from the present study. Within the *G. genetta* complex the Arabian deme displayed a substantial level of genetic divergence, which together with its apparently long history of geographic isolation, may suggest a case of either ongoing or established allopatric speciation driven by drift and possibly also by selection under a different environment (Gavrilets & Hastings 1996). Besides this finding and the detection of a highly divergent haplotype found in some individuals of the *felina* subspecies, the likely result of ancient introgression of the mtDNA from a different genet species into the *felina* genome, *G. genetta* appears to be essentially a single polytypic species, a pattern found in other continent-wide distributed species even with lower mobility (e.g. Starkey *et al.* 2003). Within *G. "rubiginosa"* a strong case for ongoing or consummated allopatric speciation of biogeographic relicts in coastal forests refuges of East Africa is derived from the mtDNA results. Furthermore, indication of a secondary diversification from rainforest habitat to savannah and grassland, judged as successful by the range size within the latter type of habitat, with admixture of haplotypes between the two ecotypes only detected in localised instances within areas where

geographical contact occurs, substantiates a scenario of parapatric ecological differentiation (e.g. Mercure *et al.* 1993). Parapatric speciation is expected when changes in the environment and selection for local adaptation overcoming the homogenising effects of gene flow act concurrently (Slatkin 1982). Invasions of new habitats may fuel rapid rates of adaptive segregation, often not reflected in neutral markers evolution, and lead to speciation via assortative mating or hybrid unfitness mechanisms (Losos *et al.* 1998; Orr & Smith 1998; Schluter 2001). The potential pace of selection-driven speciation is particularly compatible with the temporal instability that characterised both the environment and the ranges of species during the Pleistocene (Knowles 2001; Lister 2004). Whether the detected pattern within the rusty-spotted genets is developing into a speciation process remains to be assessed with additional samples and complementary types of phenotypic and genotypic markers. Similar processes of parapatric ecological divergence appear to explain the split between the three classically recognised taxa of large-spotted genets. The separation between *pardina* sensu lato and "*rubiginosa*" may have been promoted by the variable differential permeability to savannah-adapted and rainforest-adapted migrants associated with the cyclic opening and closing of the Dahomey Gap. This phylogenetic event is additionally complicated by the apparent diversification of the former in the rainforest-savannah mosaic of West Africa (Rosevear 1974; Gaubert 2003a). The differentiation of *tigrina* in the Cape Province of South Africa, a particularly distinct endemism hotspot in Southern Africa (Goldblatt 1997), from an ancestral "*rubiginosa*" stock that expanded from the contiguous Natal region also matches the profile of an instance of ecological parapatric speciation. The significant morphological divergence of *tigrina*, unparalleled by the genetic distance measured between the mtDNA haplotypes of the two taxa, and the existence of a narrow hybrid zone between *tigrina* and "*rubiginosa*" (Gaubert *et al.* in press) are compatible with an instance of parapatry between segregating taxa (Barton & Hewitt 1985; Hewitt 1989).

5.4 Comparative phylogeography, ecology and biogeography

Limited gene flow tends to preserve patterns of genetic variation that arose in the past, as the patterns are not blurred by contemporary gene flow (e.g. Pfenninger *et al.* 2003). In contrast, geographic patterns of genetic diversity may reflect, in a larger extent, recent or contemporary processes when organisms possess high mobility (e.g. Scariglia & Burns 2002). Within the latter scenario, the fact that current distribution areas may be poor

indicators of the geographical arrangement at the time of diversification events, as a result of historical range shifts with an extent and frequency usually unknown, is an additional difficulty for making inferences about past processes (Mattern & McLennan 2000; Losos & Glor 2003).

Nevertheless, by comparing the results, and biogeographic inferences derived from them, obtained for the two species complexes analysed here, within which individuals are assumed to have similar inherent dispersal abilities, the importance of the ecological requirements of each taxon in structuring their genetic variation is suggested (see Rueness *et al.* 2003). Small-spotted genets occur essentially in savannah, steppe, and semi-desert areas, in the latter being generally the sole genet and therefore free of competition with related species, whereas rusty-spotted genets are present in both forested and open habitats corresponding to one of the largest single continuous ranges among African mammals. It seems that possibly even more important than the biological asymmetries among organisms in their migration rates and distances, it is the versatility of habitats that they are able to colonise that determines how much of the current genetic variation is the product of past allopatric fragmentation throughout refuges. Indeed, the observed patterns of phylogeography, as depicted both in phylogenetic trees and haplotype networks, and sequence variation, as measured by summary statistics, indicate differences between small-spotted genets and rusty-spotted genets in their responses to changes in climate and habitat during the Pleistocene. Whereas *G. genetta* appears to have maintained multiple geographic populations across a large fraction of their present range, possibly by surviving in multiple allopatric savannah refuges during the coolest periods and secondarily adapting to drier habitats, the *G. "rubiginosa"* historic range appears linked to rainforest contraction/expansion. In the latter, adaptation to the savannah biotope allowed diversification associated with recolonisation of areas, made vacant with the previous rainforest contraction, as illustrated by the derived clades observed in Southern Africa, Tanzania and central Kenya. Habitat breadth is likely to facilitate gene flow, homogenising neutral genetic variation and reducing the fragmentation generated by variable environmental conditions, and all these factors were significantly inferred for the evolution of *G. "rubiginosa"*.

Concordance between inferences for the genet species studied here and co-distributed taxa analysed within similar frameworks elsewhere (e.g. Arctander *et al.* 1999; Flagstad *et al.* 2001; Debruyne *et al.* 2003) suggests that multiple sympatric organisms responded broadly in similar ways to topographic and climatic changes. Incongruences,

corresponding to departures from common refuge patterns, can be explained by differences in dispersal abilities, specific social systems, and man-driven unique colonisation events. However, the main reason for differences between species seems to be their capacity of adaptation to several contiguous alternative habitats, as in the case of the large-spotted genets, or of surviving in harsh environments, as illustrated by the small spotted genets in the fringes of the main African deserts and in the Arabian Peninsula.

The role of the Quaternary refuges for the survival and structuring of the biodiversity during the glacial cyclic periods in the continents of the northern hemisphere is well documented (Hewitt 2000; Willis & Whittaker 2000; Lister 2004). Although variations of common phylogeographic themes have been detected, mostly related with the inescapable fact that species have unique histories and different evolutionary responses to environmental challenges (Taberlet *et al.* 1998), it is clear that extensive ice sheets are equally unsuitable for many plants, invertebrates and vertebrates. This is true regardless of the dispersal abilities, eco-physiological resilience, life history traits, or phenotypic plasticity, as illustrated by the similar biogeographic patterns in Europe between bears, newts, hedgehogs, shrews, grasshoppers and oaks (Hewitt 1999). Nevertheless, it is likely that the climatic severity, which affected the different continents during the glacial phases, would have been variable and conforming to the latitude, size, and physiography of the landmasses. For instance, orographic features may explain the different impact of the Pleistocene glaciations in the extent of iced landscape and hence in the phylogeographic structure of North American and Eurasian biodiversity (Bernatchez & Wilson 1998; Fedorov & Stenseth 2002).

In spite of being recently questioned for South America (Colinvaux *et al.* 2000; Lessa *et al.* 2003) the importance of different types of Pleistocene refuges, accordingly with habitat type and altitude, is also amply documented in Africa (Mayr & O'Hara 1986; Vrba *et al.* 1995; Fjeldsa & Lovett 1997) and Australia (Schneider *et al.* 1998). However, in contrast with the scenario in the northern continents, the glacial periods did not generate in Africa the same extension of areas hostile to the majority of living organisms and, perhaps even more important, allowed the persistence of a greater diversity of life-supporting habitats (Adams & Faure 1997). The presence of large and long river basins, such as the Congo and Niger, possibly provided regional hydrographic moderation, in a similar way to which apparently the Amazon acted in South America, of arid periods. It may also explain why rainforest refuges seem to have a smaller relevance than previously thought, and particularly in comparison with montane habitats and isolated forest patches,

in the maintenance of allopatric phylogenetic relicts along the Quaternary (Roy 1997). The combination of a larger territory free of desert conditions, in contrast with the dominant cover of ice and permafrost in the northern continents, and the persistence of biotope gradients connected by ecotones probably meant lowered extinction of demes in the wake of suitable habitat contraction and increased opportunities for ecological radiations in parapatry (Rainey & Travisano 1998).

5.5 Future directions

The work described here provides a stimulus for further work in at least five different arenas. First, genet populations that were not included, or are poorly represented, should be sampled in order to investigate if they support biogeographic and evolutionary hypotheses that are proposed and/or suggest alternative or additional undetected patterns. In relation to the large-spotted genets, samples corresponding to a comprehensive geographic coverage of countries such as Tanzania, Ethiopia, Sudan, DRC, Congo Republic, and the area between Ghana and Senegal, would be very relevant. For the small-spotted genets samples from Zambia, Tanzania, Ethiopia, Sudan and more samples from the Sahel to western Africa and the Maghreb are the most critical to derive a clearer and more reliable picture of the phylogeography of this species complex. Second, type material from the several forms considered by Crawford-Cabral (1981a) as subspecies belonging to *G. genetta* or to *G. "rubiginosa"*, as well as of *mossambica*, is needed to allow taxonomic proposals to have formal value, particularly in the cases when elevation to the species rank was hypothesized. Third, the observed haplotype in some *felina* specimens, which is highly divergent from any other *G. genetta* haplotype including the ones found in individuals also identified as *felina*, is genetically closer to the mtDNA of a different species, *G. angolensis*, and was detected yet in another species, *G. tigrina*. The striking haplotype, although resembling more the ones found in *G. angolensis* than of any other genet taxon is nevertheless quite distinct from them (genetic divergence around 4%) and whereas *felina* and *tigrina* are sympatric both of them are allopatric with *angolensis*. A narrow contact zone between *tigrina* and *"rubiginosa"* in the Natal province of South Africa has also been reported (Pringle 1977; Gaubert *et al.* in press) and a comparable instance at the Dahomey Gap between *pardina* sensu lato and *"rubiginosa"* is suspected (Fernandes *et al.* in prep.). This suggests the occurrence of several, both in the past and present, hybridisation events between genet species in Africa, but a more detailed reconstruction of the history, spatial

architecture and implications of these phenomena in the genome and evolution of the involved taxa, requires a multiple approach, both in terms of disciplines and methods. For instance, fieldwork would be needed to carry out habitat analysis and measurement of local densities, distributions and dispersal rates of hybridising taxa in the areas where introgressed individuals were sampled. Fourth, the evolutionary and biogeographic histories for the two species complexes, as well as the introgression processes detected within them, reconstructed in this study with basis on mtDNA, should also be assessed with modern morphological methods (Dobigny *et al.* 2002; Nicola *et al.* 2003) and with nuclear DNA markers (Hare 2001; Zhang & Hewitt 2003). The areal approach in terms of grouping the samples accordingly with their geographic area of origin, which is equated to a population or geographic race, was followed in this study. This approach is dominant in the phylogeographical literature and, as attempted here, when the subdivision of the species' distribution area is carefully based on ecological or physical discontinuities and/or with the aim of testing previous taxonomies it can be indeed rewarding in their results. However, a cline analysis of both molecular and morphological data may prove to be the only way of determining if true discontinuities exist between parapatric subspecies or sister species (Waters *et al.* 2001; Salomon 2002; Pfenninger *et al.* 2003) and to examine hybrid zones (Barton & Gale 1993; Hewitt 1993), unless the hybrids possess a mosaic distribution across a patchy landscape (Harrison & Rand 1989). Although with the associated difficulty of demanding large numbers of samples from specific locations, the analysis of character gradients across contact zones could be a particularly useful next step in the evolutionary study of the large-spotted genets. As suggested by all previous work and confirmed by the results presented here, the geographic and ecological relative influences in the diversification of this widespread, successful and versatile group of carnivores have proven difficult to quantify with other approaches. The recovered pattern of isolation by distance in their mtDNA variation is often a significant indicator of clinal variation along environmental gradients (Doebeli & Dieckmann 2003). Finally, the study of other widely distributed cryptic carnivores in Africa with a similar phylogeographic framework to the one employed here is likely to allow assessing biogeographic hypotheses derived from the geographical distribution of genet haplotypes and to recover additional unsuspected patterns. For instance, good candidate species would be, with different ecological requirements and evolutionary trajectories by belonging to different genera and families, the African civet, *Viverra civetta*, the Egyptian mongoose, *Herpestes ichneumon*, the ratel, *Mellivora capensis*, and the zorilla, *Ictonyx striatus*. It is probable that one of the main

reasons why the phylogeographic analysis of several pan-African small to medium sized carnivores, of which the four referred above are just a small representation, has not been attempted before is the extreme difficulty of obtaining a relevant number of samples from the field. The present study hopes to demonstrate that, although time and effort consuming, the use of museum samples might be the only available option to circumvent such obstacle and reaching the sample size and geographical scope required for continental phylogeographies.

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Appendices

Appendix I

Martinoli et *al.* (in press). Species richness and habitat use of small carnivores in the Arusha National Park (Tanzania). *Biodiversity and Conservation.*

Running head: Carnivores in Arusha National Park, Tanzania

Species richness and habitat use of small carnivores in the Arusha National Park (Tanzania)

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Abstract

The carnivore community of Arusha National Park, Tanzania, was monitored using live trapping and diurnal and nocturnal visual counts and recording of tracks and signs along line transects. Nine carnivore species were recorded. Hyena (*Crocuta crocuta*), the most common large species, leopard (*Panthera pardus*) and African civet (*Civettictis civetta*) were detected along line transects, while the smaller species were counted and/or captured. *Mungos mungo*, *Galerella sanguinea*, and *Genetta maculata* were the most prevalent at transect counts, while *Galerella sanguinea*, *Bdeogale crassicauda*, and *G. maculata* were most frequently captured. *Ichneumia albicauda* and *Ictonyx striatus* were captured at only a single site. Carnivores selected natural habitat types, multilayered forest, open shrubs and herbaceous savannah, and were absent, or tended to avoid disturbed (man-made) habitats. Species of which sufficient data were gathered seemed to behave as habitat generalists (*Crocuta crocuta*, *Galerella sanguinea, Genetta maculata, Mungos mungo* and *Ictonyx striatus*). The need for more detailed studies on small carnivore ecology and the importance of smaller protected areas for their conservation are discussed.

Key words: Arusha National Park, carnivore community, diet, habitat use, Tanzania.

Introduction

The conservation of natural landscapes and their wildlife in Africa is closely linked to the institution of protected areas, often as national parks. Many of these have been set aside from human activities for the protection of large herbivores and carnivores, or landscape conservation (Pressey, 1994; Caro, 2001). However, it is still unclear whether variation between landscapes and protected areas in species richness of the large mammal fauna is associated with variation in species richness, abundance and diversity of small to medium-sized terrestrial mammals (e.g. Keesing, 1998; Caro and O'Doherty, 1999). Species (or community) responses to disturbance vary among mammal species, often in relation to body size and home range size (e.g. Isabirye-Basuta and Kasenene, 1987; Laurance, 1991; Andrén, 1994; Decher and Bahrian 1999, Virgós and Garcia, 2002) and with the type, duration and degree of disturbance (Jeffrey, 1977; Andrén, 1994; Estrada, Coates Estrada and Merritt, 1994). Moreover, interspecific competition among African carnivores and killing and/or predation of smaller by larger carnivores can have marked effects on distribution, habitat use and/or abundance of non-dominant species (reviewed by Caro and Stoner, 2003).

The larger carnivores and herbivores are often lacking in relatively small protected areas prone to some degree of human disturbance and/or landscape fragmentation by agriculture activity or increasing human settlements (Gehring and Swihart, 2003). However, such areas might host important populations of small to medium sized mammals, either granivores or herbivores (rodents), and carnivores. In the past two decades, several studies have investigated rodent communities and/or dynamics in parks and surrounding agriculture habitats (see above) also in relation to crop-damaging rodents (e.g. *Mastomys natalensis*, Leirs, Verhagen and Verheyen, 1994; Leirs et al., 1996). But very little is known about small carnivore distribution and relative abundance in the mildly disturbed landscapes of smaller protected areas, probably because studying these illusive species can be difficult and time consuming.

One such area is Arusha National Park, located near the Arusha Township, northern Tanzania, which covers 137 km^2 and lies in an area of a strongly increasing human population, where the need for land is high. Here we present the results of a survey of distribution, habitat use and relative abundance of small, and large, carnivores which allowed us to evaluate species richness of the carnivore community in and around the park, as part of a broader study, aimed to obtain unbiased estimates of local biodiversity. In a further step, the ecological parameters linked with carnivore distribution will be analysed in function of landscape and species conservation for future use by the park and wildlife managers. Two methods were

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used for monitoring carnivores: line transect counts for large carnivores; and capture-mark-recapture using live-traps for small carnivores.

Material and methods

The Study site

Arusha National Park is the core of Mount Meru (36°45'E - 3°15'S) natural system, Tanzania (Fig. 1: DAM ADD MAP). Rainfall and temperature data show two rainy seasons: the short rains of November-December and the long rains of February-June (rainfall on the lakes approximately 1000 mm *per* year, semi-arid grassland and savannah at lower elevations 250-500 mm *per* year). The park boundaries are delimited by rural villages, and some Masaai settlements, while a quarry for the extraction of phosphate close to Minjiingu is an important industrial site near the study area. Agriculture is becoming more and more intensive, with cultivations of beans and corn, extracting land from wild fauna and cattle. Traditional lifestock raising by the Masaai (sheep and goats) is decreasing and important revenue comes from organised hunting (Mount Meru Conservation Project, 2002). No human activity is allowed within the Park, apart from tourism, which is concentrated on alpinism at Mount Meru and guided tours for viewing wildlife (Mount Meru Conservation Project, 2002). Hence, tourism is considered to create little or no disturbance to vertebrate communities.

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The study area comprised Arusha National Park and a 10 km buffer zone around its boundaries. The landscape is very heterogeneous, and we determined 13 different habitat types. Shrubland (open shrubs, 17.5% of available habitat, closed shrubs 0.8%, shrubs on temporarily flooded land 0.03%), forest (multilayered forest 12.1%, closed forest 1.2%, open forest 0.9%) and herbaceous dry (17.5%) or temporarily flooded savannah (0.7%) are the major natural habitats. Bare land (0.8%) associated with vulcano peaks and water bodies (0.2%) are scarce. Scattered agricultural land and shrub crops (41.6%) and forest plantations or tree crops (14.5%) are the most important mildly disturbed and altered habitats, while urban, rural and industrial areas comprise only 0.13% of the study area.

Line transect counts

Line transects were distributed across the various habitat types, proportionally to their relative abundance within the study area (Fig. 1). Transect counts were carried out to collect data of observations, signs and tracks of large carnivore species, and to investigate their habitat use, and relative abundance. Five transects, each 8 km long, were surveyed by car, at a speed ranging between 5 and 15 km h^{-1}, between

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March and August 2001. Each transect was checked 10 times: for an entire week (five times) during day-time and for one week (five times) at night using spotlights. At each census, all carnivore species detected were recorded, and the locations were determined using a GPS. Additional sightings, tracks or signs of large carnivores were also used to increase sample size. In areas with a network of dustroads and paths, transect counts are a suitable method for determining the presence of medium-sized and large carnivores, such as aardwolf (*Proteles cristatus*), spotted hyena (*Crocuta crocuta*), lion (*Panthera leo*), leopard (*Panthera pardus*), cheetah (*Acinonyx jubatus*), serval (*Leptailurus serval*), caracal (*Caracal caracal*), black-backed jackal (*Canis mesomelas*), side-striped jackal (*Canis adustus*), bat-eared fox (*Otocyon megalotis*), and African wild dog (*Lycaon pictus*) (e.g. Sliwa and Richardson, 1998; Rasmussen, 1999).

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Capture-mark-recapture

Trapping was performed once at each of the 14 transect lines (5-6 traps, 200 m apart), situated in the different major habitat types of Arusha National Park, in July and August 2001. Single traps were also set near seven ranger posts. Each trapping session consisted of one week pre-baiting, using also rice to attract rodents, the carnivores' natural prey, followed by 3 to 5 nights trapping. Wooden cage traps (30 x 30 x 120 cm) baited with pieces of meat or chicken, were placed opportunistically in favourable micro-habitats for small carnivores to improve trapping success. The traps were checked early in the morning and shortly before dusk. When an animal was captured, it was transported, inside the trap, to the laboratory, where it was marked and handled with the help of a veterinary. Each captured animal was transferred into a smaller confinement cage where it was anaesthetized with ketamine (100 mg/ml, dose between 1-3 I.U. per 100g body mass), and the following data were recorded: species, sex, age (juvenile, subadult, adult), reproductive condition (anoestrus, pregnant, or lactating for females; testes position for males), head and body length (mm), tail length (mm), ear length (mm), anal-genital distance (mm), fore-foot length (mm), hind-foot length (mm), body mass (g), and tooth formula. Finally, of each captured carnivore, hair samples were collected, a skin biopsy was taken from the ear, and, if present, external parasites were collected. After manipulation, the animal was put back into the trap where it remained to recover completely from the anaesthetic. It was released at sunset near the place it had been trapped.

Skin biopsies were used for mitochondrial DNA analysis to determine animals of the genus *Genetta* at species level, since overlapping morphological variation between closely related genet species turns difficult an ascertained species ID in the field.

DNA was extracted from samples of ten different individuals using a commercial kit (DNeasy Tissue Kit, Qiagen) and following the manufacturer's protocol. Fragments, with an average size of 200 bp, of three mitochondrial regions (cytochrome b gene, ND-5 gene and control region) were amplified via PCR with genet-specific primers (Fernandes et al., in prep). Amplification took place in 25 µl (containing 2-5 µl of DNA) using Taq DNA Polymerase (Invitrogen) for one cycle in 94° C for 3 min, followed by 35 cycles in 94° C for 30s, 50-55° C (depending on the primer pair) for 45s and 72° C for 1 min. The final extension at the end of the profile was 72° C for 7 min. Negative controls were carried along the PCR reactions to detect contamination. Amplified PCR product was purified with the GeneClean Turbo Kit (Bio 101, Q-Biogene), following the manufacturer's instructions. Sequencing was carried out using the ABI Prism Dye Terminator cycle sequencing ready reaction kit (Perkin-Elmer) for ABI 377 DNA sequencer following the recommendations of the manufacturer. The sequences obtained were aligned, edited, and assembled, using Sequencher™ 3.0 (Gene Codes Corporation Inc.) software. Forward and reverse sequences were aligned for each individual. Replicates coming from different extractions and/or amplifications were performed for all individuals in order to authenticate the respective sequences.

Species identification of each genet specimen was achieved by sequence comparison with a comprehensive, both taxonomically and geographically, genet sequences database kept by one of us (C. Fernandes) and applying clustering algorithms implemented in the software PAUP version 4.0b10 (Swofford, 2002). Additionally, sequences were assessed against the available information for genets in GenBank, which in terms of mitochondrial DNA is currently restricted to the cytochrome *b* gene (Gaubert et al., 2004), using the program Blast (Altschul et al., 1997).

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Habitat use analysis

We used Ivlev's electivity index (Jacobs, 1974) to investigate habitat selection by the different carnivore species, including all records from captures and observations. For each species, the Ivlev's index E_i for habitat i is calculated with the formula: $E_i = (u_i - a_i) / (u_i + a_i)$, where u_i is the proportion of observations in habitat i (habitat utilised) of that species, and a_i is the proportion of habitat i available in the study area.

Diet composition

All carnivore faeces (scats) found were collected. The presence of guard hairs, ingested during grooming by most carnivore species, was compared with a reference collection and used to identify the species that

produced the scat. Diet analysis was carried out according to the method described by Kruuk and Parish (1981). Relative frequency of food item *a* (Fr_a) was calculated by dividing number of scats containing item *a* by total number of scats collected. Volume of food item *a* (Vm_a) was defined as the average volume of item *a* (sum of bulk-scores containing item *a* divided by total number of scats, Kruuk and Parish, 1981). Undigested remains in carnivore faecal samples were identified by comparison with reference collections made with specimens collected inside the study area. We relied on comparison with reference atlases (Debrot et al., 1982; Teerink, 1991) for hair identification of mammal at order-level, considering scale pattern and observing the composition and structure of the medulla in cross-section. Fruit consumption was determined based on the remains of undigested seeds and parts of the fruit cuticle appearing in the scats. Reference collections were used for identification of insect remains (order or family level) and seed or fruit remains.

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Results

DNA identification of genet specimens

Sequences were obtained for the three targeted genes and submitted to GenBank (accession numbers AY751466-AY751489). The ten sampled animals were determined as rusty-spotted genets - *Genetta maculata*, accordingly with the most recent nomenclatural proposal for this species, still highly debated and under revision by other experts in *Genetta* taxonomy (Gaubert et al., 2002; 2003).

Trapping and transect counts

A total of 218 records of nine different carnivore species were collected: 35 captures and 183 observations or signs (tracks) during transect counts. Leopard (3 observations), spotted hyena (68 observations) and African civet (*Civettictis civetta*, 3 observations) were only encountered during transect counts (Table 1). Thirty-one different individuals belonging to six species of small carnivores: one, the zorilla (*Ictonyx striatus*), belonging to the family *Mustelidae*, four species of the family *Herpestidae* (according to McKenna and Bell, 1997), and one rusty-spotted genet (*Genetta maculata* Gray, 1830; Gaubert et al., 2002; 2003), of the family *Viverridae*, were captured (Tables 1 and 2). Two animals, a zorilla and a bushy-tailed mongoose (*Bdeogale crassicauda*), were recaptured once, while a melanistic rusty-spotted genet was recaptured twice, for a total of 35 captures and recaptures in 337 trap-nights (overall trapping success 10.4%, Table 2). Trapping success [(number of captures/number of traps x

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nights) x 100] varied from 0% to 17% along the 14 transects, and from 0% to 100% at the ranger posts (Table 2).

The most common species, based on their frequency of occurrence among the trapped animals, were rusty-spotted genet (11 individuals), slender mongoose (*Galerella sanguinea*, 10 different animals), and bushy-tailed mongoose (5 individuals). Three zorillas, one banded mongoose (*Mungos mungo*) and one white-tailed mongoose (*Ichneumia albicauda*) were also captured. Rusty-spotted genets showed a marked sexual dimorphism, with males significantly larger and heavier than females (Table 3). This was not the case for slender and bushy-tailed mongoose (Table 3). Three species showed rare morphological anomalies. The anal glands were missing in all three zorillas, two adult males and a subadult male, that were captured at transect T6. All 10 slender mongoose (5 males, 5 females) showed asymmetry in tooth formula: the first upper premolar was present on only one side of the upper jaw. Finally, two *Genetta maculata* were melanistic.

Distribution and habitat preferences

The community of carnivores of Arusha National Park did not use the different habitats randomly, but showed strong avoidance for some habitat types, and positively selected others. We observed no signs of presence of carnivores in urban, rural and industrial areas, agriculture areas, plantations and bare land, while only one species, the slender mongoose, was found near water bodies (Table 1). Temporary flooded shrubland was avoided by all species, at least during the study period. Also closed shrub and closed forest resulted avoided by carnivores, with the exception of zorilla and spotted hyena (Table 1, Fig. 2). The habitat with the highest species-richness, thus used more than expected by chance by most species, was the multilayered forest: it was positively selected by all species except white-tailed mongoose, which was regularly observed in savannah (Table 1, Fig. 2). Also slender mongoose and leopard commonly used herbaceous savannah (Table 1, Fig. 2). Leopards, or their tracks, were also observed in open and multilayered forest (Table 1). Open forest was intensively used by rusty-spotted genet, slender mongoose and spotted hyena (Table 1, Fig. 2). Open shrubland was used more than expected by the majority of small carnivores, but apparently avoided by zorilla and leopard (Fig. 2, Table 1). The spotted hyena appeared to use most of the available, natural habitats, probably related to the species having large home ranges (Henschel and Skinner, 1991; Boydston et al., 2003).

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Food choice

We collected sufficient scats of *Genetta maculata* (n = 17) and slender mongoose (n = 20) for a quantitative analysis of their diet. (Table 4). Slender mongoose were primarily insectivorous, but took also frequently small mammals, in particular rodents. Insects belonging to the Scarabeidae were among the most important prey of slender mongoose, while remains of other insect groups occurred much less frequently (Table 4). Grass was frequently eaten but in small quantities and is probably not a real food source but serves to improve digestion. Fruits were eaten occasionally (Table 4). In contrast, genets had a more carnivorous diet, taking often rodents, and also regularly birds (Table 4). Insects are taken frequently, but their volume was less important than for mongoose: genets probably eat insects when they encounter them but do not actively search for insect prey, except maybe for Scarabeidae. Fruits are regularly consumed and in important quantities (Table 4). Thus, both species appear to be generalist carnivores, feeding on small mammals, birds and insects, but also regularly consuming fruits. Moreover, they were captured at (Table 2), and visited ranger posts to prey on chickens.

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Discussion

The conservation of the carnivore community of Arusha National Park faces two major problems: the limited size of the protected area where most natural habitats are still intact; and increasing human pressure on the areas immediately outside the park boundaries, causing loss and fragmentation of natural habitats. Nevertheless, for the moment, Arusha National Park still hosts a rich community of small carnivores. In the present study, four species of Herpestidae, one of Viverridae, and one of Mustelidae were captured using live-trapping. In addition, observations were made of two species of large carnivores: the leopard and the spotted hyena, and of the medium-sized African civet. Although the black-backed jackal was common in agriculture land and open shrubs around the park (Galanti and Kilewo, pers. obs.) it was not detected inside the park during the study period. No observations were made and no tracks or signs were found of aardwolf, or of African wild dog, although the latter has been signalled before in Arusha NP (Scheuerlein, Magotto and Schmidl, 1997). Since these species have a high probability of encounter with transect counts, we feel confident they are absent from the park and the surrounding man-made habitats that were sampled (McNutt, 1996; Sliwa and Richardson, 1998; Rasmussen, 1999).

Species richness of small carnivores in Arusha National Park was comparable to other protected areas (Waser, 1980; Maddock and Perrin, 1993; Ray and Sunquist, 2001; Admasu et al., 2004a, b). Leopards

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and hyenas, the latter common in Arusha National Park, feed mainly on medium-sized to large mammals, and thus will have little effect on viverrids and mustelids. Serval, caracal and canid predators, that might directly kill smaller carnivores (Caro and Stoner, 2003), were considered absent, since no signs of their presence had been recorded during the past 10 years (Galanti unpublished data). In general, species-richness of the Herpestidae-Viverridae clade is not related to their (small) body size (Gittleman and Purvis, 1998). Thus, more detailed studies will be necessary to test whether species richness of these small carnivores is actually related to the absence of canids and medium-sized felids.

Carnivores did not use all landscape types in the Park, but clearly avoided all man-disturbed landscapes that are mainly situated just outside the park boundaries. This avoidance does not appear to be caused by direct human disturbance, but by alteration of the original habitat into landscapes that offer less shelter and, possibly, have a lower biomass (and diversity) of food resources. We must admit that the methods used to monitor carnivore habitat use, need to be interpreted with care, and current indices of habitat selection, or avoidance, might be related to differences in the probability of contact amongst different habitat types for at least some of the smaller species.

Three of the six small carnivore species that were captured and closely examined showed rare morphological anomalies. The anal glands were missing in all three zorillas, two adult males and a subadult male, which were also smaller (head - body length, and tail length) and weighed less than animals recorded in Zimbabwe (Smithers, 1983). All 10 slender mongoose (5 males, 5 females) showed asymmetry in tooth formula: the first upper premolar was present on only one side of the upper jaw. In other studies, this anatomical characteristic was documented to occur only rarely (9% of 181 skulls investigated by Skinner and Smithers, 1990). Also, mean body mass of females from Arusha National Park was comparable with that of females from Zimbabwe, but males from Arusha weighed less (on average 150g, see Smithers and Wilson 1979, for comparison). Finally, two rusty-spotted genets were melanistic. It is unclear why carnivores from Arusha National Park had some rare morphological traits. Although we did not take measurements on both sides of the body, the asymmetry in the number of premolars is indicative for the occurrence of fluctuating asymmetry (random deviations from perfect symmetry in bilaterally paired traits), which has been shown to increase with environmental and/or genetic stress (Palmer and Strobeck, 1986; Lens and van Dongen, 1999).

As in other parts of their range, slender mongoose in Arusha National Park mainly preyed upon small mammals and Scarabeidae, the latter easily found on or near dung of large herbivores, but, in contrast with some literature data, did not show a preference for snakes (Taylor, 1975; Smithers and Wilson, 1979).

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Likewise, the generalist diet of rusty-spotted genet, with primarily rodents, some birds and insects, but also fruits, has been found in most studies of the genus *Genetta* (Smithers and Wilson, 1979; Virgos, Llorente and Cortes, 1999; Rosalino and Santos-Reis, 2002).

The data presented here indicate that the small carnivores occurring in Arusha National Park strongly select the undisturbed and protected natural habitats within the park boundaries, in particular the multilayered and open forests, open shrubs and herbaceous savannah. They seem to avoid the nearby mildly disturbed areas, such as scattered agricultural fields and crops, man-made plantations and tree crops, although some rodent species, and thus potential prey biomass, might be more common there (e.g. Caro, 2001). Although crepuscular or night-active, the small carnivores might select hunting habitats more in function of available shelters than of prey biomass. Therefore, projects including the near Mt. Meru Forest Reserve and Ngurdoto Forest Reserve to safeguard the forest habitats and species will be important for long-term conservation of the carnivore community.

We recommend that future studies should combine capture-mark-recapture methods of both carnivores and their prey (rodents, some important insect orders) in the different natural (undisturbed or slightly disturbed) and altered habitat types that occur in and around protected areas. Such monitoring should be combined with measurements of asymmetry of phenotypic traits, and intensive radio-tracking of some target species to obtain a better knowledge of their habitat use and home range size in relation to landscape structure and composition (e. g. Admasu et al., 2004a, b).

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Table 1. Total number of contacts (captures, observations, signs, tracks) per species and habitat (between brackets) and the percentage of each single species within each of the habitat types.

Habitat type	*B. crassicauda*	*C. civetta*	*C. crocuta*	*G. sanguinea*	*G. maculata*	*I. albicauda*	*I. striatus*	*M. mungos*	*P. pardus*
Multilayered forest (68)	85.7	33.3	22.1	37.0	19.4		44.4	37.9	33.3
Open forest (20)			14.7	11.1	16.1	25.0			33.3
Closed shrub (5)			2.9				33.3		
Open shrub (96)	14.3	66.7	46.5	18.5	51.6	25.0	11.1	56.1	
Herbaceous savannah (26)			11.8	25.9	12.9	50.0	11.1	4.5	33.3
Scattered agriculture (1)								1.5	
Water bodies (2)				7.4					
N. of contacts/species	7	3	68	27	31	4	9	66	3
Captures	6	0	0	10	13	1	4	1	0
Observations	1	3	68	17	18	3	5	65	3

No contacts in: closed forest, shrubs on temporarily flooded land, herbaceous savannah on temporarily flooded land, forest plantations and tree crops, urban, rural or industrialised areas, and bare land.

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Table 2. Trapping effort, trapping success and species captured at 14 transect lines and 7 ranger posts.

Transect	Trap-nights	Number of captures (% trapping success)	Species captured
T1	15	0 (0%)	
T3	15	1 (7%)	*Genetta maculata*
T4	15	1 (7%)	*Genetta maculata*
T5	15	0 (0%)	
T6	25	4 (16%)	*Ictonyx striatus*
T7	30	3 (10%)	*Mungos mungo*
			Genetta maculata
T8	30	2 (7%)	*Galerella sanguinea*
T9	30	3 (10%)	*Galerella sanguinea*
T10	24	0 (0%)	
T11	24	2 (8%)	*Galerella sanguinea*
			Ichneumia albicauda
T12	24	2 (8%)	*Genetta maculata*
			Bdeogale crassicauda
T13	24	4 (17%)	*Genetta maculata*
			Bdeogale crassicauda
T14	24	2 (8%)	*Bdeogale crassicauda*
			Galerella sanguinea
T15	24	4 (17%)	*Genetta maculata*
			Bdeogale crassicauda
			Galerella sanguinea
Ranger posts			
T99	5	0 (0%)	
Resthouse	1	1 (100%)	*Genetta maculata*
T98	2	1 (50%)	*Genetta maculata*
T97	2	2 (100%)	*Genetta maculata*
			Galerella sanguinea
T96	2	2 (100%)	*Genetta maculata*
			Galerella sanguinea
T95	4	0 (0%)	
T94	2	1 (50%)	*Genetta maculata*

Table 3. Sex, age-category and body measurements of mall carnivores captured at Arusha National Park.

Sex M = male, F = female; age J = juvenile, S = subadult, A = adult. The trapping area is located in UTM zone 37M.

Species	Sex	Age	Head-body length (mm)	Tail-length (mm)	Foot-length (mm)	Body mass (g)
Genetta maculata	M	S	480	355	79.9	1160
	M	A	460	365	83.3	1900
	M	S	415	315	77	1000
	M	A	490	410	75.3	1310
	M	J	380	400	72.6	710
	M	J	470	390	76.3	1150
Males (mean ± SD)			449 ± 43	373 ± 35	77.4 ± 3.7	1205 ± 397
	F	A	470	380	75.5	1100
	F	J	390	395	72.1	910
	F	J	390	345	57.5	510
	F	J	360	340	63.9	550
	F	A	440	400	74.8	1000
Females (mean ± SD)			410 ± 44	372 ± 28	68.8 ± 7.8	814 ± 268
Mungos mungo	F	J	285	140	68.7	410
Galerella sanguinea	M	J	260	180	55.2	320
	M	A	325	245	57.6	500
	M	A	335	315	57.2	520
	M	A	345	220	61.4	550
	M	S	330	310	58.8	510
Males (mean ± SD)			319 ± 33	254 ± 58	58.0 ± 2.3	480 ± 91
	F	A	330	270	55.8	450
	F	S	265	235	53.4	320
	F	A	250	305	57.2	550
	F	J	320	315	59.3	500
	F	A	280	240	55.2	470
Females (mean ± SD)			289 ± 35	273 ± 37	56.2 ± 2.2	458 ± 86
Ichneumia albicauda	F	J	450	410	111	1900
Bdeogale crassicauda	M	A	420	240	77.3	1600
	M	A	410	220	78.8	1120
	M	A	390	230	77	1100
Males (mean ± SD)			407 ± 15	230 ± 10	77.7 ± 1.0	1273 ± 283
	F	A	385	220	74.6	1100
	F	A	380	240	75.2	1500
Females (mean ± SD)			383 ± 4	230 ± 14	74.9 ± 0.4	1300 ± 283
Ictonyx striatus	M	J	300	165	56.2	600
	M	A	300	170	56.6	550
	M	A	310	175	56.6	550
Males (mean ± SD)			303 ± 6	170 ± 5	56.2 ± 0.2	567 ± 29

Table 4. Diet composition of slender mongoose and rusty-spotted genet based on prey remains determined from scats. Prey remains determined at different taxonomical levels.

	Slender mongoose		Rusty-spotted genet	
Prey species (group)	Relative frequency (Fr%)	Mean volume estimates(Vm%)	Relative fequency (Fr%)	Mean volume estimates (Vm%)
Mammals	24.3	38.0	25.6	38.0
Insectivora	2.4	3.0	2.5	3.0
Rodentia	14.6	15.5	15.3	38.0
Undetermined	7.3	15.5	7.6	15.5
Birds	9.7	3	17.9	15.5
Gallus domesticus	9.7	3	5.1	15.5
Other species	-	-	12.8	15.5
Insects	36.5	63	33.3	15.5
Ortoptera	4.8	3	2.5	0.5
Coleoptera	29.2	38	14.5	15.5
Tenebrionidae	-	-	2.5	0.5
Scarabeidae	29.2	38	12.8	15.5
Dermaptera	2.4	3	-	-
Heteroptera	-	-	5.1	3.0
Hymenoptera	-	-	2.5	0.5
Formicidae	-	-	2.5	0.5
Undet. Insects	-	-	5.1	3.0
Mollusca	4.8	0.5	-	-
Gasteropodidae	4.8	0.5	-	-
Vegetative matter	24.3	3.0	23.0	15.5
Fruits	9.7	3.0	12.8	15.5
Graminaceae	14.1	3.0	10.2	0.5

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Fig. 1. Study area.

Fig. 2. Ivlev's electivity index indicating habitat selection (positive values) or avoidance (negative values) for six carnivore species. Species-code: bde_cra = *Bdeogale crassicauda*, cro_cro = *Crocuta crocuta*, gal-san = *Galerella sanguinea*, gen_mac = *Genetta maculata*, ict_str = *Ictonyx striatus*, mun_mun = *Mungos mungo*. Multilayered forest = dark shaded bars; open forest = grey shaded bars; closed shrubs = hatched bars; open shurbs = dotted bars; herbaceous savannah = open bars.

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Appendix II

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Biological Journal of the Linnean Society, 2004, **81**, 589–610. With 3 figures

Genets (Carnivora, Viverridae) in Africa: an evolutionary synthesis based on cytochrome *b* sequences and morphological characters

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The taxonomy of the genets (genus *Genetta*) has long been discussed, thus hampering endeavours towards evolutionary reconstruction. Sequence data from the complete cytochrome *b* gene (cyt *b*) were generated for 50 specimens representing 15 morphological species in order to allow the production of the first exhaustive molecular phylogeny of the genets. Second, a revised morphological matrix comprising 50 characters was combined with the cyt *b* data to estimate the level of morphological homoplasy. Phylogenetic analyses were conducted using parsimony, maximum likelihood and Bayesian procedures. Our results based on cyt *b* contradict a part of the traditional taxonomy of genus *Genetta*, the servaline and small-spotted genets being paraphyletic, but confirmed the species status recently re-investigated for three genets belonging to the large-spotted complex, including the newly described *G. bourloni*. The combined analysis yielded similar results although morphological characters were clearly homoplasic. Partitioned Bremer supports indicated conflicting signals between the two data sets throughout the tree, and species-diagnostic characters, useful for delimiting species boundaries, were significantly correlated to habitat. However, morphological data supported the monophyly of clades (*G. victoriae*, other genets) (*G. servalina*, *G. cristata*), large-spotted genet complex and forest forms. Our results suggest a complex evolutionary history of the genets in Africa, with a *Poiana*-like ancestor inhabiting rain forest, and then a diversification involving two independent invasions of open habitats and one reversion to rain forest. Divergence estimates based on cyt *b* revealed that splitting events within genets partly follow a climatic speciation model during the cyclical periods of the Quaternary, although 'primitive' rain forest lineages diverged earlier, during the Late Miocene and Early Pliocene. *Biological Journal of the Linnean Society*, 2004, **81**, 589–610.

ADDITIONAL KEYWORDS: ancestral estimate – correlated evolution – divergence time – evolutionary scenario – *Genetta* – maximum likelihood – morphology – phylogeny – rain forest refugia – speciation.

INTRODUCTION

Members of the genus *Genetta* Cuvier, 1816, the 'genets', are small-spotted carnivores belonging to the family Viverridae. They are restricted to sub-Saharan Africa, with one species (*Genetta genetta*) also occurring in the Arabian Peninsula, Maghreb and south-western Europe (introduced in the latter region; Morales, 1994; Amigues, 1999). Genets occupy a wide range of habitats, from rain forest to open savannah and grassland (Kingdon, 1997). The systematics and phylogeny of *Genetta* have long been debated. Interspecific boundaries are difficult to assess on the basis of 'traditional' morphological characters, i.e. craniometry and coat pattern (Gaubert, in press). The diversity in species number and genus subdivisions described in the major classifications of the last 150 years illustrates this difficulty. The most recent authority on the taxonomy of the genus (Wozencraft,

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1993) listed nine species grouped under three subgenera, namely: *Paragenetta* (*G. johnstoni*), *Pseudogenetta* (*G. thierryi* and *G. abyssinica*), and *Genetta* (*G. angolensis*, *G. genetta*, *G. maculata*, *G. servalina*, *G. tigrina*, *G. victoriae*). However, previous investigations on the genus considered a higher number of species (e.g. Wenzel & Haltenorth, 1972; Rosevear, 1974; Crawford-Cabral, 1981; Schlawe, 1981).

The most problematic taxon in terms of species boundaries delimitation is formed by the morphologically similar species of the large-spotted genet complex. Crawford-Cabral (1981) suggested that populations of the large-spotted genets be divided into three geographical entities corresponding to valid species: west of the Volta River (*G. pardina*), east of the Volta River (*G. 'rubiginosa'*; *G. maculata sensu* Gaubert, Veron & Tranier, 2002; Gaubert *et al.*, 2003a, b; Gaubert, 2003a) and the coastal area of South Africa (*G. tigrina*). This is partly conflicting with Coetzee (1977), Kingdon (1977) and Wozencraft (1993), but craniometric analyses (Crawford-Cabral & Pacheco, 1992) and morphology (Schlawe, 1981; Powell & Van Rompaey, 1998; Gaubert, Veron & Tranier, 2001; Gaubert *et al.*, 2002; Gaubert, 2003a) support the species status of the three taxa. In addition, Crawford-Cabral (1981), Crawford-Cabral & Pacheco (1992) and Crawford-Cabral & Fernandes (1999, 2001) suggested that *G. maculata* might constitute a super-species comprising several valid species. Taxonomic uncertainties mainly concern forest 'forms' recorded within and at the boundaries of the range of the complex, such as *genettoides* in the Upper Guinean Bloc and Dahomey Gap (Rosevear, 1974), *schoutedeni* in Upper Zaire (Crawford-Cabral, 1970) and *poensis* in rain forests from Liberia to Congo (Pocock, 1908; Rosevear, 1974; Crawford-Cabral, 1981). Two other cases of debated species concern (1) the very similar servaline genets *G. cristata* and *G. servalina* (the former being either synonymized or not with the latter; see, respectively, Hayman, in Sanderson, 1940; Wozencraft, 1993; and Rosevear, 1974; Crawford-Cabral, 1981; Van Rompaey & Colyn, 1998) and (2) the widely distributed small-spotted genet *G. genetta*, for which the subspecies *G. g. felina* has been sometimes attributed the status of species, but with contradicting distribution ranges (Roberts, 1951; Cabral, 1966; Schlawe, 1981; Honacki, Kinman & Koeppl, 1982; Harrison & Bates, 1991).

The phylogeny of the genets was recently addressed by an exhaustive morphological analysis (Gaubert *et al.*, 2002), but was confounded by outgroup rooting bias and possible use of adaptive morphological characters (coat pattern). The morphological tree excluded *G. johnstoni* from the clade of the other genet species, and suggested an ecological transition from the basal rain forest genets to the derived woodland and savannah species. However, preliminary phylogenetic analyses using cytochrome *b* (cyt *b*) (Gaubert *et al.*, in press) and nuclear DNA sequences (Gaubert & Veron, 2003), which focused on other phylogenetic issues among Viverridae, strongly support the monophyly of genus *Genetta* (i.e. including *G. johnstoni* and former genus *Osbornictis*).

Our study follows a work on the large-spotted genet complex based on the combination of new diagnostic morphological characters (Gaubert, 2003a), which discriminated one new species (*G. bourloni*), re-erected *G. poensis* as a valid species and suggested a possible species status for *G. 'schoutedeni'*. In order to test for the validity of traditional and newly established morphological species in a phylogenetic framework, we present a phylogenetic analysis based on complete cyt *b* sequences from an exhaustive taxonomic sample set, involving DNA extracted from museum specimens, which represents almost all the morphological species of genus *Genetta* (50 specimens, 15 species). Second, cyt *b* and revised morphological characters are combined in a cladistic analysis, and the obtained phylogenies are used to describe for the first time an evolutionary history of the genus *Genetta* in Africa.

MATERIAL AND METHODS

TAXONOMIC SAMPLING

Nucleotide sequence variation was examined for 50 specimens representing 15 morphological species of genets (only the species *G. abyssinica* was not included; Table 1). The molecular tree was rooted with two other Viverridae, *P. richardsonii* (African linsang) and *Viverricula indica* (small Indian civet). In order to check for putative contamination (Hassanin & Douzery, 2000), nuclear copies (*Numt*; Zhang & Hewitt, 1996; Bensasson, Zhang & Hewitt, 2000) and chimeric sequences, we sequenced at least two individuals per species when possible. Replicates of different DNA extractions and/or amplifications were performed for all individuals in order to authenticate the respective sequences. The majority of the samples comprised tissue, hair or cell lines stored in 70% ethanol at –4°C. We also used canine roots, dry palatine connective tissues and small pieces of skin from 16 museum specimens, especially in the case of some crucial taxa that were not represented by fresh samples (*G. cristata*, *G. piscivora* (Gaubert *et al.*, in press), *G. angolensis*, *G. poensis*, *G. bourloni* and *G. 'schoutedeni'*).

Morphological characters were re-examined following the survey of more than 4000 specimens belonging to major European and South African museums and newly collected specimens in the Muséum National d'Histoire Naturelle of Paris (see Gaubert, 2003a, b). From this exhaustive material, the previous morpho-

Table 1. Sample set of taxa included in this study, with GenBank accession numbers, type of material and countries of origin. Numbers in square brackets correspond to museum collection catalogue numbers (KM: Amathole Museum (formerly Kaffrarian Museum), King William's Town, RSA; MNHN: Muséum National d'Histoire Naturelle, Paris, France; MRAC: Musée royal d'Afrique Centrale, Tervuren, Belgium; MVZ: Museum of Vertebrate Zoology, Berkeley, USA; NMB: National Museum, Bloemfontein, RSA; SBPUR1: Station Biologique de Paimpont, Université de Rennes 1, France)

Species	GenBank accession no.	Material	Locality
Viverricula indica 1	AY241890	hair	Taiwan
Poiana richardsonii 1	AY241891	tissue	Gabon
Genetta johnstoni 1	AF511051	hair	Ivory Coast
Genetta johnstoni 1	AY241892	hair	Ivory Coast
Genetta thierryi 1	AF511052	tissue	Mali
Genetta thierryi 1	AY241893	hair	Nigeria
*Genetta victoriae** 1	AY241894	tooth	Democratic Republic of Congo
Genetta servalina 1	AF511053	tissue	Gabon
*Genetta cristata** 2	[MRAC 95-53-M-2]/AY241887	skin	Nigeria
*Genetta cristata** 2	[MRAC 98-49-M-20]/AY241888	skin	Nigeria
*Genetta piscivora** 1	[SBPUR1 Z2544]/AF511050	tooth	Democratic Republic of Congo
*Genetta angolensis** 2	[MVZ 118448]/AY241882	skin	Zambia
*Genetta angolensis** 2	[MVZ 118449]/AY241883	skin	Zambia
Genetta genetta 1	AF511054	hair	France
Genetta genetta 1	AY241905	tissue	France
Genetta genetta 1	AY241907	hair	Spain
Genetta genetta 1	AY241922	hair	Algeria
Genetta genetta 1	AY241908	hair	Republic of South Africa
Genetta genetta 1	AY241909	hair	Republic of South Africa
*Genetta genetta** 2	[KM 27708]/AY241879	skin	Republic of South Africa
Genetta genetta 1	AY241902	tissue	Saudi Arabia
Genetta genetta 1	AY241904	tissue	Oman
Genetta genetta 1	AY241911	tissue	Mali
Genetta genetta 1	AY241903	hair	Republic of South Africa
Genetta genetta 1	AY241906	hair	Namibia
Genetta genetta 1&2	AY241910	tissue	Republic of South Africa
*Genetta genetta** 2	[NMB 8544]/AY241886	skin	Republic of South Africa
Genetta pardina 1	AY241895	hair	(unknown)
Genetta pardina 1	AY241896	tissue	Liberia
Genetta maculata 1	AY241901	tissue	Burundi
Genetta maculata 1&2	AY241917	tissue	Republic of South Africa
Genetta maculata 1&2	AY241913	tissue	Republic of South Africa
Genetta maculata 1&2	AY241919	tissue	Republic of South Africa
Genetta maculata 1&2	AY241912	tissue	Lesotho
Genetta maculata 1&2	AY241920	tissue	Republic of South Africa
Genetta maculata 1&2	AY241914	tissue	Republic of South Africa
Genetta maculata 1&2	AY241921	tissue	Republic of South Africa
Genetta maculata 1&2	AY241915	tissue	Republic of South Africa
Genetta maculata 1&2	AY241918	tissue	Republic of South Africa
Genetta maculata 1&2	AY241916	tissue	Republic of South Africa
*Genetta tigrina** 2	[KM 31276]/AY241881	skin	Republic of South Africa
*Genetta tigrina** 2	[NMB 4470]/AY241884	skin	Republic of South Africa
Genetta tigrina 1&2	AY241876	cell lines	Republic of South Africa
*Genetta tigrina** 2	[NMB 4786]/AY241885	skin	Republic of South Africa
*Genetta tigrina** 2	[KM 31185]/AY241880	skin	Republic of South Africa
Genetta tigrina 1&2	AY241877	cell lines	Republic of South Africa
Genetta tigrina 1&2	AY241878	cell lines	Republic of South Africa
Genetta tigrina 1&2	AY241889	cell lines	Republic of South Africa
*Genetta poensis** 1	[MNHN 1894-263]/AY241897	connective tissue	Congo
*Genetta bourloni** 1	[MNHN 2001-1156]/AY241898	salted skin	Guinea
*Genetta 'schoutedeni'** 1	[MNHN 2001-1158]/AY241899	skin	Congo
*Genetta 'schoutedeni'** 1	[SBPUR1 Z1588]/AY241900	tooth	Democratic Republic of Congo

*Material from museum specimens.
1 sequenced by P.G., 2 sequenced by C.A.F.

logical matrix produced by Gaubert *et al.* (2002) was redefined. Modifications are given in Appendix 1. The final matrix comprises 17 taxa and 50 characters (Appendix 2), distributed as: skull (11), dentition (6), body proportion (1), pads (1), teats (1), hair ultrastructure (4) and coat (26). The morphological tree was rooted with *P. richardsonii* because it has been proposed as the sister-species of genus *Genetta* (Gaubert *et al.*, in press). The use of the single *P. richardsonii* as outgroup allows us to avoid the rooting bias encountered in Gaubert *et al.* (2002) when using the terrestrial civets, which have acquired by convergence similar coat pattern to the genets. The ingroup is divided into 16 morphological species on the basis of Crawford-Cabral (1981)[1], Wozencraft (1993)[2], Van Rompaey & Colyn (1998)[3] and recent investigations of Gaubert (2003a)[4] and Gaubert *et al.* (in press)[5]: *G. johnstoni*[1,2], *G. thierryi*[1,2], *G. abyssinica*[1,2], *G. piscivora*[5], *G. victoriae*[1,2], *G. servalina*[1,2,3], *G. cristata*[3], *G. angolensis*[1,2], *G. genetta*, *G. felina* (see Discussion), *G. pardina*[1,4], *G. maculata*[1,4], *G. tigrina*[1,2,4], *G. poensis*[1,4], *G. bourloni*[4] and *G. 'schoutedeni'*[4].

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Sequences were obtained in two different laboratories (see Table 1), namely the Service Commun de Systématique Moléculaire, Muséum National d'Histoire Naturelle, Paris (P.G.), and the Biodiversity and Ecological Processes Group, Cardiff School of Biosciences, Cardiff University (C.A.F.). Parameters used by P.G. for the preparation of total genomic DNA, PCR and automated sequencing are described elsewhere (Kocher *et al.*, 1989; Veron & Heard, 2000; Gaubert *et al.*, in press). For tooth samples, DNA was extracted in a physically isolated box solely dedicated to 'ancient' DNA and using a modified protocol from Hardy *et al.* (1994). DNA from museum material was amplified using a set of 20 specific primers producing overlapping fragments. When running PCRs from DNA extracts of collection specimens, a less stringent *Taq* polymerase (*Q Bio Taq*) and a higher number of cycles (from 40 to 50) were used. C.A.F. extracted DNA from samples of museum skins using a commercial kit (DNeasy Tissue Kit, Qiagen) and the protocol described by Mundy, Unitt & Woodruff (1997). Overlapping fragments from the cyt *b* gene were amplified via PCR with a set of 15 specific primers described in Table 2. Reaction parameters for amplification were as follows: one step at 94°C for 3 min, followed by 45–50 cycles at 94°C for 30 s, 50–55°C (depending on the primer pair) for 45 s, and 72°C for 1 min. The final extension at the end of the profile was 72°C for 7 min. Amplified PCR product was purified with the GeneClean Turbo Kit (Bio 101, Q-Biogene). Sequencing was carried out using the ABI Prism Dye Terminator cycle sequencing ready reaction kit (Perkin-Elmer) for ABI 377 automated DNA sequencer.

PHYLOGENETIC ANALYSIS

Molecular and morphological characters were coded unordered and as having equal weights. Sequences

Table 2. List of primers used by C.A.F. L and H, respectively, refer to the light and heavy strands, and numbers refer to the position of the 3′ end of the oligonucleotide according to the numbering system for the human sequence (Anderson *et al.*, 1981). The list of primers used by P.G. is available in Gaubert *et al.* (in press)

Primer identification	Nucleotide sequence (5′→3′)
L14841 Genetta	CCC TCC AAT ATT TCA GCA TGR TGA AA
H15044 Genetta	ATG AGC CGT AGT ATA CTC CTC G
L15007 Genetta	ATA TAT WCA CGC TAA YGG AGC C
H15229 Genetta	CCT CCT CAG ATY CAT TCT
L15171 Genetta	TGR GGC CAA ATA TCC TTC TGA GG
H15350 Genetta	GGG TTG TTG GAR CCT GTT TC
L15280 Genetta	AAG CCA CCY TAA CAC GAT TC
H15493 Genetta	AAT TGT CGG GGT CTC CTA AA
H15557 Genetta	GCR AAT AGA AAA TAT CAT TC
L15442 Genetta	AAA GAY ATT CTA GGC CTC
H15684 Genetta	GGT CGG AAT ATT ATG CTT C
L15563 Genetta	CCA CCC CAT ATT AAA CCC GAA TGA T
H15762 Genetta	GTG TTC TAC TGG TTG TCC AC
L15631 Genetta	AGG AGT CCT AGC CCT YGT C
H15857 Genetta	GGG ACG RTT TTC GAT AAT

were manually aligned with BioEdit version 5.0.6 (Hall, 1999) and Sequencher 3.0 (Gene Codes Corporation Inc.), and analysed using maximum parsimony (MP; heuristic search) and maximum likelihood (ML; heuristic search and neighbour-joining method) with PAUP* Beta version 4.0b2 (Swofford, 2001), and Bayesian inference through the software MrBayes 3.0 (Huelsenbeck & Ronquist, 2001). The morphological matrix was analysed using MP (heuristic search) and multistate characters treated as 'polymorphism'. Heuristic searches were performed using stepwise-addition branch-swapping (ten random additions) and TBR swapping algorithm. The best-fitting model of sequence evolution with parameters used in the ML analyses was estimated with ModelTest 3.06 (Posada & Crandall, 1998) and was the general time-reversible model (GTR; Rodriguez *et al.*, 1990) using a gamma shape (G) of 0.3201. The distance criterion used for calculating the genetic distances between taxa was maximum likelihood. The likelihood model chosen for Bayesian analysis was the GTR model (following the estimations of ModelTest) with a gamma distribution divided into six categories and site partition-specific rates. Four independent Markov chains were run (three heated chains and one cold chain) for 200 000 Metropolis-coupled Markov chain generations (Huelsenbeck *et al.*, 2001), with tree sampling each 20 generations and burn-in after 2000 trees. Robustness of nodes was assessed with the Decay Index (DI; Bremer, 1988) in the case of the morphological cladogram and the bootstrap method (Felsenstein, 1985), with 100 (ML: heuristic search) to 1000 (MP; ML: neighbour-joining reconstruction) replicates for molecular trees. Confidence in nodes was also estimated for ML analyses with the likelihood ratio test of branch length (Felsenstein, 1993) as implemented in PAUP. Posterior probabilities were calculated in order to assess node 'reliability' of the Bayesian analysis tree topology. When the morphological and molecular matrices were combined, we estimated the conflicts in phylogenetic signals between the two data sets with the incongruence length difference test (ILD test; Farris *et al.*, 1995) as implemented in PAUP, in order not to justify data combination but to estimate the extent of conflict among the data sets (see Liu & Miyamoto, 1999). For this purpose, we ran 1000 randomizations and non-variable characters were removed. We also used the partitioned Bremer support (PBS; Baker & DeSalle, 1997), using TreeRot.v2 (Sorenson, 1999). In order to estimate the conflicting signals at nodes between and within the data sets, partitions were as follows: first codon positions – second codon positions – third codon positions (cyt *b*) – skull – teeth – body proportion – pads – teats – hair ultrastructure – coat (morphology). Phylogenetic signal value (g1; 100 000 random trees) was used as an estimation of the structuring of the signal vs. random noise (Hillis, 1991; Hillis & Huelsenbeck, 1992) for each data set alone and then combined. In order to examine statistical significance of the difference in tree topologies in a ML framework, we used the test of Kishino–Hasegawa (Kishino & Hasegawa, 1989) from a pruned tree comprising one representative per morphological species.

ANCESTRAL STATE RECONSTRUCTION, CORRELATED EVOLUTION AND ESTIMATION OF DIVERGENCE TIME

The ML cyt *b* tree topology (fully resolved) was used as a basis for ancestral state reconstruction and test of correlated evolution. We estimated the ancestral state of habitat and five diagnostic morphological characters of the genets (anterior extension of the caudal entotympanic bone, number of teats, shape of mid-dorsal spots, distribution of dorsal spots and presence of a mid-dorsal crest) using both ML and MP methods. Given the molecular-based assumption of a Late Miocene origin of the genus *Genetta* (Gaubert & Veron, in press), the poor fossil record related to the genus (Hendey, 1974; Hunt, 1996; Geraads, 1997) and the relative uncertainty surrounding habitat delimitations for this period (Maley, 1996), we restricted the coding of habitat to 'rain forest' and 'open habitat'. The morphological traits were all binary characters (Appendix 1). The ML estimates of ancestral state were made using Discrete 4.0 (Pagel, 2000). We used a two-rate model for change between states because it offered in several cases a significantly better fit than the one-rate model (see Mooers & Schluter, 1999). The methodology follows Pagel (1994, 1999) and Mooers & Schluter (1999). Values of likelihood ratio (LR) equal or superior to 2 between competing ancestral state hypotheses were considered roughly analogous to 95% confidence intervals (Pagel, 1999; Belshaw *et al.*, 2000). We compared ancestral estimations with the results obtained from the 'Ancestral states' command (Independent menu) using the 'global method' (see Pagel, 1999, 2000). This command allows the estimation of the best simultaneous set of ancestral states on the tree and may differ from the ancestral states obtained by calculating separately the most probable state at each node (Pagel, 2000). The MP estimates of ancestral states were made by mapping characters and habitat using MacClade 4.0 (Maddison & Maddison, 2000) and Wagner parsimony (see Swofford & Maddison, 1987; Maddison & Maddison, 1992; Cunningham, Omland & Oakley, 1998). In order to give a relative support to the estimates, we calculated the ratio between the number of steps for the least and most parsimonious options of ancestral character state reconstruction. We assume that the support for an MP ancestral state estimate is higher with higher values of the MP index.

The correlated evolution of the five diagnostic characters with habitat was tested through both ML and MP methods. We used Discrete in order to estimate in an ML framework whether the changes in two variables (one morphological and one habitat character) were independent (see Pagel, 1994). We first calculated the log-likelihood of the model of independent evolution for the two variables (Pagel, 1994, 1997), and then calculated the likelihood of the eight-parameter model of dependent trait evolution (see Pagel, 1994, 2000). Similarly to the estimation of ancestral states, we used LR between the likelihoods estimated from the dependent and independent tests in order to test the null hypothesis of absence of correlation between the changes of the two characters. We used MacClade and the concentrated-changes test (Maddison, 1990) in order to compare the ML estimation of evolution between characters to an MP method. The test was performed using the 'exact count' option ($n = 1000$) with 'distinguished areas' as having the habitat trait 'rain forest' (for further details about methodology, see Maddison & Maddison, 1992). The concentrated-changes test is considered to be conservative (Lorch & Eadie, 1999). Following Ortolani & Caro (1996), Lorch & Eadie (1999), Ord, Blumstein & Evans (2001) and Ord & Blumstein (2002), *P*-values less than 0.05 were considered as highly significant and *P*-values between 0.05 and 0.10 were considered as marginally significant.

In order to estimate divergence times within an ML framework, we used Rhino, a modified version of the software QDate (Rambaut & Bromham, 1998), as used in Cooper *et al.* (2001) and Paxinos *et al.* (2002). Rhino requires molecular clock-like evolution of DNA sequences. We thus tested for significance ($P < 0.05$) in branch length differences between enforced (molecular clock) and non-enforced ML trees using the test of Kishino–Hasegawa as implemented in PAUP. Because fossil remains for members of genera *Poiana* and *Genetta* are scarce or not available, we used the minimum divergence data (mdd) between the two genera – estimated by Gaubert & Veron (2003) at 8.5 (7.2–10.0) Myr – as the 'calibration point' in Rhino for estimating mdd within the *Genetta* clade. In order to avoid large confidence interval estimations, we restricted this point to 8.5 Myr.

RESULTS

PHYLOGENETIC ANALYSES AND SEQUENCE DIVERGENCE BASED ON CYT *b*

The complete cyt *b* (1140 bp) was obtained from fresh samples. Approximately 850–1000 bp were sequenced for museum specimens, depending on the set of primers used and the quality of DNA extracts.

The data matrix showed a significant structuring of the phylogenetic signal, with a shape of tree-length distribution clearly left-skewed ($g1 = -0.38319$; $P < 0.01$). The four methods of phylogenetic analysis produced topologies partly conflicting, especially at the base of the trees, but conflicting nodes were characterized by low support values (data not shown). Such incongruence is probably due to the 'noise' related to saturation of transitions at third codon positions in cyt *b* occurring at basal branchings of the genus phylogeny (Gaubert *et al.*, in press). The ML analysis (heuristic search) resulted in one tree, which is given with parameters in Figure 1. Maximum posterior probability phylograms (3858 trees retained) yielded a very similar consensus tree, even for basal branchings poorly supported by the ML analysis (see Fig. 1). The most basal species is *G. thierryi*, which represents subgenus *Pseudogenetta*. The second species to branch off is *G. victoriae*, which is sister-species of all the other genets (branch length significant and Bayesian posterior probability (PP_B) = 0.93, but bootstrap value (bp) < 70%). *G. servalina* and *G. cristata* are sister-species and group with the remaining genets (bp < 70%; $PP_B = 1.00$). Thus, the traditional servaline group, consisting of the two latter species plus *G. victoriae*, is paraphyletic. *G. piscivora* and *G. johnstoni* form a monophyletic group, which is sister-group of the small-spotted genets + *G. angolensis* + large-spotted genets (bp < 70%; $PP_B = 0.68$). One of the major phylogenetic results that arises from our study is that populations grouped under the morphological species *G. genetta* do not form a monophyletic group. Indeed, specimens from Northern Cape and Victoria West District (Republic of South Africa) form a clade that we named *G. felina* (see Discussion), which is sister-group of *G. angolensis* (bp = 100%; $PP_B = 1.00$). The other small-spotted genets group in a clade geographically structured as ((sub-Saharan Africa, (Arabian Peninsula, Maghreb + Europe)), which is the sister-group of *G. angolensis* + small-spotted genets (strictly South African group) + large-spotted genets (bp = 71%; $PP_B = 1.00$). The monophyly of the large-spotted genet complex is well supported (bp = 84%; $PP_B = 1.00$). The morphological species of the large-spotted genet complex form a monophyletic group structured as follows: ((*G. pardina*, (*G. poensis* (*G. bourloni*, *G. 'schoutedeni'*))), (*G. maculata*, *G. tigrina*)), with a *G. maculata* specimen included in the *G. tigrina* group. The NJ tree using ML as distance criterion yielded a similar topology and confirmed the well-supported phylogenetic hypotheses (minimum evolution score = 0.98288; tree not shown). Bootstrap values were high at the node joining *G. victoriae* to the other genets (82%) and very high for the clade including the small-spotted genets, *G. angolensis* and the large-spotted genets (95%). The

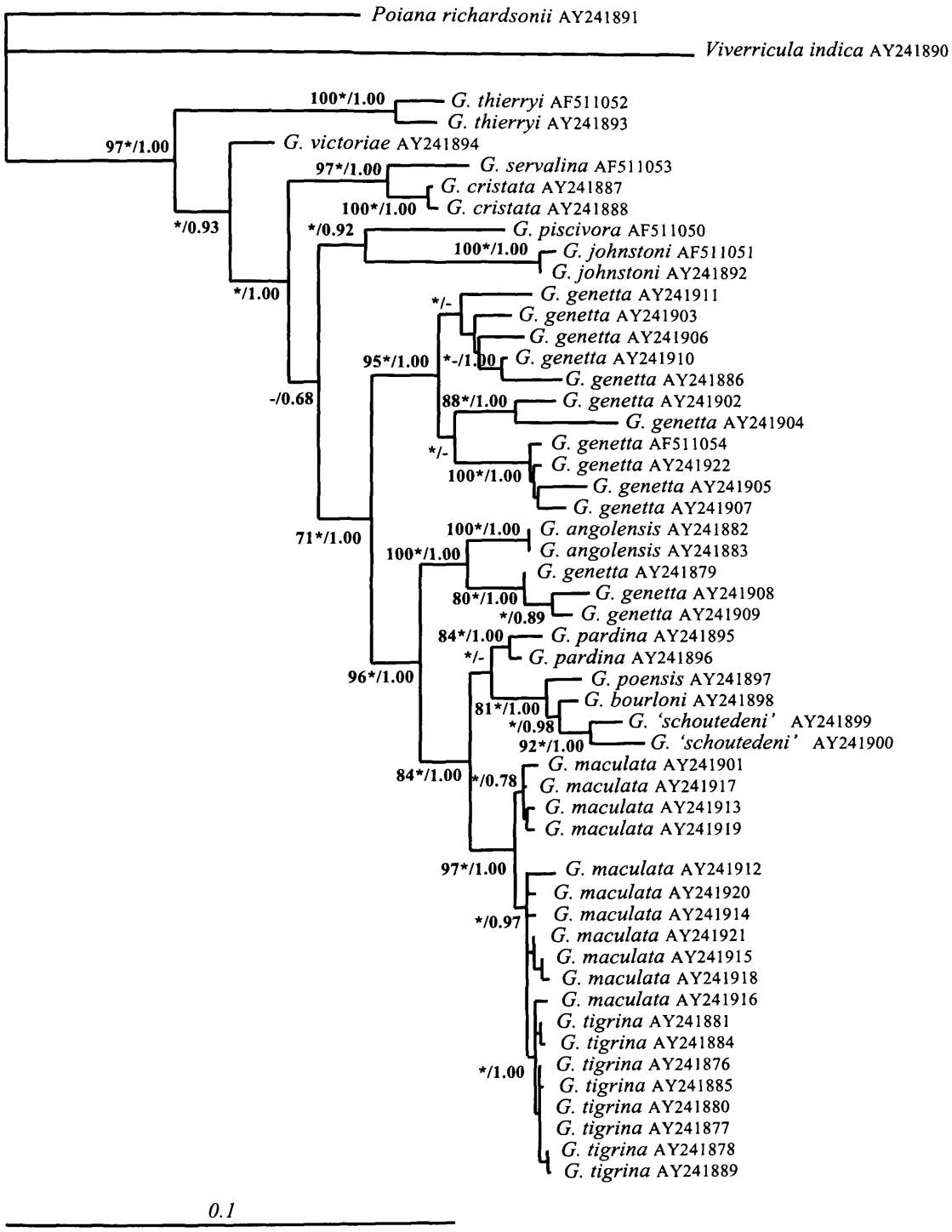

Figure 1. ML tree (heuristic search) for 50 specimens representing 15 morphological species of genus *Genetta* based on cyt *b* sequences (–ln likelihood = 6313.06275). The model of sequence evolution estimated by ModelTest is GTR (Rodriguez *et al.*, 1990) using a gamma shape (*G*) of 0.3201. The estimated substitution rates are: 1.1393 (A–T), 4.1832 (A–G), 0.7488 (A–C), 0.4114 (T–G), 8.8521 (T–C) and 1.0000 (G–C). Values at branches are bootstrap probabilities superior to 70% (see Hillis & Bull, 1993; Mason-Gamer & Kellogg, 1996) and posterior probabilities found using the Bayesian method, respectively. Asterisks at nodes mean that branch length is significant. Scale bar corresponds to 10% sequence divergence.

MP analysis yielded 186 equally parsimonious trees (985 steps; CI = 0.461; RI = 0.716), which resulted in a strict consensus tree of 1003 steps (CI = 0.453; RI = 0.706; tree not shown). Of the 1140 characters of the total matrix, 400 were variable, of which 305 were parsimony-informative. The well-supported nodes of the ML and Bayesian trees were found again in the MP tree topology. However, confidence in basal relationships between *G. thierryi*, *G. victoriae*, (*G. servalina*, *G. cristata*), *G. johnstoni* and *G. piscivora* was low (bp < 70%).

Given the paraphyly of two traditional morphological taxa (servaline and small-spotted genets) that resulted from our analyses, we tested for significance in difference with tree topologies reflecting monophyly. The Kishino–Hasegawa test supported the hypothesis of paraphyly for the small-spotted genets ($P < 0.05$). The difference in tree topologies with the servaline genets paraphyletic or monophyletic was, by contrast, not significant ($P = 0.0711$).

Pairwise distances (%) within genus *Genetta* are given in Table 3. They ranged from 13.902 (*G. 'schoutedeni'* AY241899 – *G. thierryi* AF511052) to 0.85 (*G. tigrina* AY241878 – *G. tigrina* AY241889). Between unambiguously delimited morphological species (i.e. *G. thierryi*, *G. johnstoni*, *G. piscivora*, *G. victoriae* and *G. angolensis*), ML distances varied from 12.730 (*G. piscivora* – *G. thierryi*) to 6.015 (*G. victoriae* – *G. piscivora*). Mean pairwise distance between the similar species *G. servalina* and *G. cristata* was lower (2.221), and was included in the range of variation characterizing the sibling species that constitutes the large-spotted genet complex (from 4.558 (*G. 'schoutedeni'* – *G. maculata*) to 0.770 (*G. maculata* – *G. tigrina*)). Within the large-spotted genets, mean pairwise distances were inferior to 2% in three cases: (1) *G. bourloni* – *G. 'schoutedeni'* (1.882), (2) *G. bourloni* – *G. poensis* (1.242) and (3) *G. maculata* – *G. tigrina* (0.770). Roughly speaking, pairwise distances between morphological species seem to be correlated with morphological 'divergence' (i.e. the number of differing morphological character states between pairs of species). This trend is, however, contradicted by two exceptions. First, the mean divergence was surprisingly high between *G. genetta* and the other clade of small-spotted genets (6.932) although both taxa exhibit similar morphological patterns. Second, *G. maculata* and *G. tigrina* have the lowest pairwise divergence but show clearly distinct morphological patterns. Finally, the mean variability within species ranged from 0.000 (*G. angolensis*; two specimens) to 3.624 ('true' *G. genetta*; 11 specimens), the latter value exemplifying that variability within species may be much higher than pairwise distance between species.

PHYLOGENETIC ANALYSES INCLUDING MORPHOLOGICAL CHARACTERS

The MP analysis based on the 50 morphological characters yielded seven equally parsimonious trees (201 steps; CI = 0.557; RI = 0.536), which resulted in a strict consensus tree of 225 steps (CI = 0.498; RI = 0.411). There were 43 parsimony-informative characters and seven autapomorphies. The morphological data exhibited a weak structuring of the phylogenetic signal similar to that expected at random, with a distribution of tree lengths not left-skewed (g1 = –0.425280; owing to the heterogeneity in number of character states, P is not available; see Hillis & Huelsenbeck, 1992). The consensus tree was characterized by a general lack of resolution of the branchings, with low DI values (tree and data not shown) that reached a maximum value of 2 for three monophyletic groups: (1) (*G. servalina* (*G. cristata*, *G. victoriae*)) (2) (*G. genetta*, *G. felina*) and (3) (*G. angolensis*, *G. tigrina* (*G. genetta*, *G. felina*)). Values of bootstrap (1000 replicates), all less than 60%, confirmed the very low support for nodes of the morphological tree.

The results from the ILD test between the morphological and the pruned cyt *b* matrices (one specimen per species) indicated high conflict between the two data sets ($P = 0.001$). However, we ran a combined analysis in order to estimate the conflicting signals at nodes between and within the data sets by calculating PBS. The MP analysis resulted in a single most parsimonious tree, which is shown in Figure 2. The concatenated data matrix showed a high structuring of phylogenetic signal, with a shape of tree-length distribution clearly left-skewed (g1 = –0.495250; P not available). The tree topology was very similar to the cyt *b* ML and Bayesian tree topologies. However, because of conflicts between phylogenetic signals from cyt *b* and morphological characters, some nodes were less supported (bp < 70% and low DI values). PBS values also suggest conflicting signals within cyt *b* itself at basal nodes between first and second positions, not saturated, and third positions, saturated (see Gaubert *et al.*, in press). Most morphological characters involved in signal conflicts were coat (the most important) and skull partitions, and to a lesser degree teeth, pads and teats. Phylogenetic signals from morphological and cyt *b* data were sometimes cumulative and supported the placement of *G. thierryi* at the base of the tree, the sister-relationship between *G. servalina* and *G. cristata*, and the monophyly of the large-spotted genet complex. Interestingly, morphological characters supported uncertain phylogenetic positions such as *G. victoriae* as sister-species of the other genets (excluding *G. thierryi*) and *G. pardina* sister-species of the forest forms belonging to the large-spotted

Table 3. Pairwise distances among representatives of genus *Genetta* estimated from ML parameters. Numbers correspond to the mean values, with lowest and highest estimates between parentheses. Values in bold type on the diagonal correspond to the estimated intraspecific variability

	G. thie	*G. john*	*G. pisc*	*G. vict*	*G. serv*	*G. crist*	*G. gene*	*G. feli**	*G. ango*	*G. pard*	*G. poen*	*G. bour*	*G. scho*	*G. macu*	*G. tigr*
G. thierryi	**1.900**														
G. johnstoni	12.523 (12.199–12.847)	**0.348**													
G. piscivora	12. 730 (12.657–12.803)	6.677 (6.609–6.745)	–												
G. victoriae	7.069 (6.939–7.199)	6.948 (6.713–7.183)	6.015	–											
G. servalina	11.666 (11.446–11.885)	8.989 (8.844–9.133)	6.368	5.194	–										
G. cristata	10.557 (10.396–10.719)	7.826 (7.570–8.083)	5.878 (5.723–6.032)	4.207 (4.134–4.280)	2.221 (2.157–2.284)	**0.322**									
G. genetta	12.397 (11.255–13.466)	9.322 (8.194–10.553)	8.110 (7.052–9.083)	6.605 (5.681–7.281)	8.326 (7.707–9.211)	6.798 (5.319–8.054)	**3.624 (0.899–5.929)**								
*G. felina**	12.257 (10.997–13.694)	8.867 (8.335–9.580)	7.633 (6.952–8.086)	6.483 (6.173–6.665)	7.567 (5.876–8.773)	6.360 (5.707–6.922)	6.932 (5.980–8.309)	**1.109 (0.831–1.251)**							
G. angolensis	12.384 (12.306–12.462)	9.228 (9.138–9.318)	8.117	6.605	6.347	6.723 (6.660–6.787)	6.889 (5.803–7.769)	3.277 (2.428–3.946)	**0.000**						
G. pardina	11.888 (11.406–12.233)	8.666 (8.242–9.095)	7.631 (7.261–8.000)	5.559 (5.166–5.951)	7.033 (6.728–7.338)	5.964 (5.456–6.473)	6.654 (5.435–8.589)	4.731 (3.817–5.359)	4.695 (4.413–4.976)	**0.966**					
G. poensis	11.786 (11.636–11.936)	8.536 (8.278–8.793)	7.188	4.528	6.277	5.688 (5.624–5.751)	7.426 (6.630–8.304)	5.054 (4.020–5.809)	4.864	2.624 (2.338–2.910)	–				
G. bourloni	12.231 (12.079–12.383)	8.694 (8.855–8.838)	7.356	5.098	6.427	5.467 (5.404–5.529)	6.939 (6.006–7.845)	4.690 (4.010–5.387)	4.858	2.341 (1.999–2.682)	1.242	–			
G. 'schoutedeni'	13.128 (12.525–13.902)	9.138 (8.929–9.346)	8.216 (7.918–8.513)	6.358 (6.200–6.515)	7.470 (7.095–7.844)	6.463 (5.880–7.047)	7.667 (6.334–8.622)	5.206 (4.675–6.197)	5.190 (5.115–5.265)	3.424 (2.703–4.156)	2.287 (2.228–2.345)	1.882 (1.660–2.104)	**1.754**		
G. maculata	12.098 (11.454–13.068)	8.848 (8.233–9.483)	7.738 (7.224–8.346)	6.479 (5.830–6.965)	7.686 (7.376–8.081)	6.440 (5.682–7.166)	6.351 (4.581–8.242)	5.050 (3.597–5.958)	4.657 (4.348–4.999)	2.632 (2.198–3.326)	3.611 (3.355–3.909)	3.182 (2.886–3.381)	4.558 (3.992–5.045)	**0.767 (0.171–1.442)**	
G. tigrina	12.163 (11.102–13.029)	9.486 (9.190–10.223)	8.007 (7.755–8.332)	6.973 (6.241–7.284)	6.966 (5.905–7.768)	6.349 (5.974–6.587)	6.494 (3.738–8.476)	5.134 (2.573–6.096)	4.528 (3.794–4.758)	2.773 (2.249–3.335)	3.692 (2.827–4.276)	3.145 (2.650–3.475)	4.339 (3.131–5.136)	0.770 (0.344–1.437)	**0.278 (0.096–0.707)**

*See Discussion.

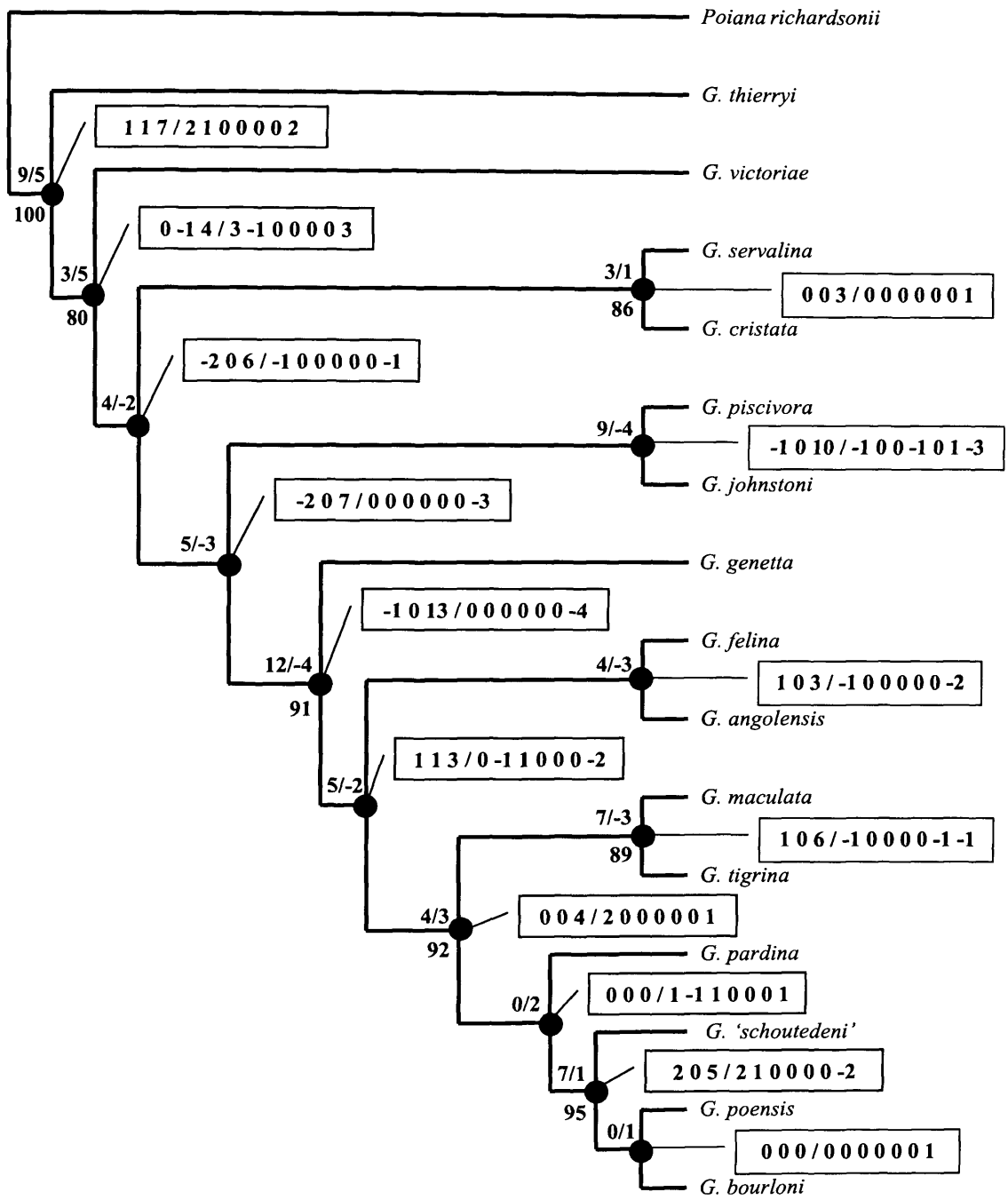

Figure 2. The most parsimonious tree based on the combined analysis of cyt *b* and morphological data (4237 steps; CI = 0.928; RI = 0.478; 519 variable characters, 203 parsimony-informative). Numbers below branches represent bootstrap values superior to 70%. Values above branches are the estimates of partitioned Bremer supports (PBS) for the following partition: cyt *b*/morphology. Numbers in boxes linked to tree nodes correspond to the estimates of PBS for the following detailed partition: first codon positions – second codon positions – third codon positions (cyt *b*)/skull – teeth – body proportion – pads – teats – hair ultrastructure – coat (morphology). A large positive PBS value indicates strong support of a partition of characters for a given node, whereas a large negative value means that the phylogenetic signal of a partition is in strong opposition with a given node. We associated the haplotypes of the clade constituted by the South African small-spotted genets to the species designated in this study as *G. felina* (see Discussion).

genet complex. The combined analysis allowed for identifying one strict morphological synapomorphy among the 50 morphological characters, namely the acquisition of a thick cortex in the spatula of dorsal guard hair (character 21) characterizing (*G. johnstoni*, *G. piscivora*).

RECONSTRUCTION OF ANCESTRAL STATES, TEST OF CORRELATED EVOLUTION AND DIVERGENCE TIME

Reconstruction of ancestral states and tests of correlated evolution gave very similar results using either ML or MP methods (Table 4). ML ancestral states

Table 4. Reconstruction of ancestral states and test of correlated evolution for/between five morphological diagnostic characters and habitat. Values of $-\ln L$ correspond to likelihood estimate of best simultaneous set of ancestral states on the tree ('Ancestral states' command). Values of $\alpha 1$ and $\alpha 2$ are the instantaneous transition rates from state 0 to 1 and from state 1 to 0, respectively. LR is the likelihood ratio statistic comparing the goodness of fit between (1) the overall tree likelihoods calculated with the two character states as ancestral and (2) the dependent and independent models of evolution. MPO and LPO mean Most and Least Parsimonious Option, respectively. Numbers of gains and losses correspond to the evolutionary events occurring in rain forest lineages

	Discrete		MacClade	
	Ancestral state	Correlation to habitat	Ancestral state	Correlation to rain forest
(11) Anterior extension of the caudal entotympanic bone	**Long** (state 1) $-\ln L$ = 22.34103 $\alpha 1$ = 0.00041 $\alpha 2$ = 1.06478 LR = 0.3456 (not significant)	**Yes** LR = 7.00491 (significant)	**Long** (state 1) MPO = 4 steps LPO = 7 steps *MP ratio* = 1.75	**Yes** gain = 1 losses = 3 P = 0.024928 (significant)
(20) Teats	**One pair** (state 0) $-\ln L$ = 28.70267 $\alpha 1$ = 0.0083 $\alpha 2$ = 0.00041 LR = 0 (not significant)	**Yes** Significant LR = 7.34935 (significant)	**One pair** (state 0) MPO = 2 steps LPO = 3 steps MP ratio = 1.5	**No** gain = 0 losses = 2 P = 0.149194 (not significant)
(27) Mid-dorsal spots	**Narrow** (state 1) $-\ln L$ = 28.40688 $\alpha 1$ = 0.00043 $\alpha 2$ = 2.08628 LR = 0.3634 (not significant)	**Yes** Significant LR = 7.99946 (significant)	**Narrow** (state 1) MPO = 4 steps LPO = 5 steps MP ratio = 1.25	**No** gain = 1 losses = 2 P = 0.079919 (marginally significant)
(29) Distribution of dorsal spots	**Densely spotted** (state 1) $-\ln L$ = 22.47135 $\alpha 1$ = 0.00037 $\alpha 2$ = 1.05199 LR = 0.44858 (not significant)	**Yes** LR = 7.02232 (significant)	**Not densely spotted** (state 0) MPO = 4 steps LPO = 5 steps MP ratio = 1.25	**Yes** gains = 2 losses = 2 P = 0.030952 (significant)
(43) Mid-dorsal crest	**Absent** (state 0) $-\ln L$ = 28.70263 $\alpha 1$ = 0.06498 $\alpha 2$ = 0.00037 LR = 0 (not significant)	**Yes** LR = 6.89535 (significant)	**Absent** (state 0) MPO = 4 steps LPO = 7 steps MP ratio = 1.75	**Yes** gain = 0 losses = 4 P = 0.013091 (significant)
Habitat	**Rain forest** $-\ln L$ = 28.70265 $\alpha 1$ = 0.12017 $\alpha 2$ = 0.00037 LR = 0 (not significant)	-	**Rain forest** MPO = 4 steps LPO = 5 steps MP ratio = 1.25	-

were not significantly more likely to characterize the ancestor of the genets than the other possible states (LR < 0.4). However, reconstructions from the two ML methodologies were identical, and were very similar with the ancestral state estimates using the MP method (with the exception of character 'distribution of dorsal spots'). Ancestral states of diagnostic morphological characters recovered by both ML and MP methods are as follows: long anterior extension of the caudal entotympanic bone (highest MP ratio: 1.75), one pair of teats (MP ratio = 1.5), narrow mid-dorsal spots (MP = 1.25) and absence of a mid-dorsal crest (MP = 1.75). In addition, the ancestral estimates support rain forest as the original habitat of the ancestor of the genets.

The test of correlation of morphological characters with habitat (see Table 4) using the ML method rejected the model of character independence (LR values between 6 and 8). The correlation with habitat was confirmed by the results from the concentrated-changes test (MP method), with numbers of gains and losses significantly correlated with rain forest in all cases but one (one pair of teats; $P > 0.1$).

The hypothesis of a clock-like behaviour of cyt *b* sequences was not rejected using either complete or pruned taxonomic sets (Kishino–Hasegawa test: $-\ln L = 6318.27070$; $P = 0.6430$, and $-\ln L = 3852.48371$; $P = 1.0000$, respectively). Estimates of minimum divergence date (mdd) are given in Table 5. There is a large gap between the estimated divergence of *G. thierryi* (8.5 Myr) and the other species of genets, because mdd of the second species to emerge (*G. victoriae*) is 4.92 (3.98–6.01) Myr. The period of divergence of the three basal rain forest lineages (*G. victoriae*, *G. piscivora* – *G. johnstoni* and *G. servalina* – *G. cristata*) are estimated to be Late Miocene and Early Pliocene, with splitting events between species of the two latter rain forest clades at Middle Pliocene and Late Pliocene – Pleistocene periods, respectively. The species *G. genetta* is estimated to diverge from the rest of the genets during the Middle Pliocene, whereas *G. felina* (sister-group of *G. angolensis*) arises *c.* 1 Myr later. The mdd estimate between *G. felina* and *G. angolensis* is between Late Pliocene and Early Pleistocene, as is dated the emergence of the large-spotted genet complex. The 'forest forms' *G. poensis*, *G. bourloni* and *G. 'schoutedeni'* diverged during the Middle Pleistocene, whereas *G. maculata* and *G. tigrina* constitute the most recent species, splitting during the Late Pleistocene.

Table 5. Estimations of minimum divergence date (mdd) between genet taxa (in Myr) using Rhino. We used 8.5 Myr as the divergence date between *G. thierryi* and the rest of the extant genet species (see Material and methods). 'Node' corresponds to mdd between a species or clade (left) and another (right) that constitute the monophyletic group defined by this node; clades are represented by one species name. Values in parentheses are confidence intervals

Node	Minimum divergence date (mdd)
G. thierryi – *G. maculata*	8.5
G. victoriae – *G. maculata*	4.92 (3.98 – 6.01)
G. servalina – *G. maculata*	4.51 (3.69 – 5.45)
G. piscivora – *G. maculata*	4.31 (3.52 – 5.21)
G. genetta – *G. maculata*	3.61 (2.86 – 4.50)
G. piscivora – *G. johnstoni*	3.56 (2.66 – 4.61)
G. felina – *G. maculata*	2.70 (2.11 – 3.42)
G. felina – *G. angolensis*	1.95 (1.36 – 2.68)
G. pardina – *G. maculata*	1.91 (1.42 – 2.51)
G. pardina – *G. 'schoutedeni'*	1.59 (1.12 – 2.16)
G. servalina – *G. cristata*	1.43 (0.85 – 2.25)
G. poensis – *G. 'schoutedeni'*	0.99 (0.65 – 1.44)
G. bourloni – *G. 'schoutedeni'*	0.86 (0.55 – 1.27)
G. maculata – *G. tigrina*	0.31 (0.13 – 0.60)

DISCUSSION

PHYLOGENETIC RESULTS AND SPECIES BOUNDARIES REVISITED

The use of museum specimens and an effective network of field collectors allowed us to present for the first time an exhaustive molecular phylogeny of the genus *Genetta*, including all but one (*G. abyssinica*) of its morphological species representatives. Combined with a revised morphological matrix, cyt *b* sequences shed new light on the evolutionary history of the genets. A synthetic phylogenetic tree is given in Figure 3.

G. thierryi, traditionally grouped with *G. abyssinica* under subgenus *Pseudogenetta*, is the most basal species, confirming the assumptions of Crawford-Cabral (1981). The hypothesis supported by our combined analysis is that *G. thierryi* is a 'primitive' genet that shares several plesiomorphic characters with genus *Poiana* (see Crawford-Cabral, 1981; Gaubert *et al.*, in press).

The second species to branch off is *G. victoriae*, the phylogenetic position of which is supported by both molecular and morphological data. The giant genet *G. victoriae* is traditionally included with *G. servalina* and *G. cristata* in the servaline genet group owing to their shared densely spotted coat and absence of continuous mid-dorsal line (Crawford-Cabral, 1981; Gaubert *et al.*, 2002), and this is the first time that the paraphyly of the group is evidenced. Observations on a great number of specimens revealed that this global similarity was misleading because the giant genet shows marked morphological differences, even in skull characteristics (see Appendix 2). The true servaline

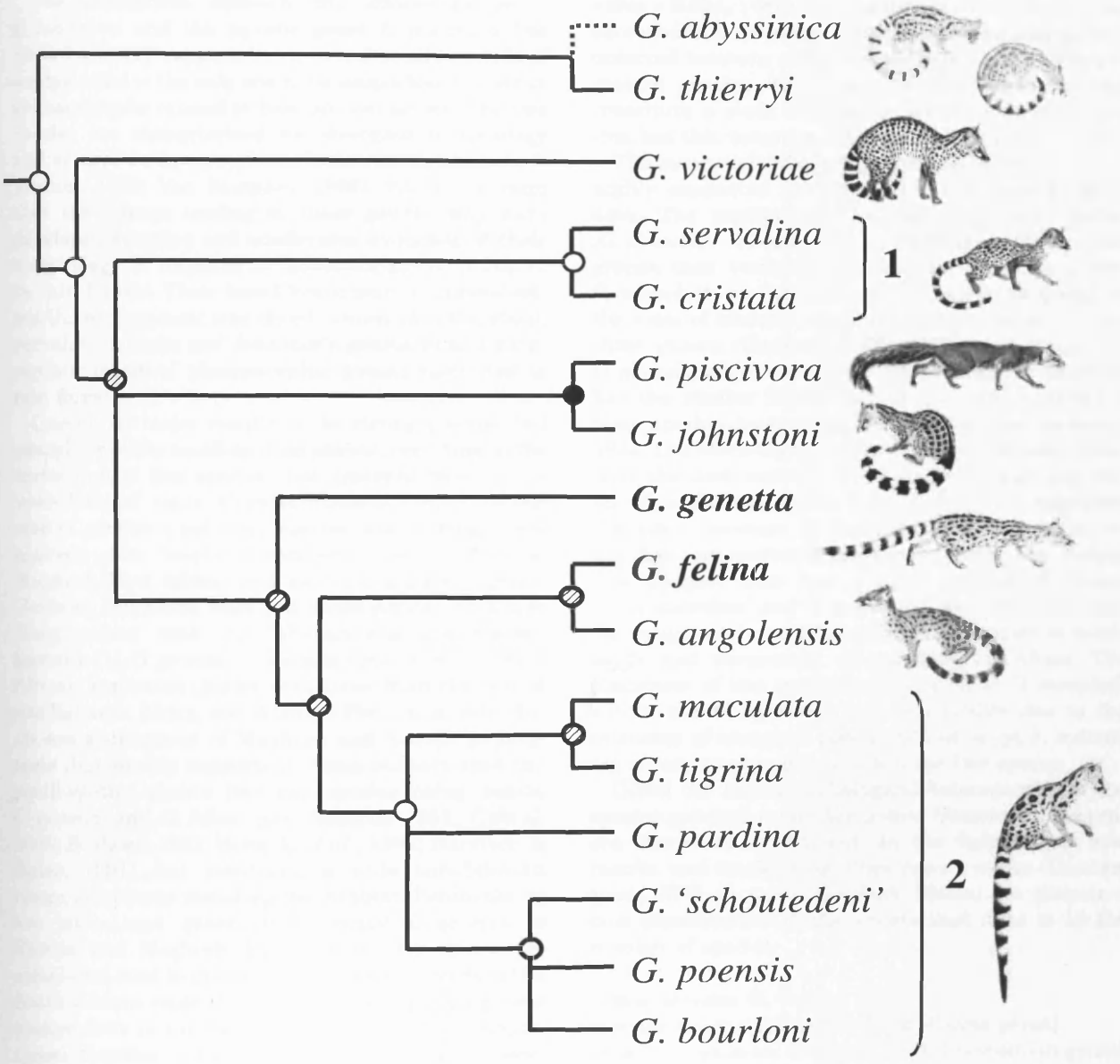

Figure 3. A synthetic phylogenetic tree of the genets. White circles indicate nodes highly supported by both cyt *b* and morphological data. Black circles with diagonal lines correspond to nodes highly supported by cyt *b* but with evidence of homoplasy of morphological characters (conflict). We maintained the weakly supported node that groups *G. piscivora* and *G. johnstoni* (black circle) because it is characterized by the only strict morphological synapomorphy of the tree (see Results). The dashed branch leading to *G. abyssinica* does not represent a hypothesis tested with our data set, but relies on diagnostic morphological synapomorphies shared with *G. thierryi* (see Gaubert *et al.*, 2002). 1 represents the 'true' servaline genets, and 2 includes the species of the large-spotted genet complex. Species in bold type represent the small-spotted genets, which constitutes a paraphyletic group. Illustrations were modified from Dorst & Dandelot (1976) and Kingdon (1997).

genets are then constituted by the morphologically similar species *G. servalina* and *G. cristata*. The genetic distance between these two genets is higher than intraspecific divergence estimates within each species (*G. genetta* excepted; but see below), thus supporting the status of valid species for *G. cristata* (Rosevear, 1974; Crawford-Cabral, 1981; Van Rompaey & Colyn, 1998; but contrary to Wozencraft, 1993).

The association between the Johnston's genet *G. johnstoni* and the aquatic genet *G. piscivora* has weak bootstrap support, but is found in all methods of analysis and is the only one to be supported by a strict synapomorphy related to hair ultrastructure. The two species are characterized by divergent morphology and several autapomorphies (Kuhn, 1959; Lamotte & Tranier, 1983; Van Rompaey, 1988). We hypothesize that the lineage leading to these genets may have developed adaptive and accelerated evolution of their morphology in response to habitat-selective pressure in rain forests. Their basal branching is unresolved, but the phylogenetic tree clearly shows that the giant, servaline, aquatic and Johnston's genets form a paraphyletic group of 'plesiomorphic' genets restricted to rain forest.

One of our major results is the strongly supported paraphyly of the small-spotted genets, resulting in the distinction of two species that emerged first in the 'open habitat' clade. Crawford-Cabral (1981) considered *G. genetta* a polytypic species, and distinguished between the 'west-and-northern group' (Europe, Maghreb, West Africa) and 'east-and-southern group' (Arabian Peninsula, East and South Africa). This is in disagreement with our phylogenetic conclusions, because (1) *G. genetta* is paraphyletic and (2) West African specimens cluster with those from the rest of sub-Saharan Africa, and Arabian Peninsula individuals are sister-group of Maghreb and Europe populations (but weakly supported). Some authors split the small-spotted genets into two species using names *G. genetta* and *G. felina* (see Roberts, 1951; Cabral, 1966; Schlawe, 1981; Honacki *et al.*, 1982; Harrison & Bates, 1991), but attributed a wide sub-Saharan range, sometimes including the Arabian Peninsula, to the latter, and generally restricted *G. genetta* to Europe and Maghreb. By contrast, the exhaustive sample set used in this study allows for restricting the South African clade that makes small-spotted genets paraphyletic to the Republic of South Africa (haplotypes), Namibia and southern Angola (collection specimens; Gaubert, 2003b). The oldest available name for designating the species is *Genetta felina* (Thunberg, 1811), which was described from the Cape of Good Hope, and we recommend its use pending further confirmation. The species shows a close morphological similarity with *G. genetta*, and a few diagnostic characters are available, such as bright ash-grey–white ground coloration, hindfeet covered by a dark 'sock' and posterior part of forefeet completely dark. We acknowledge that distinguishing between the two small-spotted genets in sympatric regions of occurrence (i.e. Namibia, Angola and north-eastern Republic of South Africa) may be ambiguous. The newly restricted *G. genetta* had intraspecific genetic divergences by far the highest among the genets (mean value = 3.624, with a maximum of 5.929), and exceeded the majority of divergence estimates that occurred between sister-species (servaline and large-spotted genets). This suggests that *G. genetta* may constitute a clade containing several allopatric species, but this assertion requires further investigation.

The monophyly of the large-spotted genet complex is highly supported by both cyt *b* and morphological data. The position of the clade of 'forest forms' (*G. poensis*, *G. bourloni*, *G. 'schoutedeni'*), which groups taxa variously related to *G. pardina* and/or *G. maculata*, confirms the specific status proposed on the basis of discrete morphological characters for the three genets (Gaubert, 2003a). The coat pattern of *G. poensis*, which could have been considered aberrant like the similar 'King Cheetah' pattern, is in fact a clear species-discriminative character (see Rosevear, 1974; Crawford-Cabral, 1981; Gaubert, 2003a). Similarly, the dark coat of *G. bourloni*, with partially coalesced mid-dorsal spots, is a good species-diagnostic character, because *G. bourloni* and *G. pardina* do not share an exclusive common ancestor. Our results also suggest that two distinct species of genets (*G. 'schoutedeni'* and *G. maculata*) having almost similar ranges and coat pattern live in sympatry in woodlands and savannahs of sub-Saharan Africa. The placement of one specimen belonging to *G. maculata* within the *G. tigrina* group is probably due to the retention of ancestral polymorphism in cyt *b*, indicating a very recent split between the two species.

Given the high morphological heterogeneity of the species grouped under the genus *Genetta*, all subgenera should be abandoned. In the light of our new results and considering other recent works (Gaubert *et al.*, 2002, in press; Gaubert, 2003a), we propose a new classification of the genets that fixes to 16 the number of species:

Genus *Genetta* G. Cuvier, 1816
Genetta thierryi Matschie, 1902 [Hausa genet]
Genetta abyssinica (Rüppel, 1836) [Abyssinian genet]
Genetta victoriae Thomas, 1901 [giant genet]
Genetta servalina Pucheran, 1855 [servaline genet]
Genetta cristata (Hayman, 1940) [crested servaline genet]
Genetta piscivora (Allen, 1919) [aquatic genet]
Genetta johnstoni Pocock, 1908 [Johnston's genet]
Genetta genetta (Linnaeus, 1758) [common small-spotted genet]
Genetta felina (Thunberg, 1811) [South African small-spotted genet]
Genetta angolensis Bocage, 1882 [Miombo genet]
Genetta tigrina (Schreber, 1776) [Cape genet]
Genetta maculata (Gray, 1830) [rusty-spotted genet]
Genetta pardina I. Geoffroy Saint-Hilaire, 1832 [pardine genet]

Genetta poensis Waterhouse, 1838 [king genet]
Genetta 'schoutedeni' (Crawford-Cabral, 1970) [Schouteden's genet]
Genetta bourloni Gaubert, 2003 [Bourlon's genet]

EVOLUTIONARY HISTORY OF THE GENETS IN AFRICA

Some of the phylogenetic hypotheses and species delimitations resulting from our analysis strongly contradict the traditional taxonomy of the genus *Genetta* and evidence the great homoplasy conveyed by morphological characters. Characters related to coat pattern and skull appear particularly labile, as shown by conflicts between Partitioned Bremer support estimations. A constant evolutionary rate of morphological characters seems to be unlikely, because our analyses evidenced important morphological differences between species with low cyt *b* divergence (*G. maculata* and *G. tigrina*) and almost similar morphotypes for species highly cyt *b*-divergent (*G. genetta* and *G. felina*). Thus, accelerated morphological evolution [as also suspected in the (*G. johnstoni, G. piscivora*) lineage] and morphological stasis might have been frequent events in the genus *Genetta*, and be responsible for the difficulty in assessing species boundaries and phylogenetic patterns.

Tests using ML and MP methods indicated in most cases a significant correlation between the diagnostic morphological characters traditionally used for genets and habitat, thus indicating that at least a part of the morphological traits used in taxonomy are adaptive. The estimates of ancestral states concerning diagnostic characters congruently suggested an ancestor of the genets characterized by morphological traits typical of *Poiana*, the sister-group of the genets (long anterior extension of the caudal entotympanic bone, one pair of teats, narrow mid-dorsal spots and absence of a mid-dorsal crest). In addition, ML and MP methods support rain forest as the ancestral habitat of the genets, thus confirming the hypothesis of an African rain forest origin for the whole group (Gaubert *et al.*, 2002), and a *Poiana*-like ancestor of genets (Gaubert *et al.*, in press).

However, the first genet species to branch off in the tree is *G. thierryi*, which is distributed in moist and dry savannahs with open woodlands from Gambia to Cameroon (Gaubert, 2003b), thus suggesting the 'exit' of an early lineage (*c.* 8.5 Myr) into African open habitats that antedates rain forest contraction and the savannah turnover of a majority of mammals around 2.8 Myr (Vrba, 1995; Wessleman, 1995). The genet tree then consists of a paraphyletic group of 'primitive' rain forest species, which diverged during the Late Miocene – Early Pliocene, at a period when equatorial Africa was warmer and more humid (Morley, 2000) and rain forest covered a larger area than today (Maley, 1996). Although these species have in their majority a restricted range that corresponds to rain forest refuges (Gaubert, 2003b), the molecular estimates of divergence time do not indicate that divergence of the lineages leading to *G. victoriae*, (*G. piscivora*, *G. johnstoni*) and (*G. servalina*, *G. cristata*) occurred during the contraction of rain forest, which started from *c.* 3.5 Myr (Maley, 1996; Morley, 2000). Thus, the phylogenetic results concerning the basal part of the genet tree reject the hypothesis of a climatically induced speciation model (Fjeldsa, 1994; deMenocal, 1995; Schluter, 2001; Via, 2002) as well as the role of rain forest refuges in species diversification by isolation (Mayr & O'Hara, 1986; Lynch, 1988). Instead, it is suggested that more complex forces of selection than simply habitat (e.g. environmental gradients, interspecific competition) acted on the evolutionary history of these rain forest genets. In this case, refuges supposed to have persisted since *c.* 3.5 Myr (Maley, 1996) have clearly influenced the current range of the species, supposing a low capacity of re-colonization after rain forest re-expansion (*G. servalina* excepted), but seem to have not played a role in speciation [see Roy, Sponer & Fjeldsa (2001) for a similar case on African forest birds]. The emergence of the derived savannah clade coincides with the starting period of rain forest contraction and increase in savannah vegetation, *c.* 3.5–3.4 Myr (Cerling, 1992; Maley, 1996; Morley, 2000), thus marking a second invasion of open habitats some 5 Myr ago after *G. thierryi*. The climatic cooling around 2.8 Myr (deMenocal & Bloemendal, 1995) corresponds to the divergence between *G. felina* and the ancestor of the large-spotted genet complex. The three lineages constituting the latter appeared during following cool–dry episodes (deMenocal & Rind, 1993). The model of speciation in savannah is thus very different from what is hypothesized for the rain forest genets, with this time a diversification of lineages linked to expansion of savannah and contraction of forested zones. One interesting point that highlights the complexity of the evolutionary history of genus *Genetta* resides in the Late Pliocene – Middle Pleistocene splitting between *G. cristata* and *G. servalina*, which are allopatric sister-species, suggesting that speciation in rain forest may also be due to isolation in refuges during the Quaternary (Mayr & O'Hara, 1986). Similarly, two species belonging to the large-spotted genets (*G. bourloni* and *G. poensis*), which diverged during the Pleistocene, exemplify a recent reversion to rain forest habitat from a savannah clade, again contradicting the common point of view that transitions in African mammals were generally made from rain forest to savannah (see Grubb, 1978).

CONCLUSIONS

The evolutionary history of the genets seems very complex and influenced by a variety of factors. Our results show that climatically induced speciation is likely to have promoted diversification within African taxa during the alternation of cool and warm Quaternary periods (Diamond & Hamilton, 1980; deMenocal, 1995) inducing displacement of ecotones (Ambrose & Sikes, 1991). However, such a model cannot explain the most ancient splitting events in the genet tree, and further investigations are needed in order to understand the evolutionary processes that yielded *G. thierryi* (with *G. abyssinica*) and basal rain forest lineages. The accuracy of divergence time estimates produced in this study will have to be improved by the sequencing of other genes in the near future in order to confirm our hypotheses. Finally, the scarcity of fossils and succession of heterogeneous morphotypes that appeared in a relatively short time suggest that the genus *Genetta* experienced regular extinction events, especially in rain forest where it started speciating. Unfortunately, the acidity of rain forest soils and the lack of caves are likely to make the fossil record unavailable (Hare, 1980; Van Neer, 1990).

The large-spotted genet complex consists of at least six species and is the most dynamic clade in terms of 'recent' evolution. Hybridizations in the wild between *G. maculata – G. tigrina* and *G. maculata – G. pardina* are strongly suspected (Pringle, 1977; Gaubert, 2003b). The large-spotted genet complex might constitute a source of ongoing and future speciation events, which should be studied in the near future with more rapidly evolving markers (C.A. Fernandes & P. Gaubert, unpubl. data). The confirmed species that are *G. bourloni* and *G. poensis*, only known from museum specimens, should urgently be the subject of field surveys in order to achieve information on their biology and status, both totally unknown.

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APPENDIX 1

List of the 50 characters constituting the morphological matrix. Numbers in parentheses correspond to the numbering used in the intrageneric analysis of Gaubert *et al.* (2002). If followed by 'inter', numbering refers to the intergeneric analysis.

SKULL

1. Crest of insertion of temporal muscles (upper part of the parietal): forming a large area slightly elevated 0 – forming a laterally narrowed area 1 – forming a narrow and elevated crest 2 – forming a narrow but not elevated area 3. (2: inter-, redefinition)
2. Premaxillary-frontal contact: absent 0 – variable 1 – present 2. (1)
3. Postorbital process: very sloped 0 – moderately sloped 1 – poorly sloped 2.
4. Posterior extension of the frontal bones: very large, almost completely overlapping the dorsal part of the interorbital constriction 0 – moderated, overlapping *c.* 50% of the dorsal part of the interorbital constriction 1 – very narrow, overlapping the dorsal part of the interorbital constriction only in its median part 1. (4: redefinition).
5. Interorbital constriction: strong 0 (inferior to 1–0.12) – intermediate 1 (equal to 1 ± 0.12) – weak 2 (superior to $1 + 0.12$). (9 inter-, redefinition). [The interorbital constriction corresponds to the ratio between the interparietal constriction (just posterior to the postorbital process) and the interfrontal constriction (just anterior to the postorbital process, vertical to the lacrymal foramen). The value 0.12 is the mean of the standard deviation for the means concerning the measures of the interorbital constriction from all the taxa, out- and ingroup included.]

6. Ventral extension of the pterygoid bones: weakly developed 0 – strongly developed 1. (7: redefinition)
7. Staphylinal fissure: indented 0 – very little marked 1. (8)
8. Curve of the anterior part of the caudal entotympanic bone (internal side): broken 0 – continuous 1.
9. Premaxillary–maxillary suture position in relation to P^1: forward 0 – at the same level 1. (33: inter)
10. Maxillary-palatine suture position in relation to main cusp of P^3: just behind 0 – at the same level 1 – anterior to 2. (21)
11. Anterior extension of the caudal entotympanic bone: short, posterior to the anterior foramen lacerum 0 – long, reaching or anterior to the anterior foramen lacerum 1. (18: inter-, redefinition).

TEETH

12. M_2: reduced 0 – developed 1. (23: redefinition)
13. M2: absent 0 – present 1. (24: redefinition)
14. Lower canines: weak 0 – strong 1. (38: inter)
15. M1 talonid: reduced 0 – developed 1. (29: redefinition)
16. P2 posterior accessory cusp: absent or extremely reduced 0 – present 1. (26)
17. P3 lingual cusp: absent or extremely reduced 0 – present 1. (25)

BODY PROPORTION

18. Ratio head + body/tail length: [superior to 1.5] 0 – [between 1.3 and 1.4] 1 – [equalling 1.2] 2 – [equalling 1.1] 3 – [equalling 1] 4. (50: inter-, redefinition)

PADS

19. Central depression of the forefeet: hairless 0 – hairy 1. (31)

TEATS

20. Number of teats: one pair 0 – two pairs 1.

HAIR ULTRASTRUCTURE

21. Spatula cortex of dorsal guard hair: thin 0 – thick 1. (60: inter)
22. Cross-section shape of the spatula base of dorsal guard hair: oblong 0 – round or slightly ovoid 1. (36)
23. Margin of the scales located in the middle of the spatula of dorsal guard hair: smooth 0 – crenelated 1 – very crenelated 2. (38)
24. Shape of the transition zone scales located between the base and the middle of dorsal guard hair: triangular 0 – rhomboid 1 – baso-distally compressed 2. (37)

COAT

25. Mid-dorsal line pattern: broken, suggested by aligned spots 0 – continuous 1. (39: redefinition)
26. Mid-dorsal line: bright and bordered by a pair of longitudinal stripes 0 – full dark 1. (39: redefinition)
27. Mid-dorsal spots: large 0 – narrow 1. (42: redefinition)
28. Longitudinal coalescence of the first row (contiguous to mid-dorsal line) of mid-dorsal spots: absent 0 – partial 1 – important 2 – complete, forming a continuous stripe 3. (41: redefinition)
29. Distribution of dorso-lateral spots: not densely spotted 0 – densely spotted 1.
30. Dark stripe longitudinally crossing the rings of the tail: very marked 0 – moderately marked 1 – just appearing 2. (44: redefinition) [the darkening of the distal part of the tail (character 47) is not related to a putative enlargement of the dark stripe, but to a darkening of the bright rings. Characters 30 and 47 are thus independent].
31. Pair of wide median nuchal stripes: absent 0 – present 1. (45)
32. Thin nuchal stripes: well defined 0 – confused 1. (46)
33. Coat of hindfoot: dark 0 – bright anterior part and dark back part (around pads) 1 – bright 2. (47)
34. Upper leg coat: uniformly dark 0 – bright anterior part and dark back part 1 – uniformly bright 2. (48: redefinition)
35. Coat of inner upper leg: bright 0 – dark 1.
36. Upper leg spots: not merged 0 – merged in partial horizontal lines 1. (49: redefinition)
37. Forefoot coat: dark 0 – bright anterior part and dark back part (around pads) 1 – bright 2. (50)
38. Upper arm coat: uniformly dark 0 – bright anterior part and dark back part 1 – uniformly bright 2. (52: redefinition)
39. Upper arm patterns: numerous spots 0 – few spots 1 – no spot 2. (53)
40. Upper arm spots: not merged 0 – merged 1.
41. Sub-ocular spots: bright and contrasting 0 – absent 1. (55: redefinition)
42. Upper labial spots: bright and very contrasting 0 – reduced and poorly contrasting 1. (56: redefinition)

43. Hairs of mid-dorsal crest: relatively short 0 – relatively long 1 [when compared with the adjacent dorsal hairs, 'relatively short' means 'similar length'].
44. Tail hairs: short (from 20 to 30 mm) 0 – long (from 40 to 45 mm) 1. (58)
45. Number of bright rings on tail: four 0 – five 1 – six 2 – seven 3 – eight 4 – nine 5– ten 6– eleven 7– twelve 8.
46. Distal part of the tail: alternation of dark and bright rings 0 – the last bright ring is hardly visible 1 – several bright terminal rings are hardly visible 2. (60)
47. Tip of the tail: dark 0 – bright 1. (59: redefinition)
48. Chin: inferior chops bordered by a large dark stripe 0 – inferior chops bordered by a thin dark stripe 1 – inferior chops uniformly bright 2.
49. Sagittal crest: present 0 – absent 1. [The sagittal crest is defined as a thin stripe of nuchal hairs orientated the opposite way to the adjacent nuchal hairs]
50. Relative width of the bright rings over the dark rings (middle of the tail): less than 20% 0 – between 50 and 75% 1 – *c.* 100% 2 – *c.* 200% 3.

APPENDIX 2

Morphological matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Poiana richardsonii	0	0	0	0	2	1	1	1	0	2	0	0	0	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	2	0	0	2	2	1	0	1	1	0	0	6/8	0	1	2	1	3
Genetta thierryi	2	0	0	1	1	1	0	1	1	2	1	1	1	1	1	0	1	3	0	1	0	1	1	0	1	0	1	1/2	0	1	0	1	2	2	0	0	2	2	1	0	0	0	0	0	4/5	1	0	2	1	2
Genetta johnstoni	1	?	0	0	1	0	0	1	1	1	1	1	1	0	1	0	0	4	1	0	1	0	1	0	1	1	0	1/3	1	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	3/4	0	1	0	1	0
Genetta servalina	1	2	0	1	2	1	0	1	0	1	1	1	1	1	1	0/1	1	3	1	0	0	0	1	0	0	0	0/1	0	1	2	0	0	0	1	1	0/1	0	1	0	0	0	0	0	0	5/7	0	1	1	1	0
Genetta victoriae	1/3	2	0	1/2	0	1	0	1	0/1	0	1	1	1	1	1	0	1	1	1	0	0	0	1	0	0	0	1	1	1	2	1	0	0	1	1	1	0	1	0	0	0	1	0	1	2/4	0	0	0	0	0
Genetta genetta	1/3	0	0	1	1	0	0	1	0	1	0	1	1	1	1	0/1	1	3	1	1	0	0	1	2	1	1	0/1	1/2	0	1	0	0	1	1	0	1	1	1	1	0/1	0	0	1	1	4/5	0	1	0	1	2
Genetta felina	1	0	0	1	1	0	0	0	0/1	1/2	0	1	1	1	1	0/1	1	4	1	1	0	0	1	2	1	1	1	2	0	1	0	0	0	1	1	0	1	1	1	0	0	0	1	1	4/5	0	1	0	1	3
Genetta angolensis	1/3	1	0	1	1	0	0	1	1	1	0	1	1	1	1	1	1	3	1	1	0	0	1	0	1	1	1	2	0	1	0	1	1	1	1	0	1	1	1	0	0	0	1	1	6/7	1	0	0	1	1
Genetta pardina	2/3	2	0	1	0	1	0	0	1	1	1	1	1	1	1	0	0	2	1	1	0	0	0	2	1	1	0	0	1	1	0	0	0	1	1	0	0	1	1	0	0	0	0	0	2	1	0	0	1	0
Genetta poensis	2	1/2	0	2	0	1	0	0	0	1	0	1	1	1	1	1	1	1	1	?	0	0	0	0	1	1	1	3	1	1	0	1	0	1/2	1	1	0	0	0	0	0	0	0	0	0/2	2	0	1	1	0
Genetta bourloni	1	1	0	0	1	0	0	0	1	1	1	1	1	1	1	1	1	2	1	?	0	0	0	0	1	1	0	2	1	1	0	0	0	1/2	1	0	0	0	0	0	0	0	0	0	1/3	2	0	1	1	0
Genetta 'schoutedeni'	2	2	0	2	0	1	0	0	0	1	1	1	1	1	1	1	1	?	1	1	0	0	?	?	1	1	0	0/1	0	1	0	0	1	1	?	0	2	2	1	0	0	0	0	0	3/4	1	0	1	1	1
Genetta maculata	2	1	0	2	0	1	0	0	0	1	0	1	1	1	1	0/1	0/1	3	1	1	0	0	0	2	1	1	0	0/1	0	1	0	0	1	1	0	0	2	2	0	0	0	0	0	0	2/5	1	0	1	1	1
Genetta tigrina	3	1	0	1	1	1	0	0	0	2	0	1	1	1	1	0	0	3	1	1	0	0	1	0	1	1	0	0	0	2	0	0	0	1	1	1	1	1	1	0	0	0	1	1	2/4	1	0	0	1	1
Genetta cristata	1	2	0	1	1	1	0	1	0	1/2	1	1	1	1	1	0	1	1	1	?	?	?	?	?	0	0	1	1	1	2	0	0	0	1	0	1	0	1	0	1	0	0	1	0	4/6	0	1	1	0	1
Genetta piscivora	2	0	1	2	0	0	0	1	1	1	1	0	1	1	1	?	0	0	0	?	1	0	0	1	?	?	?	?	?	?	0	?	0	2	0	?	0	2	?	?	0	0	0	0	?	?	?	1	1	?

Appendix III

Gaubert et *al.* (in press). Patterns of cryptic hybridization revealed using an integrative approach: a case study on genets (Carnivora, Viverridae, *Genetta* spp.) from the southern African subregion. *Biological Journal of the Linnaean Society.*

Patterns of cryptic hybridization revealed using an integrative approach: a case study on genets (Carnivora, Viverridae, *Genetta* spp.) from the southern African subregion

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Running title: cryptic hybridization in southern African genets

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Abstract.- Recent years have seen the development of molecular-based methodologies to investigate hybridization and its impact on the evolutionary process. However, morphological characterization of hybrid zones has scantily been considered, especially in zootaxa. Thus, the level of congruence between molecular and morphological characters when attempting to detect hybrids remains a poorly tackled question. The genets (genus *Genetta*) provide an ideal case study for further investigating the respective contribution of morphology and DNA in hybrid zone characterization because 1/ their morphology has recently been exhaustively explored and 2/ the existence of hybrid zones in southern Africa was proposed in the literature. We assessed levels of hybridization among the southern African genets, and questioned the role of ecological factors on the hybridisation patterns detected. We used an integrative approach involving nine discrete morphological characters and a diagnostic discriminant function (*G. "letabae" / G. tigrina* from KwaZulu-Natal), geometric morphometrics (*G. maculata, G. "letabae", G. tigrina, G. genetta* and *G. felina*) and sequences of cytochrome *b* including collection specimens (all southern African morphological species). The combination of independent materials allowed us to accurately reassess the level of hybridization in southern African genets, and revealed cryptic interspecific gene flows. Morphology unambiguously detected a low number of *G. "letabae"* x *G. tigrina* hybrids and rejected the hypothesis of a large intergradation zone in Kwazulu-Natal, thus supporting their species status. Cytochrome *b* analyses revealed 1/ cryptic, massive hybridization between *G. tigrina* and the sympatric *G. felina*, and 2/ trace of reticulation (one sequence) between *G. tigrina* and the allopatric *G. genetta*. The type specimen of *G. mossambica* Matschie, 1902 is considered a morphological hybrid between *G. maculata / G. "letabae"* and *G.*

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angolensis. Remarkably, the morphological approaches (discrete characters and morphometrics) proved complementary to conclusions derived from cytochrome *b* sequences. Whilst morphometrics was unable to accurately identify all putative hybrids, this approach revealed diagnostic cranial shape differences between recognized species as well as the cryptic *G. "letabae"*. Morphometrics also confirmed the diagnostic value and age dependency of discrete characters. Our integrative approach appeared necessary to the detection of cryptic hybridizations and to the comprehensive characterization of hybrid zones. The recurrent detection of hybrids exhibiting *tigrina*-like coat patterns may suggest 1/ asymmetric hybridization of *G. tigrina* males to females of other species and 2/ positive selection for a *tigrina*-like phenotype in South African habitats, but these hypotheses will have to be further tested using other sources of evidence. Despite the precise mosaic of hybrid zones identified in southern African genets, environmental factors that shape patterns of distribution of hybrids remain unclear. Nevertheless, in the light of our range reassessment, it appears that seasonality of precipitation and periods of annual frost may play a stringent role in the distribution of genets. The complementarity of our results based on morphology and molecules is regarded as an encouragement for further developing integrative approaches in order to better understand the complex phenomena that underlie hybridization processes.

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ADDITIONAL KEYWORDS: Africa; character combination; cytochrome *b*; discrete characters; distributional range; environmental factors; geometric morphometrics; hybrid zones; large-spotted genets; small-spotted genets.

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INTRODUCTION

Hybridization and introgression have long been recognized by botanists as crucial factors influencing the evolutionary process (e.g., Abott, 1992; Ellstrand & Schierenbeck, 2000; Broyles, 2002). However, the field of zoology still suffers from lower productivity in what concerns investigations on this peculiar issue (see Dowling & Secor, 1997). One possible explanation is that detecting animal hybrids from external morphology remains a difficult and hazardous task (Rees, Dioli & Kirkendall, 2002). Nevertheless, the arising of molecular methodologies over the last two decades has shown that interspecific gene flow occurred in various zootaxa more commonly than expected (e.g., Spolsky & Uzzell, 1984; Szymura, Spolsky & Uzzell, 1985; Lehman *et al.,* 1991; Grant & Grant, 1992; Roy *et al.,* 1994; Rohwer & Wood, 1998; Beltran *et al.,* 2002; Evans, Supriatna & Melnick, 2001; Lu, Basley & Bernatchez, 2001; Clarke *et al.,* 2002; Rees *et al.,* 2002; Good *et al.,* 2003). The stakes of studies on hybridization cover the fields of ecology, conservation, and evolution (e.g., DeMarais *et al.,* 1992; Jiggins & Mallet, 2000; Allendorf *et al.,* 2001). A trend that consists in considering hybrids as genotypic classes with various levels of "fitability" regarding environmental conditions has recently emerged (Arnold & Hodges, 1995; Rieseberg *et al.,* 1996; Vollmer & Palumbi, 2002), highlighting the complex mechanisms that underlie the maintenance of hybrid zones (Taylor & Hebert, 1993; Stone, 2000). Whereas detection of hybrids was only based on morphological characters until the mid-1960s (Allendorf *et al.,* 2001), very few recent studies have considered in detail such a material (but see, for instance, Nowak, 1992; Rohwer & Wood, 1998; Rohwer, Bermingham & Wood, 2001). The main biases attributed to the

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latter are that morphological variation has not always a genetic basis and that hybrid individuals are sometimes exactly similar to one of their parental phenotypes (Davison *et al.*, 1999; Randi *et al.*, 2001; Thulin & Tegelstrom, 2002), thus leading to instances in which hybrids are cryptic. However, characterizing morphological hybrids is a crucial point for reconstructing evolutionary processes (DeMarais *et al.*, 1992; Smith, 1992; Bowen *et al.*, 2001) and for conservation actors (Daniels *et al.*, 1998). It is surprising that morphological characterization of hybrid zones has infrequently been considered in recent years, especially since modern systematics is devoted to an integrative approach, i.e. based on the combination of multiple independent markers for reconstructing evolutionary scenarios and delimiting species (e.g., Wayne, 1992; Martin & Bermingham, 2000).

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The congruence between molecular and morphological characters when characterizing hybrid zones is thus a question insufficiently evaluated, and the degree to which each type of data may contribute to elucidate patterns of hybridization remains unclear. Genets (Carnivora, Viverridae, genus *Genetta*) provide an ideal case study to address such questions. First, their morphology has been exhaustively explored through both morphometrics and discrete characters (Crawford-Cabral, 1969, 1970, 1973, 1981a,b; Crawford-Cabral & Pacheco, 1992; Crawford-Cabral & Fernandes, 2001; Gaubert, Veron & Tranier, 2001, 2002; Gaubert, 2003a), and their phylogeny and taxonomy have been recently reexamined (Gaubert, 2003b; Gaubert *et al.*, 2004a). Second, putative hybrid zones in southern Africa have been proposed on the basis of morphological characters by Pringle (1977), thus providing the opportunity to test an *a priori* hypothesis of hybridization (in contrast with the majority of the reported studies, in

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which hybrid zones were not looked for before being detected; see Rees *et al.*, 2002). Genets are small Carnivores naturally present throughout Africa, where they occupy a variety of habitats, from rain forests to savannahs (Kingdon, 1997). Four species are generally accepted as occurring in the southern African subregion (Crawford-Cabral, 1981a; Schlawe, 1981; Crawford-Cabral & Pacheco, 1992): *Genetta angolensis* Bocage, 1882; *Genetta tigrina* (Schreber, 1776); *Genetta maculata* (Gray, 1830) (*sensu* Gaubert *et al.*, 2003a,b); *Genetta genetta* (Linnaeus, 1756). The latter two have a widespread distribution throughout sub-Saharan Africa. *G. angolensis* has a discontinuous range from Central Angola to extreme north Mozambique (Crawford-Cabral, 1969, in press). *G. tigrina* is endemic to South Africa and ranges from SW Cape Province to former Pondoland in the KwaZulu-Natal province (Schlawe, 1981; Crawford-Cabral & Pacheco, 1992). A recent phylogenetic study (Gaubert *et al.*, 2004a) demonstrated that *G. genetta* was paraphyletic in terms of mitochondrial DNA, suggesting that two distinct species of small-spotted genets may exist in the southern African subregion: *G. genetta*, which ranges from Europe to southern Namibia and northeastern South Africa (SA), and *Genetta felina* (Thunberg, 1811), present in SA, Namibia, Angola and Zambia. All these species (except *G. genetta* and *G. felina;* see below) are distinguishable through well-marked coat pattern and skull characteristics (Crawford-Cabral, 1981a; Gaubert *et al.*, 2001; Gaubert, 2003a). However, the alleged morphological similarities between *G. maculata*, *G. tigrina* and another species from West Africa (*Genetta pardina* I. Geoffroy Saint-Hilaire, 1832), which make up the large-spotted genets or "*tigrina-pardina* complex" (Crawford-Cabral & Pacheco, 1992), have made the assessment of taxonomic boundaries much debated (e.g. Schlawe, 1981a; Meester *et al.*, 1986; Kingdon, 1997). In

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addition, delimitations within *G. maculata* are difficult because of high variability in coat patterns, and several species / subspecies have been proposed for southern African populations (Crawford-Cabral, 1981a; Crawford-Cabral & Fernandes, 2001). *G. tigrina* is traditionally split into two subspecies: *G. t. tigrina*, from SW Cape Province to southern KwaZulu-Natal, and *G. t. methi*, from former Pondoland in southern KwaZulu-Natal (Roberts, 1951), the two taxa possibly intergrading together (Pringle, 1977; Meester *et al.*, 1986). The validity of the species status of *G. maculata* and *G. tigrina* is confused by reported captive crossbreeding (Gray, 1971). Moreover, Pringle (1977), while examining a large series of specimens from KwaZulu-Natal, found a predominant proportion of hybrids. Unfortunately, the subsequent work of Crawford-Cabral & Pachecho (1992) did not take into account the critical material analyzed by Pringle.

The aim of this study is to explore the question of the respective contribution of morphological and molecular data in characterizing hybrid zones. In other words, is an integrative approach including morphological characters for detecting hybrids a valuable tool? Hybridization in southern African large-spotted genets was reassessed through an integrative approach using discrete morphological characters, traditional and geometric morphometrics, and cytochrome *b* sequences. Recent collection of *G. tigrina* - *G. maculata* in the critical region of KwaZulu-Natal by PJT, provided crucial samples for both molecular and morphological / morphometric analyses. Since *G. genetta* is also reported to hybridize in captivity with members of the *tigrina-pardina* complex (Gray, 1971), we enlarged our investigation to include all five species present in the subregion in order to test for putative *in situ* hybridization. In return, taxonomic resolution of all these units is expected to be improved.

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MATERIALS AND METHODS

DISCRETE MORPHOLOGICAL CHARACTERS

More than 1500 specimens from seven museums (Durban Natural Science Museum, SA [DNSM], the Natal Museum, Pietermaritzburg, SA [NM], the South African Museum, Cape Town, SA [SAM], the Natural History Museum, London, United Kingdom [BMNH], the Museum für Naturkunde, Berlin, Germany [MFNB], the Rijksmuseum van Natuurlijke Historie, Leiden, Holland [RMNH], and the Muséum National d'Histoire Naturelle, Paris [MNHN]) were examined in order to have a broad representation of the morphological variation within genets from the southern African subregion (Gaubert, 2003b). From this material, over 400 collection localities were surveyed (Fig. 1). A total of 26 specimens from the putative *G. maculata / tigrina* hybrid zone in KwaZulu-Natal were examined (Table 1). Other specimens both from adjacent and remote parts of the subregion, and further afield for reference purposes, were examined. Following recommendations in Gaubert *et al.* (2001, 2002), we defined nine discrete characters (seven for coat pattern and two for skull) diagnostic for *G. maculata* and *G. tigrina* (Appendix 1) in order to identify putative hybrids from Kwazulu-Natal. As some character states in juvenile specimens are common to both species, only adult or old subadult specimens were used. We considered a morphological hybrid as being distinct from both species phenotypes by possessing a "mosaic" of character states (Allendorf *et*

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al., 2001; for empirical examples in Carnivorans: Bee & Hall, 1951; French, Corbett & Easterbeen, 1988).

MORPHOMETRICS

Two independent methods were used to assess the status of large-spotted genets from KwaZulu-Natal, in relation to reference samples of the four species that potentially hybridize in South Africa (*G. genetta, G. felina, G. maculata, G. tigrina*): 1) diagnostic discriminant function analysis (DFA) based on cranial distance measurements; and 2) geometric morphometric analysis of dorsal and ventral cranial images. Categories of relative ages and list of samples (n = 69) are given in Appendix 2.

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DFA

Following Crawford-Cabral & Pachecho (1992), five cranial measurements were recorded (Mitutoyo calipers - 0.01 mm) on 19 adult skulls from KwaZulu-Natal from DNSM, MFNB and NM collections (see Table 1): condylo-basal skull length (CBL) - least inter-temporal [i.e. inter-orbital] constriction (LIC) - greatest length of bulla (LOB) - interpterygoid width, taken between pterygoids, just before they flare outwards posteriorly (IPW) - width between M^1-M^1, taken at base of upper M^1 (WM1). We calculated scores of individuals based on the discriminant function equation provided by Crawford-Cabral & Pachecho (1992), which allows *a posteriori* taxonomic identification of "unknowns": Discriminant score = (-0.1797 x CBL) + (0.6705 x LIC) – (0.8934 x LOB) + (0.5530 x IPW) + (0.4697 x WM1) + 6.735. Positive discriminant scores

indicate *G. tigrina* while negative scores indicate *G. maculata*. Values close to zero were taken to be possible hybrids. These values were compared with discrete pelage and cranial characters obtained from the same specimens.

Geometric morphometrics

Dorsal (n = 69) and ventral (n = 68) images of genet skulls were obtained in a standardized fashion using a Sony Mavica FD7 digital camera. Using thin plate spline (TPS) methodology (Rohlf & Bookstein, 1990; Rohlf & Marcus, 1993), cranial shape and size variation were evaluated in *G. maculata, G. tigrina, G. genetta* and *G. felina*. The latter two species were grouped together under *G. genetta* (*sensu lato*), since the purpose of the analysis was to clarify taxonomic boundaries between *G. maculata* and *G. tigrina*. Apart from unambiguously identified "reference samples" of the four species, the sample included the problematic large-spotted specimens from KwaZulu-Natal (same specimens analyzed by discriminant analysis). The programme TPSDig (Rohlf, 1998) was used for digitizing 16 and 12 landmarks (see Corti & Fadda, 1996) from the dorsal and ventral cranium respectively (Fig. 2). Paired landmarks were included in dorsal images to give a better visualization of shape changes in the cranium as a whole. Since we were predominantly concerned with variation in symmetric structures, landmarks were taken only on the left hand side for ventral landmarks. The approach of Fadda, Faggiani & Corti (1997) was used to test for error in landmark or camera placement. Analysis of replicated samples of dorsal images (16 landmarks) from three individuals suggested that measurement error was negligible in terms of overall inter-individual variation (data not shown).

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As a first step in each analysis, Generalized Procrustes analysis (GPA; equivalent to the generalized least squares method of Rohlf & Slice, 1990) was performed to obtain a consensus configuration after scaling, rotation and translation of superimposed landmark configurations. Residual variation was then decomposed into a weights matrix (W) of partial warps scores and a uniform component comprising two vectors (U1 and U2; representing "affine" shape changes). Together W and U represent total shape changes. Using the programme tpsRelw (version 1.16; Rohlf, 1997a), relative warps analysis (RWA) was performed, and thin plate spline grids were used to visualize the distortion of landmarks. RWA was used firstly to assess intraspecific variation in dorsal cranial shape within *G. maculata*, due to measurement error, sex, age and geographic variation. This procedure resulted in the exclusion of "juveniles" in subsequent species comparisons. In the final datasets, the shape matrix (U+W) was analyzed by means of canonical variates analysis (CVA) and Multivariate Analysis of Variance (MANOVA: Wilks lambda test), to test for significance of shape differences between the three species. Using tpsRegr (version 1.13; Rohlf, 1997b), both canonical vectors were regressed against the shape matrix (U+W) so as to visualize shape changes by means of thin plate splines. Discrete morphological -pelage and cranial- criteria were used to assign, unequivocally, specimens from the disputed hybrid zone to their correct species for the purpose of CVA and to identify possible hybrids. Four suspected hybrids (see Table 1) were excluded from the *a priori* CVA analysis of species-groups, but the specimens were plotted *a posteriori* to assign each to its closest species. MANOVA and CVA were performed using the programme NTSys (Rohlf, 1997c). Centroid sizes of all specimens were extracted using tpsRegr, and differences between species were analyzed using

conventional ANOVA procedures (XLSTAT 6.1; Addinsoft, 2003). The programme tpsRegr was also used to regress the shape matrix (W+U) against centroid size, in order to test whether shape differences between and within species were allometric in nature.

CYTOCHROME *B*

Nucleotide sequence variation was examined for 60 specimens (Appendix 3). The sample set includes 19 *G. maculata* from KwaZulu-Natal -and neighbouring regions- and five from East Africa, 13 *G. tigrina* from the coastal area of SA (eastern limit: East London), five *G. pardina* from West Africa, two *G. angolensis* from Zambia and 14 *G. genetta s.l.* from SW Europe to SA. Two other species of genets (*G. johnstoni* and *G. servalina*) were used as outgroups for phylogenetic analysis. Cytochrome *b* sequences (cytb) were obtained in two different Laboratories, namely the Service de Systématique Moléculaire, MNHN (PG), and the Biodiversity and Ecological Processes Group, Cardiff School of Biosciences, Cardiff University (CAF). Extraction protocols, set of primers used for PCR amplification and sequencing steps are described in Gaubert *et al.* (2004a,b).

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Sequences were manually aligned with BioEdit version 5.0.6 (Hall, 1999) and Sequencher^{TM} 3.0 (Gene Codes Corporation Inc.), and analyzed using the criteria of maximum parsimony (MP) and maximum likelihood (ML; neighbor-joining method) under PAUP* Beta version 4.0b2 (Swofford, 2001). The best-fitting model of sequence evolution and parameters used in the ML analysis were estimated with ModelTest 3.06 (Posada & Crandall, 1998). The model selected was TrN (Tamura & Nei, 1993) using gamma shape (G) and an assumed proportion of invariable sites (I). Robustness of nodes

was assessed via the bootstrap method (Felsenstein, 1985) with 100 (MP) to 1000 (ML) replicates. Since haplotype diversity estimates are sensitive to sampling effects, we manually calculated haplotype diversity (h) within each well-supported clade by direct count of haplotypes corrected for sample size. Nucleotide diversity (π; Nei 1987) and number of polymorphic sites (S) were calculated using Arlequin 2.0 (Schneider, Roessli & Excoffier, 2000) with an allowed missing level per site of 0.05 (correction for missing data). In order to evaluate haplotype structuring within the *tigrina-pardina* complex, we used hierarchical AMOVA analyses (AMOVA 1.55; implemented in Arlequin) on a data matrix consisting of seven "samples" determined according to well-supported clades, and original morphological identification and distribution in the case of *G. maculata* and *G. tigrina*. *G. pardina* was included in order to complete the taxonomic representation of the *tigrina-pardina* complex, and *G. maculata* was split between northern (East Africa) and southern (Lesotho and KwaZulu-Natal) populations in order to test for possible geographical structuring following Crawford-Cabral & Fernandes (2001). Species representations were as follows: *G. genetta* (11 specimens), *G. felina* (7), *G. angolensis* (2), *G. pardina* (5), *G. tigrina* (8), *G. maculata* "North" (5) and *G. maculata* "South" (19). The significance of the F_{ST} values was determined with 16000 random permutations, using the model of evolution (TrN) and gamma shape parameter fixed by ModelTest.

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RESULTS

MORPHOLOGY: DISCRETE CHARACTERS AND DISCRIMINANT SCORES

The discriminant scores applied to the identification of *G. maculata* and *G. tigrina* mostly agreed with determinations based on assessment of qualitative pelage and cranial characters (Table 1). However, since hybrids are not always intermediate in morphology between parental types, discrete characters were given preference in assigning hybrids when discrepancies occurred. Five specimens originating from a restricted zone in central KwaZulu-Natal were identified as hybrids between *G. maculata* and *G. tigrina* (see Fig. 1 and Table 1). Two specimens (NM 1720 and 1721) from the area of Highflats in southern KwaZulu-Natal bore a mosaic of coat pattern character states between the two species. The specimen from the Royal Natal National Park (NM 2055) had a *tigrina*-like caudal entotympanic bone (c.e.b.), but an inter-orbital constriction / frontal width ratio (int1) characteristic of *G. maculata* (0.866). The specimen from former Pondoland (MFNB 19696) exhibited a pure *tigrina*-like coat pattern, but int1 was 0.770, characteristic of *G. maculata*. On the contrary, the specimen from Umgeni Valley Game Ranch (NM 1990) had a *maculata*-like coat pattern, but int1 > 1, which characterizes *G. tigrina*. A sixth specimen (DNSM 3335) originating from New Germany, Durban, was identified as a hybrid on the basis of mixed skull character states, although the discriminant score (-2.215) indicates a clustering within *G. maculata*. A specimen from Bishopstowe (NM 1614) had a *maculata*-like morphotype, but its int1 (1.000) was characteristic of *G. tigrina*. However, we did not consider this specimen as a hybrid and identified it as *G. maculata* instead, since it is an old subadult with the postorbital constriction wide relative to the frontal width, which might have biased the int1 ratio (see Appendix 1). The

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specimen DNSM 2232 had a non-conclusive discriminant score (-0.355), but was clearly identified as *G. maculata* on the basis of discrete characters. On the other hand, some of the identifications made by Pringle (1977) are quite incongruent with our results and these comprised half of the Pringle's specimens considered in our study (see Table 1). Only one out of four of Pringle's "hybrids" was identified as such by our analyses (NM 1990). Moreover, three specimens identified as *G. tigrina* by Pringle proved to be hybrids according to our identification criteria. Morphological evidence (discrete characters) for hybridization between *G. tigrina* and *G. felina* was found on a single specimen (BMNH 35.9.1.162; Cathcart, Eastern Cape Province; see Fig. 1). It had a pure *tigrina*-like coat pattern, but its skull was strikingly characteristic of *G. felina*. It is an old adult, with a large and quite inflated c.e.b., a very well marked lingual cusp on P^2 (only lightly marked in *G. tigrina / G. maculata*) and a shape of the inter-pterygoidal width characteristic of *G. felina* (for this last character, see Gaubert *et al.*, 2002). The type specimen of *Genetta mossambica* Matschie, 1902, which originates from Mossimboa, Mozambique (MFNB 19659 / 1106), potentially constitutes a hybrid between *G. angolensis* and *G. maculata* (Fig. 1). Indeed, it exhibits a mosaic of discrete character states of the two species: thin dorsal spots partially fused in the posterior part of the back, scapular region with spots poorly marked and posterior parts of limbs dark (*G. angolensis*), absence of erectile dorsal crest, bright inferior chops of the chin, marked inter-orbital constriction and inflated c.e.b. (*G. maculata*). In addition, it showed dark / bright contrasts less marked than in *G. angolensis,* and intermediary hair length on body and tail.

GEOMETRIC MORPHOMETRICS

Intraspecific variation

Relative Warps Analysis of dorsal image landmarks based on samples of *Genetta maculata* revealed that Class 1 skulls (juveniles) are morphometric outliers on the first relative warp axis (Appendix 4). Juveniles had a distinct cranial shape, being relatively much broader in the postorbital region, with a posteriorly diverging, pear-shaped braincase, and narrower zygomatic arches. These trends were evident to a lesser extent in Class 2 individuals (subadults), which tended to cluster much closer to the remaining adult age classes (young and older). As such, the major shape changes due to age could be described simply by the ratio of inter-orbital constriction to frontal width (i.e., character 8 in Table 1). This emphasizes the importance of excluding juvenile and subadult skulls if this character is to be used for species diagnosis. It was also apparent that adult *G. maculata* skulls from Central and East Africa plotted mostly in the top left quadrant, separate from the remaining sample from southern Africa. The difference in shape, as judged from the thin plate splines, seems mostly related to a wider inter-orbital constriction relative to frontal width, and a relatively narrower zygomatic arch, in South African samples relative to those from Central and East Africa.

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Interspecific differences and identification of hybrids

Canonical variates analysis (CVA) of the dorsal and ventral shape matrices (W+U) indicated a clear morphometric distinction between *G. maculata, G. tigrina* and *G. genetta s.l.* (Figs. 3 and 4). All individuals were correctly classified into their respective

species-groups. Wilks lambda values of 0.050 and 0.037 were obtained for dorsal and ventral analyses respectively ($p < 0.001$). Based on pairwise Mahanalobis distances calculated between pairs of species, *G. maculata* and *G. tigrina* are as distinct from one another (4.7 and 4.48 for dorsal and ventral respectively) as are *tigrina* and *genetta s.l.* (4.55 and 4.10 for dorsal and ventral respectively), supporting the hypothesis that *tigrina* and *maculata* are valid species. The analysis included, in the *tigrina* group, four specimens from former Pondoland belonging to the subspecies *G. t. methi.* Differentiation between species in dorsal shape (Fig. 4; axis 1) appeared largely to be due to changes on the relative width of the inter-orbital constriction compared with the frontal width, with *G. maculata* having inter-orbital constriction (distance from landmark # 4 to # 10) narrower than frontal width (#2 to #12), while the opposite was true for the other two taxa (*G. tigrina* and *G. genetta s.l.*). A similar picture occurred with ventral shape (Fig. 5). Variation in the first CVA axis appears to be largely due to the distance between landmarks #4 (inter-orbital constriction viewed ventrally) and #11 (midpoint of posterior margin of palate), with *G. maculata* showing a narrower distance (inter-orbital constriction) compared with the other two genets. On the other hand, the second ventral CVA axis revealed differences in the relative size of the bulla, with *tigrina* having a relatively smaller bulla, as well as a much wider zygomatic arch, and a wider skull overall, compared with *maculata* and *genetta s.l.*. Specimens identified as possible hybrids in Table 1, either between *tigrina* and *maculata* (n = 4) or between *tigrina* and *genetta s.l.* (n=1), are plotted on the dorsal and ventral CVA plots. In dorsal and ventral plots, the *tigrina x genetta s.l.* hybrid occupied a position as an outlier of the *G. genetta s. l.* group, tending in the direction of the *G. tigrina* group. The four *tigrina x maculata*

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hybrids for which skulls were available were grouped with *maculata* or as an outlier on the dorsal plot (Fig. 3), but were situated as outliers of either *G. tigrina* (one specimen) or *G. maculata* (three specimens) in Figure 5 (ventral plot). Thus, while geometric morphometric data revealed diagnostic shape differences between recognized species, including the cryptic *G. "letabae"*, they were unable to accurately identify putative hybrids.

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CYTOCHROME *B*

The complete cytochrome *b* (1140 bp) was obtained from fresh samples (n = 14). A number of 577 to 1011 nucleotidic sites were sequenced from Museum specimens, depending on the set of primers used and the quality of DNA extracts (n = 12). A total of 26 new sequences were deposited in GenBank (accession numbers from AY397697 to AY397722; see Appendix 3). The NJ tree using ML criteria (TrN model of evolution using gamma shape (G) = 0.8563 and assumed proportion of invariable sites (I) = 0.565) confirmed the clade groupings found by Gaubert *et al.* (2004a,b), each species forming a well-supported clade (Fig. 6). The only exception resides in *G. maculata* and *G. tigrina*, which hapotypes are mixed together in a single clade. The MP analysis yielded 206742 equally parsimonious trees (469 steps; CI = 0.563; RI = 0.835), which resulted in a strict consensus tree of 485 steps (CI = 0.544; RI = 0.822; tree not shown) with a very similar topology to the NJ tree (253 variable and 166 parsimony-informative characters, respectively). Hybridization was clearly identified between *G. tigrina* and 1/ *G. felina*, with *G. tigrina* specimens KM30813, A274, TM32282 and TM35133 included in the *G.*

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felina haplotype clade and 2/ *G. genetta*, with the *G. tigrina* specimen KM31133 included in the *G. genetta* haplotype clade. The unresolved branching pattern observed within the *G. maculata* - *G. tigrina* clade did not allow the identification of putative mtDNA reticulation.

Haplotype diversity (*h*) was generally high (see Table 2), and reached 1.000 in *G. pardina* and *G. maculata* "North" (n = 5 in both cases). The taxa characterized by a moderate *h* value were *G. felina* (0.571; n = 7) and *G. maculata* "South" (0.632; n = 19). Nucleotide diversity (π) was correlated to *h* values, except in *G. tigrina*, and in a less measure, *G. felina* and *G. maculata* "South", where *h* values were high or moderate and π values were very low. The two specimens representing *G. angolensis*, collected in the same locality, shared the same haplotype. The AMOVA results indicated that 69.51 % of the observed variation was explained by the differentiation among the seven taxa as defined in Materials and Methods (F_{ST} = 0.695; P < 0.0001). When the AMOVA was restricted to the taxa belonging to the *tigrina-pardina* complex, the percentage of variation among the groups (52.69 %) was still superior to that within the groups (F_{ST} = 0.527; P < 0.0001). All F_{ST} values between the seven taxa were significant, thus indicating a strong differentiation (data not shown). Pairwise F_{ST} values among the *tigrina-pardina* complex were relatively low but remained highly significant. Thus, we found a significant differentiation between *G. pardina* and the northern and southern "populations" of *G. maculata* (0.669 and 0.408; P < 0.0001 and 0.01, respectively), and between *G. tigrina* and the two latter (0.347 and 0.371; P < 0.0001 and < 0.001, respectively), which was not suggested by the phylogenetic analyses. We also found a

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low but significant F_{ST} value between the two "populations" of *G. maculata* (0.216; P < 0.01).

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DISCUSSION

PATTERNS OF HYBRIDIZATION AND TAXONOMIC BOUNDARIES IN SOUTHERN AFRICAN GENETS

The use of an integrative approach on a large set of specimens representing all southern African species of genets permitted a detailed assessment of the level of hybridization in the subregion, and revealed cryptic interspecific gene flow (see Fig. 1). All methods employed in the present study refuted the hypothesis of a large intergradation zone between *G. maculata* ("*letabae*")and *G. tigrina* in KwaZulu-Natal (Pringle, 1977), thus confirming the species status of the two genets (Crawford-Cabral, 1981a; Crawford-Cabral & Pacheco 1992; Gaubert *et al.*, 2001, 2002; Gaubert, 2003a). Notwithstanding a few incongruences, the observation of discrete morphology and use of the DFA equation (Crawford-Cabral & Pacheco, 1992) yielded concordant results in identifying a very low number of hybrids. One of the characters used by Pringle (1977) to distinguish between *G. tigrina* and *G. maculata* was defined according to the predominance of black over red-tipped hairs in dorsal spots. This might explain the lack of congruence between Pringle's and our identifications, because coat coloration has been shown to vary according to habitat across the range of *G. maculata / "letabae"* (Crawford-Cabral, 1970, 1981a,b; Gaubert *et al.*, 2003b). The geometric morphometric analysis failed to unambiguously

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confirm the status of the hybrid specimens but clearly distinguished between *G. maculata* and *G. tigrina* and *G. maculata* and *G. "letabae"*. The specimens attributed to *G. t. methi* (from southern KwaZulu-Natal: Oribi Gorge, Underberg and Donnybrook) grouped unequivocally with southwestern populations of *G. tigrina*, thus rejecting the hypothesis that *methi* may constitute a hybrid population between *G. maculata* ("*letabae*") and *G. tigrina* (see Crawford-Cabral & Pacheco, 1992) and suggesting instead its status as northeastern subspecies of *G. tigrina* (Roberts, 1951). An interesting point is that RWA of dorsal image landmarks indicated a marked differentiation between Central - East African and southern African specimens of *G. maculata* that was mainly explained by a narrower inter-orbital constriction and a wider zygomatic arch in southern African samples, these transformations being of the same nature as the ones used to distinguish *G. maculata* from *G. tigrina*. This result confirm the karyological evidence raised by Gaubert *et al.* (in press), who proposed the existence of two distinct taxa within the *G. maculata* super-species: *G. maculata sensu* Gaubert *et al.* (2003a,b; North-Central to East Africa) and *G. "letabae"* (including *zuluensis*, following Crawford-Cabral & Fernandes, 2001; South-East and South Africa). Such a "East - South" separation has already been recovered in other African mammals (Arctander, Johansen & Coutellec Vreto, 1999), suggesting the possible existence of eastern and southern 'dry' refuges during the most drastic phases of dense forest expansion at the end of the Pleistocene. The AMOVA analysis of cytochrome *b* haplotypes confirmed the structuring in distinct entities of *G. pardina, G. maculata* (East Africa), *G. "letabae"* (South Africa) and *G. tigrina*. However, putative biases related to taxonomic sampling and ancestral polymorphism, as suggested by the very low values of nucleotide diversity in *G. tigrina* and *G. "letabae"*

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from South Africa and the "mixed" branching pattern of the minimum spanning tree built from the F_{ST} matrix (data not shown), should make us cautious about the interpretation of these results. The taxonomic status of the southern genet *G. "letabae"* may be of species level, but this assertion will require analysis of additional molecular markers (e.g. microsatellites and SNPs) on a broader sample set.

Important reticulation of cytochrome *b* was detected between *G. felina* and *G. tigrina*, two sympatric species which diverged at the end of Pliocene (Gaubert *et al.*, 2004a). The important fraction of hybrids, as portrayed by the inclusion of up to 50 % of the "morphological" *G. tigrina* specimens in the haplotype clade of *G. felina*, was unsuspected, especially since the two genets are morphologically well differentiated and are likely to live in different habitats (Skinner & Smithers, 1990). The morphological observations confirmed *in situ* hybridization with one "mosaic" specimen from Cathcart. Cryptic hybridization was also detected between *G. genetta* and *G. tigrina*, two species estimated to split near the middle Pliocene (Gaubert *et al.*, 2004a) but not range-overlapping, with a specimen from Western Cape exhibiting a *G. tigrina* morphotype but having a *G. genetta* haplotype.

The morphological observation of ca. 1000 *G. maculata* and *G. angolensis* specimens allowed the identification of only one hybrid, namely the type specimen of *G. mossambica*. Considering the type specimen of *G. mossambica* as a hybrid might clarify the taxonomic debate over the "rusty-spotted genets" from Mozambique (Roberts, 1951; Crawford-Cabral, 1981a; Crawford-Cabral & Fernandes, 2001; J. Crawford-Cabral, pers.comm. 2002). The specimens of *G. angolensis* from Mozambique, Malawi and Tanzania examined by PG were exactly similar with western populations from Angola

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and Democratic Republic of Congo, and no other morphotypes related to the type-specimen of *G. mossambica* were recovered. Thus, we propose that the name *mossambica* Matschie, 1902 should be abandoned for designating specimens formerly attributed to this taxon and related to *G. maculata* or eastern populations of *G. angolensis*.

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HYBRIDIZATION PATTERNS IN GENETS AND ENVIRONMENTAL FACTORS

Recent advances in hybridization research have argued that ecological factors may play a more important role in speciation and maintenance of hybrid zones than genomic incompatibility *per se* (Arnold & Hodges, 1995; Jiggins & Mallet, 2000; Schilthuizen, 2000). Despite the comprehensively identified mosaic of hybrids in southern African genets, environmental factors that may shape patterns of genet hybrid zones in the subregion remain unclear. Although diversity exist in the spectrum of niches of genets with large distributions, the specificity of their respective habitats is likely to prevent syntopy and direct competition for food resources between genet species (Kingdon, 1977; Rautenbach & Nei, 1978; Yalden, Largen & Kock, 1980; Happold, 1987; Kingdon, 1997). Rainfall patterns for the southern African subregion indicate increasing aridity from east to west (from 1000 to <125 mm; see Mansell & Erasmus, 2002), which seems to partly influence the current distributions of *G. "letabae", G. tigrina, G. angolensis*, present in humid zones, and *G. genetta* and *G. felina*, occupying drier areas (Stuart, 1981; Skinner & Smithers, 1990; Kingdon, 1997; Crawford-Cabral, in press). Interestingly, it appears that most of the current ranges of the southern African genets are delimited by

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climatic factors such as seasonality of precipitation and periods of annual frost (see the synthetic map of O'Brien and Peters (1999: 120, Fig. 9-4)), and not by vegetation or precipitations *per se*. First, *G. "letabae"*, *G. angolensis*, *G. genetta* and *G. felina* occupy a tropical summer-rain climate zone (subhumid to desert) whereas *G. tigrina* is present mostly in the zone of winter-rain climate of the Cape and circum-Mediterranean. Second, the distributions of *G. genetta* and *G. felina* follow the "frost-susceptible region" (i.e. where frost occurs, but not annually; O'Brien & Peters, 1999), a zone in which *G. "letabae"* and *G. angolensis* seem to be absent. In some cases, ranges of southern African genets do not perfectly fit with the seasonality-of-precipitation climate regions (e.g., presence of *G. "letabae"* in the dry south west and *G. felina* sympatric with *G. tigrina* in coastal South Africa), and other cumulative factors, such as micro-habitat conditions, are likely to influence distribution.

Climatic conditions as described above might play a role in the patterns of hybrid zones identified through our study. Our results point out that despite divergences of several million years between species and climatic partitions of distributional ranges, hybridization is possible without invoking currently disturbed "environments" as a causal event (see other studies on Carnivores: Lehman *et al.*, 1991; Wayne & Jenks, 1991; Roy *et al.*, 1994; Stahl & Artois, 1995; Davison *et al.*, 1999; Kyle, Davison & Strobeck, 2003). However, it is not clear whether eco-climatic factors may influence the maintenance of the narrow hybrid zone occurring between *G. "letabae"* and *G. tigrina* in KwaZulu-Natal, a region of complex habitat mosaic (Bourquin, Vincent & Hitchins, 1971; Phillips, 1973) where the two species seem to share similar niches. The proposed infertility of *G. "letabae" x G. tigrina* hybrids based on karyological data (Kumamoto,

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1989; Gaubert *et al.*, in press) might in this case constitute a strong barrier to gene flow (see Barton & Hewitt, 1983), and be responsible of the narrow hybrid zone observed. It is likely that the split between *G. maculata* - *G. "letabae"* and *G. tigrina* was induced by a strong global cooling from 0.6 Myr associated to increasing intensity of glacial events (Denton, 1999). The contact zone between between *G. tigrina* and *G. "letabae"* may be secondary and very recent, since the estimated large extension of forest biome in KwaZulu-Natal and northern Transvaal during the Holocene altithermal (ca. 7000 yrs ago; Eeley, Lawes & Piper, 1999) could have acted as an ecological barrier. However, climatic oscillations from 0.9 Myr (Owen-Smith, 1999; Denton, 1999) might make the past history of this contact zone more complex to trace back.

Other species of genets having diverged during the Pliocene (Gaubert *et al.*, 2004a) are reported here to hybridize *in situ* (*G. tigrina x G. felina* and *G. genetta*). Although the biology of *G. felina* is almost unknown, the distributional data suggest that it occurs in South Africa in the same habitat as *G. tigrina* but has a wider tolerance to drier biotopes, since its range extends towards the dry southwest (Fig. 1). The level of hybridization for such phylogenetically distant taxa is surprisingly high. Unfortunately, karyological data are not available for *G. felina*, and the impact of hybridization on the evolution of these taxa is unknown. One hybrid was also detected between *G. tigrina* and *G. genetta*, two species currently geographically separated by arid regions like the Namib desert and Damara-Nama upland (see O'Brien and Peters, 1999). However, contact zones may have been possible in the past with the existence of a humid period in the Kalahari in the Late Pleistocene (Lancaster, 1979) yielding corridors with favorable habitats for both

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species (Denys, 1999). Given the chromosomal data available (Gaubert *et al.*, in press), it is likely that hybridization leads to unfertile individuals.

The ability to hybridize might actually constitute a symplesiomorphy of the clade studied herein, the fitness and fertility of hybrids being affected, at least partially, by derived chromosomal rearrangements.

IDENTIFYING CRYPTIC HYBRIDIZATION THROUGH AN INTEGRATIVE APPROACH

Our integrative approach appeared necessary to avoid the pitfalls related to the consideration of a single type of marker when questioning species boundaries, speciation and hybridization (e.g., Wu, 2001). Remarkably, the morphological material (discrete characters and morphometrics) proved complementary to inferences derived from cytochrome *b* sequences. The exclusively molecular approaches used in some of the recent literature for characterizing hybrid zones may have biases similar to what has been reproached to morphology, like the non-detection of "ghost" hybrids (Leary, Gould & Sage, 1996). For instance, all three morphological methodologies used in this study identified hybrids between *G. "letabae"* and *G. tigrina* in Kwazulu-Natal, and classified as a morphological mosaic between *G. angolensis* and *G. maculata / "letabae"* the type specimen of *G. mossambica*. The use of cytochrome *b* only would have failed in the clear detection of any of these cases. Despite the fact that this remark is to be downplayed in the case of *G. angolensis* (only two individuals sampled), the absence of clear phylogenetic structure between the haplotypes of the large-spotted genets constitutes a

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crucial obstacle to the detection of reticulation from mtDNA data alone. Although this statement is not applicable to all DNA-based investigations, it is likely that biases related to lineage sorting, rates of evolution and differential selections on a specific type of genome (i.e. mtDNA *versus* nDNA; see Clarke *et al.*, 2002) may lead to erroneous conclusions if not confronted with results from independent (morphological) markers (see, for a phylogenetic perspective: Degnan, 1993; Page & Charleston, 1998; Corneli & Ward, 2000; Giannasi, Malhotra & Thorpe, 2001). On the other hand, morphological evidence was weaker than cytochrome *b* data in detecting cryptic reticulation between *G. tigrina* and 1/ *G. genetta* and 2/ *G. felina* (one morphological hybrid, but large proportion of hybrids detected through haplotypes). These results dramatically illustrate the inextricable complementarity of molecules and morphology, and further clarification of hybridization patterns in southern African genets should be expected with the production of non available data for additional independent markers for the whole taxonomic set studied.

Our study also corroborated that morphological variation, when precisely delimited, can constitute a useful tool for a better characterization of processes of hybridization (Daniels *et al.*, 1998; Rohwer & Wood, 1998; Rohwer *et al.*, 2001). The delimitation of the nine discrete morphological characters used in this study was clear-cut (quasi absence of polymorphism within the species ranges), and allowed us to disregard intraspecific variability and focus on mosaics of character states truly diagnostic of morphological hybrids. The fact that each hybrid generally exhibited a proper mosaic of character states (coat pattern and skull) supports the hypothesis of epigenetic control of the phenotypic expression of the characters considered (see Rakyan *et al.*, 2001). In

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addition, the fact that all hybrids having *G. genetta* or *G. felina* cytochrome *b* haplotypes were morphologically identified as *G. tigrina* may suggest 1/ asymmetric hybridization of *G. tigrina* males to females of other species and 2/ positive selection for a *tigrina*-like coat pattern in South African habitats. However, these hypotheses will have to be further tested using for instance Y-linked markers, which already proved appropriate when combined with the maternally inherited mtDNA for revealing directionalities in hybridization processes (e.g., Kikkawa *et al.*, 2003).

The complementarity of our results based on morphology and molecules is considered as an encouragement for developing an integrative approach when questioning hybridization issues in zootaxa, as previously pursued with success in plants (e.g., Carney, Gardner & Rieseberg, 2000). Given the reciprocal illumination obtained using different (independent) materials in an integrative approach, our study strongly argues for the combination of various phenotypic and genetic markers, together with the consideration of environmental data, in order to improve our understanding of the complex phenomena that underlie the evolution of hybridization processes. Hybridization is likely to play an important role in evolutionary history, especially in what concerns speciation (e.g., DeMarais *et al.*, 1992; Moore & Price, 1993; Stone, 2000; Vollmer & Palumbi, 2002). Given the demonstrated unstability of hybrid zones in time and space (Moore, 1977; Schiltuizen, 2000; Dasmahapatra *et al.*, 2002; Rohwer *et al.*, 2002), the process of reticulation also raises the necessity of an integrative approach when dealing with conservation issues (see Graham, 1988, for an integration of a paleoecological perspective), an approach that we chart here for the case concerning the preservation of potential pools of "evolutionarity" within the southern African genets.

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Figure 1. Distributional data concerning southern African genets and detected hybrids. Gray arrows: *Genetta angolensis*; white circles: *G. maculata*; white triangles: *G. tigrina*; black circles: *G. genetta*; black triangle: *G. felina*. Hybrids are indicated as follows, with localities from north to south: black circle surrounding gray arrow (*G. angolensis x G. maculata; G. mossambica* type specimen, Mossimboa), black asterisks (*G. maculata x G. tigrina*; Royal Natal National Park, Umgeni Valley Game Ranch, New Germany, Highflats, "Pondoland"), white oval (*G. tigrina x G. felina;* Clanville, Cathcart, Western Cape, East London, Knysna) and white box (*G. tigrina x G. genetta*; Western Cape). See text for discussion about hybrids.

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Figure 2. Dorsal and ventral drawings of genet skull showing the positions of landmarks used for geometric morphometrics. The character 8 (int1; see Appendix 1) is defined by frontal width (from #2 to #12) and inter-orbital constriction (from #4 to #10).

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Figure 3. Relative Warps Analysis of dorsal image landmarks based on sample of *Genetta maculata*, showing age and sex classes and geographical groups (C, E = Central and East Africa; others from southern Africa); squares Class 1 (juvenile), asterisks Class 2 ("subadult"), triangles Class 3 (adult), diamonds Class 4 (adult).

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Figure 4. Canonical Variates Analysis of shape matrix from genet cranial dorsal images (Wilks Lambda 0.05). Species formed *a priori* groups, hybrids were added *a posteriori*. Species indicated as follows: circles *G. maculata;* squares *G. genetta;* triangles *G. tigrina;* asterisks *G. tigrina x G. maculata* hybrids; star *G. tigrina x G. genetta* hybrid.

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Deleted: Species formed *a priori* groups, hybrids were added *a posteriori*. Species indicated as follows: (+) *G. maculata;* (■) *G. genetta;* (▼) *G. tigrina;* (*) *G. tigrina x G. maculata* hybrids; (#) *G. tigrina x G. genetta* hybrid.

Figure 5. Canonical Variates Analysis of shape matrix from genet cranial ventral images (Wilks Lambda 0.037). Species formed *a priori* groups, hybrids were added *a posteriori*. Species indicated as follows: circles *G. maculata;* squares *G. genetta;* triangles *G. tigrina;* asterisks *G. tigrina x G. maculata* hybrids; star *G. tigrina x G. genetta* hybrid.

Figure 6. NJ tree (using ML criteria) based on cytochrome *b* sequences and representing phylogenetic relationships within southern African species of genets (-Ln likelihood = 4138.70308). The model of sequence evolution estimated by ModelTest was TrN (Tamura & Nei, 1993) using gamma shape (G) = 0.8563 and assumed proportion of invariable sites (I) = 0.565. The estimated substitution rates were 15.5145 (A-G) and 17.7913 (T-C), and 1.0000 in all transversions. Numbers above and below branches are boostrap values (bp) superior to 70 % (see Hillis & Bull, 1993; Mason-Gamer & Kellogg, 1996) for the NJ and MP tree, respectively. Scale bar corresponds to 1 % sequence divergence. Specimen numbers refer to Appendix 3. See Discussion for boxes indicating "North" and "South" *G. maculata*.

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Deleted: *G. genetta* and putative *G. felina* were grouped under "*G. genetta*" (see Materials and Methods).

Deleted: **Figure 6.** Minimum-spanning tree based on cytochrome *b* haplotypes for the "*tigrina-pardina*" complex (*G. pardina, G. maculata* and *G. tigrina*) using distance matrix (TrN model with Gamma a = 0.8563). DNA numbers are indicated, and size of circles is proportional to the number of identical haplotypes they contain. Grey, whitish grey and white circles represent *G. pardina, G. maculata* and *G. tigrina*, respectively. Distances between haplotypes are indicated on branches.¶

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Table 1. Details of adult large-spotted genet samples from KwaZulu-Natal province and neighbouring regions of South Africa, indicating their previous taxonomic affiliation suggested by Pringle (1977; NM specimens, marked with an asterisk) or based on Museum labels, together with discriminant scores calculated from Crawford-Cabral & Pachecho (1992) and revised discrete morphological characters. The revised taxonomic affiliation from this study is provided. Acronyms of Museums explained in text. State "a" refers to *G. tigrina* and state "b" to *G. maculata*; values between parentheses in the 8th morphological character column correspond to the inter-orbital constriction / frontal width ratio (see Appendix 1). ? means that data were not available.

Coll. Number	**Sex**	**Locality**	**Previous identification**	**Discrim. scores**	**Morphological characters**									**Identification from this study**
					1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
NM 527	F	Oribi Gorge 30°42'S,30°14'E	*G. t. tigrina**	?	a	a	a	a	a	a	a	a (1.029)	a	*G. tigrina*
NM 545	M	Oribi Gorge	*G. t. tigrina**	1.395	a	a	a	a	a	a	a	a	a	*G. tigrina*

		30°42'S,30°14'E										(1.029)			
NM	M	Oribi Gorge	*G. t. tigrina**	1.095	a	a	a	a	a	a	a	a	*G. tigrina*		
1558		30°42'S,30°14'E										(0.966)			
NM	M	Underberg	*G. t. tigrina**	0.775	a	a	a	a	a	a	a	a	*G. tigrina*		
1603		39°47'S, 29°30'E										(1.044)			
NM	?	Donnybrook	*G. t. tigrina**	?	a	a	a	a	a	a	?	?	*G. tigrina*		
1712		29°56'S, 29°52'E													
MFNB	?	Zondags River	*G. tigrina*	1.747	a	a	a	a	a	a	a	a	*G. tigrina*		
1101		33°14'S, 25°08'E										(1.063)			
MFNB	?	Zondags River	*G. tigrina*	2.337	a	a	a	a	a	a	a	a	*G. tigrina*		
1102		33°14'S, 25°08'E										(1.238)			
NM	?	Highflats	*G. t. tigrina**	?	a	b	a	b	a	a	b	?	?	Hybrid	
1720		30°16'S, 30°30'E													
NM	?	Highflats	*G. t. tigrina**	?	a	b	a	a	b	a	a	?	?	Hybrid	
1721		30°16'S, 30°30'E													

NM	F	Umgeni Valley Game	Hybrid*	-0.355	b	b	b	b	b	b	b	a	b	Hybrid
1990		Ranch										(1.049)		
		29°29'S, 30°20'E												
NM	?	Royal Natal National	*G. t. tigrina**	-0.365	?	?	?	?	?	?	?	b	a	Hybrid
2055		Park										(0.866)		
		28°40'S, 28°56'E												
DNSM	F	15 Umdoni Road, New	*G. tigrina*	-2.215	b	b	b	b	b	b	b	b	a	Hybrid
3335		Germany										(0.808)		
		29°47'S, 30°53'E												
MFNB	?	Pondoland	*G. tigrina*	-0.709	a	a	a	a	a	a	a	b	a	Hybrid
19696		31°31'S, 29°32'E										(0.770)		
NM 863		MkuziGame	Hybrid*	-1.823	b	b	b	b	b	b	b	b	b	*G. maculata*
		Reserve										(0.912)		
		27°38'S, 32°14'E												
NM	M	Bishopstowe	Hybrid*	-1.855	b	b	b	b	b	b	b	a	b	*G. maculata*

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1614		29°35'S, 30°27'E										(1.000)			
NM	?	Highflats	Hybrid*	?	b	b	b	b	b	b	b	?	?	*G. maculata*	
1719		30°16'S, 30°10'E													
DNSM	F	Iphiva Camp Site, 2 km	*G. tigrina*	-2.605	b	b	b	b	b	b	b	b	b	*G. maculata*	
1089		north of St. Lucia										(0.790)			
		Village													
		28°21'S, 32°25'E													
DNSM	?	Bayzlee Beach, South	*G. tigrina*	-3.645	b	b	b	b	b	b	b	b	b	*G. maculata*	
1286		Coast										(0.929)			
		30°27'S, 30°40'E													
DNSM	?	Kloof	*G. tigrina*	?	b	b	b	b	b	b	b	?	?	*G. maculata*	
1618		29°47'S, 30°50'E													
DNSM	M	Dbn 325 Stella Road,	*G. tigrina*	-1.985	b	b	b	b	b	b	b	b	b	*G. maculata*	
1632		Hillary										(0.860)			
		29°53'S, 30°56'E													

DNSM 1636	F	105 Carlton Avenue, Westville 29°50'S, 30°56'E	*G. tigrina*	-1.265	b	b	b	b	b	b	b	b (0.928)	b	*G. maculata*
DNSM 2232	F	Umhloti 29°39'S, 31°06'E	*G. tigrina*	-0.355	b	b	b	b	b	b	b	b (0.891)	b	*G. maculata*
DNSM 3219	F	Cowies Hill 29°49'S, 30°53'E	*G. tigrina*	-1.495	b	b	b	b	b	b	b	b (0.887)	b	*G. maculata*
DNSM 4058	F	Sunningdale 29°53'S, 31°00'E	*G. tigrina*	-2.215	?	?	?	?	?	?	?	b (0.755)	b	*G. maculata*
DNSM 4475	F	Umgeni River bank, near Bird Park 29°48'S, 31°02'E	*G. tigrina*	?	b	b	b	b	b	b	b	?	?	*G. maculata*
DNSM 4833	?	Pietermaritzburg 29°37'S, 30°23'E	*G. tigrina*	-3.495	?	?	?	?	?	?	?	b (0.857)	b	*G. maculata*

Table 2. Molecular diversity parameters for the seven "groups" (see MATERIALS AND METHODS) of southern African genets estimated from cytochrome *b* sequences. "n": number of individuals; "*h*": haplotype diversity; "π": nucleotide diversity; "θ_S": number of polymorphic sites.

Groups	**n**	***h***	π	θ_S
G. genetta	11	0,911	0.029400 ± 0.015794	64
G. angolensis	2	0,500	0.000000	0
G. felina	7	0,571	0.006547 ± 0.004167	13
G. pardina	5	1,000	0.025647 ± 0.015888	59
G. tigrina	8	0,875	0.003577 ± 0.002374	9
G. maculata "North"	5	1,000	0.010709 ± 0.006837	28
G. maculata "South"	19	0,632	0.005921 ± 0.003263	22

Deleted: Table 3. Respective contributions of morphological and molecular characters to the characterization of hybrid zones. X, hybrids detected – low rate; XX, hybrids detected – high rate; -, no hybrid detected; N, data non available.¶
¶
Hybridization ... [1]

Appendix 1. List of discriminative morphological characters used for *G. tigrina* (a) and *G. maculata* (b).

-coat pattern:

1.a. mid-dorsal spots black to dark brown, with mid-dorsal line always dark.

1.b. mid-dorsal spots rufous red, with mid-dorsal line from dark brown to rufous red.

A contrast of colouration between the mid-dorsal line and mid-dorsal spots may be present in the *G. maculata* phenotype, i.e. when spots are rufous red whereas the mid-dorsal line remains very dark (though always with rufous hairs melted). Some other specimens may have their mid-dorsal line with the same colouration as spots, i.e. rufous red. In *G. tigrina*, mid-dorsal line is always the same colouration as spots, but is restricted to a black to dark brown.

2.a. marked mid-dorsal crest.

2.b. no mid-dorsal crest.

The mid-dorsal crest is defined as present when hairs on the mid-dorsal line form a stripe of much longer hairs compared to the adjacent dorsal areas. Due to the nature and disposition of hairs, this character is difficult to quantify precisely, but is evident and reliable enough for considering it in our analysis. Although the mid-dorsal crest in *G. tigrina* is not as developed as in *G. genetta* or *G. angolensis*, it is strikingly marked in comparison to *G. maculata*.

3.a. mid-dorsal spots large and non-coalesced.

3.b. mid-dorsal spots short and often fused at the rump.

Coalescence level of mid-dorsal spots is a useful discriminative character within genet species (Gaubert *et al.*, 2001, 2002). In this case, the difference is weak but remarkable, since *G. maculata* has less large and long mid-dorsal spots than *G. tigrina* (Roberts, 1951). Besides, coat pattern looks more densely spotted in *G. maculata*. However, because of their tendency of fusing together on a same row, especially at the rump, fused spots of *G. maculata* can sometimes look similar to one single large spot of *G. tigrina*. But a more accurate examination of the spots allows the distinction between fusion events (broken outlines) and a single large spot. This character is difficulty usable with juvenile specimens, as coat pattern is often confused by a generalized coalescence of the mid-dorsal spots.

4.a. hindfoot almost completely dark, with a thin bright stripe starting from the upper part of the foot to the limb.

4.b. hindfoot bright (same as ground coloration), with a dark stripe restricted to the under part of the foot.

5.a. forelimb with a longitudinal dark stripe on the posterior part.

5.b. forelimb completely clear (same as ground coloration).

Characters 4 and 5 were, at the beginning of the study, supposed to be one single character only, related to a strong darkening of the coat (see Roberts, 1951). However,

some specimens exhibited the character states 4a and 5b at the same time (and *vice versa*).

6.a. tail with a thick aspect, and bright and dark rings clearly delimited.

6.b. tail with a thinner aspect, and bright and dark rings not clearly delimited.

Bright and dark rings have their outlines either confused (*G. maculata*) or well delimited (*G. tigrina*). In *G. maculata*, dark and bright hairs are partly overlapping with the next ring, making the annulation pattern quite confused. On the contrary, *G. tigrina* bares a clear annulation pattern (i.e. no overlapping), even though dark hairs are present and melted within bright rings.

7.a. tail with six bright rings.

7.b. tail with seven to eight bright rings.

The first bright ring is defined here as the bright ring located right after the first dark basal ring of the tail. The last bright ring is not taken into account, because the fact that it can be either almost completely masked by dark hairs or completely visible could make the counting misleading. This probably explains the difference with the counting of Roberts (1951).

-skull:

8.a. inter-orbital constriction / *frontal width* ratio $\geq 1 \pm 0{,}05$.

8.b. inter-orbital constriction / *frontal width* ratio inferior to 0,93.

The inter-orbital constriction is the narrowest point of the constriction. The *frontal width* is defined in Figure 2. Boundary values were delimited on the basis of measurements taken on non-ambiguous specimens (i.e., specimens not from the putative hybrid zone). This character, that we will name int1, is highly species-discriminative within genets (Gaubert, 2003a). Juveniles and young subadult specimens of both species have this character nearing 1 or superior to 1 because of their large inter-orbital constriction (Gaubert *et al.*, 2001).

9.a. caudal entotympanic bone not inflated compared to the ectotympanic bone (very short aspect).

9.b. caudal entotympanic bone more inflated compared to the ectotympanic bone.

This is the most "subjective" character used to discriminate the two species. The difference between the two character states was already noticed by Crawford-Cabral (1981a). *G. tigrina* bares very flat and short caudal entotympanic bone (c.e.b.), the flattest and shortest among all genet species. The difference between *G. maculata* and *G. tigrina* c.e.b. shape may sometimes be difficult to detect. However, we chose to consider this character in the analysis since c.e.b. inflation has proved to be a species-discriminant character within subfamily Viverrinae and genus *Genetta* (Gaubert *et al.*, 2002). It has been noticed that juvenile and subadult specimens of *G. maculata* tended to bare shorter c.e.b. than adults, thus excluding the use of such character states when considering juvenile and subadult age classes.

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Appendix 2. Relative age determination and list of the adult skulls used in the final geometric morphometric analyses (n = 69).

.Categories of relative age were defined by PJT using a random sample of *G. maculata* skulls (n = 33) arranged in an "age line" from smallest to largest in terms of condylobasal skull length:

-Class 1. Juveniles: M^2 not fully erupted, broad inter-orbital constriction, posterior part of the braincase widening conspicuously.

-Class 2. Subadults: M^2 fully erupted, braincase more oval, frontal-parietal and nasal-frontal sutures open, sagittal crest undeveloped.

-Class 3. Young adults: as in Class 2, but only nasal-frontal suture still visible, sagittal crest weakly developed.

-Class 4. Older adults: as in Class 3, but all sutures closed, sagittal crest well developed.

.Museum abbreviations are described in text. Juvenile skulls (marked with asterisk) were excluded from final analysis.

-*Genetta maculata* (including "*letabae*"): South Africa (KwaZulu-Natal Province): Albert Falls – DNSM 2064; Bayzlee Beach – DNSM 1286; Between St Lucia and Monzi - DNSM 2066; Bishopstowe – NM 1614; Botha's Hill – DNSM 3225; Hillary, Durban – DNSM 1632; Mkuzi Game Reserve – NM 863; Cowie's Hill, Durban – DNSM 3219; Kloof, Durban – DNSM 3499*; Hluhluwe Game Reserve – NM 1381*; Lake St Lucia, Iphiva Camp – DNSM 1089; Paradise Valley, Durban – DNSM 2189; Pietermaritzburg – DNSM 2054; Sunningdale – DNSM 4058; Umdloti – DNSM 2232; Westville, Durban –

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DNSM 1636; (Former Transvaal Province): Wangemann - MFNB 19661; Tchad: Lake Tchad – MNHN: 1904-2008, 1904-2009, 1904-2010, 1904-2011; Kenya: Kilimanjaro - MNHN1905-379; Ethiopia: Hirna (neotype) – MNHN 1972-395; "Ethiopia" (no specific locality given) - MNHN 1974-203; Zimbabwe: Sabi River – SAM 9056; Tanzania: Luganga Fringe – MFNB: 60968, 60969; Namibia: Caprivi - MFNB 19677; Mohango, Caprivi - BMNH 3591163; Swaziland: Tshaweni - BMNH 76323; Lesotho: "Lesotho"- DNSM 5176*; Mozambique: Tambarara - BMNH 81146, 81147.

-*Genetta genetta* (including *felina*)

South Africa (Eastern Cape Province): Deelfontein – BMNH 31418; Grootfontein – BMNH 3591205; (Western Cape Province): Langebaanweg – SAM: 39508, 39585, 39236, Gansbaai – SAM 38541, Matjesgat – SAM 39813; France: MNHN 1994-597, 1997-450; Mali: Emal'here – MNHN 2000-347; Ethiopia: River East of Lk Margherita - MNHN1969-465, 1969-467; Senegal: Morel – MNHN: 1961-653, 1960-3903.

-*Genetta tigrina*:

South Africa (KwaZulu-Natal Province): Oribi Gorge: NM: 1527, 1545, 1558, Underberg - NM 1603; (Western Cape Province): Rondebosch – BMNH 95935, 95936; Tamboerskloof - SAM17664; Llandudno – SAM: 39379, 39380; Cape of Good Hope Nature Reserve - SAM: 36015, 37295; Stellenbosch – 35810; Noordhoek - SAM 35639; Du Toit's Kloof – SAM 36256; Gordon's Bay - SAM 37710; (Eastern Cape Province):

Sunday's River – MFNB: 1101, 1102; Knysna – BMNH 55739, 55741; King William's Town – BMNH 7710125.

Hybrids detected from discrete morphological characters (Appendix 1):

-*G. tigrina/maculata*: South Africa (KwaZulu-Natal Province): Royal Natal National Park – DNSM 2055, New Germany, Durban – DNSM 3335, Umgeni Valley Ranch – NM 1990; "Pondoland"- MFNB 19696.

-*G. tigrina/genetta:* South Africa (Eastern Cape Province): Cathcart – BMNH 3591162.

Appendix 3. List of species and specimens used in the molecular phylogenetic analysis, with collection and GenBank accession numbers, and locality names. The asterisk refers to voucher specimens. DNA extracts from museum collection specimens (i.e. not fresh material) are indicated by "M". Specimens used in both molecular and morphometric analyses have their numbers in bold. Collection numbers in italic means that the sequences were obtained both from PG and CAF. Collection numbers underlined indicate sequences obtained by CAF, the rest of the sequences having been produced by PG. New sequences have their GenBank accession numbers in bold.

Deleted: *G. genetta* and putative *G. felina* were grouped under "*G. genetta*" (see MATERIALS AND METHODS).

Species	Collection number	GenBank accession number	Locality
G. johnstoni	E-74	AY241892	Taï National Park, Ivory Coast
G. servalina	C-20	AF511053	Kouala Moutou, Gabon
G. angolensis	MVZ-118448 *M	AY241882	Luanshya, Zambia
G. angolensis	MVZ-118449 *M	AY241883	Luanshya, Zambia
G. genetta	C-9	AY241905	Puéchabon, France
G. genetta	E-03	**AY397700**	Africa
G. genetta	E-10	**AY397697**	Donana National Park, Spain
G. genetta	E-16	AY241911	Emnal'here, Mali
G. genetta	E-48	**AY397701**	Gironde, France
G. genetta	E-64	**AY397699**	France
G. genetta	E-65	**AY397698**	Dakar, Senegal

G. genetta	E-88	AY241922	Jijel, Algeria
G. genetta	E-17	**AY397722**	Beaufort West, RSA
G. genetta	*E-19*	AY241910	Chelmsford, Kwazulu-Natal, RSA
G. genetta	NMB-8544 *M	AY241886	Northern Cape, RSA
G. felina	E-04	AY241908	Victoria West District, RSA
G. felina	E-05	AY241909	Victoria West District, RSA
G. felina	KM-27708 *M	AY241879	Northern Cape, RSA
G. pardina	C-47	**AY397706**	West Africa
G. pardina	C-62	AY241896	Tappita, Liberia
G. pardina	E-07	AY241895	West Africa
G. pardina	E-84	**AY397707**	near Laminia and Kédougou, Senegal
G. pardina	E-106	**AY397708**	Cavally River, Liberia
G. maculata	C-87	**AY397711**	Laneata, Kenya
G. maculata	***E-32 (DNSM-1089)****	AY241920	St. Lucia Village, Kwazulu-Natal, RSA
G. maculata	*E-30 (DNSM-1617)**	**AY397719**	Albert Falls National Reserve, Kwazulu-Natal, RSA
G. maculata	*E-33 (DNSM-1618)**	AY241916	Kloof, Kwazulu-Natal, RSA
G. maculata	***E-28 (DNSM-1632)****	AF511055	Hillary, Kwazulu-Natal, RSA
G. maculata	***E-34 (DNSM-1636)****	**AY397721**	Westville, Kwazulu-Natal, RSA
G. maculata	*E-25 (DNSM-2178)**	**AY397714**	Waterfall, Kwazulu-Natal, RSA
G. maculata	***E-35 (DNSM-2189)****	AY241921	Paradise Valley, Kwazulu-Natal, RSA
G. maculata	*E-31 (DNSM-2190)**	**AY397720**	Gillits, Kwazulu-Natal, RSA
G. maculata	***E-29 (DNSM-2232)****	**AY397718**	Umhloti, Kwazulu-Natal, RSA
G. maculata	*E-24 (DNSM-3219)**	AY241917	Westville, Kwazulu-Natal, RSA
G. maculata	***E-27 (DNSM-3225)****	AY241913	Botha's Hill, Kwazulu-Natal, RSA
G. maculata	*E-21 (DNSM-3335)**	**AY397716**	New Germany, Kwazulu-Natal, RSA

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G. maculata	***E-20 (DNSM-3499)****	AY241919	Kloof, Kwazulu-Natal, RSA
G. maculata	*E-37 (DNSM-4475)**	**AY397717**	Umgeni River Mouth, Kwazulu-Natal, RSA
G. maculata	***E-53 (DNSM-5176)****	AY241912	Lesotho
G. maculata	*E-36 (DNSM-5611)**	AY241914	Eshowe, Dlinza Forest, Kwazulu-Natal, RSA
G. maculata	E-06	**AY397710**	Thika, Kenya
G. maculata	E-86	**AY397712**	Kibwezi, Kenya
G. maculata	E-70'	AY241901	Bukinayana, Kibira National Park, Burundi
G. maculata	E-113	**AY397713**	House, Ethiopia
G. maculata	*E-22 (TM-39910)**	AY241915	Blidschap Private Reserve, Northern Province, RSA
G. maculata	*E-23 (TM-39551)**	AY241918	Blidschap Private Reserve, Northern Province, RSA
G. maculata	*E-26 (TM-39726)**	**AY397715**	Pietermaritzburg, Kwazulu-Natal, RSA
G. tigrina	*A-270*	AY241889	East London, RSA
G. tigrina	*A-271*	AY241876	East London, RSA
G. tigrina	*A-274*	**AY397705**	East London, RSA
G. tigrina	A-272	AY241878	East London, RSA
G. tigrina	A-273	AY241877	East London, RSA
G. tigrina	KM-30813 *	**AY397702**	Western Cape, RSA
G. tigrina	KM-31133 *	**AY397709**	Western Cape, RSA
G. tigrina	KM-31185 *	AY241880	Eastern Cape, RSA
G. tigrina	KM-31276 *	AY241881	Eastern Cape, RSA
G. tigrina	NMB-4470 *	AY241884	Eastern Cape, RSA
G. tigrina	NMB-4786 *	AY241885	Eastern Cape, RSA
G. tigrina	TM-32282 *	**AY397704**	Knysna, RSA
G. tigrina	TM-35133 *	**AY397703**	Clanville, RSA

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Table 3. Respective contributions of morphological and molecular characters to the characterization of hybrid zones. X, hybrids detected – low rate; XX, hybrids detected – high rate; -, no hybrid detected; N, data non available.

Hybridization	Discrete characters	Traditional morphometrics	Geometric morphometrics	Cytochrome *b*
G. "letabae" x G. tigrina	X	X	X	-
G. genetta x G. tigrina	-	N	-	X
G. felina x G. tigrina	X	N	X	XX
G. maculata / "letabae" x G. angolensis	X	N	N	-

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G. genetta E-04 AY241908 Victoria West District, RSA

G. genetta E-05 AY241909 Victoria West District, RSA

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G. genetta NMB-8544 *M AY241886 Northern Cape, RSA

Appendix IV

Fernandes & Crawford-Cabral (in press). Comment on the proposed conservation of *Viverra maculata* Gray, 1830 (currently *Genetta maculata*; Mammalia, Carnivora). *Bulletin of Zoological Nomenclature.*

Comment on the proposed conservation of *Viverra maculata* Gray, 1830 (currently *Genetta maculata*; Mammalia, Carnivora)

(Case 3204; see BZN **60**: 45-47, **61**: 119-122))

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1. We agree that an application to conserve a junior primary homonymy may be submitted to the Commission under Article 23.9.5 of the Code. Since Article 23.9.5 applies only to names in use, we need to ponder what exactly 'in use' means and consider the fact that *V. maculata* (Gray, 1830) has been used so far by only a minority of the authors concerned with the relevant taxa. Indeed, after Coetzee's (1967) reference to the homonymy of *V. maculata* and its status as an invalid senior synonym of *Genetta pardina* I. Geoffroy Saint-Hillaire, 1832 (the pardine genet), the usage of *V. maculata* Gray, 1830 for any genet species has not been prevalent (see Grubb, BZN **61**: 119-122). We think that instead of using the specific name in question to identify a particular genet species, the author of the alternative proposals (BZN **61**: 121) should have waited for the Commission's ruling and maintained prevailing usage as stated in Article 82.

2. It appears that the main objective of the proposal to validate the name *Viverra maculata* Gray, 1830 is to use it for a genet species (the rusty-spotted genet) whose valid name is currently controversial. However, a list of arguments both in favour and against attributing *maculata* Gray, 1830 to the rusty-spotted genet is essentially absent. We regard this matter as critical, both for consideration of *maculata* as a valid name and for its assignment to the rusty-spotted genet. When stating our objections to Grubb's alternative proposals and suggesting different alternative proposals for providing a valid name to the rusty-spotted genet, we will refer to Gaubert et al. (2003), where the present issue is discussed in more detail and the 'neotype' of *V. maculata* Gray, 1830 was designated.

3. The former usage of *Viverra maculata* Gray, 1830 for the rusty-spotted genet was mostly related to the acceptance of its conspecificity with the pardine genet and that *maculata* was regarded as the valid senior synonym of the latter (Schlawe, 1981). This view was followed by some ecologists (Fuller et al. 1990; Angelici et al. 1999; Angelici, 2000) and taxonomists (Wozencraft, 1993) who were not experts in the systematics of the genus *Genetta* or fully acquainted with the imbroglio under discussion here. Only recently has *maculata* Gray, 1830 been used to denote the rusty-spotted genet alone and, even then, only by the applicants of the Case (Gaubert et al. 2002; Gaubert, 2003). Previous usage of the name *maculata* for the rusty-spotted genet should not be invoked if we now know that such usage had been essentially mistaken. Moreover, the previous usage of *maculata* as a valid name for the pardine genet is a good argument for opposing its transfer to another species since it would be a source of confusion and justification for requesting its suppression.

4. We accept as pertinent the arguments presented by Gaubert et al. (2003) that the type locality and species identification traditionally assigned to the original

illustration of *V. maculata* are uncertain, although a West Africa locality and identity with the species *G. pardina* has always been indicated in the literature. However, an important problem with the proposals is that although the original figured specimen may not represent a pardine genet from Senegal. It is not beyond doubt that the specimen may indeed represent that species after all. Below we describe why Gray's drawing may represent equally well any of two or three species regarded as separate today and, in consequence, why *maculata* is a nomen dubium in the context of *Genetta* taxonomy, and hence subjectively invalid. In order to state that the original figured specimen *maculata* represents a rusty-spotted genet, the proposal relies only on the observation and interpretation of the illustration. This is because the type locality and type specimen are both unknown and the descriptions attached to the image do not provide for an accurate determination of the species. The 'diagnostic' character invoked by Gaubert et al. (2003) to ascribe the drawing by Gray to a rusty-spotted genet is far from clear. Crawford-Cabral (1981) figured a specimen of *G. pardina* from Guinea-Bissau kept at the Zoology Centre of the Scientific Institute of Tropical Research (CZ-IICT) in Lisbon, Portugal (collection number 1945-340) that matches Gray's illustration for *maculata* perfectly well. In another pardine genet specimen from the same collection (number 1945-68) this situation is even clearer. The light colour of the limbs is usually accepted as the character state that distinguishes *G. pardina* from the rusty-spotted genet (Gaubert, 2003). However, considering the example given above, this difference does not seem to be universal and hence is not diagnostic but more just a question of degree or frequency. The original figure of *G. maculata* (Gray, 1830) may not represent a rusty-spotted genet but instead a pardine genet, as stated by Schwarz (1930), or even *G. genettoides*

Temminck, 1853, a form of dubious taxonomic status but closely related to both pardine and rusty-spotted genets.

5. We cannot accept the neotype designation, which accompanies the intent to use *G. maculata* (Gray, 1830) as the species name of the rusty-spotted genet. We believe that some of the qualifying conditions in Article 75.3 are not met. In view of the points given in para. 4 above, the neotype designation does not clarify the taxonomic status of the nominal taxon (required in Article 75.3.1) since the characters in Gray's drawing which are supposed to differentiate the nominal taxon from other taxa are not diagnostic (as required in Article 75.3.2). Furthermore, when addressing Article 75.3.6, Gaubert et al. (2003) followed a series of apparently sensible criteria in the suggesting a type locality, but do not address one important issue (see para. 6 below). The taxonomic status of the rusty-spotted genets described from the region of the proposed type locality (*Genetta matschiei* Neumann, 1902, *G. schraderi* Matschie, 1902 and *G. deorum* Funaiolo & Simonetta, 1960) should have been assessed prior to designation of a neotype. If one or more of the nominal species in question are not conspecific with the neotype, then that neotype does not represent the whole of what we colloquially call rusty-spotted genets. If the authors of the Case had followed Recommendation 75B of the Code they would have been aware of the serious objection from other *Genetta* specialists to the neotype designation.

6. There is a problem in assigning *maculata* or any other name to the rusty-spotted genet at the moment. It fails to acknowledge the potential occurrence of more than one species within an assemblage which is clearly polytypic, reflected in the 17 names given to forms of the rusty-spotted genet (listed in Crawford-Cabral, 1981), most regarded as subspecies and distributed across a vast range of heterogeneous habitats. For instance, Crawford-Cabral & Fernandes (2001) have found evidence

supporting the existence of three morphological species of rusty-spotted genets in Southern Africa alone. It is indeed possible that rusty-spotted genets constitute a superspecies and then, since superspecies are not to be given formal nomenclatural recognition (Mayr, 1969), the search for a single valid name for this group of forms is a false quest. The conspecificity of some or all of the described forms of rusty-spotted genet, and consequently the synonymy of the corresponding names, has not been established. The uncertainty about speciation within the rusty-spotted genets, together with the absence of information other than morphological data for this species, suggests that it would be more advisable to wait for additional data before deciding upon such a difficult nomenclatural issue. At the moment we are undertaking DNA-based research on the population structure, phylogeography, and putative speciation within the rusty-spotted genets. The results, when put together with the extensive available morphological data, will allow a much more comprehensive and sound decision on the systematics of the species.

7. Schlawe (1980) discovered that the type specimen of *Genetta rubiginosa* Pucheran, 1855, the name traditionally given to the rusty-spotted genets, following Roberts (1951) and Crawford-Cabral (1966), is an individual of a completely different species, *G. thierryi* Matschie, 1902. This led Crawford-Cabral & Fernandes (1999) to recommend a search for a new scientific name for the rusty-spotted genets, eventually following Article 23.3.5, but they warned that a thorough investigation was necessary, as outlined in para. 6 above, before making a decision. We follow the same line of reasoning here and furthermore state that we will have, in the very near future, results from our current research that we believe will be central to the rusty-spotted genet's taxonomy. This approach is the soundest in terms of taxonomy as well as for nomenclatural stability in this variable group of genets.

8. We strongly recommend that the Commission reject the proposals published in BZN **60**: 46. We also agree with Grubb (BZN **61**: 120) that the neotype designation by Gaubert et al. (2003) is invalid under Article 75.3 of the Code.

Acknowledgements

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