The Population History of Extant and Extinct Hyenas

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We have analyzed partial DNA sequences of the mitochondrial cytochrome b gene from extant striped, brown, and spotted hyenas as well as from Pleistocene cave hyenas. Sequences of the Pleistocene cave hyenas from Eurasia and modern spotted hyenas from Africa are intermixed in phylogenetic analyses, questioning any taxonomic delineation between the two groups. Contrary to cave hyenas in Eurasia, spotted hyenas in Africa show a phylogeographic pattern with little geographical overlap between two mitochondrial DNA (mtDNA) clades, suggesting two Pleistocene refugia in the north and south of Africa. Our results, furthermore, suggest three waves of migration from Africa to Eurasia for spotted hyenas, around 3, 1, and 0.3 MYA. A recent emigration of striped hyenas from Africa to Eurasia took place less than 0.1 MYA, resulting in a dramatic expansion of the geographical range of striped hyenas. In striped hyenas and within the geographical range of mtDNA clades in spotted hyenas, we found identical sequences several thousand kilometers apart, indicating a high rate of migration during the Pleistocene as well as the Holocene. Both striped and brown hyenas show low amounts of genetic diversity, with the latter ones displaying just a single haplotype.

Introduction

The four extant hyena species (spotted hyena, striped hyena, brown hyena, and aardwolf) are the remnants of a large radiation that reached its peak about 5 MYA and contains more than 100 fossil species. (Supplementary Material online). While the aardwolf (*Proteles cristatus*) is highly specialized, in that it feeds only on termites (Bothma 1998; Bothma and Walker 1999) and belongs to its own subfamily (Protelinae), the other three species, spotted hyena (*Crocuta crocuta*), striped hyena (*Hyaena hyaena*), and brown hyena (*Parahyaena brunnea*) (subfamily Hyaeninae), hunt and scavenge (Bothma and Walker 1999). The spotted hyena, genus *Crocuta*, which currently occurs only in Africa, inhabited large parts of Eurasia during most of the Pleistocene (Kurten 1968; Werdelin and Solounias 1991; Kahlke 1994). The Eurasian members of the genus *Crocuta* are usually described as "cave hyenas." While a close relationship between spotted and cave hyenas is widely accepted, both subspecies (*Crocuta crocuta spelaea*, e.g., Kurten 1957, 1968; Turner 1984; Werdelin and Solounias 1991) and species status (*Crocuta spelaea*, e.g., Soergel 1937; Musil 1962; Markova et al. 1995; Baryshnikov 1999) are discussed for cave hyenas. The fossil record of the striped hyena is controversial, as some authors (e.g., Kurten 1957, 1968) have argued that striped hyenas occurred in the Mediterranean during the Pleistocene, while others argue for an exclusively African record from Ethiopia to South Africa (e.g., Werdelin and Solounias 1991; Bothma 1998). In contrast, fossil remnants of brown hyenas are known only from southern Africa, and thus they seem never to have inhabited a large area (Turner 1990).

Key words: ancient DNA, migration, out-of-Africa, Pleistocene, phylogeography.

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We have assessed the population history of the extinct cave hyena as well as the extant spotted, striped, and brown hyenas by sequencing 340–366 bp of the mitochondrial cytochrome b gene.

Materials and Methods

DNA Extraction

We extracted DNA from 78 Pleistocene cave hyena (*C. crocuta spelaea*) samples, originating from 27 locations across Europe and Asia (Supplementary Table 1, Supplementary Material online) and from 46 museum samples (23 spotted hyenas, 13 striped hyenas, and 10 brown hyenas; Supplementary Table 1, Supplementary Material online), covering the current and historical ranges of the three extant species. DNA from 40 museum samples were extracted following the methods of Rohland, Siedel, and Hofreiter (2004), whereas 6 additional museum samples and the Pleistocene samples were extracted as described by Hofreiter et al. (2004*a*).

Polymerase Chain Reaction and Analyses

Amplification of Pleistocene DNA was done as described by Hofreiter et al. (2004), using annealing temperatures between 42°C and 52°C, depending on the primer pair. We reconstructed a 366-bp fragment of the mitochondrial cytochrome b gene, either in four overlapping fragments or, when only amplification of shorter fragments was possible, in seven overlapping fragments (Supplementary Material online). Reamplification (when necessary), cloning, and sequencing were done as described previously (Hofreiter et al. 2004*a*). For each segment, a minimum of six clones, three from each of two independent amplifications, were sequenced. If all clones from the first amplification consistently differed from all clones from the second amplification at one or more positions, a third amplification was done from the extract to determine which sequence was reproducible (Hofreiter et al. 2001*a*). DNA amplification and

sequencing for the museum specimens were done as described by Rohland, Siedel, and Hofreiter (2004). Mock extractions without sample and polymerase chain reaction (PCR) blanks were performed throughout all experiments to monitor contamination.

Altogether, 366 bp of the cytochrome b gene were amplified for spotted hyenas and 340 bp for brown and striped hyenas. For striped and brown hyenas, we amplified a shorter fragment as the sequence information available in GenBank and necessary for primer design was limited in length for these taxa when the experiments were done. Some of the Pleistocene spotted hyena sequences have been described elsewhere (AJ809318–AJ809332; Hofreiter et al. 2004*b*). Spotted hyena sequences from GenBank (accession numbers AY048805, AY048806, AY048810, AY048811, and AY048812), one striped hyena (AY153054) and one brown hyena (AY048789), were included in the analyses. All newly determined DNA sequences were deposited in GenBank (accession numbers DQ157554–DQ157592).

Sequences were aligned by eye, and phylogenetic analyses were performed using the program package PAUP* (Swofford 1998). Neighbor-joining and maximum likelihood trees were constructed using a Hasegawa-Kishino-Yano substitution model with gamma-distributed rates (Hasegawa, Kishino, and Yano 1985; Yang 1996) inferred by the program Modeltest (Posada and Crandall 1998). To estimate the time to the most recent common ancestor (MRCA), we used the program r8s (Sanderson 2003). Phylogenetic trees used for molecular dating were rooted using domestic cat (*Felis sylvestris*) as out-group. A median-joining network was constructed using the program Network (Bandelt, Forster, and Röhl 1999).

Dating

Several of the Pleistocene hyena fossils have been dated previously (Hofreiter et al. 2004*b*). In addition to these samples, two hyena fossils originating from Asia were dated at the Leibniz Laboratory, University of Kiel, Germany, using accelerator mass spectroscopy dating.

Results

We amplified and sequenced 366 bp of the mitochondrial cytochrome b gene from 23 spotted hyena museum specimens and 340 bp from 7 brown and 13 striped hyena museum specimens, either as a single piece or in two overlapping fragments. These specimens cover the current and historical range of spotted, striped, and brown hyenas.

The spotted hyenas carried 12 haplotypes (unique sequences), whereas the striped and brown hyenas carried only 4 and 1 haplotype, respectively. We also amplified the 366-bp piece in four overlapping fragments (93–121 bp excluding primers) for 23 Pleistocene cave hyenas. For three additional samples, we reconstructed the 366-bp piece using shorter fragments (41–89 bp excluding primers, Supplementary Material online). For 15 of the Pleistocene samples, we found consistent changes between the first and the second amplification in one or several fragments, indicating that in these cases, amplifications started from a single strand of damaged DNA (Hofreiter et al. 2001*a*).

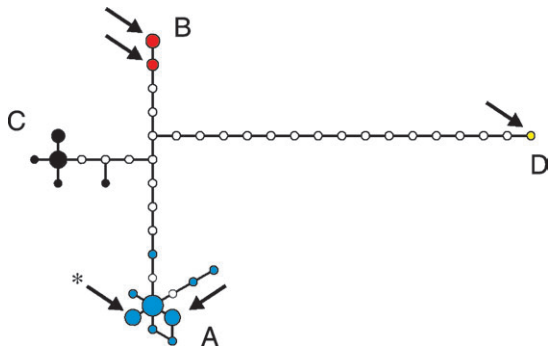

FIG. 1.—Median-joining network based on 366 bp of spotted/cave hyena cytochrome b mtDNA sequences. Yellow: East Asian cave hyena; blue: northern African spotted/cave hyena group; black: southern African spotted hyenas; red: exclusive European cave hyena group. Arrows indicate cave hyena sequences. The haplotype also found in the Altai is marked with an asterisk.

All affected fragments were amplified a third time to determine the correct nucleotides for these positions. We reconstructed the complete 366-bp piece for 26 Pleistocene samples originating from 17 locations from Western Europe to East Asia. As we were mainly interested in the species-wide variation, we amplified the complete 366-bp piece from only a single specimen from all except one location. At this location (Teufelslucke, Austria), we amplified the 366-bp fragment from 10 different samples (representing a minimum of three individuals), all of which yielded the same sequence. This result together with the observation that partial sequences from additional samples from other locations were always identical to the complete sequence from the respective locations (data not shown) indicates that local variation was small. Overall, we found five haplotypes for Pleistocene cave hyenas from Eurasia, none of which was shared with extant spotted hyenas. All previously dated cave hyena fossils gave Late Pleistocene dates, ranging from 37,000 to >50,000 years B.P. (before present) (Hofreiter et al. 2004*b*). The two newly dated fossils from the Altai and East Asia fall into the same age range with dates of 48,650 +2,380/−1,840 and 42,300 +940/−840 years B.P., respectively.

A network analysis (fig. 1) shows four groups of haplotypes (A–D) for spotted and cave hyenas, where a maximum of 2 deduced haplotypes separate observed haplotypes within a group, while between 6 and 20 deduced haplotypes separate the different groups from each other (fig. 1). In the tree analyses, three (A–C) of these four haplotype groups are recovered as monophyletic clades (bootstrap support 68%–96%, fig. 2 and Supplementary Material online), while group D consists of a single sequence falling basal to other spotted hyena sequences. This sequence comes from the single East Asian sample and differs by 18–22 differences from the other spotted/cave hyena sequences. However, it clearly is a spotted hyena sequence, as it differs at 41–45 positions from striped and brown hyena sequences. Each possible pair of clades A–C is distinguished by eight mutational steps (fig. 1), arguing for a temporally close divergence of these three clades. In clade A, Pleistocene and extant spotted hyena mitochondrial DNA (mtDNA) sequences are intermingled, whereas two

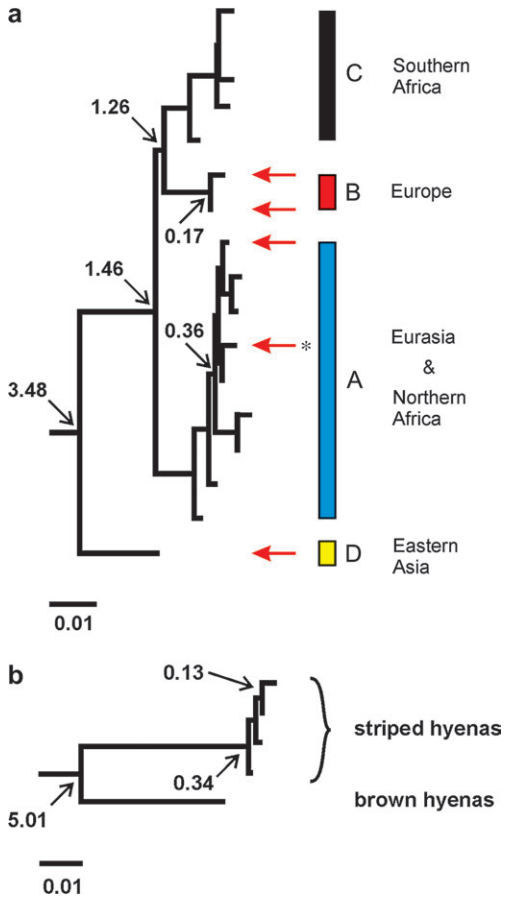

FIG. 2.—Neighbor-joining trees for the hyena sequences analyzed. The numbers show the estimated age of some of the nodes in million years B.P. The bar represents 1% sequence divergence. (*a*) Tree for spotted hyenas, based on 366 bp of mitochondrial cytochrome (cyt)-b DNA sequence. The positions of cave hyenas are shown by red arrows. The haplotype also found in the Altai is marked with an asterisk. Bootstrap values that support the different clades are 92%, 94%, and 79% for clades A, B, and C, respectively. The monophyly of clades A–C to the exclusion of clade D is supported by 94% bootstrap value. Bootstrap values for maximum likelihood and maximum parsimony trees are provided in Supplementary Fig. 2 (Supplementary Material online). (*b*) Tree for brown and striped hyenas, based on 340 bp of mitochondrial cyt-b DNA sequence.

other clades contain exclusively Pleistocene (B) or extant (C) hyena mtDNA sequences.

As a maximum likelihood ratio test did not reject the assumption of a molecular clock, we estimated the age of divergence events, using a point estimate of 10 Myr for the divergence between spotted and striped/brown hyenas as a calibration date (Wayne et al. 1989; Werdelin and Solounias 1991). The MRCA of all spotted hyena mtDNA sequences was estimated to be about 3.48 MYA (confidence interval [CI]: 2.25–5.09 MYA), whereas the MRCA of the remaining three clades A–C was estimated to be 1.26–1.46 MYA (CI: 0.83–2.4 MYA). Finally, the age of the youngest node containing all Eurasian Pleistocene and modern DNA sequences within clade A was estimated to be about 0.36 MYA, while the node of clade C was estimated to be about 0.17 MYA (fig. 2*a*). For striped hyenas, we found a date for the MRCA of about 0.34 MYA (fig. 2*b*). Within striped hyenas, the most recent sequence divergence was dated to be about 0.13 MYA. For the lower values, it is not possible to estimate CIs using r8s.

In Europe, the cave hyena haplotypes from the two clades (A and B) show complete geographic overlap (fig. 3 and Hofreiter et al. 2004*b*). Contrary to this, spotted hyenas in Africa show a strong phylogeographic pattern (fig. 3*a*), where one clade (C) occurs in the south and the other clade (A) in the north of Africa. Both clades show some overlap in their geographic distribution around the equator. In striped hyenas, no phylogeographic pattern exists as all four haplotypes were found in Africa and three of the four haplotypes outside Africa (fig. 4). Finally, all seven brown hyenas analyzed had identical mtDNA sequences (fig. 5).

Discussion

Reliability of the Pleistocene Sequences

Due to the fact that Pleistocene bones usually contain only small amounts of DNA, sequences obtained from such samples may be affected by contamination (Kolmann and Tuross 2000; Hofreiter et al. 2001*b*; Wandeler et al. 2003; Serre et al. 2004) or DNA damage (Hansen et al. 2001; Hofreiter et al. 2001*a*; Gilbert et al. 2003). For several reasons, this is unlikely in our study.

First, no haplotypes are shared between Holocene and Pleistocene spotted hyenas. Second, we amplified the fragments using several overlapping PCRs. If cross-contamination with PCR products from previous amplifications had affected our results, we would expect to see mosaic haplotypes, which is not the case. Third, all sequences show an open reading frame as expected for a part of the cytochrome b gene. Moreover, for those amino acid positions that differ between Pleistocene and Holocene samples, the Pleistocene state was found in other extant mammal species. Finally, we amplified each sequence position at least twice for all Pleistocene samples. When we observed consistent substitutions, a third amplification was performed to determine the correct nucleotide, making it highly unlikely that incorrect sequence positions were determined (Hofreiter et al. 2001*a*). We therefore conclude that neither contamination nor template damage is likely to have affected our results.

Phylogenetic Position of Cave Hyenas

Cave hyenas, that is, Pleistocene specimens from Eurasia that belong to the genus *Crocuta*, are usually considered a subspecies, *C. crocuta spelaea*, of the spotted hyena (Kurten 1957, 1968; Turner 1984; Werdelin and Solounias 1991) or a different species (*C. spelaea*; Soergel 1937; Musil 1962; Markova et al. 1995; Baryshnikov 1999). Although some substructure is present with monophyletic clades with either some Eurasian or some African hyena sequences (figs. 1 and 2), neither of the two groups is monophyletic as a whole. Thus, our data support neither of these classifications. Interestingly, despite this lack of phylogenetic separation, there are a number of morphological characters that distinguish Eurasian spotted hyenas from African ones such as different body proportions (Kahlke 1994), a slightly different cranial morphology, and

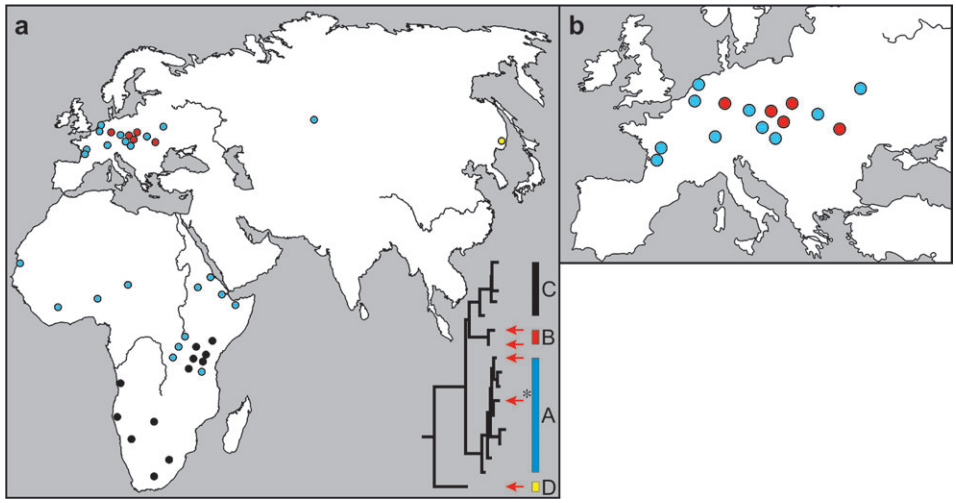

FIG. 3.—Geographical distribution of mtDNA sequences from spotted and cave hyenas. The colors correspond to clades A–D. Red arrows show the phylogenetic position of cave hyena mtDNA sequences. The haplotype also found in the Altai is marked with an asterisk. Each dot represents one individual. (*a*) View of the complete area investigated. The inserted tree is the same tree as in figure 2*a*. (*b*) Enlarged view of cave hyena mtDNA sequence distribution in Europe.

differences in jaw and teeth (Nagel, Pacher, and Morlo 2005). It has been shown that neutral markers such as mtDNA sequences can be poor predictors for ecological adaptations (reviewed in McKay and Latta 2002), which may persist even in the presence of high gene flow between populations (Smith et al. 1997; Saint-Laurent, Legault, and Bernatchez 2003). Thus, Pleistocene spotted hyenas from Eurasia may have preserved certain adaptive traits despite gene flow from African populations.

Spotted Hyena Dispersal

Based on the topology of both the network and the phylogenetic tree, a panmictic African-Eurasian spotted hyena population is unlikely, as under such a scenario, we should not have found mtDNA clades belonging exclusively to either Eurasian (B and D) or African hyenas (C). Moreover, none of the nine haplotypes is shared between Africa and Eurasia even in clade A, which contains mtDNA sequences from both continents. Thus, it is likely that Eurasian and African populations were connected only temporarily during their history when the climatic conditions allowed migrations between the continents.

The occurrence of sequences from clade A in both Africa and Eurasia is evidence for a recent dispersal event as the divergence of the Eurasian from the African sequences was estimated to be about 0.36 MYA (figs. 2*a* and 6*c*). As the number of African haplotypes in clade A is larger than that of Eurasian ones (seven vs. two) and the Eurasian haplotypes are nested within the African haplotypes, it is likely that this migration took place from Africa to Eurasia. The oldest spotted hyena fossils in Europe, dated at 0.8 MYA (Garcia and Arsuaga 1999), argue for at least one additional, earlier dispersal of spotted hyenas from Africa to Europe. In the mtDNA sequences, this event is most

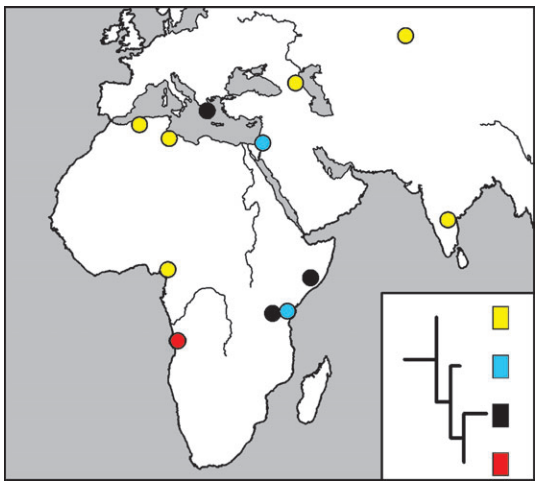

FIG. 4.—Map showing the geographical distribution of mtDNA sequences from striped hyenas. One dot represents two individuals. The four sequences are shown in different colors. The inserted tree is the same tree as in figure 2*b*.

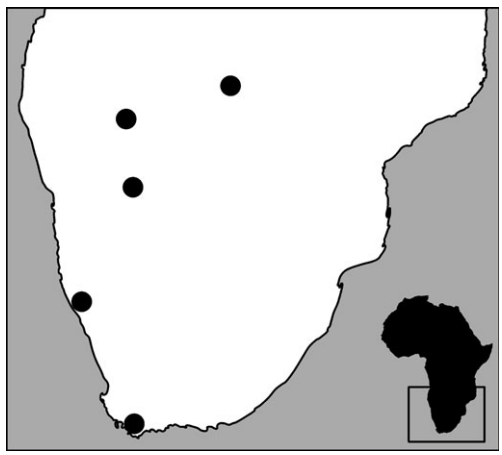

FIG. 5.—Map showing the location of the brown hyena samples. Some dots represent more than one individual.

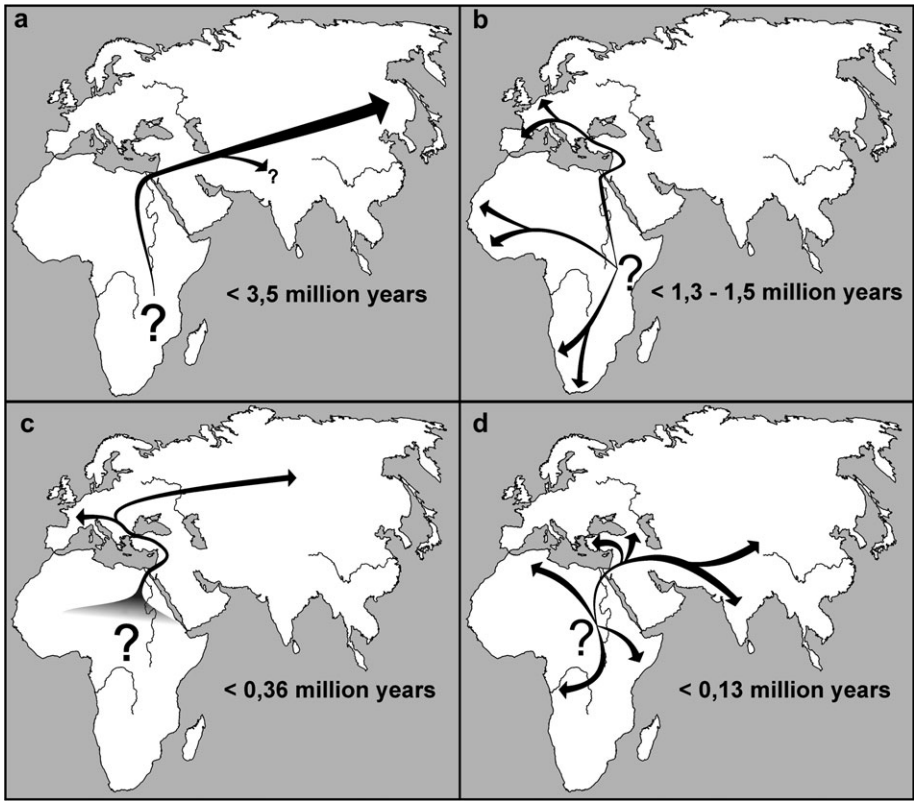

FIG. 6.—Timing and likely routes of migration of spotted and striped hyenas. (*a*) The earliest migration of spotted hyenas started less than about 3.5 MYA, most likely from the area where the first spotted hyena fossils were found, reaching East Asia and most likely also Pakistan. (*b*) The second migration of spotted hyenas, less than 1.3–1.5 MYA, resulted in the first arrival of hyenas in Europe and a separation of African spotted hyenas in a southern and a northern population. (*c*) The third spotted hyena migration took place after 0.36 MYA, starting from the northern African population and reaching both Europe and Asia. (*d*) Striped hyenas expanded their habitat from a small refugia population in Africa after 0.13 MYA, spreading across Africa and to Asia.

likely recorded by clade B, as the divergence of clades A–C took place about 1.3–1.5 MYA and molecular divergence predate population divergence (Nei 1986). Finally, the oldest spotted hyena fossils in Africa at 3.46 MYA (Barry 1987; Turner 1990) and in Asia at 2.6–3.7 MYA (de Vos, Leinders, and Hussain 1987) argue for a third dispersal event separating clade D from the three other clades. Two reasons argue for a geographical origin of both clade B and clade D in Africa rather than in Asia (fig. 6*a* and *b*). First, the divergence of clades A–C took place around the same time, arguing for a single cause that separated an ancestral population into three subpopulations which gave rise to clades A–C. As this event eventually resulted in the phylogeographic separation of African spotted hyenas into a northern and a southern group, it is likely to have taken place in Africa. Second, there is no evidence that after the initial divergence between clade D and all other lineages, descendants of clade D migrated from Asia to Europe or Africa. However, it should be noted that neither the topology of the phylogenetic tree nor the fossil record precludes the possibility that the origin of the genus *Crocuta* was in Asia, as originally proposed (Kurten 1956). Independent of the direction of migration, the deep divergence between clade D and the other lineages raises the possibility that the earliest *Crocuta* populations in Asia dating to more than 3 MYA were the ancestors of the Late Pleistocene hyenas from East Asia (Baryshnikov 1999). However, as the conclusions with regard to clade D are based on a single sequence, further sampling in Asia may well lead to the detection of additional dispersal events both between Africa and Asia and within Asia itself and thus complicate the scenario proposed above.

Despite being based on just 366 bp mtDNA sequence, the estimated divergence dates correlate well with the fossil record. The age of the oldest fossils in both Asia and Africa are about 3.5 Myr, in good agreement with the estimate of dispersal between these two areas less than about 3.5 MYA. Similarly, considering the fact that sequence divergence may considerably predate population divergence (Edwards and Beerli 2000), a first migration from Africa to Europe about 1 MYA (Turner 1992; Kahlke 1994; Lahr and Foley 1998) is consistent with the estimated age of the divergence of clades A–C about 1.3–1.5 MYA.

Phylogeography of Spotted Hyenas in Africa

Contrary to spotted hyenas in Europe (Hofreiter et al. 2004*b*), the mtDNA sequences of spotted hyenas in Africa show a strong phylogeographic pattern, with clade C in the south and clade A in the north of Africa and limited geographical overlap of the two clades around the equator (fig. 3). As cytochrome b is less variable than the mitochondrial

control region, it is possible that the latter marker would reveal additional structure within spotted hyenas, similar to other African species, which were found to show a variety of phylogeographic patterns (e.g., Simonsen, Siegismund, and Arctander 1998; Arctander, Johansen, and Coutellec-Vreto 1999; Matthee and Robinson 1999; Flagstad et al. 2001; Girman et al. 2001; Nersting and Arctander 2001; Uphyrkina et al. 2001; Muwanika et al. 2003). Unfortunately, only some of these studies cover a geographical range similar to our sampling of spotted hyenas, as most studies concentrate on the more southern parts of Africa. Of three species sampled across a similar geographical range as the spotted hyena, warthog and hartebeest (Arctander, Johansen, and Coutellec-Vreto 1999; Flagstad et al. 2001; Muwanika et al. 2003) also show the deepest phylogeographic split between northern and southern populations, while leopard shows no phylogeographic subdivision for mtDNA sequences in Africa (Uphyrkina et al. 2001). For two more species, roan (Matthee and Robinson 1999) and African wild dog (Girman et al. 2001), similarly a deep phylogenetic split between northern and southern Africa was found, although the two wild dog clades have a large geographical overlap.

Two major explanations have been offered for north-south phylogeographic patterns in Africa, the formation of the rift valley and restriction of animal populations into refugia during glacial cycles (e.g., Flagstad et al. 2001; Girman et al. 2001). For two reasons, we consider the restriction of populations to glacial refugia more likely. First, the formation of the African rift valley started approximately 25 MYA (Beyene and Abdelsalam 2005) and was completed long before the genetic split between the two African populations occurred. The formation substantially predates the separation of northern and southern clades in both hartebeest (Flagstad et al. 2001) and wild dog (Girman et al. 2001), too. Second, it separates the middle part of Eastern Africa from the rest of the continent.

Despite the strong phylogeographic pattern, we found identical sequences up to 4,500 km apart from each other in African spotted hyenas. Thus, within phylogeographic groups, extant spotted hyenas from Africa resemble Pleistocene spotted hyenas from Eurasia in that identical sequences are found at great geographical distances. However, contrary to the situation in Europe, almost no overlap exists in the geographical range of the two spotted hyena clades in Africa. Two factors may have contributed to these patterns. First, the time available for gene flow between different clades after expansion from refugia may play a critical role (Hofreiter et al. 2004*b*). If African hyenas expanded from two Pleistocene refugia after the end of the last glacial maximum, the 10,000 years since then may not have allowed for sufficient gene flow to result in large-scale geographical mixing of the two mtDNA clades as observed in Europe. Second, rapid expansions into empty habitats immediately after the end of a glacial maximum could explain the occurrence of identical haplotypes over large geographical distances. Both lower population densities and empty habitats have been shown to result in presaturation dispersal in several large mammals, that is, dispersal of animals before the carrying capacity of a habitat is reached (Cheeseman et al. 1988; Swenson, Sandegren, and Soderberg 1998). This scenario would explain both the large geographical distance between identical genotypes within a clade and the limited geographical overlap between clades of spotted hyenas in Africa. Unfortunately, our sampling in Africa is too limited in numbers to pinpoint possible areas for Pleistocene refugia by searching for clines in genetic diversity. Similarly, due to the limited sampling, we cannot draw conclusions about possible phylogeographic patterns of cave hyenas in Asia.

Striped Hyenas

Although striped hyenas occur across a large geographical area, we found only four different sequences, with two variable positions in the 13 striped hyenas studied. The low genetic diversity and the fact that all haplotypes that were carried by more than one individual occurred across large geographical distances suggest that the current habitat of striped hyenas is a result of a recent expansion from a single Pleistocene refugium, as it has been suggested for a number of European species with similarly low amounts of genetic diversity, such as wolverine (Walker et al. 2001), otter (Cassens et al. 2000; Ferrando et al. 2004), and lynx (Hellborg et al. 2002). As only few Pleistocene fossils of striped hyenas are known from outside of Africa (Kurten 1965; Werdelin and Solounias 1991), the glacial refugium of striped hyenas was most likely in Africa.

Similar to the results for spotted hyenas, identical sequences were found large distances apart from each other, up to more than 7,000 km. The most common haplotype in striped hyenas was found from West Africa across North Africa to Siberia, and the other two haplotypes that were detected in more than one individual also occurred over large geographical distances (fig. 4). Thus, the expansion of the geographical range, including the emigration from Africa, must have been rapid. Although our data only allow estimating the striped hyena migration to younger than 0.13 Myr (figs. 2*b* and 6*d*), this event must have been more recent as all sequences found in Asia have identical counterparts in Africa (fig. 4). Kurten (1965) argued that size differences between fossil striped hyenas of different ages from the Levant show an emigration of striped hyenas from Africa as recently as in Neolithic times. If the expansion of the geographical range indeed took place so recently it would explain that, given the small sample number and sequence length, we do not see a signal of a demographic expansion in the striped hyena data.

Brown Hyenas

Brown hyenas are even more depleted in genetic diversity than striped hyenas, as all seven individuals we sequenced plus the one from the database carry identical mtDNA sequences. Similar to their current distribution, fossil remains of brown hyenas are found only in southern Africa (Turner 1990). Thus, it is likely that brown hyenas always occupied a comparatively small geographic area and may have been particularly vulnerable to environmental changes. However, brown hyenas are not unique with regard to low genetic diversity. There is in fact increasing evidence that Pleistocene climate shifts had a strong

influence not only on Holarctic species (Hewitt 2000) but also on African species (e.g., Matthee and Robinson 1997; Flagstad et al. 2001; Matthee and Flemming 2002; Leonard et al. 2005). In addition to brown and striped hyenas, two more large African carnivores, cheetahs (Freeman et al. 2001) and lions (Burger et al. 2004), show low levels of mtDNA sequence diversity. Thus, of six species of large African carnivores only leopard (Uphyrkina et al. 2001) and spotted hyenas (this study) do not show evidence for a recent decrease in population size. It would be interesting to investigate whether the reduction in genetic diversity in the other four species took place around the same time and may therefore have a common cause.

Conclusions

The phylogeographic distribution of mtDNA haplotypes indicates three migration events from Africa to Eurasia for spotted hyenas and a recent habitat expansion of striped hyenas across Africa and Asia from a small African population. The data also indicate rapid expansions of the geographical range in both species. However, extant spotted hyenas in Africa show a marked phylogeographic pattern with one mtDNA sequence clade in the north and a second in the south, similar to several other African species. Little genetic diversity in striped and brown hyenas indicates population bottlenecks in these species during the Pleistocene, a pattern shared with other African carnivores. These results are further evidence of the importance of glacial cycles in shaping genetic diversity of animal populations also in Africa. Finally, we found that spotted hyena from Africa and cave hyenas from Eurasia are intermingled in phylogenetic analyses. This result questions a taxonomic delineation of the Pleistocene spotted hyenas as a subspecies or even species distinct from extant spotted hyenas.

Supplementary Material

Supplementary Table 1 and Figure 2 and details are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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