Supplementary information

Primers used for amplifications from Pleistocene samples:

Cr F1	5`-GAA AAT CTC ACC CAC TCA TTA AAA-3`
Cr F2	5`-GAA ATT TCG GGT CAC TAT TAG GAA-3`
Cr F3	5`-CAA CAA CCG CCT TCT CAT CAG-3`
Cr F4	5`-GAG CTT CCA TAT TCT TCA TCT GTC TA-3`
Cr F5	5`-CAA CAT CTC GGC ATG GTG AA-3`
Cr F6	5`-CAC TAT TAG GAA TCT GCT TAA TCT TA-3`
Cr F7	5`-CAA CCG CCT TCT CAT CAG TG-3`
Cr F8	5`-CGA TAC ATA CAC GCC AAC GGA-3`
Cr F9	5`-TAT ATA CAT ATC GGC CGA GGA A-3`
Cr F10	5`-CGT GAA ACA TCG GAA TCC TAC TA-3`
Cr R1s	5`-GAT TAA GCA GAT TCC TAA TAG TGA C-3`
Cr R1l	5`-GTA TGG CTA GGA ATA GAC CTG TCA G-3`
Cr R2	5`-CCG TAG TTT ACG TCT CGG C-3`
Cr R3	5`-GTA AGA TCC GTA GTA TAT TCC TCG G-3`
Cr R4	5`-CAC CTC AGA ATG ATA TTT GGC CTC-3`
Cr R5	5`-TAT ATA TAG ACA GAT GAA GAA TAT G-3`
Cr R6	5`-TTC ACG TCT CTG GGA GTG TGT AA-3`
Cr R7	5`-GAC ATA ACC TAT GAA TGC GGT A-3`

Primer pairs used and length of amplified fragments (see also suppl. Fig. 1)

Primer pair	product lengths in bp with (without) primers
Cr F1 – Cr R1l	147 (98)
Cr F2 – Cr R2	136 (93)
Cr F3 – Cr R3	146 (100)
Cr F4 – Cr R4	171 (121)
Cr F1 – Cr R1s	113 (64)
Cr F5 – Cr R1l	86 (41)
Cr F6 – Cr R2	124 (79)
Cr F7 – Cr R5	110 (65)

Cr F8 – Cr R6	103 (59)
Cr F9 – Cr R7	114 (70)
Cr F10 – Cr R4	93 (46)

Primers used for historical spotted hyena samples were Cr F1 in combination with Cr R4 (414bp) or Cr F1 together with Cr R2 (214bp) and Cr F3 together with Cr R4 (252bp). All fragment lengths include primers.

Primers used for striped and brown hyenas were

Hy F1	5`-GAA AAT CTC ACC CGC TCA TTA AAA-3`
Hy R1	5`-CAG CCA TAG TTG ACG TCT CGG C-3`
Hy F2	5`-CAA CCG CCT TTT CAT CAG TA-3`
Hy R2	5`-GTA AGA CGT AAC CTA TGA ATG CG-3`

Primer Hy F1 in combination with Hy R1 results in a 217 bp long fragment, Hy F1 with Hy R2 in a 387 bp fragment and Hy F2 with Hy R2 in a 222 bp fragment. All fragment lengths include primers.

Overview about the fossil history of the investigated genera.

Crocuta crocuta spelaea, Goldfuss 1828

Holotype: Complete skull with left and right P4 to I2, I1 missing and lower jaws with complete tooth row (GOLDFUSS 1828: tab. 55)

Paratype: skull with right P4 to P2, P1 to I1 missing and lower jaws with right m1-i3 and left m1-cinf (GOLDFUSS 1828: tab 56, 57).

Differences of *C.c.spelaea* to *C.c.crocuta*: The skull of the cave hyena is build more powerful, (e.g. stronger sagital crest). The processi occipitalii for the attachment of the first vertebra are stronger. The palatinal bone is broader between P3 and P4 than in extant *Crocuta*. The jugale bone is enlarged to allow the attachment of stronger chewing muzzles. The cave hyena was about 25% larger than its living relative.

Occurrence of fossil *C. crocuta*: The oldest remains of the spotted hyena are probably from Laetoli (older than 3.46 ma, Barry, 1987). This species is listed in

Werdelin & Solounias (1991) as *C. Crocuta* while Petter & Howell (1989) described them as a separate subspecies *C.c.dietrichi*, because of their smaller size but morphological similarities compared to recent forms. Turner (1984, 1990) argued that the natural size variation of the spotted hyena is quite large and *Crocuta* from the southern part of Africa is larger then their relatives from eastern Africa. Therefore the specimens from Laetoli fit well into the *Crocuta* distribution when compared with material from East Africa. We follow Turner and recognise only two subspecies of *Crocuta*, *C. c. crocuta* and *C. c. spelaea*. A synonymy list is given in Werdelin & Solounias (1991).

If Africa is the place where *Crocuta* first appeared, then the Sivalik specimen (*C. sivalensis*, de Vos et al. 1987) are the oldest evidence for the first immigration wave of *Crocuta* from Africa into Asia (Turner 1992), where descendents of this group survived till the end of the Pleistocene.

Crocuta co-existed in Africa with a large number of other predators between 3.0 and 1.5 to 1 Ma years (Turner 1990): *Homotherium, Megantereon, Dinofelis, Panthera leo, Panthera pardus, Acinonyx, Felis, Chasmaporthetes,* and probably *Pachycrocuta*. After 1 Ma, more canid species arrived and the fauna changed towards the extant guild structure with jackals, foxs, lion, leopard, cats, aardwolf, striped, brown and spotted hyenas.

In Europe between 3.5 and 1.5 Ma, the guild structure consisted of *Homotherium*, *Megantereon, Acinonyx, Pliocrocuta* and *Chasmaporthetes*. The carnivore guild was much smaller compared to the African one. Between 1.5 and 1 Ma, *Chasmaporthetes* and *Pliocrocuta* disappeared and *Panthera gombaszögenis* and *Pachycrocuta* arrived in Europe, as well as several *Canis* species, migrating from Asia and first appearance of *Crocuta* in Europe (Atapuerca, Rodriquez 2001). Locations like Ubeidiya (Israel, ca 1.5 Ma) indicate an increase of African species in the Levantine Corridor, while the major part of the fauna is still of Eurasian origin (Tchernov E. & Guérin 1986, Belmaker 2004).

The carnivore guild structure from 1.0 to 0.5 Ma on was similar to the East African fossil record (Turner 1995): *Crocuta* co-existed with *Homotherium*, *Acinonyx*, *Panthera gombaszögenis, Panthera leo, Panthera pardus*, *Pliocrocuta*, *Pachycrocuta* and two canids (Turner 1990, Kahlke 1994, Lahr 1998). These immigration of new carnivores

from Africa and Asia mark a climatic change as well. *Pliocrocuta*, *Pachycrocuta* and *P.gombaszögenis* are not present any more during the Upper Pleistocene.

In Asia *Crocuta crocuta* was described from the Upper Pleistocene, similar to the *C.c.spelaea* forms from Europe, meaning only larger in size.

Hyaena appeared around 3.0 Ma (Usno and Shungura B10), slightly after *Crocuta* in the fossil record of Africa. It never made its ways into Pleistocene Europe but migrated into Asia only recently. European fossil forms like *H. striata* seem to be synonymous with *Pliocrocuta* (Howell & Peter 1980, Turner, 1990, Werdelin & Solounias 1991). Remains from Palestine caves (Kurten 1965) indicate a migration of striped hyenas at least to Asia minor in the Upper Pleistocene.

Parahyaena is present in a very restricted area (parts of Namibia and South Africa) today. As shown by different authors (Pohle 1928, Howell & Petter 1976, Turner 1990, Werdelin & Barthelme 1997) it is possible that its range shifted as far as to the Ethiopian region, starting with Omo Shungura (younger than 2.6 Ma). The question, if a sympatric occurrence between striped hyena and brown hyena existed is still not answered.

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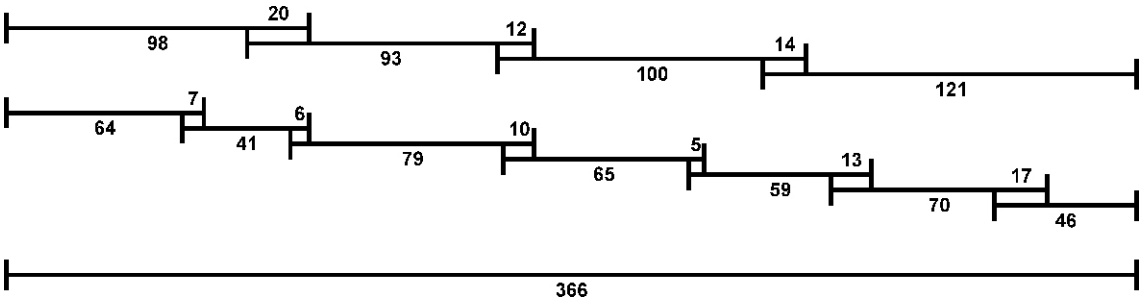

Suppl. Fig. 1: Schematic view of the amplification of 366 bp of the cytochrome B gene for the Pleistocene samples using four and seven PCR fragments, respectively. The numbers below show the length of the amplification products without primers, the numbers above the overlap between individual fragments. The upper part of the figure shows amplification of the 366 bp piece in long PCR fragments, the lower part in short fragments.