

- Proc. Natl. Acad. Sci. USA 101, 1626-1631.
11. Hallstrom, I., Blanck, A., and Atuma, S. (1984). Genetic variation in cytochrome P-450 and xenobiotic metabolism in *Drosophila melanogaster*. *Biochem. Pharmacol.* 33, 13-20.
 12. Snook, R.R., Cleland, S.Y., Wolfner, M.F., and Karr, T.L. (2000). Offsetting effects of *Wolbachia* infection and heat shock on sperm production in *Drosophila simulans*: analyses of fecundity, fertility and accessory gland proteins. *Genetics* 155, 167-178.
 13. Hoffmann, A.A., Hercus, M., and Dagher, H. (1998). Population dynamics of the *Wolbachia* infection causing cytoplasmic incompatibility in *Drosophila melanogaster*. *Genetics* 148, 221-231.
 14. Collier, S., Chan, H.Y., Toda, T., McKimmie, C., Johnson, G., Adler, P.N., O'Kane, C., and Ashburner, M. (2000). The *Drosophila* *embargoed* gene is required for larval progression and encodes the functional homolog of *Schizosaccharomyces Crm1*. *Genetics* 155, 1799-1807.
 15. Drnevich, J.M., Reedy, M.M., Ruedi, E.A., Rodriguez-Zas, S., and Hughes, K.A. (2004). Quantitative evolutionary genomics: differential gene expression and male reproductive success in *Drosophila melanogaster*. *Proc. Biol. Sci.* 271, 2267-2273.
 16. McKenzie, J.A. (1994). Selection at the diazinon resistance locus in overwintering populations of *Lucilia cuprina* (the Australian sheep blowfly). *Heredity* 73, 57-64.
 17. Davies, A.G., Game, A.Y., Chen, Z., Williams, T.J., Goodall, S., Yen, J.L., McKenzie, J.A., and Batterham, P. (1996). Scalloped wings is the *Lucilia cuprina* Notch homologue and a candidate for the modifier of fitness and asymmetry of diazinon resistance. *Genetics* 143, 1321-1337.
 18. Pasyukova, E.G., Nuzhdin, S.V., Morozova, T.V., and Mackay, T.F. (2004). Accumulation of transposable elements in the genome of *Drosophila melanogaster* is associated with a decrease in fitness. *J. Hered.* 95, 284-290.
 19. Pasyukova, E.G., Belyaeva, E.S., Kogan, G.L., Kaidanov, L.Z., and Gvozdev, V.A. (1986). Concerted transpositions of mobile genetic elements coupled with fitness changes in *Drosophila melanogaster*. *Mol. Biol. Evol.* 3, 299-312.

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Evolution of the extinct Sabretooths and the American cheetah-like cat

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The sabretooths (*Smilodon* and *Homotherium*) and the American cheetah-like cat (*Miracinonyx*) were the top predators in Late Pleistocene America, but became extinct about 13 thousand years ago [1]. As the evolutionary history of these taxa remains poorly understood [1,2], we analysed their phylogenetic relationship to extant felids. In contrast to previous molecular studies [3], our results show that the sabretooths diverge early and are not closely related to any living cats. This supports their morphological placement in a separate subfamily (Machairodontinae) [1]. Despite its remarkable morphological similarity to the African cheetah (*Acinonyx jubatus*), *Miracinonyx* appears to have evolved from a puma-like ancestor, presumably in response to similar ecological pressures [4,5].

Even though the evolutionary relationships within the cat family (Felidae) have been analysed using morphological, molecular and palaeontological data [1], many proposed relationships remain contentious, including those of recently extinct species. For example, the sabretooth cats are known from plentiful Late Pleistocene fossil material and have been placed, based on morphological criteria, outside of all extant cats (subfamily Felinae) as a separate subfamily, the Machairodontinae [1]. In contrast, an early ancient DNA study tentatively suggested that *Smilodon* was a member of the Felinae and thus part of the modern cat radiation [3].

The relationships of the American cheetah-like cat,

Miracinonyx trumani, are similarly confused as it had previously been mistaken for an ancestral or modern puma (e.g. *Puma trumani*). It was only recognised as a truly distinct group after abundant post-cranial fossil material became available [6]. Morphologically, *M. trumani* strongly resembles the modern African cheetah (*Acinonyx jubatus*) [4], which has led to uncertainty about whether it is more closely related to the Old World cheetah or the New World puma [2,4,6,7].

To address these questions, we used ancient DNA techniques, including independent replication, to generate mitochondrial DNA sequences from several specimens of *Smilodon populator* from Patagonia and a *M. trumani* specimen from Wyoming. In total, 1302 bp of *cytochrome b* (*cytb*), *NADH5*, *ATP8*, as well as 12S and 16S ribosomal RNA genes were recovered (Supplemental Data). We also obtained a short fragment of *cytb* from a Yukon scimitar-toothed cat (*Homotherium serum*), another recent member of the Machairodontinae.

Phylogenetic analyses (Figure 1; Supplemental Data) show that *Smilodon* and *Homotherium* fall outside the extant cats, the Felinae. This is in agreement with their traditional morphological placement [1]. The deep genetic split between the two sabretooth taxa is also consistent with morphological analyses [1].

These phylogenetic analyses also reveal that *M. trumani* is the sister taxon to the puma, rather than the African cheetah. Another endemic American cat, the jaguarundi, groups with *Miracinonyx* and the puma. This result corroborates the first morphological studies using complete specimens of *Miracinonyx* [6,7], but also raises questions about the anatomical structures used to classify *Miracinonyx* as a cheetah [4]. Re-analysis of these characters [2,7] has emphasised that many of them are associated with a highly cursorial lifestyle, such as elongated limbs and enlarged nares [2,4], which increase running speed and air-intake efficiency, respectively. In contrast, other limb

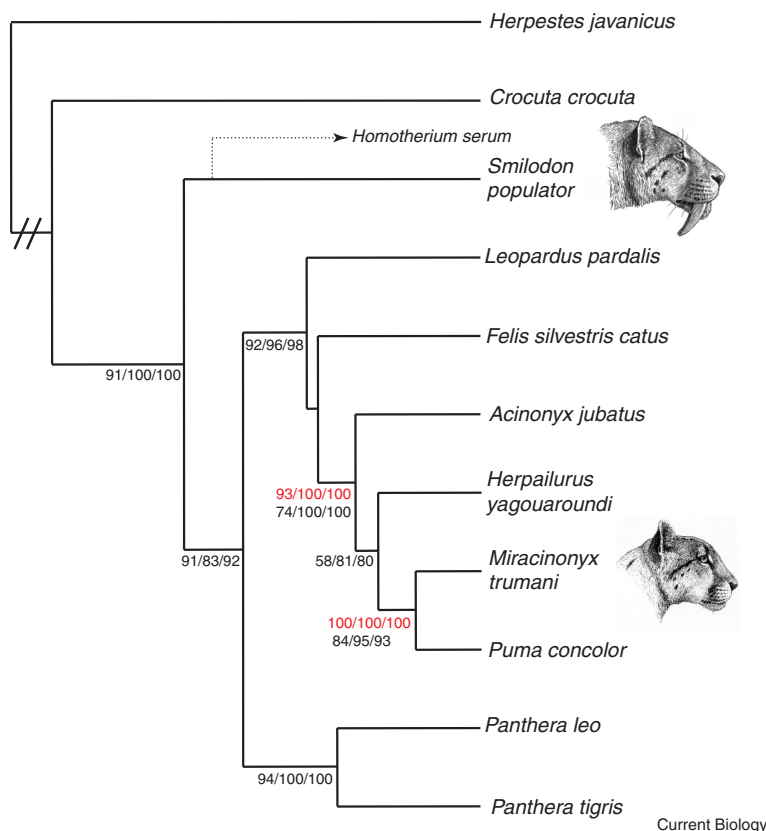


Figure 1. Consensus tree for members of the Carnivora analysed in this paper.

Maximum parsimony bootstrap replicates and Bayesian posterior values are given for nodes. The numbers at the nodes show: maximum parsimony bootstrap support (1000 replicates), the Bayesian GTR+I+G posterior probabilities (5 million generations, burnin at 1 million) and the Bayesian GTR+cov+G posterior probabilities (5 million generations, burnin at 2.5 million). The values given in red represent results after omission of the jaguarundi from analysis (Supplemental Data). The maximum likelihood position of *Homotherium serum* is based on a short dataset (Supplemental Data). Figures of *Smilodon* and *Miracinonyx* courtesy of M. Anton.

characters, such as a well-developed anteriorly projecting flange on the head of the fibula, link *Miracinonyx*, puma and jaguarundi [2,7].

It has been suggested that the cheetahs originated in the New World [4] and later migrated to the Old World. However, the mitochondrial sequence analysis together with recent fossil data (Supplemental Data) suggests that they originated in the Old World and that a puma-like cat then invaded North America around six million years ago [5,7,8]. Around 3.2 million years ago, this ancestor diverged into *Miracinonyx* and *Puma*, which is broadly contemporaneous with increasing prairie in North America [9]. The expansion of this habitat and its effect on ungulate prey, e.g. the pronghorn antelope (*Antilocapra americana*), may have driven the

evolution of cursoriality in *Miracinonyx*, allowing it to excel in high-speed pursuit [1].

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Supplemental data

Supplemental data including Experimental Procedures are available at

<http://www.current-biology.com/cgi/content/full/15/15/R589/DC1/>

References

1. Turner, A., and Anton, M. (1997). *The Big Cats and Their Fossil Relatives* (New York: Columbia University Press).
2. Herrington, S.J. (1986). Phylogenetic relationships of the wild cats of the world. Ph.D thesis, University of Kansas.
3. Janczewski, D.N., Yuhki, N., Gilbert, D.A., Jefferson, G.T., and O'Brien, S.J. (1992). Molecular phylogenetic inference from saber-toothed cat fossils of Rancho La Brea. *Proc. Natl. Acad. Sci. USA* 89, 9769–9773.
4. Adams, D.B. (1979). The Cheetah: Native American. *Science* 205, 1155–1158.
5. Hemmer, H., Kahlke, R.D., and Vekua, A.K. (2004). The Old World puma - *Puma pardoides* (Owen, 1846) (Carnivora: Felidae) - in the Lower Villafranchian (Upper Pliocene) of Kvabebi (East Georgia, Transcaucasia) and its evolutionary and biogeographical significance. *Neues Jahrbuch für Geologie und Paläontologie* 233, 197–231.
6. Martin, L.D., Gilbert, B.M., and Adams, D.B. (1977). A Cheetah-like cat in the North American Pleistocene. *Science* 195, 981–982.
7. van Valkenburgh, B., Grady, F., and Kurten, B. (1990). The Plio-Pleistocene Cheetah-like Cat *Miracinonyx inexpectatus* of North America. *Journal of Vertebrate Palaeontology* 10, 434–454.
8. Zhanxiang, Q. (2003). Chapter 2: Dispersals of Neogene Carnivorans between Asia and North America. *Bull. Am. Mus. Nat. Hist.* 279, 18–31.
9. Janis, C.M., Damuth, J., and Theodor, J.M. (2002). The origins and evolution of the North American grassland biome: the story from the hoofed mammals. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 177, 183–198.

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