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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

