

Paleodiet study based on isotopic ratio analysis of bone collagen from Malagasy extinct species

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Madagascar is well known for the unique ecological system and approximately 80% of plants and animals in Madagascar are the endemic species. During the past 2000 years, at least 17 genera of vertebrates became extinct, which led to demise of animals weighting over 12 kg excepting crocodile *Crocodylus niloticus* (Burney and MacPhee, 1988). The main factors of the megafaunal extinction in the island of Madagascar would be human activities (e.g. over-kill, fire) or environmental changes (e.g. aridification, vegetational transition) (Crowley, 2010; Burney et al., 2004). Previous research reveals that the vegetational shift coincided with the extermination of megafauna (Geoffrey Clark, personal communication). However, the relationship between them has remained less understood.

Here we reconstructed the changes in the feeding habits of extinct species at the level of C3 or C4 plants to evaluate the correlation between feeding habitats and vegetational shift as well as human arrival. Fossil bones of extinct pygmy hippo *Choeropsis liberiensis* were collected from Taolambiby, Ambolisatra and Itampolo in southwestern Madagascar and used for isotopic ratio analysis. Collagen was extracted from the bones, and carbon and nitrogen stable isotopic ratios of collagen were measured using EA/IRMS at Japan Agency for Marine-Earth Science and Technology. Radiocarbon dating of bone collagen was also employed using Single-Stage AMS at Atmosphere and Ocean Research Institute, The University of Tokyo.

Radiocarbon dates of bone collagen show the range from 2750-1130 cal BP. The $\delta^{15}\text{N}$ values were 9.6-10.1‰ in Taolambiby ($n=2$), 11.1-12.3‰ in Itampolo ($n=6$) and 12.48-12.57‰ in Ambolisatra ($n=3$) without the date of 2750 cal BP, suggesting that the trophic level of hippos would not change in the same region. The $\delta^{13}\text{C}$ values were -18.9--19.7‰ in Taolambiby, -17.2--17.9‰ in Ambolisatra and -12.7--14.8‰ in Itampolo. The range of $\delta^{13}\text{C}$ values in fossil bones of pure C3 feeders would be approximately -31--17‰ through the $\delta^{13}\text{C}$ values in modern C3 plants from Beza Mahafaly, located about 20 km off eastern Taolambiby (Crowley et al., 2011). To take into account of the isotopic fractionation, the values in C4 feeders should be higher than that of C3 feeders. In comparison with the range of C3 feeders and our results, hippos in Taolambiby and Ambolisatra would be C3 feeders, while hippos in Itampolo would feed on both C3 and C4 plants, indicating that the diet of hippos in southwestern Madagascar did not change at the level of C3 or C4 plants in the same region. Previous studies revealed that human migrated to Taolambiby ca. 2300 cal BP (Burney et al., 2004). The extinction window of hippos in southwestern Madagascar is estimated 1250-950 cal BP (Geoffrey Clark, personal communication). Furthermore, pollen records in Ambolisatra show that the vegetational change occurred around ca. 1250 cal BP (Geoffrey Clark, personal communication). Therefore, our results suggest that hippos would not shift their diet at the level of isotope composition in their tissues from 2600 cal BP to 1100 cal BP, corresponding the timing after human arrival until hippos' extinction. Moreover, the $\delta^{13}\text{C}$ values different among locations suggest that hippos would feed on local plants without distinction of C3 or C4 plants. Hence, vegetational shift would not have an impact on the isotope composition of their tissues, and food shortage may not directly cause hippos' extinction.

Keywords: Madagascar, Holocene, extinction, bone collagen, stable isotope ratio, feeding habit