Fossil Mice and Rats Show Isotopic Evidence of Niche Partitioning and Change in Dental Ecomorphology Related to Dietary Shift in late Miocene of Pakistan

Kimura et al.

Supporting Information S1

Effect of Nursing on Carbon Isotope Composition in Murine Rodents

Developmental timing of molars greatly overlaps with lactation in murine rodents, in which deciduous cheek teeth are lacking. In mice, enamel mineralization of m1 and m2 begins only a few days after birth and completes within 16 to 18 days while mother's milk was their only food source, and m3 begins enamel mineralization in Day 11 to 12 and erupts in Day 28 to 29 [1]. Thus, isotope compositions in m1 and m2 would reflect nutrients from mother's milk, and $\delta^{13}C$ values of m3 would partially reflect solid diet after weaning. Milk is depleted in ^{13}C by 2.1 ‰ relative to the mother's plasma [2] because milk has a high lipid content, which is depleted relative to bulk diet [3,4]. Teeth formed before weaning have lower $\delta^{13}C$ values than those formed after weaning by ~0.5 ‰ in humans [5] to ~2 ‰ in sea lions [6]. The varying degree of the milk effect probably comes from varying fat contents of milk [7]. In fossil theridomyid rodents, the deciduous premolar is depleted in ^{13}C by 1.5 ‰ relative to the permanent molar [8].

We measured isotope ratios in lower molars (m1, m2, m3) of three specimens of Recent *Rattus* sp. from Pakistan, whose diets are unknown (Figure S1). The result that $\delta^{13}C$ values in m1 were more negative than $\delta^{13}C$ in m3 (Figure S1A) is consistent with the timing of enamel mineralization. The isotope enrichment between m1 and m3 ($^{13}\delta^*_{m1\text{-}m3}$) varies from -0.6 ‰ (n=1) to -2.7 and -2.9 ‰ (n=2), which encompasses the total variation in teeth formed before and after weaning of different animals. The isotope enrichment of m3-m2 (1.9 ‰, n=2) is larger than that of m2-m1 (0.9 ‰), suggesting that carbon isotope ratios in m3 reflect, if not fully, post-weaning diet. A slight enrichment of m2-m1 may come from an analytical uncertainty or a partial signal of prenatal nutrients transported through mother's placenta. Thus, it suggests that carbon isotope compositions in m1of murine rodents are influenced by mother's milk.

The result that the mean $\delta^{18}O$ value of m1 is more positive than that of m3 by 1.3 ‰ (Figure S1B) is also congruent with the fact that isotope composition in m1 is more influenced by mother's milk. Milk is synthesized from mothers' body water, whose isotope composition is more enriched in ^{18}O than that of drinking water due to water intake from plants and metabolic reactions in the body [9,10]. Based on a model modified from Bryant and Froelich [10] to describe the influence of seasonal birth on oxygen isotope composition in different dentitions, Bryant et al. [11] demonstrated that, if individuals are born in the same season, oxygen composition in teeth formed prior to weaning are more enriched in ^{18}O than teeth formed after weaning.

Carbon isotope values in large herbivorous mammals are often used to estimate the proportion of C_3 plants (all trees, most shrubs, and some grasses) versus C_4 grasses in their diet based on a two-source linear mixing model [12,13,14,15] with isotope enrichment between diet and bioapatite [16]. Our brief analysis on a sequence of lower molars of Recent *Rattus* sp. suggests that the isotopic effect of nursing on molars must be understood well to reconstruct the proportion of C_3 to C_4 diet of small mammals using molars rather than ever-growing incisors.

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