Baboons, Water, and the Ecology of Oxygen Stable Isotopes in an Arid Hybrid Zone

Gillian L. Moritz[1]
Nicolaas Fourie[2]
Justin D. Yeakel[3]
Jane E. Phillips-Conroy[4]
Clifford J. Jolly[5]
Paul L. Koch[6]
Nathaniel J. Dominy[1,7,*]

[1]Department of Biological Sciences, 103 Gilman Hall, Dartmouth College, Hanover, New Hampshire 03755; [2]Center for the Advanced Study of Hominid Paleobiology, Department of Anthropology, George Washington University, 2110 G Street NW, Washington, DC 20052; [3]Department of Ecology and Evolutionary Biology, 1156 High Street, University of California, Santa Cruz, California 95064; [4]Department of Anatomy and Neurobiology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110, and Department of Anthropology, Washington University in St. Louis, One Brookings Drive, St. Louis, Missouri 63130; [5]Department of Anthropology, New York University, Rufus D. Smith Hall, 25 Waverly Place, New York, New York 10003; [6]Department of Earth and Planetary Sciences, 1156 High Street, University of California, Santa Cruz, California 95064; [7]Department of Anthropology, 6047 Silsby Hall, Dartmouth College, Hanover, New Hampshire 03755

ABSTRACT

Baboons regularly drink surface waters derived from atmospheric precipitation, or meteoric water. As a result, the oxygen isotope ($\delta^{18}O$) composition of their tissues is expected to reflect that of local meteoric waters. Animal proxies of the oxygen isotope composition of meteoric water have practical applications as paleoenvironmental recorders because they can be used to infer aridity and temperature in historic and fossil systems. To explore this premise, we measured the $\delta^{18}O$ values of hair from two baboon species, *Papio anubis* and *Papio hamadryas*, inhabiting Awash National Park, Ethiopia. The hybridizing taxa differ in their ranging behavior and physiological response to heat. *Papio hamadryas* ranges more widely in the arid thornbush and is inferred to ingest a greater proportion of leaf water that is enriched in ^{18}O as a result of evaporative fractionation. It is also better able to conserve body water, which reduces its dependence on meteoric waters depleted in ^{18}O. Taken together, these factors would predict relatively higher $\delta^{18}O$ values in the hair ($\delta^{18}O_{hair}$) of *P. hamadryas*. We found that the $\delta^{18}O_{hair}$ values of *P. hamadryas* were higher than those of *P. anubis*, yet the magnitude of the difference was marginal. We attribute this result to a common source of drinking water, the Awash River, and the longer drinking bouts of *P. hamadryas*. Our findings suggest that differences in $\delta^{18}O$ values among populations of *Papio* (modern or ancient) reflect different sources of drinking water (which might have ecological significance) and, further, that *Papio* has practical value as a paleoenvironmental recorder.

Introduction

The influx of oxygen into an animal determines the oxygen isotope ($\delta^{18}O$) composition of its body water. For terrestrial mammals, such influxes include ingested water (>50%), which is not fractionated during uptake, and the inhalation of atmospheric O_2 (~25%) and water vapor (~15%), which undergo isotopic fractionation during diffusion across the lung lining. Metabolic water, formed from food during digestion, is generally a minor flux (<10%) when drinking water is readily available (Bryant and Froelich 1995; Kohn 1996). External sources of oxygen, such as dietary water and atmospheric O_2, thus provide a baseline from which the $\delta^{18}O$ of body water deviates (Longinelli 1984; Luz et al. 1984; Podlesak et al. 2008; Tuross et al. 2008). The isotopic variation in these sources can range from very low for atmospheric O_2 (Kroopnick and Craig 1972) to very high for atmospheric water vapor, meteoric water (surface water derived from atmospheric precipitation), and plant water (Flanagan et al. 1991; Gat 1996; Bowen and Wilkinson 2002). In addition, the physiological differences among animals that affect metabolic rate and water economy (i.e., sweating, panting, urine production, and fecal water loss) can shift the balance among these fluxes, leading to differences among species within a community (Kohn 1996). As a result, the $\delta^{18}O$ of animal body water can vary across large spatial scales and within communities of species with different diets, drinking behaviors, and physiologies.

Body water is the source of oxygen in the biological apatite of bones and teeth (Kohn 1996) as well as the carbonyl oxygen in protein (Ehleringer et al. 2008). Environmental and physi-

ological variation in the $\delta^{18}O$ value of body water is thus strongly reflected in the $\delta^{18}O$ of biogenic tissues (Ayliffe and Chivas 1990; Kohn et al. 1996; Clementz and Koch 2001; Sponheimer and Lee-Thorp 2001; Cerling et al. 2003, 2004, 2008; Hobson et al. 2004; Bowen et al. 2005b, 2009; Levin et al. 2006; Ehleringer et al. 2008; Chenery et al. 2011). For example, species such as giraffes that derive much or all of their water from leaves have ^{18}O-enriched tooth enamel (high $\delta^{18}O$ values) because leaf water is subject to ^{18}O enrichment from evaporation (Flanagan et al. 1991; Kahmen et al. 2011). Species that rely on meteoric water, termed "obligate drinkers," have ^{18}O-depleted tooth enamel (low $\delta^{18}O$ values) because their body water is regularly recharged with water that has experienced less evaporation (and is therefore less ^{18}O enriched; Cerling et al. 2003, 2008; Levin et al. 2006).

This general pattern has been instructive for studying the diet, physiology, and evolution of fossil species (Koch et al. 1995; Quade et al. 1995; Sponheimer and Lee-Thorp 1999; Clementz et al. 2006; Thewissen et al. 2007; Fourie et al. 2008b; Liu et al. 2008; Secord et al. 2008; Bedaso et al. 2010; Eagle et al. 2011). Perhaps of special relevance is the increasing use of stable oxygen isotopes in studies focused on the diet and ecology of early hominins (Bocherens et al. 1996; Schoeninger et al. 2003; Yeakel et al. 2007; White et al. 2009; Cerling et al. 2010, 2011). These studies have called attention to the ecological implications of $\delta^{18}O$ values in primates, yet the extent to which $\delta^{18}O$ values can vary in a population of contemporary primates is practically unknown (Smith et al. 2010; Chenery et al. 2011).

Baboons as Obligate Drinkers

Baboons (*Papio* spp.) are obligate drinkers, in part because thermal stress causes sweating (Funkhouser et al. 1967; Hiley 1976). To replenish their body water and maintain homeothermy (Mitchell et al. 2009), baboons in arid habitats ingest water almost daily (Stoltz and Saayman 1970; Barton et al. 1992; Altmann 1998; Brain and Mitchell 1999); in fact, some populations excavate and drink subsurface meteoric waters (Crook and Aldrich-Blake 1968; Kummer 1968; Sigg 1980; Hamilton 1985; Biquand et al. 1992). Such behavior suggests that the tissues of baboons should be ^{18}O depleted, reflecting the isotopic composition of local meteoric waters. Animal proxies of meteoric water, or evaporative insensitive (EI) taxa (Levin et al. 2006), can be used to infer aridity and temperature in historic and fossil systems (Koch 1998). Thus, baboons have potential practical applications as paleoenvironmental recorders. At the same time, if *Papio* show isotopic fidelity to local meteoric waters and if regional differences exist in the $\delta^{18}O$ of drinking waters, it should be possible to use isotopic data from their tissues to study dispersive and ranging behaviors.

Awash National Park, Ethiopia: A Model System

To explore the validity of these ideas, we focus on two baboon species (*Papio anubis* and *Papio hamadryas*) whose ranges meet in the semiarid thornbush and riparian woodland of Awash National Park (ANP). Although similar enough that natural hybridization occurs, producing viable and fertile offspring and a distinct hybrid zone, these taxa exhibit subtle differences in development and physiology (Jolly and Phillips-Conroy 2003). Some experimental evidence suggests that *P. hamadryas* is more tolerant of heat stress and better able to conserve body water than is *P. anubis* (Zurovsky and Shkolnik 1993).

ANP is located in the northern Rift Valley, ~150 km east of Addis Ababa. At 1,000 m elevation, it lies in the middle of a steep topographic and ecological gradient between the central Ethiopian plateau, now largely agricultural but once consisting of moist forests, woodlands, and savannas inhabited by *P. anubis*, and the arid thornscrub and semideserts of the eastern lowlands, the domain of *P. hamadryas*. ANP includes both taxa and their hybrids (fig. 1). The diets of the two species in the ANP hybrid zone are qualitatively similar, including fruit, flowers and seeds from trees, shrubs, and herbaceous plants, grass rhizomes, and sedge corms as well as some insects and small vertebrates. The leaves, flowers, exudates, and seeds of shrubby and arborescent *Acacia* species (especially *A. senegal*, *A. mellifera*, *A. nubica*, *A. brevispica*, and *A. tortilis*) are important foods for both species. The habitat of *P. anubis*, in the level floodplain above the Awash Falls, includes narrow evergreen woodland strips along the Awash River and its ephemeral tributaries; as a result, the diet of *P. anubis* includes a higher proportion of fruits from nonleguminous trees and shrubs, such as *Ficus*, *Dobera*, *Grewia*, *Celtis*, and *Ziziphus* (= *Zizyphus*), many of which are confined to or concentrated in riparian habitats. In contrast, the diets of *P. hamadryas* and the hybrids are derived mainly from plants in the thornscrub and the walls of the Awash canyon below the Awash Falls (Aldrich-Blake et al. 1971; Nagel 1973; Nystrom et al. 2004). Both species and their hybrids drink from the Awash River, from puddles and seeps during the rainy season, and, in the case of *P. hamadryas* Group A, from the mineral Lake Beseka (fig. 1d).

In studies of hair keratin from mid-twentieth- and early twenty-first-century human populations, the r^2 for the correlation between the $\delta^{18}O$ values of local drinking water and hair ($\delta^{18}O_{hair}$) is high, from 0.77 to 0.86. These strong correlations occur despite the fact that some fraction of the water present in food and ingested fluids has a distant source, especially in the early twenty-first century. Deviations from these strong linear relationships distinguish some populations, where food water is very different from local drinking water (e.g., Inuit, who consume marine prey with relatively high $\delta^{18}O$ values but ingest local ^{18}O-depleted drinking water; Ehleringer et al. 2008; Bowen et al. 2009). Generalizing from these observations on humans, we conjecture that if *P. hamadryas* is more dependent on plant foods in the arid thornbush, its hair will be relatively ^{18}O enriched (high $\delta^{18}O_{hair}$ values) as a result of the ingestion of evaporated leaf water. Moreover, if *P. hamadryas* is indeed more tolerant of heat stress and better able to conserve body water than is *P. anubis* (Zurovsky and Shkolnik 1993), a reduced dependence on meteoric water is also expected to result in higher $\delta^{18}O_{hair}$ values. Alternatively, daily drinking of ^{18}O-
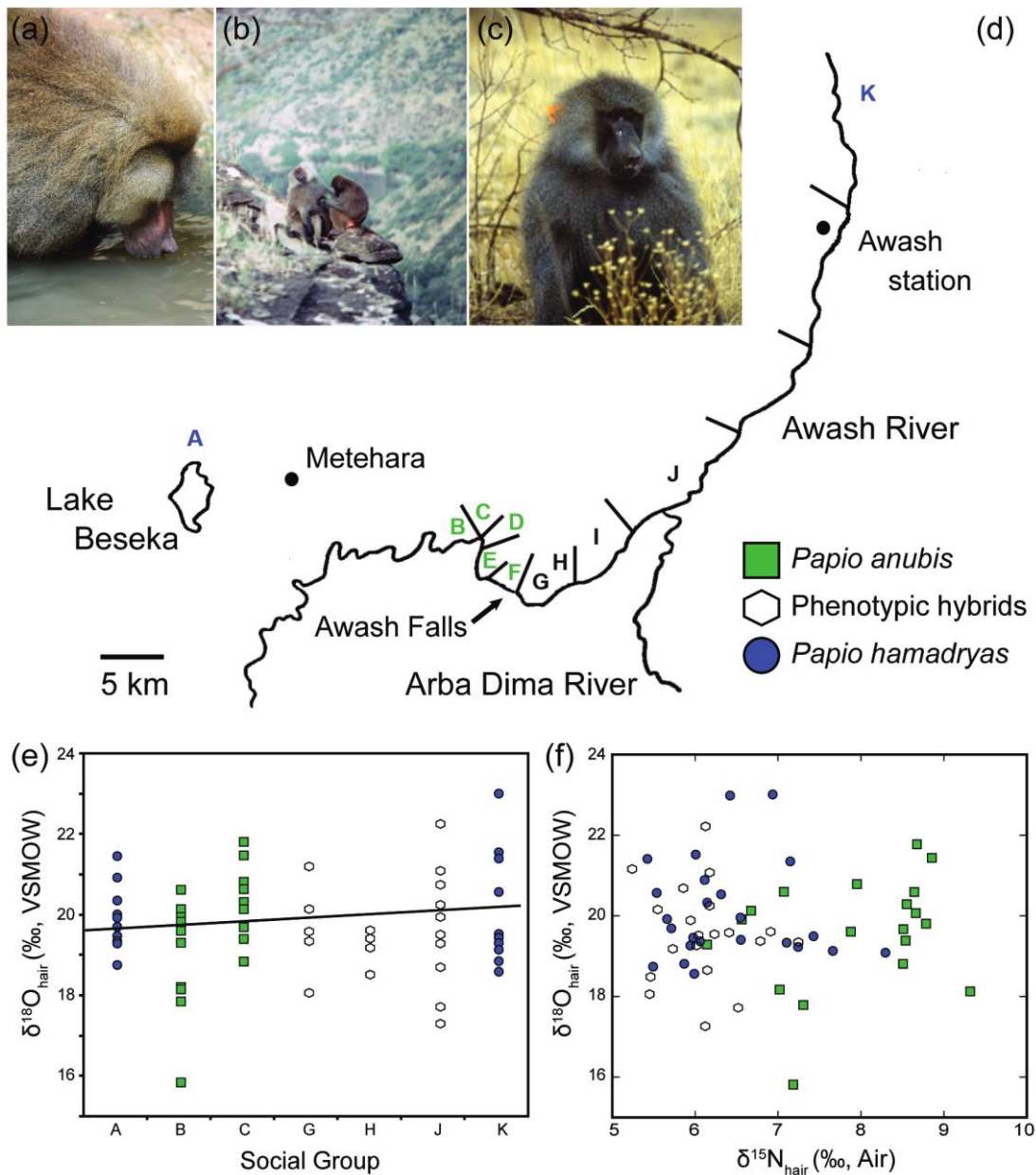
Figure 1. *A*, *Papio hamadryas* and the consumption of meteoric water; *b*, a *Papio anubis* female grooms and consorts with a hybrid male; *c*, phenotypic hybrid; *d*, sketch map of the study area, indicating the location of all social groups (redrawn from Phillips-Conroy and Jolly 1986); *e*, taxonomic variation in $\delta^{18}O_{hair}$ as a function of social group membership; *f*, variation in $\delta^{18}O_{hair}$ as a function of variation in $\delta^{15}N_{hair}$.

depleted meteoric waters might be enough to overwhelm the enrichment effects of plant water and physiology, resulting in relatively uniform $\delta^{18}O_{hair}$ values across populations. These potential outcomes are tested here.

Nitrogen Stable Isotopes

Environmental water dynamics are also reflected in the isotopic composition of other elements present in biogenic tissues, for example, nitrogen. Soils and plants in arid habitats tend to be enriched in ^{15}N due to the loss of ^{15}N-depleted forms of mineral N, and plants in arid habitats often preferentially take up soil ammonium, which is enriched in ^{15}N relative to nitrate (Amundson et al. 2003). For herbivores, a diet of such plants, in addition to water stress, can result in tissues that are enriched in ^{15}N (Ambrose and DeNiro 1986; Sealy et al. 1987; Crowley et al. 2011). This logic predicts higher $\delta^{15}N_{hair}$ values in *P. hamadryas* than in *P. anubis*. The opposite effect, however, can result if a species consumes large amounts of *Acacia*. Many plants in the order Fabales, including *Acacia*, depend on symbiotic bac-

teria to fix atmospheric N_2, resulting in $\delta^{15}N$ values that resemble that of air (~0‰; DeNiro and Epstein 1981; Schmidt and Stewart 2003). Given that *Acacia* is a central plant food for *P. hamadryas* in Ethiopia (Kummer 1968; Swedell 2006; Swedell et al. 2008; Schreier 2010), relatively low $\delta^{15}N_{hair}$ values can be expected for *P. hamadryas* in the Awash canyon. This prediction was supported by a recent investigation of stable carbon and nitrogen isotopes over nearly three decades (Fourie et al. 2008*a*). This study found both a positive association between $\delta^{15}N_{hair}$ values and proxies of habitat productivity and lower $\delta^{15}N_{hair}$ values in *P. hamadryas* than in *P. anubis*.

Material and Methods

Sample Acquisition

Baboons were caught in individual cage traps, anesthetized, sampled, and released (Brett et al. 1982) as part of the Awash National Park Baboon Research Project (Phillips-Conroy and Jolly 1981, 1986, 1994; Phillips-Conroy et al. 1991, 1992; Jolly and Phillips-Conroy 2003). The 63 hair specimens examined here were clipped from the shoulder and represent members of seven social groups: A (*Papio hamadryas*; $n = 10$), B (*Papio anubis*; $n = 10$), C (*P. anubis*; $n = 9$), G (mixed, mostly hybrids; $n = 5$), H (hybrids; $n = 5$), J (hybrids; $n = 10$), and K (*P. hamadryas*; $n = 14$). All groups ranged along the Awash River, except Group A, which foraged north of Lake Beseka outside of the Awash Valley proper (fig. 1*d*). Within hybrid groups, the influence of *P. anubis* admixture diminished downstream, so that "hamadryasness" increases from Group G to Group H and from Group H to Group J (Phillips-Conroy and Jolly 1986). All samples were collected in 1973, except those from Group C, which were collected in 1993. For samples collected in 1973, rainfall during the preceding 12 months was 297 mm; for 1993, it was 656 mm.

Sample Preparation and Analysis

Isotope ratios are presented as δ values, where $\delta = 1,000[(R_{sample}/R_{standard}) - 1]$ and $R = {}^{18}O/{}^{16}O$ or ${}^{15}N/{}^{14}N$; reference standards are Vienna standard mean oceanic water (VSMOW) for oxygen and atmospheric N_2 for nitrogen. Units are expressed as parts per thousand (‰). For measurements of $\delta^{18}O_{hair}$, two or three strands of hair were cleaned of debris, washed three times in a 2 : 1 mixture of chloroform and methanol, cut into 3-mm segments, and weighed ($150 \pm 15\ \mu g$) into precombusted silver foil capsules. Next, the samples were vacuum-dehydrated for a minimum of 6 d to remove oxygen under active exchange with atmospheric water vapor (Bowen et al. 2005*a*, 2009). The dried samples were immediately combusted and analyzed with a Thermo-Chemical Elemental Analyzer interfaced with a Delta Plus XP isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany) located in the Stable Isotope Laboratory of the University of California, Santa Cruz. Analytical precision (± 1 SD) based on 65 IAEA-601 (benzoic acid) replicates was 0.1‰, and that of the repeated analysis of a check reference for keratin (local horse hair) was 0.3‰.

Our measurements of $\delta^{15}N_{hair}$ were drawn from the larger data set of Fourie et al. (2008*a*; N. H. Fourie, D. Codron, J. Lee-Thorp, C. J. Jolly, and J. E. Phillips-Conroy, unpublished manuscript). For measurements of $\delta^{15}N_{hair}$, hair samples were washed using a 1 : 200 liquid detergent solution, rinsed three times in distilled water, and freeze-dried overnight. Next, the hair was homogenized into 1–2-mm segments with a pair of surgical scissors and weighed (~600 μg) into precombusted tin capsules. The samples were then combusted in an automated Carlo-Erba device (Carlo-Erba, Milan, Italy) and analyzed with a continuous-flow inlet system attached to a Finnigan MAT 252 mass spectrometer. Sample results were calibrated using multiple determinations of three internal standards of known isotopic composition. The analytical precision of these internal standards was 0.1‰.

Data Analysis

We assessed the assumptions of normality and homoscedasticity of variance for all analyses, and we used ANOVAs and Tukey's post hoc HSD test to determine the extent to which variation in $\delta^{18}O_{hair}$ can be explained by baboon classification (*P. anubis*, *P. hamadryas*, or hybrid) and year of sample acquisition (1973 or 1993). We ran all analyses in JMP-SAS, version 8.0.2 (SAS Institute, Cary, NC), and R, version 2.14.1 (R Development Core Team 2011). All probability levels are two-tailed, and the significance for all tests was set at $\alpha = 0.05$.

Subsample Size Calculations

Hartigan's dip test (Hartigan and Hartigan 1985) was used to determine whether the distribution of $\delta^{18}O_{hair}$ values was unimodal. The P values for the dip test statistic (D_n) were obtained from the dip test package (Maechler 2011) in R, version 2.14.1 (R Development Core Team 2011). To examine the minimum sample size necessary to represent with a stated degree of confidence the mean $\delta^{18}O_{hair}$ value of a population, we performed a subsampling analysis of all samples from within the Awash Valley, thus excluding those from Group A. We calculated the mean and SD of randomly generated subsamples without replacement increasing in size from 3 to a maximum of 53 individuals. This process was repeated for 1,000 iterations, from which the mean and standard error (SE), normalized to the actual mean and SD, were calculated for both measurements.

Results

We measured the $\delta^{18}O_{hair}$ values of 63 baboons (table A1). The mean $\pm$ SD of $\delta^{18}O_{hair}$ values in this sample was 19.8‰ $\pm$ 1.3‰ (range: 15.8‰–23.0‰). In general, we detected little intergroup variation in $\delta^{18}O_{hair}$ values ($F_{6, 56} = 1.55$, $P = 0.18$; fig. 1*e*), but we found that baboon classification and the year of sample acquisition explained a significant amount of the variation in the total data set (nested ANOVA: $F_{3, 59} = 3.36$, $P = 0.04$). To control for sampling year, which explained 48% of the variation in our model, we excluded the samples collected

in 1993 (*Papio anubis*, Group C) and detected differences between the baboon taxa. As predicted, *Papio hamadryas* evinced higher $\delta^{18}O_{hair}$ values than did *P. anubis* (Tukey HSD: $q = 2.41$, $P = 0.05$; fig. 1*f*), although the magnitude of the mean difference was small (20.1‰ ± 1.2‰ vs. 19.6‰ ± 1.4‰). This difference was influenced by a single male (no. 10039, $\delta^{18}O_{hair}$ value = 15.8‰), which fell 2σ outside of the mean for the 1973 samples and almost 3σ outside of the mean for all *P. anubis* samples. When individual 10039 was excluded from the analysis, the $\delta^{18}O_{hair}$ values of *P. hamadryas* and *P. anubis* did not differ statistically ($F_{2,50} = 2.00$, $P = 0.15$). The hybrids exhibited intermediate $\delta^{18}O_{hair}$ values that fell within the range exhibited by *P. anubis* and *P. hamadryas* (fig. 1*f*).

We also report $\delta^{15}N_{hair}$ values from each of the 63 subjects (table A1); these data represent a subsample of the much larger data set reported by Fourie et al. (2008*a*). In our subsample, we calculated a mean ± SD of 6.8‰ ± 1.1‰ (range: 5.2‰–9.3‰), and we found significant variation between taxa regardless of when the samples were collected ($F_{3,59} = 31.91$, $P < 0.0001$; fig. 1*f*). The mean $\delta^{15}N_{hair}$ values of *P. hamadryas* (6.4‰ ± 0.8‰) and the hybrids (6.1‰ ± 0.5‰) were similar to each other and significantly lower than those of *P. anubis* (7.9‰ ± 0.9‰; Tukey HSD: $q = 2.40$, $P = 0.04$). These findings do not differ significantly from those of the larger study by Fourie et al. (2008*a*; N. H. Fourie, D. Codron, J. Lee-Thorp, C. J. Jolly, and J. E. Phillips-Conroy, unpublished manuscript).

Finally, we found that our $\delta^{18}O_{hair}$ values exhibited a unimodal variance structure (Hartigan dip test: $D_{62} = 0.03$, $P > 0.99$). Our subsampling analysis showed a sharp decrease in the SE of the estimated mean and SD for increasing subsample sizes. Figure 2 illustrates that a subsample size of at least 10 individual baboons is required to decrease the SE to within 0.1‰, while a subsample size of at least 35 individuals is required to decrease the SE to within 0.01‰ of the estimated mean and SD.

Discussion

The mean $\delta^{18}O_{hair}$ value of the baboon populations in ANP (Groups B–K) was 19.8‰ ± 1.4‰, which is ca. 19‰ higher than that of precipitation in ANP (0.8‰; Bowen and Revenaugh 2003; Bowen 2012) and ca. 18‰ higher than that of the Awash River itself (range: 1.3‰–2.4‰; Levin et al. 2004). This latter isotopic difference (ε^*) is 3‰ higher than that between tap water and human hair in the United States (Ehleringer et al. 2008), but it is a close match to the $\varepsilon^*_{drinking water-hair}$ of mid-twentieth-century hunter-gatherers with limited access to nonlocal bottled water or food water (Bowen et al. 2009).

As we predicted, the $\delta^{18}O_{hair}$ values of *Papio hamadryas* were higher than those of *Papio anubis*. This difference cannot be attributed to the fact that Group A drank from the ^{18}O-enriched waters of Lake Beseka (range: 2.2‰–10.2‰; Levin et al. 2004; Kebede et al. 2009; Bretzler et al. 2011), as *P. hamadryas* Group K had similar values despite its proximity to and ingestion of waters from the Awash River. Instead, the elevated $\delta^{18}O_{hair}$ values of *P. hamadryas* are most likely due to the consumption of ^{18}O-enriched leaf waters in the arid thornbush. This dietary interpretation of our $\delta^{18}O_{hair}$ values agrees well with our analysis of $\delta^{15}N_{hair}$ values, which also links *P. hamadryas* to plant foods that abound in the thornbush habitat.

The relatively depleted ^{15}N values of *P. hamadryas* indicate a diet based on *Acacia* tissues with low $\delta^{15}N$ values (DeNiro and Epstein 1981; Yeakel et al. 2009). Although comparative dietary studies in the ANP hybrid zone are lacking, the arid thornbush exploited by *P. hamadryas* Groups A and K and hybrid Groups H–J has few plant species that produce moisture-rich fruits. Accordingly, these groups are relatively more dependent on the seed pods, fruits, flowers, and leaves of *Acacia* than are *P. anubis* Groups B and C, which often forage near the Awash River (Nagel 1973). This dependence on *Acacia* re-
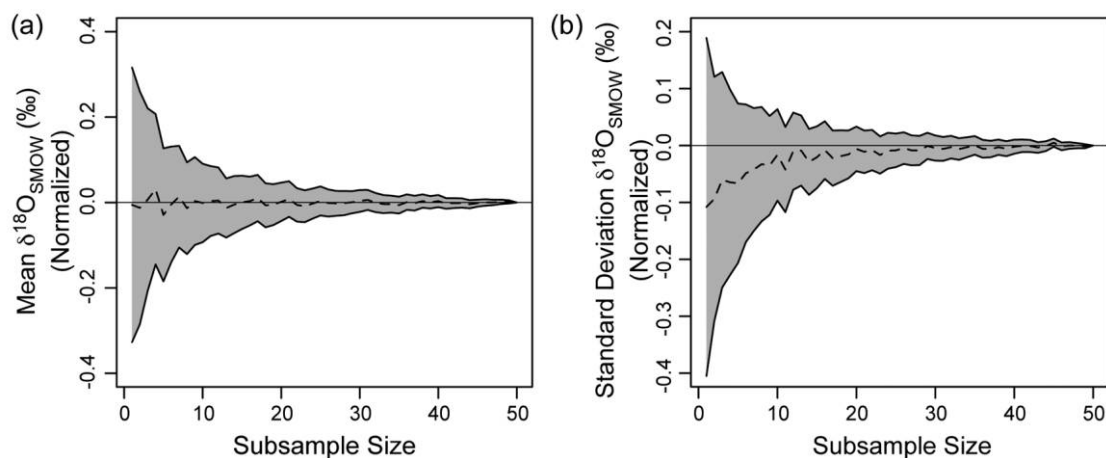

Figure 2. Standard error for the estimated mean (*a*) and standard deviation of $\delta^{18}O_{hair}$ values (*b*) for 1,000 randomly selected subsamples of size $n = 3$–53 from baboons within the Awash Valley. Dashed lines represent the normalized mean for each subsample, and solid lines signify the standard error around the mean.

sembles that of other populations of *P. hamadryas* in Ethiopia, where *Acacia* tissues can account for a large proportion (39%–84%) of time spent feeding (Kummer 1968; Schreier 2010).

Yet the magnitude of the difference distinguishing the mean $\delta^{18}O_{hair}$ values of *P. anubis* and *P. hamadryas* is relatively small (0.5‰) and perhaps of little biological significance. We attribute our finding of similar $\delta^{18}O_{hair}$ values to three factors. First, there are few categorical differences in the diets of ANP baboons, despite differences in the consumption of non-*Acacia* plant tissues. Second, all baboons in the Awash Valley drink water from the same sources, namely, the Awash River and its tributaries as well as rain-filled pools and puddles (Aldrich-Blake et al. 1971; Nagel 1973). Third, *P. hamadryas* tends to drink for longer durations than other baboons, with typical drinking bouts of 21.0 s (vs. 5.3 and 11.4 s for *P. anubis* and the hybrids, respectively; Nagel 1973). If longer drinking bouts correspond to the ingestion of larger volumes of water, a relatively greater recharge of ^{16}O might mitigate the ^{18}O-enrichment effects of ingesting evaporated leaf waters.

Taken together, our findings support the classification of baboons as EI taxa (Levin et al. 2006). All baboons drink meteoric water (Barton et al. 1992), and we conclude that the $\delta^{18}O$ of meteoric water is reflected in the $\delta^{18}O$ of baboon hair tissues, despite subtle species differences in diet, ranging behavior, and physiology. Thus, *Papio* has practical promise as a paleoenvironmental recorder (Koch 1998), especially if a fossil assemblage is devoid of more conventional EI species, such as the hippopotamus (Levin et al. 2006; Cerling et al. 2008).

Recommended sample size for $\delta^{18}O_{hair}$ analyses. Any study involving the destructive sampling of fossil or zoological specimens should develop an experimental design that balances the need to minimize the effect on individual specimens and collections against the need for sufficiently large sample sizes to address paleoecological and ecological questions with statistical confidence. To address this sampling issue, Clementz and Koch (2001) resampled their estimates of $\delta^{18}O$ in the tooth enamel of black-tailed deer (*Odocoileus h. hemionus*) living near Monterey, California. Their analysis of 42 individuals showed that a subsample size of ca. 5 individuals decreased the SE to within 0.01‰ of the estimated mean and SD measurements; however, their method did not address the $\delta^{18}O$ of hair, which is expected

to be more variable, as hair samples are less time averaged (representing weeks to months) than typical enamel samples milled off mammal teeth (representing months to several years). As the number of studies measuring $\delta^{18}O$ values in historic hair samples expands (e.g., Bowen et al. 2009; Chenery et al. 2011), it becomes increasingly important to develop an informed sampling protocol.

In our data set, we found that sampling at least 10 individuals was necessary to reduce the SE of the population to within 0.1‰. Although the variance structure of our data was unimodal, the greater overall variance is perhaps unsurprising, given the rapid isotopic turnover of hair and the fact that Awash baboons have a much more varied diet and a higher level of water stress than black-tailed deer from coastal California. Indeed, the variance in our data might represent an upper limit for populations of terrestrial mammals. We therefore suggest that a minimum sample size be set at 10 and increased as materials and curators allow. If such sample sizes are impossible to obtain, researchers should shift to nonparametric statistical approaches.

Acknowledgments

APPENDIX

Table A1: Source data used in this analysis

Social group and taxon, animal ID	Sex	Year sampled	$\delta^{15}N_{hair}$ (‰)	$\delta^{18}O_{hair}$ (‰)
A. *Papio hamadryas*:				
10203	Male	1973	6.11	20.91
10204	Male	1973	5.41	21.42
10205	Female	1973	5.66	19.93
10207	Male	1973	5.71	19.69
10228	Female	1973	5.95	19.28

Table A1 (*Continued*)

Social group and taxon, animal ID	Sex	Year sampled	$\delta^{15}N_{hair}$ (‰)	$\delta^{18}O_{hair}$ (‰)
10239	Male	1973	5.97	19.47
10240	Male	1973	5.49	18.74
10251	Female	1973	6.14	20.35
10258	Female	1973	6.54	19.98
10279	Female	1973	6.06	19.38
B. *Papio anubis*:				
10002	Male	1973	8.79	19.82
10003	Male	1973	9.30	18.15
10004	Male	1973	6.67	20.16
10011	Female	1973	7.05	20.58
10012	Male	1973	6.14	19.29
10029	Female	1973	7.02	18.18
10030	Male	1973	7.30	17.81
10039	Male	1973	7.18	15.82
10058	Female	1973	7.87	19.62
10062	Female	1973	6.55	19.89
C. *P. anubis*:				
10805	Male	1993	8.64	20.61
10879	Male	1993	8.53	19.40
10899	Female	1993	8.50	18.83
10900	Male	1993	8.67	20.10
10914	Male	1993	8.54	20.30
10970	Female	1993	8.85	21.45
10972	Female	1993	8.68	21.80
10979	Female	1993	7.94	20.79
10982	Male	1993	8.50	19.66
G. Phenotypic hybrids:				
10191	Male	1973	5.52	20.14
10193	Female	1973	7.22	19.33
10194	Male	1973	5.45	18.05
10199	Male	1973	6.03	19.57
10202	Female	1973	5.24	21.18
H. Phenotypic hybrids:				
10382	Male	1973	6.90	19.62
10395	Male	1973	5.74	19.20
10396	Male	1973	5.46	18.49
10398	Female	1973	6.40	19.57
10407	Male	1973	6.77	19.40
J. Phenotypic hybrids:				
10418	Male	1973	6.00	19.31
10420	Female	1973	6.52	17.70
10422	Female	1973	6.11	17.28
10426	Female	1973	6.22	19.52
10427	Female	1973	6.15	18.68
10429	Female	1973	6.18	20.24
10430	Female	1973	5.94	19.91
10438	Male	1973	5.85	20.71
10439	Female	1973	6.11	22.23
10440	Female	1973	6.16	21.07
K. *P. hamadryas*:				
10307	Female	1973	6.42	22.99
10308	Female	1973	5.52	20.58
10309	Female	1973	7.42	19.50

Table A1 (*Continued*)

Social group and taxon, animal ID	Sex	Year sampled	$\delta^{15}N_{hair}$ (‰)	$\delta^{18}O_{hair}$ (‰)
10337	Male	1973	7.24	19.25
10338	Female	1973	7.10	19.34
10339	Female	1973	6.00	21.52
10340	Female	1973	7.14	21.38
10342	Female	1973	6.94	23.02
10349	Female	1973	5.99	18.57
10350	Female	1973	5.86	18.83
10353	Male	1973	8.30	19.11
10360	Female	1973	6.30	20.56
10366	Female	1973	6.55	19.42
10368	Female	1973	7.65	19.14

Note. The δ values, where $\delta = 1{,}000[(R_{sample}/R_{standard}) - 1]$ and $R = {}^{15}N/{}^{14}N$ or ${}^{18}O/{}^{16}O$, are expressed relative to atmospheric nitrogen or standard mean ocean water.

Literature Cited

Aldrich-Blake F.P.G., T.K. Bunn, R.I.M. Dunbar, and P.M. Headley. 1971. Observations on baboons, *Papio anubis*, in an arid region in Ethiopia. Folia Primatol 15:1–35.

Altmann S.A. 1998. Foraging for survival: yearling baboons in Africa. University of Chicago Press, Chicago.

Ambrose S.H. and M.J. DeNiro. 1986. The isotopic ecology of east African mammals. Oecologia 69:395–406.

Amundson R., A.T. Austin, E.A.G. Schuur, K. Yoo, V. Matzek, C. Kendall, A. Uebersax, D. Brenner, and W.T. Baisden. 2003. Global patterns of the isotopic composition of soil and plant nitrogen. Global Biogeochem Cycles 17:1031.

Ayliffe L.K. and A.R. Chivas. 1990. Oxygen isotope composition of the bone phosphate of Australian kangaroos: potential as a palaeoenvironmental recorder. Geochim Cosmochim Acta 54:2603–2609.

Barton R.A., A. Whiten, S.C. Strum, R.W. Byrne, and A.J. Simpson. 1992. Habitat use and resource availability in baboons. Anim Behav 43:831–844.

Bedaso Z., J.G. Wynn, Z. Alemseged, and D. Geraads. 2010. Paleoenvironmental reconstruction of the Asbole fauna (Busidima Formation, Afar, Ethiopia) using stable isotopes. Geobios 43:165–177.

Biquand S., V. Biquand-Guyot, A. Boug, and J.-P. Gautier. 1992. The distribution of *Papio hamadryas* in Saudi Arabia: ecological correlates and human influence. Int J Primatol 13:223–243.

Bocherens H., P.L. Koch, A. Mariotti, D. Geraads, and J.-J. Jaeger. 1996. Isotopic biogeochemistry (${}^{13}C$, ${}^{18}O$) of mammalian enamel from African Pleistocene hominid sites. Palaios 11:306–318.

Bowen G.J. 2012. The online isotopes in precipitation calculator (OIPC). Version 2.2. http://www.waterisotopes.org. Accessed January 17, 2012.

Bowen G.J., L. Chesson, K. Nielson, T.E. Cerling, and J.R. Ehleringer. 2005a. Treatment methods for the determination of δ^2H and $\delta^{18}O$ of hair keratin by continuous-flow isotope-ratio mass spectrometry. Rapid Commun Mass Spectrom 19:2371–2378.

Bowen G.J., J.R. Ehleringer, L.A. Chesson, A.H. Thompson, D.W. Podlesak, and T.E. Cerling. 2009. Dietary and physiological controls on the hydrogen and oxygen isotope ratios of hair from mid-20th century indigenous populations. Am J Phys Anthropol 139:494–504.

Bowen G.J. and J. Revenaugh. 2003. Interpolating the isotopic composition of modern meteoric precipitation. Water Resour Res 39:1299.

Bowen G.J., L.I. Wassenaar, and K.A. Hobson. 2005b. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia 143:337–348.

Bowen G.J. and B. Wilkinson. 2002. Spatial distribution of $\delta^{18}O$ in meteoric precipitation. Geology 30:315–318.

Brain C. and D. Mitchell. 1999. Body temperature changes in free-ranging baboons (*Papio hamadryas ursinus*) in the Namib Desert, Namibia. Int J Primatol 20:585–598.

Brett F.L., T.R. Turner, C.J. Jolly, and R.G. Cauble. 1982. Trapping baboons and vervet monkeys from wild, free-ranging populations. J Wildl Manag 46:164–174.

Bretzler A., K. Osenbrück, R. Gloaguen, J.S. Ruprecht, S. Kebede, and S. Stadler. 2011. Groundwater origin and flow dynamics in active rift systems: a multi-isotope approach in the Main Ethiopian Rift. J Hydrol 402:274–289.

Bryant J.D. and P.N. Froelich. 1995. A model of oxygen isotope fractionation in body water of large mammals. Geochim Cosmochim Acta 59:4523–4537.

Cerling T.E., J.M. Harris, J.A. Hart, P. Kaleme, H. Klingel, M.G. Leakey, N.E. Levin, R.L. Lewison, and B.H. Passey. 2008. Stable isotope ecology of the common hippopotamus. J Zool 276:204–212.

Cerling T.E., J.M. Harris, M.G. Leakey, and N. Mudida. 2003. Stable isotope ecology of northern Kenya, with emphasis on the Turkana Basin. Pp. 583–603 in M.G. Leakey and J.M. Harris, eds. Lothagam: the dawn of humanity in eastern Africa. Columbia University Press, New York.

Cerling T.E., J.A. Hart, and T.B. Hart. 2004. Stable isotope ecology in the Ituri Forest. Oecologia 138:5–12.

Cerling T.E., N.E. Levin, J. Quade, J.G. Wynn, D.L. Fox, J.D. Kingston, R.G. Klein, and F.H. Brown. 2010. Comment on the paleoenvironment of *Ardipithecus ramidus*. Science 328: 1105.

Cerling T.E., E. Mbua, F.M. Kirera, F.K. Manthi, F.E. Grine, M.G. Leakey, M. Sponheimer, and K.T. Uno. 2011. Diet of *Paranthropus boisei* in the early Pleistocene of East Africa. Proc Natl Acad Sci USA 108:9337–9341.

Chenery C.A., A.L. Lamb, H.J. O'Regan, and S. Elton. 2011. Multi-tissue analysis of oxygen isotopes in wild rhesus macaques (*Macaca mulatta*). Rapid Commun Mass Spectrom 25:779–788.

Clementz M.T., A. Goswami, P.D. Gingerich, and P.L. Koch. 2006. Isotopic records from early whales and sea cows: contrasting patterns of ecological transition. J Vertebr Paleontol 26:355–370.

Clementz M.T. and P.L. Koch. 2001. Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. Oecologia 129:461–472.

Crook J.H. and P. Aldrich-Blake. 1968. Ecological and behavioural contrasts between sympatric ground dwelling primates in Ethiopia. Folia Primatol 8:192–227.

Crowley B.E., S. Thorén, E. Rasoazanabary, E.R. Vogel, M.A. Barrett, S. Zohdy, M.B. Blanco, et al. 2011. Explaining geographical variation in the isotope composition of mouse lemurs (*Microcebus*). J Biogeogr 38:2106–2121.

DeNiro M.J. and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351.

Eagle R.A., T. Tütken, T.S. Martin, A.K. Tripati, H.C. Fricke, M. Connely, R.L. Cifelli, and J.M. Eiler. 2011. Dinosaur body temperatures determined from isotopic (^{13}C-^{18}O) ordering in fossil biominerals. Science 333:443–445.

Ehleringer J.R., G.J. Bowen, L.A. Chesson, A.G. West, D.W. Podlesak, and T.E. Cerling. 2008. Hydrogen and oxygen isotope ratios in human hair are related to geography. Proc Natl Acad Sci USA 105:2788–2793.

Flanagan L.B., J.F. Bain, and J.R. Ehleringer. 1991. Stable oxygen and hydrogen isotope composition of leaf water in C_3 and C_4 plant species under field conditions. Oecologia 88:394–400.

Fourie N.H., D. Codron, J. Lee-Thorp, C. Jolly, and J. Phillips-Conroy. 2008*a*. Stable isotopes and dietary variability in extinct and extant papionins. Am J Phys Anthropol 135(S46): 98.

Fourie N.H., J.A. Lee-Thorp, and R.R. Ackermann. 2008*b*. Biogeochemical and craniometric investigation of dietary ecology, niche separation, and taxonomy of Plio-Pleistocene cercopithecoids from the Makapansgat Limeworks. Am J Phys Anthropol 135:121–135.

Funkhouser G.E., E.A. Higgins, T. Adams, and C.C. Snow. 1967. The response of the savannah baboon (*Papio cynocephalus*) to thermal stress. Life Sci 6:1615–1620.

Gat J.R. 1996. Oxygen and hydrogen isotopes in the hydrologic cycle. Annu Rev Earth Planet Sci 24:225–262.

Hamilton W.J. 1985. Demographic consequences of a food and water shortage to desert chacma baboons, *Papio ursinus*. Int J Primatol 6:451–462.

Hartigan J.A. and P.M. Hartigan. 1985. The dip test of unimodality. Ann Statist 13:70–84.

Hiley P.G. 1976. The thermoregulatory responses of the galago (*Galago crassicaudatus*), the baboon (*Papio cynocephalus*) and the chimpanzee (*Pan stayrus*) to heat stress. J Physiol 254: 657–671.

Hobson K.A., G.J. Bowen, L.I. Wassenaar, Y. Ferrand, and H. Lormee. 2004. Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. Oecologia 141:477–488.

Jolly C.J. and J.E. Phillips-Conroy. 2003. Testicular size, mating system, and maturation schedules in wild anubis and hamadryas baboons. Int J Primatol 24:125–142.

Kahmen A., D. Sachse, S.K. Arndt, K.P. Tu, H. Farrington, P.M. Vitousek, and T.E. Dawson. 2011. Cellulose δ^{18}O is an index of leaf-to-air vapor pressure difference (VPD) in tropical plants. Proc Natl Acad Sci USA 108:1981–1986.

Kebede S., Y. Travi, and K. Rozanski. 2009. The δ^{18}O and δ^2H enrichment of Ethiopian lakes. J Hydrol 365:173–182.

Koch P.L. 1998. Isotopic reconstruction of past continental environments. Annu Rev Earth Planet Sci 26:573–613.

Koch P.L., J.C. Zachos, and D.L. Dettman. 1995. Stable isotope stratigraphy and paleoclimatology of the Paleogene Bighorn Basin (Wyoming, USA). Palaeogeogr Palaeoclimatol Palaeoecol 115:61–89.

Kohn M.J. 1996. Predicting animal δ^{18}O: accounting for diet and physiological adaptation. Geochim Cosmochim Acta 60: 4811–4829.

Kohn M.J., M.J. Schoeninger, and J.W. Valley. 1996. Herbivore tooth oxygen isotope compositions: effects of diet and physiology. Geochim Cosmochim Acta 60:3889–3896.

Kroopnick P. and H. Craig. 1972. Atmospheric oxygen: isotopic composition and solubility fractionation. Science 175:54–55.

Kummer H. 1968. Social organization of hamadryas baboons: a field study. University of Chicago Press, Chicago.

Levin N.E., T.E. Cerling, B.H. Passey, J.M. Harris, and J.R. Ehleringer. 2006. A stable isotope aridity index for terrestrial environments. Proc Natl Acad Sci USA 103:11201–11205.

Levin N.E., J. Quade, S.W. Simpson, S. Semaw, and M. Rogers. 2004. Isotopic evidence for Plio-Pleistocene environmental change at Gona, Ethiopia. Earth Planet Sci Lett 219:93–110.

Liu A.G.S.C., E.R. Seiffert, and E.L. Simons. 2008. Stable isotope evidence for an amphibious phase in early proboscidean evolution. Proc Natl Acad Sci USA 105:5786–5791.

Longinelli A. 1984. Oxygen isotopes in mammal bone phosphate: a new tool for paleohydrological and paleoclimatological research? Geochim Cosmochim Acta 48:385–390.

Luz B., Y. Kolodny, and M. Horowitz. 1984. Fractionation of oxygen isotopes between mammalian bone-phosphate and environmental drinking water. Geochim Cosmochim Acta 48:1689–1693.

Maechler M. 2011. Hartigan's dip test statistic for unimodality: corrected code. Version 0.75-1. http://cran.r-project.org/web/packages/diptest/diptest.pdf. Accessed January 18, 2012.

Mitchell D., A. Fuller, and S.K. Maloney. 2009. Homeothermy and primate bipedalism: is water shortage or solar radiation the main threat to baboon (*Papio hamadryas*) homeothermy? J Hum Evol 56:439–446.

Nagel U. 1973. A comparison of anubis baboons, hamadryas baboons and their hybrids at a species border in Ethiopia. Folia Primatol 19:104–165.

Nystrom P., J.E. Phillips-Conroy, and C.J. Jolly. 2004. Dental microwear in anubis and hybrid baboons (*Papio hamadryas*, sensu lato) living in Awash National Park, Ethiopia. Am J Phys Anthropol 125:279–291.

Phillips-Conroy J.E. and C.J. Jolly. 1981. Sexual dimorphism in two subspecies of Ethiopian baboons (*Papio hamadryas*) and their hybrids. Am J Phys Anthropol 56:115–129.

———. 1986. Changes in the structure of the baboon hybrid zone in the Awash National Park, Ethiopia. Am J Phys Anthropol 71:337–350.

———. 1994. Male dispersal and philopatry in the Awash baboon hybrid zone. Primate Report 68:27–52.

Phillips-Conroy J.E., C.J. Jolly, and F.L. Brett. 1991. Characteristics of hamadryas-like male baboons living in anubis baboon troops in the Awash hybrid zone, Ethiopia. Am J Phys Anthropol 86:353–368.

Phillips-Conroy J.E., C.J. Jolly, P. Nystrom, and H.A. Hemmalin. 1992. Migration of male hamadryas baboons into anubis groups in the Awash National Park, Ethiopia. Int J Primatol 13:455–476.

Podlesak D.W., A.-M. Torregrossa, J.R. Ehleringer, M.D. Dearing, B.H. Passey, and T.E. Cerling. 2008. Turnover of oxygen and hydrogen isotopes in the body water, CO_2, hair, and enamel of a small mammal. Geochim Cosmochim Acta 72:19–35.

Quade J., T.E. Cerling, P. Andrews, and B. Alpagut. 1995. Paleodietary reconstruction of Miocene faunas from Pasalar, Turkey using stable carbon and oxygen isotopes of fossil tooth enamel. J Hum Evol 28:373–384.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org.

Schmidt S. and G.R. Stewart. 2003. $\delta^{15}N$ values of tropical savanna and monsoon forest species reflect root specialisations and soil nitrogen status. Oecologia 134:569–577.

Schoeninger M.J., H. Reeser, and K. Hallin. 2003. Paleoenvironment of *Australopithecus anamensis* at Allia Bay, East Turkana, Kenya: evidence from mammalian herbivore enamel stable isotopes. J Anthropol Archaeol 22:200–207.

Schreier A.L. 2010. Feeding ecology, food availability and ranging patterns of wild hamadryas baboons at Filoha. Folia Primatol 81:129–145.

Sealy J.C., N.J. van der Merwe, J.A.L. Thorp, and J.L. Lanham. 1987. Nitrogen isotopic ecology in southern Africa: implications for environmental and dietary tracing. Geochim Cosmochim Acta 51:2707–2717.

Secord R., S.L. Wing, and A. Chew. 2008. Stable isotopes in early Eocene mammals as indicators of forest canopy structure and resource partitioning. Paleobiology 34:282–300.

Sigg H. 1980. Differentiation of female positions in hamadryas one-male-units. Z Tierpsychol 53:265–302.

Smith C.C., M.E. Morgan, and D. Pilbeam. 2010. Isotopic ecology and dietary profiles of Liberian chimpanzees. J Hum Evol 58:43–55.

Sponheimer M. and J.A. Lee-Thorp. 1999. Oxygen isotopes in enamel carbonate and their ecological significance. J Archaeol Sci 26:723–728.

———. 2001. The oxygen isotope composition of mammalian enamel carbonate from Morea Estate, South Africa. Oecologia 126:153–157.

Stoltz L.P. and G.S. Saayman. 1970. Ecology and behaviour of baboons in the northern Transvaal. Ann Transvaal Mus 26:99–143.

Swedell L. 2006. Strategies of sex and survival in hamadryas baboons: through a female lens. Pearson, Upper Saddle River, NJ.

Swedell L., G. Hailemeskel, and A. Schreier. 2008. Composition and seasonality of diet in wild hamadryas baboons: preliminary findings from Filoha. Folia Primatol 79:476–490.

Thewissen J.G.M., L.N. Cooper, M.T. Clementz, S. Bajpai, and B.N. Tiwari. 2007. Whales originated from aquatic artiodactyls in the Eocene epoch of India. Nature 450:1190–1194.

Tuross N., C. Warinner, K. Kirsanow, and C. Kester. 2008. Organic oxygen and hydrogen isotopes in a porcine controlled dietary study. Rapid Commun Mass Spectrom 22:1741–1745.

White T.D., S.H. Ambrose, G. Suwa, D.F. Su, D. DeGusta, R.L. Bernor, J.-R. Boisserie, et al. 2009. Macrovertebrate paleontology and the Pliocene habitat of *Ardipithecus ramidus*. Science 326:67, 87–93.

Yeakel J.D., N.C. Bennett, P.L. Koch, and N.J. Dominy. 2007. The isotopic ecology of African mole rats informs hypotheses on the evolution of human diet. Proc R Soc B 274:1723–1730.

Yeakel J.D., B.D. Patterson, K. Fox-Dobbs, M.M. Okumura, T.E. Cerling, J.W. Moore, P.L. Koch, and N.J. Dominy. 2009. Cooperation and individuality among man-eating lions. Proc Natl Acad Sci USA 106:19040–19043.

Zurovsky Y. and A. Shkolnik. 1993. Water economy and body fluid distribution in the hamadryas baboon (*Papio hamadryas*). J Therm Biol 18:153–157.