Stable isotope canopy effects for sympatric monkeys at Taï Forest, Côte d’Ivoire

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This study tests the hypothesis that vertical habitat preferences of different monkey species inhabiting closed canopy rainforest are reflected in oxygen isotopes. We sampled bone from seven sympatric cercopithecid species in the Taï forest, Côte d’Ivoire, where long-term study has established taxon-specific patterns of habitat use and diet. Modern rib samples (n = 34) were examined for oxygen (δ18Oap) and carbon (δ13Ccap) from bone apatite (bioapatite), and carbon (δ13Cco) and nitrogen (δ15Nco) from bone collagen. Results are consistent for C3 feeders in a closed canopy habitat. Low irradiance and evapotranspiration, coupled with high relative humidity and recycled CO2 in forest understory, contribute to observed isotopic variability. Both δ13Cco and δ13Ccap results reflect diet; however, δ13C values are not correlated with species preference for canopy height. By contrast, δ18Oap results are correlated with mean observed height and show significant vertical partitioning between taxa feeding at ground, lower and upper canopy levels. This oxygen isotope canopy effect has important palaeobiological implications for reconstructing vertical partitioning among sympatric primates and other species in tropical forests.

1. Introduction

Woody plants in tropical rainforest follow the C3 photosynthetic pathway and exhibit patterned isotopic variability. For example, under closed canopy conditions, understory plants show marked depletion in 13C such that a vertical gradient exists with low carbon isotope ratios (δ13C) at ground level compared with average δ13C values at the top of the canopy [1]. This gradient of increasing δ13C values from forest floor through canopy is known as the ‘canopy effect’ and is attributed to environmental factors characteristic of dense forest, including recycled CO2 and low irradiance [2]. A similar vertical gradient in dense forest exists with oxygen isotope ratios (δ18O) owing to variations in relative humidity and evapotranspiration [3].

One consequence of these isotopic trends in dense forest is that resident consumers should exhibit similar patterns of vertical stratification within their tissues. To examine isotopic stratification in canopy use in consumer tissue, we analyse δ18Oap and δ13Cap from bone apatite (bioapatite) and δ13Cco and δ15Nco from bone collagen derived from ribs of seven species of sympatric cercopithecid monkeys from Taï forest, Côte d’Ivoire. Isotopic analysis of bone offers time-averaged signatures that reflect individual food/water consumption [4], and these biogeochemical tools provide an independent means of characterizing primate diet and local ecology [5]. We test the hypothesis that the stable carbon and oxygen isotope ratios of primate species that feed at different average heights within the Taï forest [6] will reflect the isotopic canopy effect.
The results show that $\delta^{13}C$ values do not but $\delta^{18}O$ values do reflect the canopy effect.

The Tai forest (approx. 330,000 ha) is a World Heritage site where ongoing research investigates linkages among primate morphology, habitat use and activity patterns [17], electronic supplementary material. Cercopithecid monkeys comprise a substantial component of the mammalian biomass of this humid tropical forest.

Seven sympatric species were sampled, including three colobines (king colobus (Colobus polykomos), olive colobus (Procolobus verus), western red colobus (Procolobus badius)); three guenons (diana monkey (Cercopithecus diana), Campbell’s monkey (Cercopithecus campbelli), lesser spot-nosed monkey (Cercopithecus petruroides); and one papionin (sooty mangabey (Cercocebus atys)). Diet varies among species with respect to degree of frugivory, folivory and entomophagy [6]; electronic supplementary material, table S1 and preferred feeding locations within the canopy [8] (figure 2).

Isotopically, $\delta^{13}C_{co}$ values principally reflect dietary protein components, whereas $\delta^{13}C_{ap}$ values reflect whole diet [9]. The canopy effect in $\delta^{13}C$ values of leaves and CO₂ in closed canopy forest [2] is reflected in ground-dwelling browsers with low $\delta^{13}C$ values compared with arboreal species [10,11]. However, for sympatric primates, there is a surprisingly poor correlation between canopy and $\delta^{13}C$ values owing to environmental factors affecting dietary $\delta^{13}C$ [12], including $\delta^{13}C$ variation of food items spatially, and taxon-specific differences in digestive physiology (e.g. microbial gut fermentation in folivorous colobines). Enrichment studies of bone collagen and bioapatite do suggest that $\Delta^{13}C_{ap-co}$ spacing is broadly comparable across primates [13].

With respect to oxygen isotopes, $\delta^{18}O_{ap}$ values in bioapatite are directly related to body water that is formed from food and drinking water [4,14]. In perhumid forest, these values should broadly correspond to $\delta^{18}O$ values of foods consumed and meteoric surface water. The increased humidity on the forest floor is associated with low leaf $\delta^{18}O$ values, with enrichment of $\delta^{18}O$ observed along a vertical gradient [3], analogous to the carbon isotope canopy effect [15]. The proportion of leaves and fruits in the diet certainly contributes to $\delta^{18}O$ variation in consumer tissues, such that leaves have high surface area/low volume (higher $\delta^{18}O$) compared with fruits, which have low surface area/high volume (lower $\delta^{18}O$). However, in perhumid conditions, since $\delta^{18}O$ in consumer tissues is related to body water [14], $\delta^{18}O_{ap}$ values on average should reflect source waters consumed [4,5].

Carbon and oxygen isotope canopy effects may reflect foraging and strata use of sympatric primates within a closed canopy forest. Because Tai primate diets vary spatially and compositionally [6], we hypothesize that $\Delta^{13}C$ values will not covary with strata use. Further, since Tai primates consume food/water at known heights above the forest floor [8], we hypothesize that $\Delta^{18}O_{ap}$ values for the seven species under study will covary with preferred foraging position in the canopy.

### Table 1. Summary statistics of mean observed height and isotopic data for Tai cercopithecids sampled in this study.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Mean observed height (m)</th>
<th>$\delta^{13}C_{co}$ ‰ (PDB)</th>
<th>$\delta^{13}C_{ap}$ ‰ (PDB)</th>
<th>$\Delta^{13}C_{ap-co}$ ‰ (PDB)</th>
<th>$\delta^{18}O_{ap}$ ‰ (PDB)</th>
<th>$\delta^{15}N_{co}$ ‰ (AIR)</th>
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<tbody>
<tr>
<td>Papionins</td>
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<tr>
<td>Cercopithecus neglectus</td>
<td>6 2.4 (1348)</td>
<td>−18.7 ± 0.3 (−19.3 to −17.9)</td>
<td>−24.1 ± 0.4 (−24.8 to −23.6)</td>
<td>5.4 ± 0.7 (5.1–5.7)</td>
<td>23.2 ± 5.0 (22.0–24.0)</td>
<td>−2.0 (−2.4 to −1.6)</td>
</tr>
<tr>
<td>Cercopithecus ascanius</td>
<td>6 19.7 (1359)</td>
<td>−18.4 ± 0.3 (−18.8 to −18.0)</td>
<td>−23.6 ± 0.4 (−24.4 to −23.1)</td>
<td>5.2 ± 0.4 (4.9–5.7)</td>
<td>23.8 ± 5.0 (22.6 to 24.6)</td>
<td>−2.0 (−2.5 to −1.5)</td>
</tr>
<tr>
<td>Cercopithecus campbelli</td>
<td>3 8.4 (1437)</td>
<td>−17.9 ± 0.6 (−18.2 to −16.1)</td>
<td>−23.3 ± 0.8 (−24.6 to −22.3)</td>
<td>5.3 ± 0.2 (5.1–5.6)</td>
<td>24.0 ± 5.0 (23.4 to 24.5)</td>
<td>−2.0 (−2.5 to −1.5)</td>
</tr>
<tr>
<td>Cercopithecus petaurista</td>
<td>1 13.3 (2042)</td>
<td>−18.6 ± 0.2 (−18.8 to −17.5)</td>
<td>−23.0 ± 0.5 (−24.0 to −21.7)</td>
<td>5.4 ± 0.5 (5.0–5.9)</td>
<td>24.0 ± 5.0 (23.4 to 24.5)</td>
<td>−2.0 (−2.5 to −1.5)</td>
</tr>
<tr>
<td>Colobus polykomos</td>
<td>6 27.5 (3538)</td>
<td>−19.0 ± 0.6 (−19.6 to −18.5)</td>
<td>−23.8 ± 0.4 (−24.3 to −22.8)</td>
<td>4.9 ± 0.3 (4.6–5.2)</td>
<td>24.9 ± 5.0 (24.6 to 23.6)</td>
<td>−2.0 (−2.5 to −1.5)</td>
</tr>
<tr>
<td>Procolobus badius</td>
<td>7 26.05 (4196)</td>
<td>−18.9 ± 0.7 (−19.2 to −17.5)</td>
<td>−23.7 ± 0.4 (−24.2 to −22.8)</td>
<td>5.3 ± 0.2 (4.9–5.7)</td>
<td>24.9 ± 5.0 (24.6 to 23.6)</td>
<td>−2.0 (−2.5 to −1.5)</td>
</tr>
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aData for mean observed height based on hours (in parentheses) observed in the field [8].

bPECDEE Belemnite standard.
cAtmospheric nitrogen standard.
Once oxidized, each sample was rinsed with DI-dH2O to neutral pH, and approximately 12 ml 0.125 M NaOH was added to remove organic contaminants. After 16 h in solution, the samples were neutralized by adding 10–3 M HCl to maintain acidity and prevent absorption of atmospheric CO2. Pre-treated bioapatite carbonate samples (n = 34) were then weighed into vials, and samples were run on a Finnigan MAT 252 IRMS with a Kiel device. NBS-19 standard (n = 7) for δ13Cap was 1.95 ± 0.08‰ and for δ18Oap was −0.5 ± 1.0‰. Bioapatite carbonate samples (n = 34) were prepared using a modified Krueger method [9] with approximately 0.25 g of the smaller bone fraction placed in a 15 ml centrifuge tube with approximately 12 ml 0.2 M HCl. Acid was refreshed every 24 h, and all samples had completely demineralized by approximately 5 days. Each sample was rinsed with DI-dH2O to neutral pH, and approximately 12 ml 0.125 M NaOH was added to remove organic contaminants. After 16 h in solution, each sample was rinsed to neutral pH, transferred to a 20 ml glass scintillation vial with approximately 10 ml 10–3 M HCl, and added to maintain acidity and prevent absorption of atmospheric CO2. Samples remained at 95°C for 4–5 h. The demineralized solution was transferred to a 15 ml tube, centrifuged and transferred to its glass vial, and reduced to approximately 2 ml at 65°C. Purified bone collagen was frozen, lyophilized and weighed to calculate % collagen yield. The sample was then weighed and loaded for δ13C and δ18O analysis using a Finnigan MAT DeltaPlus XL isotope ratio mass spectrometer (IRMS). Weight %C and %N were determined (n = 32) using a Carlo Erba 1500 elemental analyser after IRMS analysis. USGS40 standard (n = 7) for δ13CVPD = −26.39 ± 0.08‰ and for δ18OVPDB = −4.52 ± 0.15‰. Bioapatite carbonate samples (n = 34) were prepared using a modified Krueger method [9] with approximately 0.25 g of the smaller bone fraction placed in a 15 ml centrifuge tube with approximately 12 ml 2.5% NaOHCl for 16 h to remove organics. Once oxidized, each sample was rinsed with DI-dH2O to neutral pH and approximately 12 ml 0.2 M CH3COOH was added for 16 h to remove secondary carbonates. Pre-treated bioapatite (n = 33) was then weighed into vials, and samples were run on a Finnigan MAT 252 IRMS with a Kiel device. NBS-19 standard (n = 16) for δ13CVPD = 1.95 ± 0.03‰, and for δ18OVPDB = −2.20 ± 0.05‰.

**Figure 1.** Bivariate plots of (a) model II regression of taxon mean δ13Cco and δ13Cap values, (b) mean (± 1 s.d.) δ13Cco and δ18Oap and (c) model II regression of taxon mean δ18Oap and δ13Cap values with mean observed height.

### 3. Results

Table 1 presents summary statistics, with individual isotope data listed in the electronic supplementary material, Table S2. Average δ13Cco (n = 34; −23.9 ± 0.8‰) and δ13Cap values (n = 33; −18.7 ± 0.8‰) show a positive correlation (Spearman’s ρ = 0.88; p < 0.008) as do taxon-specific δ13Cap and δ13Cco mean values (R2 = 0.8767; p = 0.002; figure 1a). These results support the relationship between protein/whole diet and bone collagen/bioapatite identified in controlled feeding studies [9]. Average Δ13Ccap–co spacing values (n = 33; 5.2 ± 0.4‰) suggest that dietary protein and whole diet δ13C values for ‘omnivorous’ primates are intermediate between Δ13Ccap–co spacing observed for herbivores (approx. 7‰) and for carnivores (approx. 3‰) [13]. Average Δ18Nco values (n = 34; 8.2 ± 0.8‰) do not correlate with Δ13Cap (Spearman’s ρ = 0.142; p = 0.78), nor do they correlate with canopy height (R2 = 0.022; p = 0.375).

Figure 1b presents taxon-specific means for δ13Capp and δ18Oap values (± 1 s.d.), and figure 1c presents mean δ18Oap and δ13Cap values plotted against mean observed canopy height. A positive correlation characterizes mean δ18Oap values and mean observed height (R2 = 0.9197; p = 0.0006), but no correlation is found between δ13Cap values and mean observed height (r = −0.0912).

Δ18Oap values (n = 33; 0.9 ± 0.7‰) differ significantly by taxon. Average δ18Oap values range from 0.1 ± 0.3‰ with C. atys to 1.5 ± 0.3‰ with P. indius. Colobus polykomos is also enriched with a mean δ18Oap value of 1.4 ± 0.4‰. Procolobus verus and C. campbelli have intermediate mean δ18Oap values (each with 0.6 ± 0.7‰), as does the C. petuaria sample (0.6‰). δ18Oap values reflect the isotopic composition of body water and the temperature at which the bioapatite is
formed [4,14] and are thus likely to reflect environmental factors independent of diet and physiology. These δ18O values are strongly correlated to strata use (Spearman’s $r = 0.93; p, 0.003$) in terms of mean overall height observed [8].

4. Discussion

Stable carbon isotope ratios show positive correlations with each other but no correlations with species’ mean observed height at Taï; thus, δ13Cco and δ13Cap values broadly reflect taxon-specific patterns of diet (figure 1a), but δ13C values do not strictly correlate to stratified habitat use expected under the carbon isotope canopy effect (figure 1c). By contrast, oxygen isotope data partition taxa along a vertical gradient and strongly correlate to strata use (mean observed height) at Taï (figure 1c).

Patterns of feeding and foraging are reflected in δ13Cap and δ18Oap values (figures 1b and 2). For example, arboreal colobines tend to be more folivorous than guenons and the ground foraging sooty mangabey; however, colobines vary in terms of proportion of leaves in their diet and preferred foraging location that would influence their δ13Cap and δ18Oap values, respectively [6,8]. *Procolobus verus* feeds primarily on young leaves in the lower canopy, so δ13Cap and δ18Oap are more negative compared with upper-canopy colobines such as *P. badius*. The less negative δ13Cap values observed in cercopithecines (guenons and mangabey) reflect their more omnivorous habits relative to colobines, while similarity to *P. badius* is explained by its feeding higher in the forest canopy. The sooty mangabey exhibits low δ18Oap values relative to guenons, which reflects the mangabey’s terrestrial foraging habits.

These data demonstrate the utility of stable isotope ratio analysis of consumer tissue to delineate ecological variables.
prior to the isotopic analysis of their foodweb (e.g. water sources and food parts). Further, these data underscore the potential of oxygen isotope data from bioapatite to vertically discriminate foraging patterns in sympatric communities. The vertical gradient of $\delta^{18}O_{\text{ap}}$ values in Tai cercopithecids is consistent with the canopy level at which each monkey species feeds. This oxygen isotope canopy effect observed in modern bioapatite should also be apparent in tooth enamel bioapatite and has great palaeobiological potential [15] to test models of foraging and habitat use among sympatric taxa in forested contexts.

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References