21 Vervet Monkeys (*Chlorocebus pygerthrus*), Chimpanzees (*Pan troglodytes*), and Humans (*Homo sapiens*): Studying Interactions Using Stable Isotope Analysis

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INTRODUCTION

Approximately half of the earth's nonhuman primates are considered threatened by the International Union for Conservation of Nature (IUCN). This is due primarily to anthropogenic influences, including habitat destruction or modification, hunting, and capture for trade (Mittermeier et al., 2012). Interactions between humans and nonhuman primates are often viewed negatively given that they often result in our nonhuman kin being displaced from their natural habitats and/or faced with local extirpation. However, primatologists who focus on human–nonhuman primate interconnections have revealed a diversity of human and nonhuman primate relationships demonstrating that simple positive or negative characterizations are too simplistic (Fuentes, 2012, 2014). Traditionally, these approaches have fused a variety of behavioral observational techniques with cultural anthropological methodologies, including interviews and questionnaires (but see Riley & Ellwanger, 2013).

A few studies have used stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses to examine the impacts of humans on sympatric nonhuman primate behavior (Loudon et al., 2007, 2014; Schurr

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et al., 2012; Schillaci et al., 2014). Stable isotope ratios are expressed as δ values relative to international standards in parts per thousand (permil), as in the following example for carbon isotopes: $\delta^{13}C$ (‰) = $(R_{sample} / R_{standard} - 1) \times 1000$, where $R = {}^{13}C/{}^{12}C$. The $\delta^{13}C$ and $\delta^{15}N$ values of animals reflect the $\delta^{13}C$ and $\delta^{15}N$ values of the foods they consume with some additional fractionation that varies depending on the tissue (e.g., dental enamel, bone, hair) of interest. Most primates consume primarily plant foods, and there are three types of plant photosynthetic pathways (C_3, C_4, and Crassulacean acid metabolism [CAM] plants) that typically, or in many cases always, have different carbon isotopic compositions. C_3 plants include dicot trees, shrubs, and temperate grasses, C_4 plants include most tropical grasses and some sedges, and CAM plants include succulents like cacti and some euphorbias (Kluge & Ting, 1978). The carbon isotope compositions of C_3 and C_4 plants do not overlap (C_4 plants have higher $\delta^{13}C$ values), and therefore the stable isotope compositions of an animal's tissues reveal the relative amounts of C_3 or C_4 plants it consumes (Lee-Thorp & van der Merwe, 1987; Cerling et al., 2006). Nevertheless, among C_3 plants, there exists a considerable degree of carbon isotopic variation. Plants growing underneath a dense canopy have lower $\delta^{13}C$ values than those growing in more open areas due to incorporation of ${}^{13}C$-depleted CO_2 produced by decaying leaves (Medina & Minchin, 1980) and lower light intensities (Ehleringer et al., 1986). As a result, when humans degrade forest habitats, resulting in a loss of canopy closure, the $\delta^{13}C$ values of the remaining C_3 plants tend to increase.

Most naturally occurring plants consumed by primates use C_3 photosynthesis, but throughout much of Africa, people grow C_4 crops (e.g., corn, millet, sorghum, and sugarcane) that are opportunistically consumed by crop-raiding primates. Given the isotopic difference between most wild primate foods (C_3) and the human crops (often C_4) they raid, stable carbon isotope analysis should allow us to trace consumption of human foods by nonhuman primates. Moreover, since the degradation of forest habitats also leads to higher $\delta^{13}C$ values, anthropogenic influences in general would tend to result in higher primate $\delta^{13}C$ values. Nitrogen isotopic compositions may also reveal anthropogenic influences, most notably because crops fertilized with natural fertilizers will typically have higher $\delta^{15}N$ values than other local foods (Bateman & Kelly, 2007) and because consumption of animal food products, which are ubiquitous in human food refuse, also tends to result in higher $\delta^{15}N$ values (Schoeninger & DeNiro, 1984). Thus, all else being equal, one might expect higher $\delta^{15}N$ values to be indicative of anthropogenic influence. However, plant and animal $\delta^{15}N$ values are much more difficult to interpret because they are highly influenced by local and regional nitrogen cycles and are therefore most readily interpreted when good data on local plant $\delta^{15}N$ values are available (see Loudon et al., 2016).

Here, we seek to build upon previous work using stable isotopes to investigate the impact of humans on nonhuman primate $\delta^{13}C$ and $\delta^{15}N$ values (Loudon et al., 2007, 2014; Schurr et al., 2012; Schillaci et al., 2014). We use hair from two primate species, vervet monkeys and chimpanzees, both of which can be found in African "savanna" environments (Wrangham & Waterman, 1981; Pruetz & Bertolani, 2009), and for which there are $\delta^{13}C$ and $\delta^{15}N$ data from environments with both high and low anthropogenic disturbance. Following the above, we expect to find that groups of vervets and chimpanzees with significant anthropogenic influence will have higher $\delta^{13}C$ values. There is also reason to believe that anthropogenic influence might lead to a similar increase in $\delta^{15}N$ values, although we expect that unknown baseline differences in plant $\delta^{15}N$ values (e.g., Craine et al., 2009) will make the central tendencies of high- versus low-disturbance sites difficult to distinguish.

METHODS

We collected 100 hair samples from ten groups of vervet monkeys (*Chlorocebus pygerythrus*) throughout South Africa (Loudon et al., 2014) and four hair samples from a population of vervet monkeys (*Chlorocebus sabaeus*) in St. Kitts. Samples were collected from populations that were known to be significantly impacted by humans and from others where human influence was minimal. Monkeys were baited with corn or fruit and captured in traps designed to minimize the risk of injury to the monkeys and humans and to facilitate administration of tranquilizers (Grobler & Turner, 2010). All field protocols were approved by the Interfaculty Animal Ethics Committee of the University of the Free State and the Institutional Animal Care and Use Committee at the University of Wisconsin-Milwaukee. Hair was collected from the upper shoulder of each individual as a part of a larger study examining vervet monkey population genetics, genomics, phylogeography, and biology (Jasinska et al., 2013). We compared the $\delta^{13}C$ and $\delta^{15}N$ hair values of these vervet monkey groups to those of free-ranging chimpanzee (*Pan troglodytes*) communities from three "savanna" sites (Ishasha and Ugalla: Schoeninger et al., 1999; Fongoli: Sponheimer et al., 2006), three forest communities in Uganda (Kanyanchu, Kanyawara, and Chambura Gorge: Loudon et al., 2016), and three highly anthropogenically influenced sites in Uganda (Miranga Village, Lake Kerere, and Uganda Wildlife Education Center [UWEC]: Loudon et al., 2016). We chose the forest sites we did (rather than sites from Ivory Coast or Cameroon, for instance) because we felt they were most appropriate for comparison with our three anthropogenically influenced sites, all of which are in Uganda.

Hair samples were cleaned with ethyl alcohol, cut into segments with a razor blade, weighed (~700 μg), and placed in tin capsules. Samples were combusted in an elemental analyzer and analyzed for stable carbon and nitrogen isotope abundances using a flow-through inlet system on a continuous-flow isotope ratio mass spectrometer. We determined the degree of anthropogenic disturbance (high versus low) for each vervet group and chimpanzee community based on our observations or published accounts. We used Welch's analysis of variance (ANOVA) for all comparisons between species, groups with different levels of disturbance, and, for chimpanzees, habitat. We examined the effects of species and disturbance level on isotopic compositions using the Fit Model feature (least squares method) in JMP Pro12 with $\delta^{13}C$ and $\delta^{15}N$ as response variables and disturbance (high versus low) and species (vervet versus chimpanzee) as effects

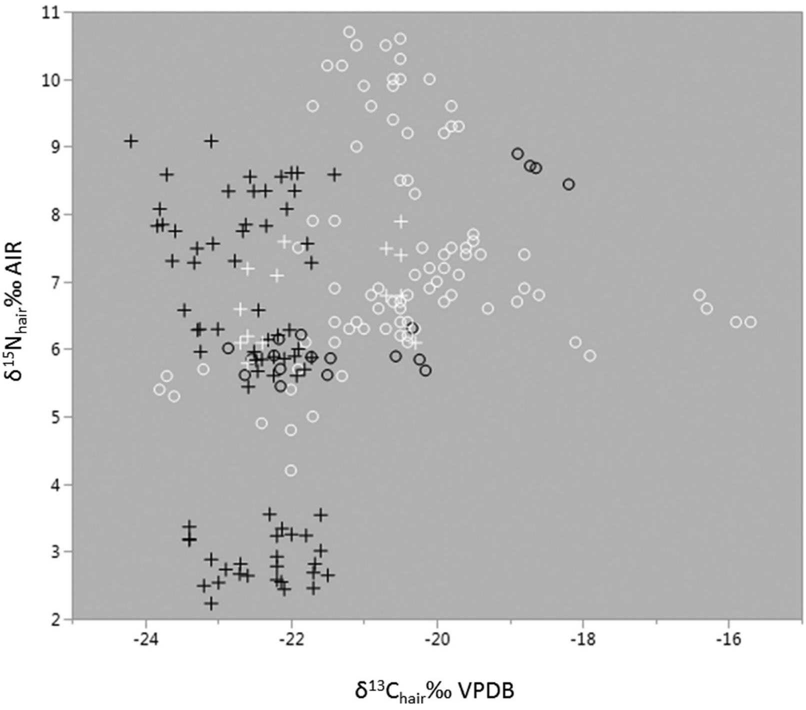

FIGURE 21.1 δ^{13}C and δ^{15}N biplot for vervet monkey and chimpanzee populations. Light symbols represent vervet monkeys and dark symbols represent chimpanzees. Circles illustrate high levels of anthropogenic disturbance and crosses represent low levels of or little disturbance. VPDB = Vienna Pee Dee Belemnite.

(interaction terms were included initially). In some cases, data were log transformed or ranked prior to analyses in order to avoid violating assumptions of the tests employed, but as these transformations did not alter the significance of the results appreciably relative to non-transformed values, we present results for the non-transformed data set below. Data used for this paper are available at https://doi.org/10.6084/m9.figshare.7545158.

RESULTS

Figure 21.1 shows the δ^{13}C and δ^{15}N values for each vervet and chimpanzee specimen. Welch's ANOVA revealed differences in the mean δ^{13}C ($F_{1,190.35} = 82.97$; $p < 0.0001$) and δ^{15}N ($F_{1,170.67} = 35.29$;

$p < 0.0001$) values of vervets and chimpanzees. Among the vervet monkeys, we found significant differences in the $\delta^{13}C$ ($F_{1,24.61} = 14.59$; $p < 0.001$) and $\delta^{15}N$ ($F_{1,47.97} = 6.11$; $p < 0.05$) values of populations living in habitats with high versus low anthropogenic disturbance. Among chimpanzees, we found differences in both $\delta^{13}C$ ($F_{1,20.91} = 16.02$; $p < 0.001$) and $\delta^{15}N$ values ($F_{1,58.94} = 5.77$; $p < 0.05$) for communities living in habitats with high versus low levels of anthropogenic disturbance. We also found that "savanna" and forest chimpanzees (excluding high disturbance sites) have different $\delta^{13}C$ ($F_{1,22.54} = 32.18$; $p < 0.0001$) and $\delta^{15}N$ ($F_{1,63.01} = 54.36$; $p < 0.0001$) values. Two models (one for $\delta^{13}C$ and another for $\delta^{15}N$) exploring the effects of species and disturbance on $\delta^{13}C$ and $\delta^{15}N$ values were significant ($p < 0.0001$), as were the effects species ($p < 0.01$) and disturbance ($p < 0.05$) in both models (these models do not include interaction terms as initial runs revealed no disturbance and species interaction). However, R^2 values were low for both models ($\delta^{13}C$, $R^2 = 0.40$ and $\delta^{15}N$, $R^2 = 0.18$), such that between 60 and 82 percent of the variance in $\delta^{13}C$ and $\delta^{15}N$ remained unexplained. The unexplained variance is probably linked to unknown habitat or behavioral differences between populations.

DISCUSSION

It is not surprising that there are differences in the $\delta^{13}C$ values of vervets and chimpanzees (vervet $\delta^{13}C$ values are higher), especially as some of the chimpanzee sites are from closed environments (e.g., Kanyawara, Kanyanchu) where we would expect a canopy effect. The same mechanism can explicate differences in the $\delta^{13}C$ values of the "savanna" and forest chimpanzee groups. Only a handful of vervet values fall within the range of forest chimpanzees (without high anthropogenic disturbance). In contrast, there are no differences in the $\delta^{13}C$ values of vervets and chimpanzees with high levels of anthropogenic influence ($F_{1,26.81} = 3.22$; $p = 0.084$), suggesting that disturbance in these environments and anthropogenic contexts tends to impart a similar isotopic signature to both primate species. We also found differences in the $\delta^{15}N$ compositions of vervets and chimpanzees and in the $\delta^{15}N$ values of forest and "savanna" chimpanzees, but these are much more difficult to interpret given baseline differences in the $\delta^{15}N$ values of vegetation from place to place (Craine et al., 2009; see Loudon et al., 2016). Consequently, it is difficult to ascribe any ecological or biological significance to these differences in central tendency.

Of particular note, and as predicted, both vervets and chimpanzees show trends toward higher $\delta^{13}C$ and $\delta^{15}N$ values in disturbed habitats. This makes sense as in these contexts disturbance often includes clearing of landscapes, leading to less canopy-driven depletion in the $\delta^{13}C$ values of plants, or the use of human crops, which are typically ^{13}C-enriched C_4 plants like corn, sorghum, or sugarcane. Since both vervets and chimpanzees are expected to consume few C_4 resources under normal circumstances, their consumption would be expected to significantly alter the isotopic compositions of consumers, and this study appears to bear that out. The higher $\delta^{15}N$ values in disturbed habitats make sense, as opening of landscapes, consumption of foods grown with natural fertilizers, and access to greater amounts of animal foods, all of which can happen as a result of human disturbance, may also lead to elevated $\delta^{15}N$ values. Once again, however, this trend in $\delta^{15}N$ values is difficult to interpret, as consumption of anthropogenic resources might also decrease $\delta^{15}N$ values if synthetic fertilizers are used (Bateman & Kelly, 2007) or if legume crops such as peanuts are raided (Schoeninger et al., 1999). Moreover, differences in baseline plant $\delta^{15}N$ values from place to place due to local nitrogen cycling add another complicating factor. The latter problem can be overcome if local plants are sampled in addition to hair, but this was not done during the present study or the other studies from which we derived the isotopic data used here.

Visual inspection of the $\delta^{13}C$ data for both species suggests that values of –21.0‰ or greater are usually indicative of anthropogenic disturbance. Yet, individuals with lower values could also be from disturbed environments. As for the $\delta^{15}N$ data, values that approach

9.0‰ are likely to indicate anthropogenic inputs, at least across the range of environments represented by our samples. This is not an overly useful measure as the vast majority of the specimens sampled fall below this threshold, making $\delta^{13}C$ a much more sensitive indicator of anthropogenic influence, at least in the absence of plant baseline data.

CONCLUSIONS

This study investigated the impacts of species and anthropogenic disturbance on the $\delta^{13}C$ and $\delta^{15}N$ values of two primate species. There is a strong pattern where $\delta^{13}C$ values above –21.0‰ likely indicate an anthropogenic signal. While $\delta^{13}C$ values are not perfect indicators of disturbance, they are quick and inexpensive, and are an especially useful supplement if hair is collected for DNA analyses or other purposes. We believe that stable isotope analysis can be a powerful tool for revealing cryptic feeding behaviors (i.e., crop raiding) and nonhuman primate and human interplays when long-term behavioral studies are not feasible.

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