Geophagy for micronutrients or protection? A geophagy field experiment with black-and-white colobus monkeys in the Budongo Forest Reserve, Uganda

Running title: Budongo Geophagy Field Experiment

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Abstract

Geophagy, the intentional consumption of soil, has been observed in humans and numerous animal species. Geophagy has been posited to be adaptive, *i.e*., consumed soil protects against gastrointestinal distress and/or supplements micronutrients. We conducted a field experiment in the Budongo Forest, Uganda to investigate soil preference and quantity of soil eaten. We placed pairs of artificial tree stumps with different types of soil in each at two existing geophagy sites. We placed iron-rich soil from the surrounding area in one stump and clay-rich soil from a neighboring community in the second stump. After five days, we reversed the type of soil that was in the stumps at both sites (*i.e*., a cross-over design). We monitored activity and engagement with the stumps using camera traps for ten days. Only *Colobus guereza* (black-and-white colobus monkeys) interacted with the stumps on three of the ten days and only ate the clay-rich soil. In total, monkeys consumed 9.67 kg of soil over 4.33 hours. Monkeys competed for access to the stumps and videos captured aggression in 13% of videos, including pushing, excluding, and chasing other individuals from the experimental stumps. Nine episodes of vigilance and flight behavior were also observed, demonstrating their apprehension of being on the ground. These monkeys used visual and olfactory cues to select novel soil that can bind pathogens and plant toxins and adjust gut pH over known soil that could supplement bioavailable iron. Given that geophagic soil may confer health benefits, geophagy sites should be conserved and protected.

Keywords: Soil eating ・ Detoxification ・Bioavailable iron ・Nonhuman primates ・Field experiment

Introduction

Understanding nonhuman primate culture, language, learning, and social interaction is inherently challenging. Unlike human subjects, whom researchers can interview, wildlife researchers need creative techniques to understand the motivation for and consequences of complex animal behaviors. One such technique is field experiments (Gruber et al. 2009). Previous nonhuman primate field experiments have been transformative for understanding differences in cultural knowledge between populations of chimpanzees (Gruber et al. 2009), responses to potentially contaminated material in Japanese macaques (Sarabian and MacIntosh 2015), transmission of tool use in chimpanzees (Matsuzawa 1994; Biro et al. 2003; Sirianni et al. 2018; Lamon et al. 2018), variation in calls using bio-acoustic playback (Cheney and Seyfarth 1982; Hauser 1998; Zuberbühler 2000; Fischer et al. 2013; Caselli et al. 2018), efficiency of nut-cracking in capuchin monkeys (Fragaszy et al. 2010), social relationships and cognition (Cheney et al. 1986), and social learning (Botting et al. 2018; Bono et al. 2018). Despite their importance, few field experiments have been conducted because they have the potential to alter an animal's social and physical world.

One primate behavior that remains an enigma is geophagy, the purposive consumption of earth (*e.g.*, soil from the forest floor, termite and other insect mounds, earthen bricks). The behavior is common across the animal kingdom; it has been observed in humans on all inhabited continents and in over 300 species of mammals, birds, and reptiles (Young et al. 2011). Among nonhuman primates, 136 species are known to eat earthen materials (Pebsworth et al. 2019b). Within the genus *Colobus,* researchers have observed geophagy in four of five recognized species and several subspecies (Pebsworth et al. 2019b). Yet despite its ubiquity, little is known about the motivations for and the consequences of geophagy.

Two adaptive hypotheses have been posited to explain the motivation for geophagy. The first, and perhaps most intuitive, is that soil supplements micronutrients that may be lacking in the diet (Kreulen 1985; Pebsworth et al. 2019b). Micronutrient deficiencies can adversely affect overall primate health, growth, reproduction, and disease resistance (Rode et al. 2003). Previous studies have therefore suggested that soil consumed by nonhuman primates can supplement a variety of micronutrients, although more research is needed to understand bioavailability (*i.e.*, the proportion of nutrients that are absorbed by the body and enter circulation) (Pebsworth et al. 2019b). We primarily focused on iron as a key micronutrient because it was low in the black-and-white colobus monkeys' diet (Rode et al. 2003), it has been hypothesized as a geophagy stimulus (Mahaney et al. 1990), and we had assessed bioavailability (Pebsworth et al. 2019a).

The second hypothesis is that soil protects against gastrointestinal (GI) distress (*e.g.*, nausea, diarrhea, vomiting) and/or infection (Young 2010; Pebsworth et al. 2019b). Previous studies have demonstrated that clay minerals can bind directly to agents that cause GI distress (Gilardi et al. 1999; Dominy et al. 2004), strengthen the luminal epithelium of the GI tract (Said et al. 1980; González et al. 2004), and lyse bacterial cells (Papaioannou et al. 2005).

Soils with a high proportion of clay minerals have the capacity to adsorb polar plant secondary compounds (PSCs), like alkaloids and phenolics (Johns 1986; Ta et al. 2018; Pebsworth et al. 2019a) that can negatively affect plant palatability and the function of proteolytic enzymes necessary for digestion (Hladik 1977; DeGabriel et al. 2009). Indeed, several species of nonhuman primates regularly consume clay-rich soils that can adsorb PSCs (Pebsworth et al. 2019b). Although the supplementation and protection hypotheses are often considered separately, several authors have concluded that geophagy is multifunctional and that both protection and supplementation may co-occur (Davies and Baillie 1988; Pebsworth et al. 2019a). Previous nonhuman primate studies have not, however, been able to rigorously test these hypotheses concurrently due to imprecise estimation of the amount and type of soil consumed.

Accurate measurement of soil consumption is critical for quantifying exposure and determining potential physiological impacts. In human geophagy studies, soil ingestion has been estimated using 1) the "tracer element" method, which measures the amount of common soil elements (*i.e*., Al, Ce, La, Si) in an individual's feces and urine; 2) the "biokinetic model comparison" method, which measures the concentration of an element (*e.g*., Pb) in blood; and 3) the "survey response" method, in which questions about soil ingestion are combined with a tracer element (Doyle et al. 2012). None of these techniques are practical for free-ranging animals. Previous nonhuman primate geophagy studies have therefore used qualitative descriptions, like a "handful," "mouthful," or "a bite," to estimate the amount of soil consume (Krishnamani 1994; Klein et al. 2008). These descriptions, however, are imprecise. Field experiments can overcome these limitations through direct observation and weighing the amount of soil consumed.

Black-and-white colobus monkeys (*Colobus guereza)* have particular behaviors and diets that make them an ideal species to examine multiple hypotheses about geophagy. For instance, black-and-white colobus monkeys eat highly digestible foods that are broken down via anaerobic fermentation in the foregut, creating volatile fatty acids. These compounds can lead to a decrease in the forestomach pH and may cause fatal acidosis (Goltenboth 1976). Further, the diet of black-and-white colobus monkeys also contains PSCs that can cause GI distress. Although there is evidence that clay minerals may offer some protection against acidosis (Davies and Baillie 1988) and PSCs (Hladik and Gueguen 1974; Oates 1978), no studies have investigated this in colobus monkeys. Additionally, while previous studies have analyzed the chemical and mineralogical content of soils consumed by colobus monkeys (Oates 1978; Fashing et al. 2007), none have assessed bioavailability, which is essential for testing the supplementation hypothesis.

We, therefore, sought to fill these knowledge gaps by conducting a field experiment in which we concurrently offered known amounts of iron- and clay-rich soils from existing geophagy sites to black-and-white colobus monkeys living in the Budongo Forest Reserve, Uganda. Given that the soils had the potential to confer beneficial micronutrients and/or protection from GI distress, we hypothesized that black-and-white colobus monkeys would compete for access (*i.e.*, show aggression) because soil is a valued resource. We also hypothesized that clay-rich soils would be preferentially selected and consumed, in support of the protection hypothesis.

Materials and Methods

Study site and subjects

We conducted a geophagy experiment across ten days (October 14 - 23, 2016) in the Budongo Forest Reserve (1.617 – 2.0° N, 31.367 – 31.766° E) (Fig. 1). This reserve is a moist, semi-deciduous tropical forest located in the Masindi District of western Uganda (Eggeling 1947; Plumptre and Reynolds 1996). The reserve is home to over 95 vertebrate species (Plumptre, unpublished data), some of whom eat soil from permanent geophagy sites and termite mounds (*e.g.*, chimpanzees, bushbuck, duiker, black-and-white colobus). We previously identified four geophagy sites in the reserve by direct observation and camera trap monitoring in 2015-2016 (Reynolds et al. 2015, 2019; Pebsworth et al. 2019a) (Fig. 1).

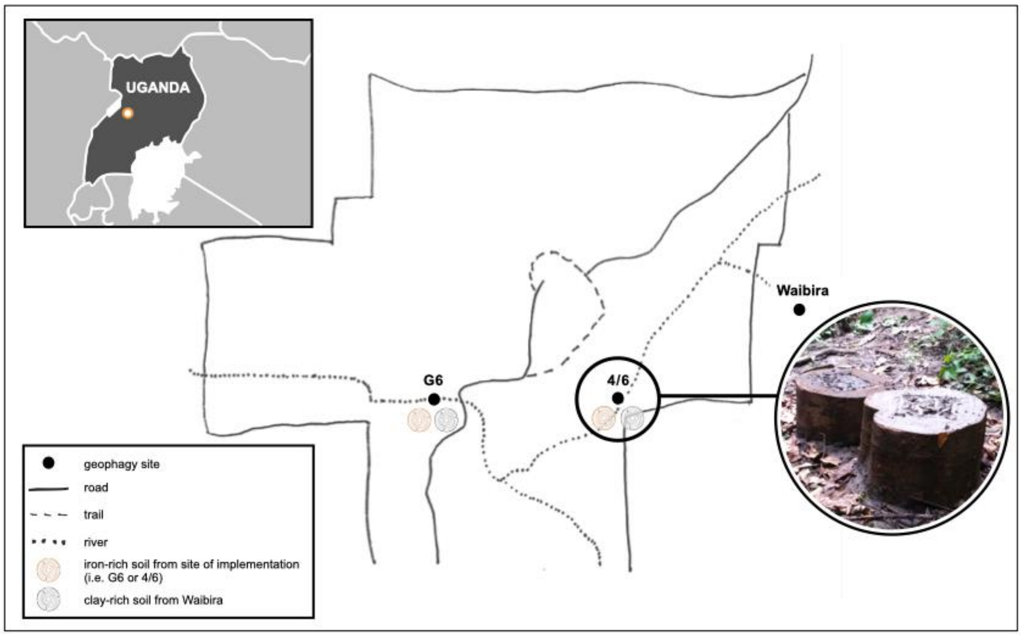

Figure 1. Two experimental stumps were placed at each permanent geophagy study site (G6, 4/6) in the Budongo Forest Reserve. One stump contained soil higher in clay minerals and the other contained soil higher in bioavailable iron. Black-and-white colobus monkeys were observed routinely eating both soils in prior studies.

Black-and-white colobus monkeys live in small troops containing multiple females and one or more adult males. In multi-male troops, one male is dominant, and interaction between males is agonistic (Bocian 1997). The average troop size in the Budongo Forest is 8-10 individuals (Marler 1969; Schel et al. 2010). Intergroup aggression among colobus monkeys functions to directly defend mates and food resources (Fashing, 2001). Between 2008-2009, researchers identified approximately 60 different colobus groups within the Budongo Forest Reserve. These groups were found 150-200 m apart (Schel et al. 2010), indicating that there was considerable home range overlap among colobus troops (*i.e.*, there is potential for troops to interact and compete for resources).

Colobus monkeys are folivore/frugivores and possess a chambered stomach (Chivers and Hladik 1980). Leaves and leaf buds typically dominate their diet (Oates 1978). There is no published dietary information for black-and-white colobus monkeys from Budongo. However, data from eight groups within Kibale National Park (located in Uganda) showed that 78.5-94.0% of foraging effort is spent on leaves (Harris and Chapman 2007). At Budongo, black-and-white colobus monkeys are preferred prey for chimpanzees and crown eagles (Newton-Fisher et al. 2002; Schel et al. 2010; Hobaiter et al. 2017).

Experimental design

Researchers have conducted several field experiments at Budongo (Gruber et al. 2009), but this was the first geophagy field experiment. We first created four similar-looking experimental stumps made out of dead wood found in the Budongo Forest Reserve by drilling a 15 x 15 x 10 cm hole on the flat surface; stumps were cleaned with an antiseptic liquid to prevent transmission of human pathogens (Fig. 2A). We then selected two sites where geophagy was regularly occurring and placed two stumps at each (Fig. 1). We partially buried the stumps with the surrounding soil and placed leaves and branches around the base to make them look natural (Fig. 2B).

At each study site, one stump was filled with approximately 5kg of iron-rich soil from that particular area and the other was filled with approximately 5kg of clay-rich soil from a neighboring community, Waibira (Fig. 2B). On the sixth day, we reversed the type of soil that was in the stumps at both sites (*i.e.*, a cross-over design), keeping the stump position the same to ensure that any observed differences in behavior were related to soil type, not stump design or stump preference. We checked the stumps daily and replenished the soil in the evening or the day after animals consumed it. The first five-day period was October 14-18, and the second was October 19-23.

Figure 2. We created experimental stumps of a known weight. (A) On the surface of the stump, we carved a hole to house a known quantity of soil from two permanent geophagy sites. A smaller hole was drilled on the side to allow moisture to drain. (B) One stump was filled with approximately 5kg of iron-rich soil from that particular area (stump on the left), and the other was filled with approximately 5kg of clay-rich soil from a neighboring community, Waibira (stump on the right).

The soils from the study sites (G6 and 4/6) were physically, chemically, and mineralogically similar, but different from Waibira soil. Specifically, we classified the soil from Waibira and G6 as “Gley 1”. Waibira had a neutral hue (7/N), while G6 soil had a green-yellow hue (7/5GY) (Munsell Color 2010). Soil color is indicative of minerals from the parent material, organic matter, iron, and moisture. As such, soil from Waibira contained a higher percentage of 2:1 clay minerals (*e.g.*, montmorillonite), which we measured with X-ray diffraction (XRD) (Table 1). In the laboratory, soil from Waibira had a stronger capacity to adsorb phenolic and alkaloid compounds than soil from sites G6 and 4/6. We measured the adsorption of plant secondary compounds (expressed as average gallic acid equivalent adsorbed (μg/mg soil) using the Folin-Ciocalteu method (Table 1) (Pebsworth et al. 2019a).

We removed approximately 95% of micronutrients found in the soil with a HNO_3 / $HCLO_4$ extraction and measured the concentration of iron using Inductively Coupled Plasma (ICP) (Table 1). We also analyzed the soils for bioavailable iron using Caco-2 cell experiments.

Table 1

Characteristics of soils placed in artificial tree stumps used in a geophagy field experiment conducted in the Budongo Forest Reserve. We determined 2:1 clay minerals by X-ray diffraction, adsorption of plant secondary compounds using the Folin-Ciocalteu method, 95% of iron (ppm) using Inductively coupled plasma (ICP), and bioavailable iron using Caco-2 cell experiments.

Site	**2:1 clay minerals** (mean %)	**Average gallic acid equivalent adsorbed** (µg/mg soil)	**Fe** (ppm)	**Bioavailable iron** (ng/ferritin/mg cell protein)
G6	3.5	12	4653.2	19.73
4/6	2.0	15	1754.0	20.68
Waibira	22	29	8007.2	3.97

Monitoring behavior

The field experiment was actively monitored for all ten days with Bushnell Trophy camera traps (one at each site) that were activated by infrared motion and heat detection. We positioned the cameras approximately 20 cm above the ground near the experimental stumps, and programmed them to take 59-second videos with a 1-second interval between videos, for a total of 480 hours. Cameras were synchronized by date and time.

For each video, the first author documented the stump from which the animal(s) consumed soil, how many animals were present, age-class and sex (when possible), and signs of aggression. We defined "aggression" as an individual pushing, hitting, excluding, and chasing away others from the stumps. Aggression that was not associated with competition for access to the stump was not coded.

Statistical analysis

We used chi-squared tests to evaluate our first hypothesis that if soil was a valued resource intra- and inter-troop aggression would increase over time. Using chi-squared tests, we determined whether the number of aggressive events at the experimental stumps were statistically different between the first five days and the last five days ($p<0.01$). We ran basic statistics in Stata 15 (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC).

Ethical note

We obtained ethical permission for this study from the University of Texas at San Antonio's Institutional Animal Care and Use Committee (IACUC). We followed all applicable international, national, and institutional guidelines, and complied with all recommendations from the University of Texas at San Antonio. All study activities conformed to the ASAB/ABS guidelines for the Treatment of Animals in Behavioral Research and Teaching. For instance, we used camera traps to reduce human contact and limited the number of experimental days to minimize any potential effects of the study.

Results

Geophagic behavior

Although other species of animals have been observed eating soil in the Budongo Forest Reserve (*e.g.*, chimpanzee, bushbuck, red duiker, blue duiker) (Pebsworth, unpublished data), the camera traps only recorded black-and-white colobus monkeys eating soil at the experimental stumps. Additionally, while monkeys were present at both sites, interaction with the stumps was only observed at site G6 (Fig. 1).

A camera trap captured 260 videos of black-and-white colobus interacting with the experimental stumps on three days. Of these videos, one or more monkeys were eating soil in 242 (93%) videos. In the remaining 18 videos, monkeys were smelling the soil (n=6), licking the soil and stump (n=3), or chewing bark on the experimental stump (n=9). For example, one monkey was recorded smelling both stumps and then eating only the clay-rich soil from Waibira. Two of the three licking events occurred at the end of the experiment, apparently to remove remaining soil from the outside of the stump that housed clay-rich soil. When the clay-rich soil was fully exhausted, two monkeys were recorded smelling the iron-rich soil and then departed while another smelled the iron-rich soil, placed its head into the stump that had contained clay-rich soil, and proceeded to lick the outside of the stump.

The number of individuals varied considerably at the stumps, from 1 to 11 (Fig. 3A, Table 2). In total, the black-and-white colobus monkeys spent 4.33 h interacting exclusively with the experimental stumps and consumed only the clay-rich soil from Waibira. Sex identification was difficult, as distinguishing features (*e.g.*, nipples, penis, ischial callosities) were often concealed. Nonetheless, adult females were obvious in 51% of videos, adult females with an infant in 47%, juveniles in 18%, and adult males in 1% (Fig. 3B). During this experiment, only one female chimpanzee with an infant interacted with an experimental stump; however, by the time she arrived, all the clay-rich soil had been eaten.

Figure 3. Two experiment stumps were visited by two troops of black-and-white colobus monkeys. (A) The number of individuals at the stumps varied from 1-11 during the video clip, (B) adult females with an infant were present in 47% of videos, (C) aggression occurred between adults and juveniles, and (D) monkeys exhibited vigilant behavior.

There were more geophagy videos recorded during the second five days than the first five days of the experiment (Table 2). Even though more time was spent at the stump during the second period and the monkeys ate more soil, the rate of soil consumption during the first and second period remained the same (0.04 kg/min). Observed aggression increased across the two periods and the difference in the number of events was statistically significant ($p = 0.012$) (Table 2). In these videos, some individuals had less access, appeared younger, and were pushed away, excluded, and chased from the experimental stumps (Fig. 3C) (Table 2).

Table 2. Camera trap data from the G6 site in the Budongo Forest Reserve, where black-and-white colobus monkeys interacted with an experimental stump that contained soil from Waibira.

	Days 1-5 (n=121)	Days 6-10 (n=139)
Individuals at stumps	1-8	1-11
Videos with geophagy	121	139
Amount of soil consumed (kg)		
Clay-rich (from Waibira)	4.52	5.15
Iron-rich (from G6)	0	0
Number of aggressive events	9	25

Soil consumption and quantity eaten

After a brief exploration of both stumps (looking, smelling), black-and-white colobus monkeys exclusively ate the clay-rich soil from Waibira, even after reversing the soils in the stumps (Table 1). The monkeys ate 4.52 kg of soil on October 14-15, 2016, and 5.15 kg of soil on October 22, 2016. On average, the monkeys ate approximately 2.23 kg of soil/hr. Although we were unable to determine when stumps were depleted, there is evidence that the monkeys continued to interact with the stump even when empty (*e.g.*, licked remaining clay-rich soil from the stump).

Discussion

In this first geophagy field experiment, we used camera trap videos and well-characterized soil to examine soil preference in *Colobus guereza*. We allowed monkeys to choose between clay-rich and iron-rich soils, and they exclusively consumed the clay-rich soil. To distinguish between soils, monkeys looked at and smelled both experimental stumps, but few licked the soil before eating it. This suggests that soil selection was primarily based on visual and olfactory cues.

Wet earth has a distinct smell that human geophagists most commonly cite as the main attraction for soil eating (Young et al. 2010; Huebl et al. 2016). Our experiment demonstrated that soil with higher clay content was selected over soil with lower clay content. We cannot say why these monkeys chose the clay-rich soil but suggest that the smell of clay could have been a stimulus for geophagy. In addition to clay, it has been hypothesized that soil containing salts, lime, and organic matter may provide olfactory stimulation for geophagy (Stambolic-Robb 1997; Krishnamani and Mahaney 2000). The soil provided in this experiment lacked organic matter, but monkeys may have been responding to the smell of micronutrients, such as iron within the soil. It has been suggested that the smell of organically bound iron may lead gorillas to soluble iron (Mahaney et al. 1990). Other studies have also reported the importance of olfactory cues in locating and assessing food quality (Nevo and Heymann 2015).

It has never been demonstrated that the taste of salt was a stimulus for primate geophagy (Krishnamani and Mahaney 2000). In fact, when chacma baboons selected between soil of varying degrees of saltiness, they chose soil with the least amount of sodium and the highest percentage of clay minerals (Pebsworth et al. 2012). In response to sodium deficient diets, black-and-white colobus monkeys may or may not preferentially select sodium-rich foods (Oates 1978; Rode et al. 2003). Given that some monkeys were observed licking the soils, soil texture (*i.e.*, sand, silt, clay content) may also be important.

We primarily focused on iron as a key micronutrient because it is generally low in natural foods and may be deficient in the black-and-white colobus monkeys' diet (Rode et al. 2003). It is clear that despite large amounts of iron in the Waibira soil, very little was bioavailable, which suggests that the clay fraction can bind up trace iron. (Table 1). The Caco-2 cell experiments revealed that the amount of bioavailable iron present in the Waibira soils was below the level of a quality control cell that lacked soil (Pebsworth et al. 2019a). Naturally occurring plant compounds can decrease micronutrient bioavailability; oxalate and phytate inhibit calcium absorption (Gibson et al. 2010), phytate inhibits zinc absorption, and polyphenols, phytate, calcium, legume proteins, and casein inhibit iron absorption (Hambidge 2010). Future research should determine the bioavailability of calcium, and other micronutrients found in soil eaten by non-human primates.

Wild black-and-white colobus monkeys competed for access to soil from Waibira containing a higher percentage of 2:1 clay minerals than from soil within their home range (G6) containing more bioavailable iron. Camera trap videos documented that more monkeys were present, more soil was eaten, and more time was spent at the experimental stumps during the second half of the experiment (Table 2). The videos also documented intra-troop aggression at the experimental stumps. Juveniles and seemingly less privileged individuals had fewer opportunities to consume the clay-rich soil in the stump. The experimental stumps represented a poorly distributed, novel, food source that could be monopolized, which caused feeding competition (Harris 2006). These observations support our first hypothesis that black-and-white colobus monkeys would compete for access (*i.e.*, show aggression) for this soil because it was a valued resource.

In addition to aggression, black-and-white colobus displayed vigilance, perhaps in response to competition and risk of predation. Vigilance is defined as “any visual search directed beyond arm’s reach” (Treves 1999). Typical vigilant behaviors include a heightened state of awareness, watching, listening intently, and looking up (Cords 1990). Based on the presence/absence of recognizable individuals and behavior, it appeared that two troops of black-and-white colobus monkeys interacted with the geophagy experiment. Camera traps did not record inter-troop interactions at the stumps, but they could have taken place out of the camera’s view.

That soil was a valued resource is further supported by risk of predation to access the soil. Descending to the ground might also be perceived as a risky behavior. Colobus monkeys are highly arboreal and locomotion is awkward while on the ground. Furthermore, black-and-white colobus monkeys are a preferred prey item for chimpanzees at Budongo, so vigilance could be in response to risk of predation (Schel et al. 2010; Hobaiter et al. 2017). While in the trees, escape would be much easier than on the ground. Similar results were observed with brown spider monkeys, who descended to saladeros (licks) to eat soil even though there was risk of jaguar, puma, and ocelot predation (Link et al. 2011).

In sum, our findings provide support for our second hypothesis that clay-rich soils would be preferentially selected and consumed, in support of the protection hypothesis. Colobus monkeys preferentially consumed clay-rich soil that had the potential to bind polar PSCs common in the black-and-white colobus diet (Ta et al. 2018; Pebsworth et al. 2019a) and adjust gut pH (Oates 1978; Davies and Baillie 1988). During the experiment, some individuals ate the novel clay-rich soil in the stump while others continued to eat iron-rich soil in the natural setting. Curiously, the monkeys did not eat the iron-rich soil in the experimental stump, even once the clay-rich soil was exhausted. What remains unclear is whether the monkeys ignored the iron-rich soil in the stump because they knew where to find it, they preferred the clay-rich soil, or they preferred a novel food item.

Why chimpanzees did not interact with the experimental stumps is curious. Over the last decade, both habituated and non-habituated chimpanzees have engaged with these types of field experiments (Gruber et al. 2016). One possibility is that the chimpanzees perceived the stumps as unnatural and avoided them. Another possibility is that chimpanzees in the Budongo Forest Reserve do not eat the iron-rich soil but instead drink standing water at G6 and 4/6 that is infused with clay and iron (Pebsworth et al. 2019a).

This experiment had several strengths: soils eaten by the monkeys were well described, *i.e.*, a laboratory ran a battery of soil analyses, and there was a marked difference between the two soils with regards to potential for detoxification and iron supplementation (Pebsworth et al. 2019a). We used camera traps to document geophagy continually (*e.g.*, which species, duration) without introducing observer bias. To our knowledge, this was the first field experiment to establish not only soil preference but the quantity of soil consumed.

This experiment also had several weaknesses, including that these monkeys were not habituated to human presence, nor were they routinely followed, so we do not know their diet and the social hierarchy or composition of the troop members. As well, we may have selected soil different from what the colobus monkeys would have selected for themselves. Additionally, soil from Waibira would have been outside the monkeys' home range and, therefore, novel to them. As such, this experiment would have been more appealing to less neophobic monkeys. The hole carved into the stump was also small, which may have prevented less privileged monkeys from participating.

Despite these shortcomings, this experiment yielded insights about geophagy and its role as a form of self-medication for black-and-white colobus. We cannot definitively conclude why one soil was selected and the other was not. The behavior observed at the stumps, however, suggests that consumed soil was chosen based on color, smell, and in limited cases, taste and texture. Once eaten, the soil had the potential to serve multiple functions – protection and supplementation of micronutrients.

Regarding future geophagy experiments, we recommend that the number of experimental trials is limited to avoid altering patterns of movement and increasing intra and inter-troop aggression. Research at Budongo has demonstrated that clay-rich soil can adsorb high concentrations of plant secondary compounds that can result in gastrointestinal distress. We recommend that this geophagy field experiment be repeated and conducted at other research sites to ensure that these findings are not due to stochastic variation.

Research has shown that a consequence of global climate change is a decline in the nutritional composition of leaves and an increase in plant secondary compounds (Marsh et al. 2013). This experiment demonstrates that soil is an important dietary resource, and we advocate for the conservation and preservation of geophagy sites where animals congregate to eat soil. In a time of unprecedented habitat destruction and intra- and interspecies competition for finite resources, geophagy sites may become increasingly vital to animal health and survival.

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Competing interests

No competing interests to declare.

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References

Biro D, Inoue-Nakamura N, Tonooka R, et al (2003) Cultural innovation and transmission of tool use in wild chimpanzees: evidence from field experiments. Anim Cogn 6:213–223. https://doi.org/10.1007/s10071-003-0183-x

Bocian CM (1997) Niche separation of black-and-white colobus monkeys (*Colobus angolensis* and *C. guereza*) in the Ituri Forest. Doctoral dissertation, The City University of New York

Bono AEJ, Whiten A, van Schaik C, et al (2018) Payoff- and Sex-Biased Social Learning Interact in a Wild Primate Population. Curr Biol 28:2800-2805.e4. https://doi.org/10.1016/j.cub.2018.06.015

Botting J, Whiten A, Grampp M, van de Waal E (2018) Field experiments with wild primates reveal no consistent dominance-based bias in social learning. Anim Behav 136:1–12. https://doi.org/10.1016/j.anbehav.2017.11.025

Caselli CB, Ayres PHB, Castro SCN, et al (2018) The role of extragroup encounters in a Neotropical, cooperative breeding primate, the common marmoset: a field playback experiment. Anim Behav 136:137–146. https://doi.org/10.1016/j.anbehav.2017.12.009

Cheney DL, Seyfarth R, Smuts B (1986) Social relationships and social cognition in nonhuman primates. Science 234:1361–1366. https://doi.org/10.1126/science.3538419

Cheney DL, Seyfarth RM (1982) How vervet monkeys perceive their grunts: Field playback experiments. Anim Behav 30:739–751. https://doi.org/10.1016/S0003-3472(82)80146-2

Chivers DJ, Hladik CM (1980) Morphology of the gastrointestinal tract in primates: Comparisons with other mammals in relation to diet. J Morphol 166:337–386. https://doi.org/10.1002/jmor.1051660306

Cords M (1990) Vigilance and mixed-species association of some East African forest monkeys. Behav Ecol Sociobiol 26:. https://doi.org/10.1007/BF00178323

Davies AG, Baillie IC (1988) Soil-eating by red leaf monkeys (*Presbytis rubicunda*) in Sabah, northern Borneo. Biotropica 20:252. https://doi.org/10.2307/2388241

DeGabriel JL, Moore BD, Foley WJ, Johnson CN (2009) The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. Ecology 90:711–719

Dominy NJ, Davoust E, Minekus M (2004) Adaptive function of soil consumption: an in vitro study modeling the human stomach and small intestine. J Exp Biol 207:319–324

Doyle JR, Blais JM, Holmes RD, White PA (2012) A soil ingestion pilot study of a population following a traditional lifestyle typical of rural or wilderness areas. Sci Total Environ 424:110–120. https://doi.org/10.1016/j.scitotenv.2012.02.043

Eggeling WJ (1947) Observations on the Ecology of the Budongo Rain Forest, Uganda. J Ecol 34:20. https://doi.org/10.2307/2256760

Fashing PJ (2001) Male and female strategies during intergroup encounters in guerezas (Colobus guereza): evidence for resource defense mediated through males and a comparison with other primates. Behav Ecol Sociobiol 50:219–230. https://doi.org/10.1007/s002650100358

Fashing PJ, Dierenfeld ES, Mowry CB (2007) Influence of plant and soil chemistry on food selection, ranging patterns, and biomass of *Colobus guereza* in Kakamega Forest, Kenya. Int J Primatol 28:673–703. https://doi.org/10.1007/s10764-006-9096-2

Fischer J, Noser R, Hammerschmidt K (2013) Bioacoustic Field Research: A Primer to Acoustic Analyses and Playback Experiments With Primates: Bioacoustic Field Methods. Am J Primatol 75:643–663. https://doi.org/10.1002/ajp.22153

Fragaszy D, Pickering T, Liu Q, et al (2010) Bearded capuchin monkeys’ and a human’s efficiency at cracking palm nuts with stone tools: field experiments. Anim Behav 79:321–332. https://doi.org/10.1016/j.anbehav.2009.11.004

Gibson RS, Bailey KB, Gibbs M, Ferguson EL (2010) A Review of Phytate, Iron, Zinc, and Calcium Concentrations in Plant-Based Complementary Foods Used in Low-Income Countries and Implications for Bioavailability. Food Nutr Bull 31:S134–S146. https://doi.org/10.1177/15648265100312S206

Gilardi J, Duffey S, Munn C, Tell L (1999) Biochemical functions of geophagy in parrots: detoxification of dietary toxins and cytoprotective effects. J Chem Ecol 25:897–922. https://doi.org/10.1023/A:1020857120217

Goltenboth R (1976) Non human primates (apes, monkeys and prosimians). Handb Zoo Med 46–85

González R, de Medina FS, Martínez-Augustin O, et al (2004) Anti-inflammatory effect of diosmectite in hapten-induced colitis in the rat. Br J Pharmacol 141:951–960. https://doi.org/10.1038/sj.bjp.0705710

Gruber T, Muller MN, Strimling P, et al (2009) Wild chimpanzees rely on cultural knowledge to solve an experimental honey acquisition task. Curr Biol 19:1806–1810. https://doi.org/10.1016/j.cub.2009.08.060

Gruber T, Zuberbühler K, Neumann C (2016) Travel fosters tool use in wild chimpanzees. eLife 5:. https://doi.org/10.7554/eLife.16371

Hambidge KM (2010) Micronutrient bioavailability: Dietary Reference Intakes and a future perspective. Am J Clin Nutr 91:1430S-1432S. https://doi.org/10.3945/ajcn.2010.28674B

Harris TR (2006) Between-group contest competition for food in a highly folivorous population of black and white colobus monkeys (<i>Colobus guereza<i/>). Behav Ecol Sociobiol 61:317–329. https://doi.org/10.1007/s00265-006-0261-6

Harris TR, Chapman CA (2007) Variation in diet and ranging of black and white colobus monkeys in Kibale National Park, Uganda. Primates 48:208–221. https://doi.org/10.1007/s10329-006-0036-8

Hauser MD (1998) Functional referents and acoustic similarity: field playback experiments with rhesus monkeys. Anim Behav 55:1647–1658. https://doi.org/10.1006/anbe.1997.0712

Hladik CM (1977) A comparative study of the feeding strategies of two sympatric species of leaf monkeys: *Presbytis senex* and *Presbytis entellus*. In: Clutton-Brock TH (ed) Primate ecology: studies of feeding and ranging behaviour in lemurs, monkeys and apes. Academic Press, London, UK, pp 324–353

Hladik CM, Gueguen L (1974) Géophagie et nutrition minérale chez les primates sauvages. CR Acad Sc Paris Ser D 279:1393–1396

Hobaiter C, Samuni L, Mullins C, et al (2017) Variation in hunting behaviour in neighbouring chimpanzee communities in the Budongo forest, Uganda. PLOS ONE 12:e0178065. https://doi.org/10.1371/journal.pone.0178065

Huebl L, Akello G, Kutalek R, et al (2016) Geophagy in Northern Uganda: Perspectives from Consumers and Clinicians. Am J Trop Med Hyg 95:1440–1449. https://doi.org/10.4269/ajtmh.15-0579

Johns T (1986) Detoxification function of geophagy and domestication of the potato. J Chem Ecol 12:635–646. https://doi.org/10.1007/BF01012098

Klein N, Fröhlich F, Krief S (2008) Geophagy: soil consumption enhances the bioactivities of plants eaten by chimpanzees. Naturwissenschaften 95:325–331. https://doi.org/10.1007/s00114-007-0333-0

Kreulen DA (1985) Lick use by large herbivores: a review of benefits and banes of soil consumption. Mammal Rev 15:107–123

Krishnamani R (1994) Diet composition of the bonnet macaque (*Macaca radiata*) in a tropical dry evergreen forest of southern India. Trop Biodivers 2:285–302

Krishnamani R, Mahaney WC (2000) Geophagy among primates: adaptive significance and ecological consequences. Anim Behav 59:899–915. https://doi.org/10.1006/anbe.1999.1376

Lamon N, Neumann C, Gier J, et al (2018) Wild chimpanzees select tool material based on efficiency and knowledge. Proc R Soc B Biol Sci 285:20181715. https://doi.org/10.1098/rspb.2018.1715

Link A, Galvis N, Fleming E, Di Fiore A (2011) Patterns of mineral lick visitation by spider monkeys and howler monkeys in Amazonia: are licks perceived as risky areas? Am J Primatol 73:386–396. https://doi.org/10.1002/ajp.20910

Mahaney WC, Watts D, Hancock RGV (1990) Geophagia by mountain gorillas (*Gorilla gorilla beringei*) in the Virunga Mountains, Rwanda. Primates 31:113–120

Marler P (1969) *Colobus guereza* : Territoriality and Group Composition. Science 163:93–95. https://doi.org/10.1126/science.163.3862.93

Marsh LK, Chapman CA, Arroyo-Rodríguez V, et al (2013) Primates in Fragments 10 Years Later: Once and Future Goals. In: Marsh LK, Chapman CA (eds) Primates in Fragments. Springer New York, New York, NY, pp 505–525

Matsuzawa T (1994) Field experiments on use of stone tools by chimpanzees in the wild. Chimpanzee Cult 351–370

Munsell Color (2010) Munsell soil color charts : with genuine Munsell color chips. Grand Rapids, MI

Nevo O, Heymann EW (2015) Led by the nose: Olfaction in primate feeding ecology: Olfaction in primate feeding ecology. Evol Anthropol Issues News Rev 24:137–148. https://doi.org/10.1002/evan.21458

Newton-Fisher NE, Notman H, Reynolds V (2002) Hunting of Mammalian Prey by Budongo Forest Chimpanzees. Folia Primatol (Basel) 73:281–283. https://doi.org/10.1159/000067454

Oates JF (1978) Water-plant and soil consumption by guereza monkeys (*Colobus guereza*): A Relationship with minerals and toxins in the diet? Biotropica 10:241. https://doi.org/10.2307/2387676

Papaioannou D, Katsoulos PD, Panousis N, Karatzias H (2005) The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: A review. Microporous Mesoporous Mater 84:161–170. https://doi.org/10.1016/j.micromeso.2005.05.030

Pebsworth PA, Bardi M, Huffman MA (2012) Geophagy in chacma baboons: patterns of soil consumption by age class, sex, and reproductive state. Am J Primatol 74:48–57. https://doi.org/10.1002/ajp.21008

Pebsworth PA, Hillier S, Wendler R, et al (2019a) Geophagy among East African Chimpanzees: consumed soils provide protection from plant secondary compounds and bioavailable iron. Environ Geochem Health. https://doi.org/10.1007/s10653-019-00366-8

Pebsworth PA, Huffman MA, Lambert JE, Young SL (2019b) Geophagy among nonhuman primates: A systematic review of current knowledge and suggestions for future directions. Am J Phys Anthropol. https://doi.org/10.1002/ajpa.23724

Plumptre AJ, Reynolds V (1996) Censusing chimpanzees in the Budongo Forest, Uganda. Int J Primatol 17:85–99. https://doi.org/10.1007/BF02696160

Reynolds V, Lloyd AW, English CJ, et al (2015) Mineral acquisition from clay by Budongo Forest chimpanzees. PloS One 10:e0134075. https://doi.org/10.1371/journal.pone.0134075

Reynolds V, Pascual-Garrido A, Lloyd AW, et al (2019) Possible mineral contributions to the diet and health of wild chimpanzees in three East African forests. Am J Primatol e978. https://doi.org/10.1002/ajp.22978

Rode KD, Chapman CA, Chapman LJ, McDowell LR (2003) Mineral resource availability and consumption by colobus in Kibale National Park, Uganda. Int J Primatol 24:541–573. https://doi.org/10.1023/A:1023788330155

Said SA, Shibl AM, Abdullah ME (1980) Influence of various agents on adsorption capacity of kaolin for *Pseudomonas aeruginosa* toxin. J Pharm Sci 69:1238–1239

Sarabian C, MacIntosh AJJ (2015) Hygienic tendencies correlate with low geohelminth infection in free-ranging macaques. Biol Lett 11:20150757. https://doi.org/10.1098/rsbl.2015.0757

Schel AM, Candiotti A, Zuberbühler K (2010) Predator-deterring alarm call sequences in Guereza colobus monkeys are meaningful to conspecifics. Anim Behav 80:799–808. https://doi.org/10.1016/j.anbehav.2010.07.012

Sirianni G, Wittig RM, Gratton P, et al (2018) Do chimpanzees anticipate an object’s weight? A field experiment on the kinematics of hammer-lifting movements in the nut-cracking Taï chimpanzees. Anim Cogn 21:109–118. https://doi.org/10.1007/s10071-017-1144-0

Stambolic-Robb A (1997) Geophagy amongst free-ranging Sumatran orang-utans (*Pongo pygmaeus abelii*) of Gunung Leuser National Park, and rehabilitated Bornean orang-utans (*Pongo pygmaeus pygmaeus*) of Sungai Wain Forest, Indonesia. Masters thesis, York University

Ta CAK, Pebsworth PA, Liu R, et al (2018) Soil eaten by chacma baboons adsorbs polar plant secondary metabolites representative of those found in their diet. Environ Geochem Health 40:803–813. https://doi.org/10.1007/s10653-017-0025-4

Treves A (1999) Within-group vigilance in red colobus and redtail monkeys. Am J Primatol Off J Am Soc Primatol 48:113–126

Young SL (2010) Pica in pregnancy: new ideas about an old condition. Annu Rev Nutr 30:403–422. https://doi.org/10.1146/annurev.nutr.012809.104713

Young SL, Sherman PW, Lucks JB, Pelto GH (2011) Why On Earth?: Evaluating Hypotheses About The Physiological Functions Of Human Geophagy. Q Rev Biol 86:97–120. https://doi.org/10.1086/659884

Young SL, Wilson MJ, Hillier S, et al (2010) Differences and commonalities in physical, chemical and mineralogical properties of Zanzibari geophagic soils. J Chem Ecol 36:129–140. https://doi.org/10.1007/s10886-009-9729-y

Zuberbühler K (2000) Causal cognition in a non-human primate: field playback experiments with Diana monkeys. Cognition 76:195–207. https://doi.org/10.1016/S0010-0277(00)00079-2

FIGURE LEGENDS

Figure 1. At permanent geophagy sites, we placed two experimental stumps. One stump contained soil high in clay minerals and the other contained soil high in bioavailable iron. Both soils were routinely eaten by black-and-white colobus in the Budongo Forest Reserve.

Figure 2. Figure 2. We created experimental stumps of a known weight. (A) On the surface of the stump a hole was carved to house a known quantity of soil from two permanent geophagy sites. A smaller hole was drilled on the side to allow moisture to drain, (B) one stump was filled with approximately 5kg of iron-rich soil from that particular area (stump on left) and the other was filled with approximately 5kg of clay-rich soil from a neighboring community, Waibira (stump on right). [2B filled stumps on Figure 1].

Figure 3. Two experiment stumps were visited by two troops of black-and-white colobus monkeys. A) The number of individuals at the stumps varied during the 59-second videos from 1-11, B) adult females with an infant were present in 47% of videos, C) we saw aggression

among adults and between adults and juveniles, and D) monkeys exhibited highly vigilant behavior.