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Geophagy of Yunnan snub-nosed monkeys (*Rhinopithecus bieti*) at Xiangguqing in the Baimaxueshan Nature Reserve, China

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Abstract. Geophagy is common in extant nonhuman primate species, but the exact reasons for it across species remain unclear. Previous diet studies on Yunnan snub-nosed monkeys (*Rhinopithecus bieti*) were only focused on organic materials (plants and small animals). There are no reports on *R. bieti* exhibiting geophagy in the field before this study. This study was carried out at Xiangguqing in the Baimaxueshan Nature Reserve from June 2008 to May 2009. We recorded the behavior of geophagy and collected samples of soil consumed by the monkeys there and analyzed their content in a laboratory. We identified a total of eight sites where the monkeys consumed soil in the home range during the study period. The total time spent ingesting soil was 13,690 seconds. 20 adult males, 34 adult females except lactating mothers, and 12 immatures without infants were seen to eat soil throughout this study. Average time spent in soil-eating bouts differed significantly among age/sex classes. This study suggests that particular age/sex classes or individuals in certain states of society and health will predictably display a behavioral pattern of geophagy. Our data indicate that geophagy in *R. bieti* is best explained as a response to nutrient deficiency, as soil consumed by the monkeys was significantly higher in calcium, copper, zinc, iron, manganese, and sodium. Although *R. bieti* consumes iron-rich soil, control samples that were not consumed also had high levels of iron, suggesting that high altitude alone is not a sufficient explanation for geophagy in this species.

Key words: Yunnan snub-nosed monkeys (*Rhinopithecus bieti*), geophagy, age/sex classes, mineral.

Introduction

Nutritional requirements of non-human primates are determined by their food choices, which are in turn influenced by the spatio-temporal availability of food, their energy needs, and the presence of toxins in potential forage (Mckey et al. 1981, Milton 1984). The potential breadth of primate diets is well documented (Chapman & Chapman 1990, Grueter et al. 2009). Despite this fact, it is puzzling that many primates have been observed to eat soil or clay (Oates 1978, Heymann & Hartmann 1991, Overdorff 1993, Mahaney et al. 1997). The ingestion or consumption of soil is termed geophagy (Krishnamani & Mahaney 2000). Geophagy has been reported in some primates, as 39 extant species have been reported to eat or ingest soil (Martin 1990).

A number of hypotheses have been put forward to help explain geophagy in non-human primates. First of all, geophagy may supplement a diet otherwise deficient in minerals (Wheatley 1980, Davies & Baillie 1988, Mahaney et al. 1990). Deficiencies and imbalances of minerals are well recognized as important factors affecting animal physiology, fertility, productivity and mortality (Robbins 1983). High concentrations of minerals such as calcium, magnesium, potassium, and phosphorus can occur in soil, which could lead primates to benefit from geophagy (Zippin 1998). Mahaney (1993) reported that mountain gorillas (*Gorilla beringei*) eat clay in the Virunga Mountains of Rwanda. Second, there is also the possibility that high altitude may play a particular role in geophagy, an alternative hypothesis suggested by the behavior of mountain gorillas (*G. beringei*). The soil ingested by the gorillas contained elevated concentrations of sodium, iron and bromine. Iron is the essential element present in haemoglobin. Iron depletion may explain geophagy in gorillas living at higher altitudes, especially since they may ascend or descend up to 1000 m in elevation over short (24-h) periods (Mahaney, 1993). Thirdly, there is the hypothesis that particular age/sex classes or individuals in certain rank of society or health will predictably display a behavioral pattern of geophagy. Pebsworth et al (2012) reported that different age/sex classes of chacma baboons (*Papio cynocephalus ursinus*) have different likelihoods of consuming soil. Pregnant chacma baboons spent more time eating soil than any other baboons. Shieh et al (2001) also suggested that both the individual and social group status of Formosan macaques (*Macaca cyclopis*) played a particularly important role in soil-eating behavior at Shoushan Nature Park in southern Taiwan. Geophagy in non-human primates may also buffer toxicity of alkaloids in food (Jones 1957, Daykin 1960, Freeland & Janzen 1974, Gurian et al. 1992), and serve as an antacid within the stomach after feeding on large amounts of leaves (Goltenboth 1976, Oates 1978, Davies & Baillie 1988). Finally, regular ingestion of soils may also help to reduce primates' parasitic loads. Knezevich (1998) reported that the rhesus macaques (*M. mulatta*) of Cayo Santiago ingested soil that could counteract endoparasitic infections and increase their fitness.

Yunnan snub-nosed monkeys (*Rhinopithecus bieti*) are highly endangered non-human primates and have a total population size of approximately 2,000 animals inhabiting high-altitude forests (3000-4400 m above M.S.L.) in the Hengduan Mountains, which border the Himalaya Range (Long et al. 1994). This is a species of diurnal colobine primate with a diet based on lichens and the leaves of angiosperm plants (Kirkpatrick 1996, Ding & Zhao 2004, Xiang et al. 2007, Grueter et al. 2009). This primate lives in a very large band which is composed of a single male's core families (Grueter 2009). Starting in 2003, we occasionally observed *R. bieti* digging in the dirt during a long-term ecological study, but due to their fear of humans and the poor visibility in the dense forests, we could not confirm whether the monkeys consumed the soil. With increased habituation by 2008, we were able to confirm that monkeys indeed engaged in geophagy. This paper, not only reports this geophagic behaviour, but evaluates possible hypotheses for its occurrence.

Material and Methods

Study site and subjects

We conducted field work between June 2008 and May 2009 at Xiangguqing (27°37'N, 99°22'E) in the Baimaxueshan National Nature Reserve, Yunnan, China. The research area (90 km^2) consists of subtropical and temperate forests, previously clear-cut areas, and cattle grazing lands. Forest cover is a mosaic of mixed coniferous and deciduous broadleaf forests, subalpine fir forest, montane sclerophyllous oak forest, subtropical evergreen broadleaf forest, and pine forest. The study area is characterized by distinct seasonality in precipitation and temperature. Annual rainfall is 1,371 mm over the course of the study, with 70% of the rain falling between June and October. Mean annual temperature is 9.8°C. Temperatures may drop to – 9.3°C in winter (Li et al. 2010).

We studied geophagy in one large wild group of *Rhinopithecus bieti* at Xiangguqing, which included 480 individuals and an adult sex ratio of males to females of 1.0: 2.9 (Li 2010). The group had been well-habituated since 2006 and could be observed on occasions from as little as 20-30 m distance. We classified individuals into four age/sex categories based on body size and pelage color: adult male, adult female, juvenile, and infant (Li et al. 2010).

Data collection and soil analysis

During the study period we were able to observe animals on 120 days, for an average of 10 days per month. We used instantaneous scan sampling to record feeding behaviour with a 15 min interval, the length of this interval being very important for reliable data collection (Altmann 1974, Martin & Bateson 2001). Each scan was carried out either horizontally, alternating between "left to right" and "right to left", or between "ground to tree" and "tree to ground", depending on how the troop were dispersed. If instances of geophagy occurred, we used focal sampling to record information about geophagy. Data collected at each scan included in the topographic positions (valley, hillside or ridge), vegetation types, altitude for geophagy sites, age/sex classes of individuals, and time that each recorded individual actually eating soil (fresh mud on teeth and lips) spent doing so. The observation distance was often from far away (>100 m) to the position of the monkey group, and it was impossible to record all instances of geophagy. Meanwhile, group members were often spread out over large distances (>100 m) in the forest, and we could not collect data on each members of the group during a single scan (Grueter et al. 2009). Thus we are not sure if all geophagy activity was recorded in the field. In the course of study, some individuals were observed involved in geophagy on 61 days, and the total duration of soil consumption summed to 13,690 seconds. It should be noted that geophagy is also very different from the tuber-digging behavior that has previously been described for the same group (Ren et al. 2008). When the group left the feeding site, we collected samples of both soil that had been consumed and nearby soil that did not show evidence of soil eating. The distance was approximately 30 cm between the two soil sites (Bolton et al. 1998).

We analyzed soil samples at Soil and Fertilizer Institute in Sichuan Academy of Agricultural Science of China. We cleaned and processed soil samples by removing debris, air-drying, crushing, and finally passing through different sieves before we performed chemical analysis. Soil passed through a 2-mm sieve was used to analyze water content, pH, exchangeable element content, and available element content. Soil passed through a 0.25-mm sieve was used to analyze soil organic matter and total nitrogen. Soil passed through a 0.149-mm sieve was used to analyze total phosphorus and total potassium. All tests of the air-dried samples were conducted using the standard analytical methods described in Lu (2000).

Data analysis

We used SPSS® 17.0 and Microsoft Office Excel® 2003 to analyze the data. All statistical analyses were two-tailed with significance set at $p<0.05$. Descriptive statistics were used to examine the characteristics of geophagy sites and the individual instances of soil consumption. We used a Mann-Whitney *U* test to compare the time spent eating soil between mixed coniferous and deciduous broadleaf forest and the other vegetation types. We also used this test to compare the chemical composition of soil that had been eaten with soil that had not. The Kruskal-Wallis test was used to examine differences in time spent eating soil by age/sex class.

Results

Soil consumption

We recorded instances of geophagy in *R. bieti* on 61 days at eight different sites in the home range during the study period. The total duration of soil consumption summed to 13,690 seconds (Table 1). The range of time spent eating soil by particular individuals was 3 to 600 seconds, with an average duration of 207±129 seconds (n=66). We observed that monkeys ate soil soon after arrival at sites where geophagy occurred - they did not feed on leaves first and then eat soil.

The eight geophagy sites at Xiangguqing were scattered among four vegetation types (Table 1). The study group spent the most time eating soil (11,484 seconds) in mixed coniferous and deciduous broadleaf forest; this preference was statistically significant ($Z=-2.309$, $n_1=4$, $n_2=4$, $p=0.029$).

Geophagy by age/sex classes

We recorded 20 adult males, 34 adult females, and 12 immature individuals eating soil at all 8 sites where geophagy occurred, which accounted of 13.8% of group members. We did not ever observe infants or lactating females consuming soil (Table 1). For recorded individuals, adults of both sexes ate soil more frequently (81.8%) than immatures did (18.2%) (Fig. 2a).

Analyses of data indicated a significant difference in the average time spent per consumption of soil among adult males, adult females and juveniles ($x^2=7.561$, $df=2$, $p=0.023$) (Fig. 1). Adult males spent more time (285.3 sec) eating soil than adult females (165.3 sec) ($Z=-2.083$, $n_1=20$, $n_2=34$, $p=0.040$). Adult males usually found the geophagy site (Fig. 2b) first and spent the most time eating soil. Among the 12 immatures who practiced geophagy, 9 individuals were sub-adult males and the other 3 were juveniles of unknown sex.

Chemical characteristics of ingested vs. uneaten soil

We collected 16 soil samples in total from the 8

Table 1. Characteristics of the geophagy sites and the different sex/age classes who consumed soil at Xiangguqing.

Geophagy site	Vegetation type	Topographic position	Alt. (m)	Visited days	Total duration of soil consumption (second)	Adult male	Adult female	Juvenile	Infant
1	subtropical evergreen broadleaf forest	hillside	2731	4	523	1	3	0	0
2	pine forest	hillside	2854	8	789	2	4	2	0
3	mixed coniferous and deciduous broadleaf forest	hillside	2912	11	2568	2	6	3	0
4	mixed coniferous and deciduous broadleaf forest	valley	3039	12	4573	5	8	0	0
5	mixed coniferous and deciduous broadleaf forest	hillside	3094	12	1126	3	5	4	0
6	mixed coniferous and deciduous broadleaf forest	hillside	3216	10	3217	4	5	2	0
7	subalpine fir forest	hillside	3478	3	648	1	3	1	0
8	subalpine fir forest	ridge	3624	1	246	2	0	0	0
In total	---	---	---	61	13690	20	34	12	0

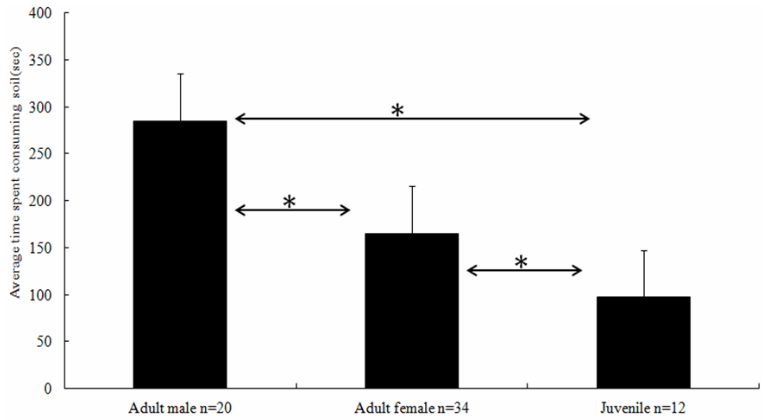

Figure 1. The average time spent per bout of soil consuming by individuals of different age/sex classes at Xiangguqing. * p<0.05.

Figure 2. a) An adult male is eating soil; b) A geophagy site in mixed coniferous and deciduous broadleaf forest.

Table 2. Chemical characteristics of eaten soil vs. uneaten soil at Xiangguqing in the Baimaxueshan Nature Reserve.

Chemical characteristics of soil sample	Ingested soil		Uneaten soil		Z	p
	Mean	SD	Mean	SD		
pH	4.16	0.30	4.17	0.55	-0.263	0.793
Water content (%)	4.50	1.10	2.74	0.87	-3.057	0.002
Available nitrogen (mg/kg)	420.06	73.02	102.29	104.74	-3.151	0.002
Available phosphorus (mg/kg)	8.66	1.44	2.82	1.14	-3.361	0.001
available potassium (mg/kg)	88.86	34.97	89.04	32.96	-0.210	0.834
Organic matter (%)	4.76	1.18	3.81	2.54	-1.892	0.059
Saline minerals (g/kg)	1.53	1.19	0.23	0.11	-2.735	0.006
Na^+ (mg/kg)	30.55	27.56	27.95	3.69	-2.521	0.012
Ca [l(1/2Ca^{2+})]	10.08	1.32	7.12	0.25	-3.258	0.001
Mg [l(1/2Mg^{2+})]	0.36	0.12	0.31	0.05	-1.422	0.155
Cu (mg/kg)	5.78	3.38	1.47	1.15	-3.153	0.002
Zn (mg/kg)	2.30	1.46	0.39	0.08	-3.257	0.001
Fe (mg/kg)	66.20	14.31	59.36	7.12	-2.100	0.036
Mn (mg/kg)	25.57	7.34	6.31	2.34	-3.363	0.001

geophagy sites, one of ingested soil and one uneaten control sample from each. Results from the chemical analysis are summarized in Table 2. Water content of ingested soil was significantly higher than that in control samples (Z=-3.057, n_1=8, n_2=8, p=0.002). Ingested soil was acidic, with pH values ranging from 3.83 to 4.71 (mean= 4.16±0.30, n=8). However, the mean pH value of the control samples was nearly the same at 4.17 (range: 3.05-4.83) and no significant difference was detected between the two ($Z=-0.263$, $n_1=8$, $n_2=8$, $p=0.793$). Available nitrogen ($Z=-3.151$, $n_1=8$, $n_2=8$, $p=0.002$) and available phosphorus ($Z=-3.361$, $n_1=8$, $n_2=8$, $p=0.001$) were found to be significantly higher in ingested soil than in the controls, but available potassium was not significantly different ($Z=-0.210$, $n_1=8$, $n_2=8$, $p=0.834$). Percentage composition of organic matter was similar between ingested and control soil samples ($Z=-1.892$, $n_1=8$, $n_2=8$, $p=0.059$). Saline mineral content of ingested soil (1.53±1.19 mg/kg, n=8) differed significantly from the controls (0.23±0.11 mg/kg, n=8) ($Z=-2.735$, $n_1=8$, $n_2=8$, $p=0.006$).

The detailed differences for some metal elements are as follows: Ingested soil had extremely significantly higher calcium ($Z=-3.258$, $n_1=8$, n2=8, $p=0.001$), copper ($Z=-3.153$, $n_1=8$, $n_2=8$, $p=0.002$), zinc ($Z=-3.257$, $n_1=8$, $n_2=8$, $p=0.001$), manganese ($Z=-3.363$, $n_1=8$, $n_2=8$, $p=0.001$) and sodium ($Z=-2.521$, $n_1=8$, $n_2=8$, $p=0.012$) content than those in the control samples. Although iron had significantly different in ingested soil than it in control samples ($Z=-2.100$, $n_1=8$, $n_2=8$, $p=0.036$), the mean iron values of control samples was 59.36 mg/kg ranging from 52.46 mg/kg to 70.63 mg/kg, which was not much lower than the 66.20 mg/kg (range: 32.28-75.24) in ingested soil. The only exception to this trend of higher mineral content in ingested soil was magnesium ($Z=-1.422$, $n_1=8$, $n_2=8$, $p=0.155$).

Discussion

Age and sex-related differences in patterns of geophagy

Our data confirm that Yunnan snub-nosed monkeys (*Rhinopithecus bieti*) consume soil at this field site. However, we did not record all monkeys consuming soil, and these individuals ingesting soil did not all consume soil as frequently as one another.

Rhinopithecus bieti also commonly dig up the tubers of hemlocks (*Tsuga dumosa*) at this study site, but this behavior was not associated with geophagy (Ren et al. 2008). Notably, all age/sex classes of the Xiangguqing group have been observed digging tubers, including lactating mothers with their infants (Ren et al. 2008). Yet lactating mothers did not consume the mineral-rich soil. This conclusion is not the same for all primates (Knezewich 1998, Pebsworth et al. 2012). All age/sex classes in chacma baboons (*Papio cynocephalus ursinus*) have been found to eat soil at special geophagy sites, and in fact, pregnant females spent more time consuming soil (Pebsworth et al. 2012). However, only adult females of *Rhinopithecus roxellana* were observed to eat soil in Qinling Mountains (Zhao et al. 2013).

Adult males spent more time engaging in geophagy than other age-sex classes in this study This may indicate that adult males might be able to handle greater amounts of certain kinds of toxins than females and immature individuals due to their larger body size. This trend was reported in Sichuan snub-nosed monkeys (*R. roxellana*) (Ren et al. 2007), which like *R. bieti*, display marked sexual dimorphism in body weight (Kirkpatrick 1996).

Geophagy as mineral supplementation

Lichens and leaves make up the bulk of the diet (50.6% and 16.3% respectively, totaling 66.9%) of *R. bieti* at Xiangguqing (Li et al. 2011), which are foods with relatively poor protein and mineral contents (Kirkpatrick 1996, Kirkpatrick et al. 2001, Fashing et al. 2007). The soil consumed was both acidic and moist. Geophagy occurred only at eight particular locations throughout the entire home range of the study group during the study of period. Owing to the difference in mineral content between ingested and control samples but the comparative uniformity of moisture and organic content, we assert that those minerals were responsible for the presence of geophagy in our study animals, rather than organic nutrients.

It is well known that mineral supplementation plays an important role in the geophagy of nonhuman primates (Wheatley 1980, Davies & Baillie 1988, Heymann & Hartmann 1991, Ketch et al. 2001). Minerals can play a key role in animal physiology, fertility, productivity, and mortality (Robbins 1983, Brightsmith et al. 2008). Other studies have found that soil consumed by primates often has higher levels of zinc (Zn) (King & Keen 1999), calcium (Ca), potassium (K), phosphorus (P), sodium (Na), and iron (Fe) (Fossey 1983, Mahaney et al. 1996, Smith et al. 2000, Brightsmith et al. 2008). Our result suggests that ingested soil for *R. bieti* has higher mineral contents; therefore we thought that some important minerals may be supplied when *R. bieti* ate soil in the wild.

Geophagy high altitude hypothesis

Mahaney et al. (1990) analyzed the geophagic sites of mountain gorillas (*Gorilla beringei*) and found that the soil eaten by those gorillas contained elevated concentrations of sodium, iron, and bromine. Humans living at high altitudes need iron-rich food to increase erythrocytes in the blood (Stickney & van Liere 1953). The explanation of geophagy as a strategy for coping with higher iron requirements at altitude is evidently supported by Mahaney's (1993) study of *G. beringei* and Mahaney and Hancock's (1990) study of African buffaloes (*Syncerus cafer cufer*), which also range at high altitude (>2400m above sea level). In the present study, we found that Yunnan snub-nosed monkeys, which live in mountain forests at elevations between 3000 and 4400m (Long et al. 1996), consumed iron-rich soil. However, iron concentrations were high in both ingested and control soil samples. If iron deficiency induced *R. bieti* to engage in geophagy, then individuals could eat soil from anywhere, and their site fidelity would not make sense. Thus, we conclude that geophagy in *R. bieti* cannot be explained solely as a reaction to iron deficiency.

This study only tested why Yunnan snub-nosed monkeys consumed soil at particular locations. We did not collect any data on mineral intake at the individual level. We cannot currently analyze the mineral requirements of wild *R. bieti*. In summary, we believe that the pattern of geophagy exhibited by our study group is most consistent with explanations that behavioral plasticity by age, sex, and reproductive status, and inconsistent with iron supplementation required by a high-altitude lifestyle. We also conclude that the reasons for geophagy in *R. bieti* are particular to this species in this habitat and may be different from those found for other primate species. Our results may also indicate a role for mineral supplementation in cases where provisions from humans make up a significant portion of the diet of wild monkey groups (Li et al. 2012).

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