Forest Pharmacy

by Michael A. Huffman

Michael Huffman, a professor at Kyoto University's Primate Research Institute in Japan, has been studying the chimpanzees of Mahale since 1985. He observed and documented the first scientific evidence for self-medication in animals. He also found that the plant in question was used by humans for treating similar ailments - with similar outcome to that of the chimpanzees.

As a young boy growing up in Denver Colorado in the 60's, summer for me was a time to run barefoot, climb trees and explore the wonders of urban nature. I was always putting something into my mouth - young grass shoots, roots, berries, seeds, clay, you name it, everything yet unexplored was fair game. Although they never said so, I think my parents were probably a little concerned about my gustatory tendencies, especially when I would lick a rock or eat a small chunk of dirt! Had I known what I do today, I would have calmed their fears and explained that I was simply behaving like any respectable young primate.

I look back fondly on those days of summer, freedom from homework and the confines of the four walls of a classroom. Had someone pointed it out to me then, I would have no doubt denied it but in retrospect, during those balmy days of summer, I remained 'in school and learning' - learning about the tastes and smells of my environment. Now, more than thirty years later, I am still climbing into trees and putting new things into my mouth and smelling new smells - yes, even bits of termite mound clay and the occasional pith or leaf. My childhood cravings and serendipitous foraging in the suburban jungles have transformed into scientific curiosity -

investigating why animals in the wild medicate themselves and how they learn to do so.

My interest in the topic of animal self-medication came about quite accidentally while routinely following a habituated group of wild chimpanzees in the Mahale Mountains of Western Tanzania. It all started on November 21, 1987. Mohamedi and I had been following a small group of females and their offspring when it became apparent that one of them, an adult female, named Chausiku, was patently ill. For the next hour, Chausiku quietly slept in a tree nest while her companions moved off to feed

on figs and succulent ginger pith - taking her young son Chopin along with them. When they returned, Chausiku slowly

Chausiku and Chopin

climbed down and they all moved on together. Chausiku's progress was painfully slow as she frequently had to stop and rest. The others continued on ahead, but always remained in sight. At one point in the afternoon, Chausiku went directly to and sat down in front of a shrub. She pulled down several new growth branches about the diameter of my little finger, placed them all on her lap and removed the bark and leaves of the first branch to expose the succulent inner pith. She then bit off small portions and chewed on each for several seconds at a time as she extracted and swallowed the juice, frequently spitting out most of the remaining fibres. She had eaten nothing else for the last two hours since we had been following her.

Not being familiar with the plant I asked Mohamedi in Swahili, our common language of communication, what it was called in the local Tongwe vernacular. He replied, "Mjonso". Mohamedi Seifu Kalunde, now a game officer of the Mahale Mountains National Park worked with the Kyoto University

chimpanzee research project since it started in the early 1960's and is widely acknowledged by researchers and fellow Tongwe tribesman for his vast knowledge of the local flora. He was trained by his mother and grandfather in the art of herbal medicine.

While Chausiku continued to feed, her three adult companions and their offspring sat nearby feeding on hefty elephant grass shoots and ginger pith but showed no interest in the plant she was eating. Chopin was interested, however, and begged for a piece of pith hanging from his mother's mouth. He picked up a piece, which fell from her mouth and a section of the discarded leaf and bark from the ground. After briefly sniffing and putting these things in his mouth he quickly discarded them and continued chewing on his little piece of ginger. This is a typical response for all young primates, and is one way that they learn what foods the group eats. However, Chopin's interest in this bitter tasting new plant was thwarted as he quickly discarded it after only a small taste.

Puzzled about the plant, I asked Mohamedi if chimpanzees consumed it very often, adding that I had not seen them, before today, to show any interest in it during my ten months of observations. He nodded and agreed that it is indeed not eaten frequently, but that chimpanzees

probably would not eat too much because it is very bitter. I next asked Mohamedi if the Tongwe have a traditional use for it. Ever patient of my questions, he smiled and said, "Yes, it is very strong Dawa (medicine)." "What kind of Dawa?", I ask in amazement. He replied saying it is most commonly used for stomach upset, malarial fever and intestinal parasites.

Chausiku climbed up in the trees and made her night nest unusually early that day, long before the sun began to sink behind the landscape on the opposite side of Lake Tanganyika. Early the next day we were able to find Chausiku again and followed her until late afternoon. Up until midday, her condition did not improve much. She rested frequently, moved slowly and ate little. However, after about an hour rest, nearly 24 hours after ingesting the bitter piths of Mjonso, her behaviour abruptly changed. For the next 38 minutes we had to run at times, just to keep up with Chausiku, as she rapidly moved through the forest leaving Chopin and his caretaker for the day trailing behind. They ended up in the swampy grassland near the lake where Chausiku then feed voraciously

An adult male eating pith of ginger

on ginger pith, figs, and large succulent shots of elephant grass.

Later investigation of the ethnomedicinal literature and personal interviews showed that for numerous ethnic groups across Africa, a concoction made from Vernonia amygdalina is a valued treatment for malaria, schistosomiasis, amoebic dysentery, various intestinal parasites, stomach upset and a host of other ailments. Phytochemical analysis by my colleagues, Professors Koichi Koshimizu and Hajime Ohigashi (Kyoto University), using samples we collected at Mahale, revealed the presence of two major classes of biologically active compounds. They found a total of four previously known sesquiterpene lactones and nine steroid glucosides new to science. Laboratory analyses by other collaborators in Japan, France and the UK using these isolated compounds, verified their effects against parasites responsible for malaria, dysentery and bilharzia. From extracts of the leaves, Koshimizu's laboratory found significant inhibition of the tumor promotion process and immunosuppressive activities. The cytotoxic sesquiterpene lactones were found to be most abundant in the leaves and bark, the parts that chimpanzees always avoid. This is quite interesting given the leaves can be lethal if ingested in large amounts, as is sometimes observed among domestic goats in West Africa. Uganda farmers feed their pigs branches of Vernonia amygdalina in controlled amounts to treat intestinal parasites. A number of bitter Vernonia species found across Africa and Central America are known both for their wide ethnomedicinal use and pharmacological effectiveness against gastrointestinal related ailments, including parasite infections.

The observations Mohamedi and I made those two days in November were to become the first documented evidence of a sick animal consuming a plant with demonstrated medicinal properties and recovering from that illness - the first striking scientific evidence for self-medication in animals. However, the

The daily ritual of grooming

story does not end here. Since then we have documented other instances of the use of Vernonia, again showing improvement in health and identifying the parasite infection likely responsible. Not only do Mahale chimpanzees and local humans use this same medicinal plant, but they also appear to use it for the same illnesses and show signs of recovery over the same time period.

Throughout human history, animals have captured our imagination and been a source of legends and practical knowledge. The Navaho and other indigenous North Americans, credit the sacred brown bear to their acquisition of 'Osha' (Ligusticum porteri) - a medicinal plant with demonstrated antiviral and antibacterial properties. Bears will deliberately dig up the roots and chew on them. Going into a 'catnip-like' frenzy, they spread the mixture of juice and saliva on themselves. In South America capuchins do the same with aromatic plants, as do coati bears with tree sap. The meaning of this behaviour is not known, but some believe it functions to help repel ectoparasites. Mohamedi's grandfather credited his

discovery of an important treatment for dysentery to his observations of a sick young porcupine ingesting the roots of plant found outside the park. This plant thus far has been identified to me only by it's local name 'mulengele'. These are but a few examples and there is still much to be learned. Steps must simultaneously be taken to insure that both the environment and animals are protected so that they are still around to be studied.

How do animals learn to medicate themselves? In non-human primates important benefits come from social learning which allow naive individuals to acquire information through the experience of others, and over time to perfect the behaviour themselves. Once an effective self-medicative behaviour is recognized, it will likely spread through the group, first slowly, but after a time increasingly faster as it is passed onto the youngest members. At Mahale, and presumably elsewhere, initial exposure to self-medicative behaviours take place by individuals at an early age, not when ill themselves, but by observing the behaviour and tasting the items used by others that are ill - most often their mothers.

In the case of Vernonia amygdalina, it is not just what species of plant to use, but also the context, appropriate plant part, and in what amount they are to be ingested, that must be learned for the plant to be effective and safe to use. It is argued that bitter taste perception has evolved as a means of avoiding toxic substance. In plants, such substances are produced to prevent them from being eaten by insects and large herbivores. The observational learning process is undoubtedly an important component. Given the high degree of conservatism in chimpanzee feeding habits, the random sampling of novel items, especially when ill, does not seem likely to occur very frequently. If so, these

traditions must have started sporadically, perhaps as a result of ill, hungry chimpanzees trying new foods during periods of extreme food scarcity, recovering their health, and associating their improvement with the new item.

Several points taken from the observation of Chausiku and others help to illustrate what I feel are some important elements of the learning process. Chimpanzees have a strong capacity for empathy and good long-term memory. Any associations made between the change in a sick individual's feeding habits and subsequent recovery are likely to reinforce the learning of both context and the details of plant use. On numerous occasions Chausiku's travel companions coordinated their activities to be near her and thus had ample opportunity to observe her self-medicative behavior in proper context. This probably works strongly for naive dependent offspring like Chopin. The medicinal context of bitter pith chewing appears to be understood. While all of the adults in the group have been observed to use this plant at some time or another, they never seem to show interest in it simply because it is available along the trail or because somebody else is eating it. Learning in chimpanzees is basically a unidirectional process - that is information is only acquired by observational learning and duplication, not through instruction. Chausiku made no attempt to either encourage or prevent her son from tasting the bitter plant. While the connection between his mother's illness and her using this bitter plant to relieve discomfort may not be made by young Chopin in one single observation, over time, he will learn the context in which the plant is eaten and begin to use it properly. Contextually correct self-medicative behaviour should

thus begin at different ages for different individuals depending upon such factors as individual experience, the health of one's mother, seasonal and yearly patterns of disease transmission. This may begin as early as two to three years of age.

Taste perception and physiological feedback in response to the ingestion of bitter plants are likely to play a supportive role in the learning process. Historically, herbalists have emphasized the importance of taste and smell in the evaluation of plant medicinal properties. In a study of the criteria of medicinal plant selection by the Tzeltal Mayans of Highland Chiapas in Mexico, John Brett (University of Colorado-Denver) noted taste and smell were consistently used to select or evaluate a plant to treat related illnesses. Regardless of taxonomy, bitter tasting plants were selected and predominantly used to treat gastrointestinal upsets, parasites and stomach pains. This close correspondence between bitter taste and pharmacological activity may also aid chimpanzees in their selection of plants for treating parasite infection and related gastrointestinal illness based on previous experiences.

Selective association between taste and gastrointestinal illness is a widely accepted principal of taste aversion learning among mammals and the learning mechanism of food aversion in response to induced sickness has been well-documented in a number of animal species. The capacity to associate improved health after eating novel plants that have medicinal properties has received less attention, but the highly adaptive significance of this process is self-evident. Such biological and psychological processes in conjunction with observational learning are argued to constitute the core of non-human primate self-medicative behaviour and are surely the roots of the human cultural practice of medicine.

Chimps eating figs in trees.

The fundamentals of associating the medicinal properties of a plant by its taste, smell and texture have their roots deep in our primate background. A major turn of events in the evolution of medicine is likely to have come about in early humans with the advent of language, enabling us to share and pass on detailed experiences about plant properties and their effect against disease. Another major event in human history is considered to have been the attainment of food preparation and detoxification technologies, which allowed humans to exploit a wider range of plant life to be used as food. Timothy Johns (McGill University, Quebec) argues that it was this turning point that might actually have increased our dependence on secondary compounds of plants, because of their disappearance from the daily diet. In this way perhaps, a greater specialisation of plant use, specifically as medicine, came about.

The current level of sophisticated medicinal practices in traditional human societies is likely the product of the greater variety of diseases and stress, brought about by a subsistence revolution - from hunting and gathering

to a greater dependence on domestic crops and livestock. Our early modern human hunting and gathering ancestors may have had a smaller pharmacopoeia, but this was not because of any lack of technical sophistication. More likely it was because of fewer diseases and stress to respond too.

Over the history of humankind, people have developed elaborate health-care systems based on experimentation and observations of nature. We in the 'developed world' have much to learn from the people of traditional-living cultures as well as from our primate cousins who continue to live closely with nature. What Mohamedi and I witnessed over those two days in 1987 was the beginning of a very productive study that has continued to this day. I have continued to expand the scope of this research and I am now collaborating with colleagues working at a number of other field sites throughout Africa and more recently in Southeast Asia. The laboratory side of this research now includes collaborations with pharmacologists, parasitologists, veterinarians, physiologists, ethnobotanists and others in countries around the globe including Japan, Tanzania, Uganda, Kenya, Sweden, United States, Canada, United Kingdom, Australia, France, Holland, Denmark, Germany and the Czech Republic. Our goal is not only to understand better how animals learn to deal with illness, but also to search for new medicines and most importantly ecologically sound strategies for the treatment of disease inflicting those living in the tropical countries of the world.

Thanks to the technological advancement of modern medicine, the lives of millions of people are saved or prolonged every year. However, it is also technological advancement - that brings about the changes in our diets and lifestyles - that is responsible for the modern diseases on which nations spend fortunes every year, trying to find cures for. We do not have to turn back the clock or abandon civilization to regain a piece of that paradise lost. However, we have much to re-learn and gain from the ancient wisdom of our primate ancestors and the wealth of traditional plant medicine being practiced by a large, but often ignored, majority of the world's people. ✿

Michael Huffman will be in New Zealand to speak at the Holistic Animal Health International Conference and Expo, 2 - 5 June. For details contact the Conference Organiser: NZHATA, PO Box 906, Tauranga, email: nzhata@clear.nz

Photos in text taken by Michael A. Huffman

VIVAMUS

Self-induced Increase of Gut Motility and the Control of Parasitic Infections in Wild Chimpanzees

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*When physiological adaptation is insufficient, hosts have developed behavioral responses to avoid or limit contact with parasites. One such behavior, leaf-swallowing, occurs widely among the African great apes. This behavior involves the slow and deliberate swallowing without chewing of whole bristly leaves. Folded one at a time between tongue and palate, the leaves pass through the gastro-intestinal (GI) tract visibly unchanged. Independent studies in two populations of chimpanzees (*Pan troglodytes schweinfurthii*) showed significant correlations between the swallowing of whole leaves and the expulsion of the nodule worm* Oesophagostomum stephanostomum *and a species of tapeworm (*Bertiella studeri*). We integrate behavioral, parasitological and physiological observations pertaining to leaf-swallowing to elucidate the behavioral mechanism responsible for the expulsion and control of nodule worm infections by the ape host. Physical irritation produced by bristly leaves swallowed on an empty stomach, increases motility and secretion resulting in diarrhea which rapidly moves leaves through the GI tract. In the proximal hindgut, the site of third-stage larvae (L3) cyst formation and adult worm attachment, motility, secretion and the scouring effect of rough leaves is enhanced by haustral contractions and peristalsis-antiperistalsis. Frequently, at the peak of reinfection, a proportion of nonencysted L3 is also predictably vulnerable. These factors should result in the disruption of the life cycle of* Oesophagostomum *spp. Repeated flushing during peak periods of reinfection is probably responsible for long-run reduction of worm burdens at*

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certain times of the year. Accordingly, leaf-swallowing can be viewed as a deliberate adaptive behavioral strategy with physiological consequences for the host. The expulsion of worms based on the activation of basic physiological responses in the host is a novel hitherto undescribed form of parasitic control.

INTRODUCTION

We integrated new findings and current knowledge of leaf-swallowing behavior in chimpanzees with gut function and life-histories of *Oesophagostomum* spp. to ascertain the mode(s) of action likely responsible for their expulsion by chimpanzee hosts.

Parasitism has played an important role in the evolution of host behavior (Anderson and May, 1982; Clayton and Moore, 1997; Futuyma and Slatkin, 1983). Coevolution between host and parasite has resulted in the development of mechanisms by which the host limits parasitic infection and the parasite increases its chance of infecting the host (Toft *et al.*, 1991; Ewald, 1994). At the primary level, a host's immune response—innate and acquired—normally controls infections; however, some parasites invariably establish themselves by undergoing antigenic variation, thus disguising themselves with the hosts antigens, or by interfering directly with the immune response (Cox, 1993; Wakelin, 1996).

When physiological adaptation is not enough, hosts have developed behavioral responses to avoid or to limit contact with endo- and ectoparasites and other pathogens. These behaviors, which are widespread among the vertebrates, include regularly changing sleeping or feeding sites, differential use of drinking sites, use of antiparasitic leaf material to line nests or dens occupied over long periods, and the application of aromatic substances to repel fur and feather infesting parasites (Baker, 1996; Clark, 1991; Freeland, 1980; Gompper and Holyman, 1993; Hart, 1990; Hausfater and Meade, 1982; Kummer in Nelson, 1960; Seigstadt in Cowen, 1990; Sengputa, 1981). Learned aversion of foods or tastes associated with illness, parasitic infection, and compensatory changes in host dietary preferences induced by parasites are exhibited in the laboratory and field by a wide range of vertebrates (Gustavson, 1977; Kyriazakis *et al.*, 1994; Keymer *et al.*, 1983). The learned aversions are another level at which the host avoids prolonged exposure to pathogens. Dietary modification is another possible means to alter or control internal parasitic load. Rausch (1954, 1961) suggested a causal relationship between a sudden change in diet and reduced tapeworm load in brown bears (*Ursus americanus*). Another example of this type of behavior

involves the ingestion of specific plant parts that have little or no nutritional value for their antiparasitic qualities, which may be either pharmacological (Huffman *et al.*, 1993; 1998; Ohigashi *et al.*, 1994) or physical (Huffman *et al.*, 1996). We will discuss the latter.

Swallowing of whole leaves and subsequent defecation of undigested material was first reported in chimpanzees by Wrangham (1977) and has been investigated subsequently in greater detail by Wrangham and Nishida (1983), Wrangham and Goodall (1989) and Huffman *et al.*, (1996; 1997). Chimpanzees frequently swallowed leaves early in the morning, often as the first or one of the first items ingested (Wrangham and Nishida, 1983; Wrangham and Goodall, 1989). These observations led us to define leaf-swallowing as the slow and deliberate ingestion one by one of whole leaves, which are folded between tongue and palate, and pass through the gastro-intestinal tract visibly unchanged. Similar behavior occurs widely in the African great apes; chimpanzees (*Pan troglodytes*), bonobos (*Pan paniscus*) and lowland gorillas (*Gorilla gorilla gorilla*) (Huffman, 1997).

Independent studies of the behavior in two populations of eastern long-haired chimpanzees (*Pan troglodytes schweinfurthii*) showed significant correlation between swallowing whole leaves and the expulsion of the strongyle nematode *Oesophagostomum stephanostomum* at Mahale (Tanzania), and a species of tapeworm (*Bertiella studeri*) at Kibale, Uganda (Huffman *et al.*, 1996; Wrangham, 1995). Similar behavior may also have evolved convergently in at least two other vertebrate taxa; the snow goose (*Anser caerulescens*) and the Alaskan brown bear (*Ursus arctos*: Huffman, 1997).

Several ideas have been advanced to explain the early morning habit of leaf-swallowing. All were proposed to explain the effective absorption of a pharmacologically active agent into the body without damage by bright light or stomach pH or to avoid dilution of the agent's effective strength by other foods in the stomach (Newton and Nishida, 1990; Rodriguez and Wrangham, 1993; Wrangham and Goodall, 1989). However, unlike the pharmacological action of pith of *Vernonia amygdalina* that is chewed by chimpanzees apparently for its pharmacological antiparasitic properties (Huffman *et al.*, 1993; 1998; Ohigashi *et al.*, 1994), a primarily pharmacological mode of action for leaf-swallowing, proposed earlier by Rodriquez and Wrangham (1993) has been ruled out for the great apes (Huffman *et al*, 1996; Messner and Wrangham, 1996; Page *et al.*, 1997). The mode of action appears instead to be based on physical properties of the leaves leading to the expulsion of parasites. However, the significance of swallowing leaves in the early morning and the details of the mechanism of worm expulsion remain unclear (Huffman *et al.*, 1996). Elucidation of this mechanism is crucial to understand this behavior and is expected to enhance understanding of host-parasite interactions.

METHODS AND MATERIALS

Study Site and Subjects

Huffman observed the M group of chimpanzees in the Mahale Mountains National Park, Tanzania with the assistance of Game Officer Mohamedi Seifu Kalunde between 23 November 1993 and 25 February 1994. The study site is on the eastern shore of Lake Tanganyika. The climate is influenced by weather from the lake and the mountainous terrain, which ranges from 772 m at lakeside to 2,500 m above sea level on the mountains. Chimpanzees are supported mainly by the semideciduous gallery forests between 780-1,300 m in elevation. There is a rainy season from approximately mid-October to mid-May (Takasaki *et al.*, 1990). We worked in the early rainy season months because of seasonal peaks in the occurrence of leaf-swallowing behavior (Huffman *et al.*, 1997; Wrangham and Nishida, 1983) and the correlation between the rainy season and a rise in parasitic infections (Huffman *et al.*, 1997), most notably by *Oesophagostomum stephanostomum*.

Behavioral Data and Fecal Collection

We observed subjects by focal-animal and *ad libitum* observations, recording in detail the occurrence of all behaviors. We followed subjects continuously throughout the day until lost or the night nest was made. We noted visible cues to state of health, paying specific attention to deviations in normal activity patterns—frequent resting, suppressed foraging activity, early nesting at night and late exit from the night nest in the morning—involuntary behaviors diagnostic of illness and/or discomfort—coughing, sneezing, flatulence, wheezing and stool type.

We monitored fecal output of the focal subject continuously and as closely as possible. When possible we also monitored the nearest neighbors. We inspected faeces quickly for presence or absence of whole leaves and adult parasitic worms. We collected feces with whole leaves or worms and stored it in plastic bags for further inspection at camp, where we counted the leaves and worms and stored the worms in ethanol.

Concurrently with behavioral notes, we collected fecal samples for parasitological analysis immediately after discharge and placed them individually in 5.0 ml Corning sterile vials. We weighed vials and their contents at camp and prepared and fixed 1-g samples with a 10% neutral formalin solution. We thoroughly mixed contents in the vial before sealed and stored it in a cool dark room. The samples were later microscopically examined at the Kyoto University Primate Research Institute by S. Gotoh. Via the MGL and

McMaster's techniques (expressed as eggs/g feces: EPG), we identified species and measured parasitic loads. We made 3 egg counts for each sample and calculated the EPG for each sample as the mean value derived from those trials.

Oesophagostomum stephanostomum in Mahale Chimpanzees

The nematode parasites of *Oesophagostomum* (Strongyloidea, Oesphagostominae)—nodular worms—are common parasites in the proximal hindgut of pigs, ruminants, primates and rodents. Confusion still exists regarding their taxonomy, but several species of *Oesophagostomum* occur in gorillas (g), chimpanzees (c), and occasionally humans (h) including; *O. bifurcum*, (h, c), *O. aculeatum*, (h), *O. polydentatum* (c, g), and *O. stephanostomum* (c, g, h) (Brack, 1987; Polderman and Blotkamp, 1995; Yamashita, 1963). The species in the gastro-intestinal tract of Mahale chimpanzees has been morphologically identified as *Oesophagostomum stephanostomum* from adult worms by R.C. Anderson, Dept. of Zoology, University of Guelph, Canada and from larvae by J. Blotkamp, Dept. of Parasitology, University of Leiden, The Netherlands (Huffman *et al.*, 1997; Gasser *et al.*, 1999).

A brief summary of the life cycle of *Oesophagostomum*, based on Anderson (1992), is essential to understand the likely impact of leaf-swallowing on the control of infections by the of nodular worms (Fig. 1). Eggs are laid at the 16–32 cell stage. While in the feces, the eggs rapidly develop into L1 rhabditiform larvae, hatching as early as 24 h under optimal conditions. The larvae feed on bacteria and molt to the L2 stage within 24 h of hatching. Within 3–4 days of hatching the L2 molt to become infective L3. L3 retain the protective cuticle of L2 and are capable of surviving long periods of adverse environmental conditions, e.g., hot-dry conditions of the dry season, in a state of dormancy. Infection occurs via ingestion of filariform L3 larvae that are on vegetation eaten by the host. After ingestion, L3 pass to the cecum, where they exsheath within approximately 3 days of ingestion. They then invade the tunica mucosa, stimulating the formation of separate cysts around individual larvae in the gut wall. The larvae develop there to L4 stage, eventually returning to the lumen of the hindgut as immature adults. Adults attach by suction to the mucosal wall, but detach and move freely in search of food and mates.

In adult hosts, which may have developed some degree of immunity to the parasite, tissue reaction may cause larvae to remain in their mucosal cysts. The presence of mature worms in the lumen also inhibits the emergence of
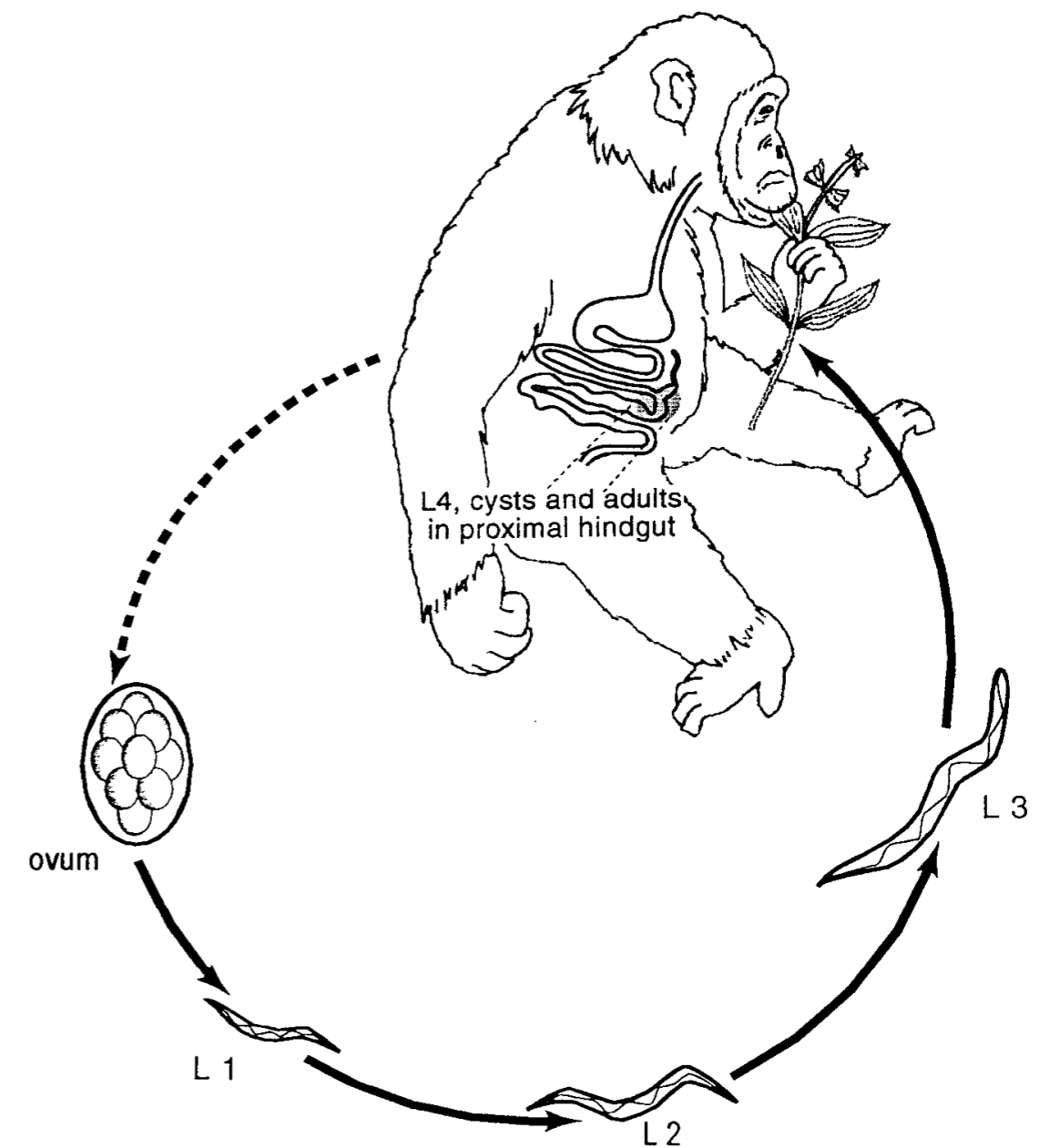
Fig. 1. Life cycle of *Oesophagostomum* spp.

L4 larvae from the nodules (Boch, 1956 cited in Anderson, 1992; Taylor & Michel, 1953). Once in the lumen, the larvae molt and reach the adult stage. Females begin to reproduce and deposit eggs after about one month postinfection.

While infections are carried year-round, reinfection occurs mainly during the rainy season in the Mahale chimpanzees (Huffman *et al.*, 1997). Reinfection, noted by a significant elevation in individual eggs-per-gram (EPG) counts, occurs approximately 1-2 mo after the onset of the rainy season, regardless of which month the rains first begin (Huffman *et al.*, 1997). This lapse

in time corresponds with the 1 mo prepatent period of *Oesophagostomum* spp. (Anderson, 1992). Arrested larval development occurs in *Oesophagostomum* spp. (Armour and Duncan, 1987; Krepel *et al.*, 1994), and at Mahale, shortly after the onset of the rainy season, the external environmental conditions of rising humidity and temperature become optimal for rapid development of the eggs and larvae. The marked rise in individual EPG levels at the beginning of the rainy season is probably due to the increase in transmission by infecting L3 larvae from the environment. However, it is possible that larvae that underwent arrested larval development in the mucosa also play a role. Some nodular worm species are a significant pathogen in domestic livestock, nonhuman primate and occasionally human hosts (Brack, 1987; Karim and Yang, 1992; Lumb *et al.*, 1985; McClure and Guilloud, 1971; Polderman and Blotkamp, 1995; Rousselot and Pellissier, 1952). In humans symptoms of moderate to heavy infections of *Oesophagostomum stephanostomum, O. bifurcum and O. aculeatum* reportedly range from weight loss, enteritis, diarrhea, anemia and lethargy to anorexia and intense pain simulating appendicitis. Pathology includes hemorrhagic cysts containing larvae, septicimia resultant from bacterial invasion of lesions in the colon, blockage of the colon due to gross thickening of the wall, epigastric or periumbilical masses clearly visible from the outside. Gross lesions in submucosal or subserosal nodules (5–20 mm diameter) are associated with the larvae. These cysts or nodules contain caseous, necrotic centers in a fibrous capsule. Histological findings typically includes intense inflamatory cell reaction accompanied by high levels of neutrophils and eosinophils associated with the nodules (Brach, 1987; Polderman and Blotkamp, 1995; McClure and Guilloud, 1971).

Pathology is believed to be caused only by the encapsulated juvenile worms, not by the adult worms, so a low EPG does not necessarily mean that the host is not affected by some form of pathology. Subsequent reinfection by larvae developing from eggs newly deposited in the feces is no doubt also a contributing factor. Dry season rises in EPG and leaf-swallowing can occur. They are presumably the result of the cessation of arrested larval development within the mucosa (Taylor and Michel, 1953) due to the depression of the host's immune response caused by such factors as secondary illness and food-stress (Huffman *et al.*, 1997), rather than reinfection. Larvae of *Oesophagostomum* spp. can remain dormant within the host for ≤1 year (Gordon, 1949). The trends in seasonality of reinfection at Mahale are in close agreement with seasonality in life cycle data from clinical observations of a rural human population in Togo and Ghana infected with *Oesophagostomum bifurcum* (Krepel *et al.*, 1995). Given the severe pathology associated with *Oesophagostomum* species in general, the removal of encysted L3 and L4 stages (Polderman & Blotkamp, 1995; Taylor & Michel, 1953),

or maintenance at low levels or both is a highly-adaptive host strategy for controlling the level of damage caused by the parasites.

RESULTS

Time of Swallowing, Transit Time of Leaves and Stool Type

We recorded 14 cases of leaf-swallowing performed by 12 individuals (Table I). The time of leaf swallowing in the morning or the time leaves first appeared in the feces in the afternoon was closely associated with weather conditions of the previous night and that morning. In general, whole leaves appeared in the feces of chimpanzees later on days when the morning was cloudy and rainy or heavy rains had fallen the previous night than on clear days. Typically chimpanzees stay in their night nests longer on rainy days, likely to avoid the cold and dampness of the underbrush and also because they are likely to have been kept awake by heavy rains in the night. On such days, we observed leaf-swallowing between 0912 and 1035 h (n = 9; cases 1–10), and subsequently leaves were in the faeces between 1542 and 1638 h.

A conservative duration calculated from the lower and upper time limits of swallowing and defecation of whole leaves is 6–6.5 h. Subtracting these figures from the lower and upper time range that leaves were in the feces on clear days (ca. 1200–1535 h), an estimated swallowing time on clear mornings is between 0530 and 0900 h. This period fits precisely in the typical waking schedule of Mahale chimpanzees on clear mornings, i.e. between dawn and the time radiant sunlight has reached most areas of the group's home range. This also conforms closely to the data available for Gombe. On average, leaf-swallowing at Gombe occurred around 0715 h and often leaves were the first ingesta of the day in 75% (n = 40) of the observations (Wrangham and Goodall, 1989).

Effect on Gastro-Intestinal Function and Transit Time

Leaf-swallowing temporarily induces diarrhea and reduces transit time. Among the 14 cases of leaf-swallowing in Table I, there is a significant relationship between the presence of whole undigested leaves in feces and the occurrence of diarrhea (Fisher's exact test, p = 0.0472, n = 24). No long-term adverse effects were evident because diarrhea did not persist and the individual's stool type varied throughout the day (Table I).

In 3 cases (3, 9, 13) we closely monitored individuals from the time of leaf-swallowing to the first feces containing leaves (Table I). This

Table I. Time of leaf swallowing, defaecation without leaves and first time with leaves, stool type and estimate of leaf transit times

Case	Date	Weather[1]		Subject, observation period starts - ends//	Time swallowed (no. leaves)	Time of defaecation[2] before leaves appear	Time first leaves appear (leaves, worms)	Estimated TT[3] (h)
1.	10 Jan. 1994	rainy night	AL	09:12//	09:12 - (9+)	no data	no data	
2.	10 Jan. 1994	rainy night	MJ	09:39–13:11	09:39 - (14)	11:19, 12:06	no data	
3.	7 Feb. 1994	rainy, dark	NT	09:55// 13:35–17:16	09:55 - (5+)	not observed	15:56*, 15:57* (55:21)	6.02
4.	7 Feb. 1994	rainy, dark	BE	10:00// 10:58–17:31	10:00 / (6+)	12:28, 12:32, 15:56	not observed	
5.	7 Feb. 1994	rainy, dark	IW	10:20// 12:00	10:20 / (9+)	12:00	not observed	
6.	10 Jan. 1994	dark, rainy	LD	10:30//	10:30//	no data	no data	
7.	7 Feb. 1994	rainy, dark	JL	10:32//	10:32 - (20)	no data	no data	
8.	7 Feb. 1994	rainy, dark	CY	10:33//	10:33 - (33)	no data	no data	
9.	7 Feb. 1994	rainy, dark	BB	10:35–10:51 // 13:35–17:16	10:35 - (55)	15:54	16:38*	6.3
10.	8 Feb. 1994	dark, cloudy	BE	11:41–16:10	not observed	not observed	15:42* (4, 0)	4.02 → 8.5[5]
11.	19 Feb. 1994	clear	NT	11:40–15:30	not observed	not observed	11:58* (1, 2) 15:27	
12.	6 Feb. 1994	clear	DE	09:23–14:51	not observed	12:40	12:45* (not counted, many)	3.4 → 5.5[5]
13.	25 Nov. 1993	clear	MU	08:11–15:10	08:00–08:10	8:37, 9:45, 9:47, 12:14, 12:23	13:44* (not counted, many)	5.6
14.	27 Dec. 1993	clear	TL	09:31–17:50	not observed	10:50	15:07, 15:36* (18 : 18)	5.6

[1]Weather conditions of previous night and early morning leaf swallowing was observed.
[2]Stool type: italicized times are diarrhea otherwise stools were firm. *worms found in dung.
[3]Estimation of TT calculated from the observed time of leaf-swallowing (or the onset of focal observations) to first time defaecation of leaves was observed. There estimates (cas nos. 3, 9, 13) are considered the best approximations of TT in this study based on direct evidence.
[4]Observations started approximately 10 m after subject climbed down from night nest.
[5]Adjusted estimate of TT derived by subtracting average leaf swallowing time of 7:15 on clear mornings from first time leaves were observed in dung.

measurement is the digesta transit time (Warner, 1981). The estimated transit time (TT) for these 3 cases ranged from 5.5 to 6.3 h, which agrees closely with the conservative calculated range based on observed morning swallowing times and independently observed afternoon defecation times on rainy days. Two of the estimates for TT on clear days were notably shorter (3.5–4.8 h). This may be due to the fact that observations did not begin until late morning (Table I). If the figures are corrected by calculating TT based on an average clear day, swallowing time of 0715 h (mean of the estimated range of clear day swallowing time), the resultant TT in both cases (5.5 h; 8.5 h) closely fits the general trend. The mean of estimates of TT in Table I is TT 6.2 h (SD 1.1, n = 6) for swallowed whole leaves. When leaves are swallowed whole first thing in the morning on an empty stomach, there is ≥ 17.4 h reduction in TT versus the digesta transit of 23.6 h (SD 2.4, n = 8) for a fruit meal, fed to captive chimpanzees (Idani, 1986). The estimated (6.23 ± 1.15 h, n = 6) and best approximate TT (5.97 ± 3.5 h, n = 3) means are in close agreement.

It cannot be overstressed that these are estimates of the upper and lower limits of TT for whole leaves swallowed by chimpanzees in our study. TT modified by leaf-swallowing is expected to vary depending upon the total number of leaves swallowed and certainly upon whether or not the individual had eaten other foods before leaf-swallowing. Due to small samples size we are unable to evaluate the possible effect of the number of leaves swallowed on the variability of TT.

Impact of Leaf-Swallowing on Parasitic Load

The presence of adult *Oesopghagostomum stephanostomum* in the feces of Mahale chimpanzees is an extremely rare event in any situation (3.7%, 9/245, Huffman *et al.*, 1997). However, when in feces of individuals on days when they swallowed leaves, they were most likely in the feces with whole leaves (Fisher's Exact Test, p = 0.0001, n = 24; Table I). Live adult *Oesophagostomum stephanostomum* were in the liquid fecal material or trapped within the folded leaves. On average, there were 10.3 worms (SD 10.8, range 2–21) with 19.8 leaves (SD 25.6, range 1–55) per stool, at a rate of 0.54 worms per leaf expelled via leaf-swallowing (n = 4; Table I). There are discrepancies, however, in each case between the number of leaves we saw subjects swallow and the number of leaves subsequently recovered from their feces (Table I). Accordingly, the figures are conservatively low estimates of the possible immediate impact of leaf-swallowing on worm burden.

We know of no data on total worm burdens of *Oesopghagostomum stephanostomum* in wild chimpanzees. For *Oesophagostomum bifurcum* in

humans, an average burden of 96 worms (SD 89.06, range 12–300, n = 12) can occur in rural people of Togo and Ghana (calculated from Table I. of Kreple and Polderman, 1992). Extrapolating from our data, theoretically a chimpanzee would need to pass at least 176 leaves or engage in leaf-swallowing on average 10 times over the rainy season to rid itself of a comparable worm burden. The actual number of leaves swallowed by an individual in a single case ranged from 5 to 55. This scenario suggests that repeated swallowing of leaves by chimpanzees over consecutive days or weeks could have a significant impact on their overall worm burden. A more decisive approximation of total impact would require controlled clinical testing of chimpanzees infected with predetermined worm burdens, but, due to ethical considerations such a study is not feasible.

DISCUSSION

Hypothesized Mechanism of Leaf-Swallowing for the Expulsion of *Oesopghagostomum stephanostomum*

Three factors are important to understand the effects of leaf-swallowing on the gastro-intestinal tract of the chimpanzees and ultimately on the parasites in the proximal hindgut, namely, the timing of leaf-swallowing, increased rate of digesta transit, and the diarrhea associated with the appearance of leaves in the feces. Much is known about the function of the gastro-intestinal compartments of *Homo sapiens*, and it is reasonable to assume that the chimpanzee gut functions similarly. This assumption is based on the similarities in gastro-intestinal morphology, and the close phylogenetic relationship of the two species. The gastro-intestinal tract can be divided into three distinct units. Regulation of the activity of each of them is independent of the others, nevertheless the physiological state of another gut compartment influences the others through neurochemical mechanisms (Guyton, 1976; Weisbrodt, 1987).

The patterns of motility in each gastro-intestinal units and the timing of leaf-swallowing behavior are crucial to understand the effect of the leaves on the gut. Leaf-swallowing commonly occurs before the first meal of the day (Wrangham and Goodall, 1989; Huffman *et al.*, 1996). The stomach and small intestine of the chimpanzees would have emptied overnight, so that the interprandial pattern of slow waves of contraction—myo-electrical activity—would have been established in them (Weisbrodt, 1987; Malgelada and Azpiroz, 1989). Swallowing the leaves would immediately stimulate the full stomach pattern of increased secretion and motility. The contractions of the external muscle layer would thus bring the rough surfaces of the leaves

into direct contact with the gastric mucosa causing physical irritation. This effect would be enhanced by emptiness of the stomach. The presence of the mucosal irritant would have two effects, which are part of a protective mechanism for the removal of potentially damaging substances (Guyton, 1976):

1. increased secretion of highly acidic gastric juice;
2. increased motility of the hindstomach allowing the leaves and secretions to pass quickly through the pyloric sphincter into the small intestine.

The rate of movement of the leaves through the small intestine would also be rapid and would be accompanied by an increase in secretion (Guyton, 1976; Malagelada and Azpiroz, 1989). Clearly digesta transit time is increased by leaf-swallowing (Table I). The TT of seeds from fruit meals in captive chimpanzees was approximately 23.6 h (Idani, 1986). The mean transit time of a particle marker (chromium mordanted to cell wall constituents) in chimpanzees fed a commercial diet ranged from 37.7 to 48.0 h, depending on the fiber content of the diet (Milton and Demment, 1988). Comparison of these results with times in wild chimpanzees after leaf-swallowing shows that there is a marked reduction in the estimated transit time for a single meal (Table I).

Whole leaves, with their abrasive surfaces and the corrosive mixture of secretions that accompany them, would be emptied into the proximal hindgut, thereby stimulating increased secretion and motility there. The pattern of movement of the cecum and proximal colon is different from that of the small intestine. It consists of alternating bouts of peristalsis and antiperistalsis, as well as localized mixing movements within individual haustra (Elliott and Barclay-Smith, 1904; Christensen, 1989; Caton, 1997). The movements of the leaves back and forth, as well as within the haustra, would help to dislodge the worms. Also, even subtle changes in the environment of the lumen can have significant impact on establishment and persistance of *Oesophagostomum* in the host (Petkevicius *et al.*, 1999). Any resultant change in the contents of the lumen, caused by secretions of acid and enzymes from the stomach, plus enzymes from the small intestine, are likely to affect adult worms as well as any pre-encysted L3 larvae in the lumen. The contents of the hindgut would be evacuated rapidly before there is a chance for the reabsorption of water, resulting in the diarrhea that is often associated with leaf-swallowing. Prolonged bouts of leaf-swallowing would produce a continual source of irritation.

The control-of-nematode-infection hypothesis (Huffman *et al.*, 1996; 1997) predicts that since nodular worm infections are typically self-limiting (Beaver *et al.*, 1984), the total infection may be controllable if a chimpanzee

responds to the symptoms during the most likely period of reinfection. Based on the observed physiological response of the gut to whole rough leaves and details of the life cycle of *Oesophagostomum*, we conclude that the most probable mechanism explaining the control of nematode infection (Huffman *et al.*, 1996) results in at least three areas of antiparasitic action (Fig. 2); 1) adult worm detachment and expulsion, 2) flushing out a proportion of the incoming infective L3 larvae before they are able to exsheath and penetrate the mucosa, and 3) decreasing pathology of cysts (and reducing discomfort) by inducing the emergence of juvenile larvae into the lumen. This process, repeated continually during peak periods of reinfection should result in a lowering of overall infection and control the number of dehabilitating cysts.

A Convergent Evolutionary Strategy for the Expulsion of Intestinal Parasites?

Leaf-swallowing has also been associated with the presence of tapeworm proglottids in feces of chimpanzees, geese and bear and with roundworms in dogs. Wrangham (1995) demonstrated that passing whole leaves through the gut increases the probability of tapeworm fragments being shed by chimpanzees at Kibale.

Observations of undigested plant leaf material (*Carex* sp. Cyperaceae) and large masses of tapeworms in the feces of Alaskan brown bears in the late fall by Barrie Gilbert (Department of Fisheries and Wildlife, Utah State University) led him to suggest that grass swallowing may be responsible (Huffman, 1997). In hibernating grizzly bears, Parasitic levels, in particular tapeworms and ascarids, are quite low or absent (Rausch, 1954; 1961). The fact that grass-swallowing in bears occurs before hibernation suggests a causal relationship.

John Holmes (Parasitology, Department of Biological Sciences, University of Alberta) has observed a similar phenomena in Canadian snow geese. In the summer prior to their migration south, juvenile birds in particular, carry significant tapeworm burdens. Also at this time of year, Holmes and his colleagues observed large boluses of undigested grass and tapeworms in goose faeces. When they measured the parasite loads of these same flocks after migrating south, the middle to lower guts were found to be completely void of tapeworm infection; no scolices were found (J. Holmes, personal communication in Huffman, 1997).

AE Makundi (Veterinarian, Faculty of Veterinary Medicine, Sokoine University, Morogoro, Tanzania) has observed the expulsion of roundworms (*Ascaris toxicara*) by dogs swallowing grass (personal communication to M.A.H).
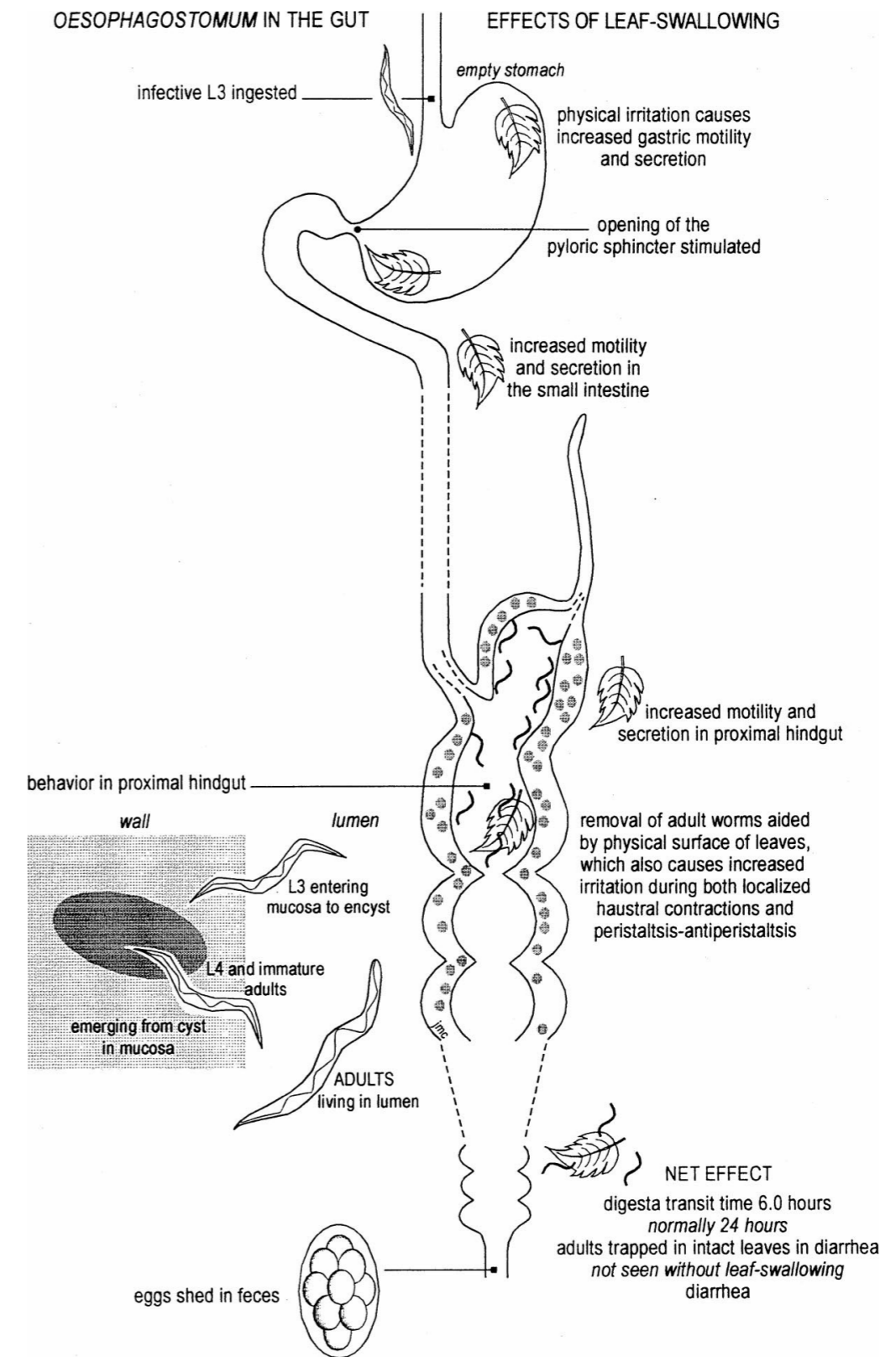
Fig. 2. The proposed mechanism of leaf-swallowing and its effect on infections with *Oesophagostomum* at different stages of the life cycle.

Given the wide phylogenetic diversity of these three species it is possible that such an adaptation occurs among a much wider range of vertebrates infected with these and other gastro-intestinal parasites. We predict that increased transit time induced by a leaf-swallowing like behavior will play a significant role in any host with similar gut function infected with *Oesophagostomum* spp.

CONCLUSIONS

1. Control of infections by repeatedly flushing out worms during peak periods of infection is thought to lead to the long-run reduction of worm burdens.
2. Repeated flushing of the GI tract is predicted to disturb the life cycle of *Oesophagostomum* spp. at three key stages of the life cycle; reducing new L3 infections taking hold in the mucosa, removing mature adult worms from the lumen and, encouraging encysted L4 and immature adults to exit into the lumen, whereupon they are removed from the gastro-intestinal tract of the host.
3. Leaf-swallowing is a deliberate action that is an adaptive behavioral strategy with physiological consequences for the host apparently lacking an efficient immunological defense against a parasite's virulence to the host.
4. Leaf-swallowing is a strategy which may have convergently evolved in several vertebrate taxa.
5. This non-pharmacological expulsion of worms based on the activation of basic physiological responses in the host is a novel hitherto undescribed mechanism of parasite control.

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VIVAMUS

The Adaptive Significance of Geophagy for Japanese Macaques (*Macaca fuscata*) at Arashiyama, Japan

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*We gathered data on the amount, composition, and rate of ingestion of foods and soils by the provisioned Japanese macaques (*Macaca fuscata fuscata*) at Arashiyama, Japan. Behavioral observations spanned one year on 8 adult females, using focal animal sampling. We analyzed a subsample of their foods for nutritional and toxic secondary compound content. We also analyzed soils eaten by the macaques for several physical-chemical properties and tested their adsorption affinity to tannins and alkaloids. Geophagy occurred at a high rate of 2.97 g/indiv./day with an elevated frequency in the afternoon. About two-thirds of their foods (by fresh weight) were provisioned items, which are extremely rich in proteins and soluble carbohydrates. The soils that they ingested were generally poor in mineral elements, the bio-availability of which was low. The soils had a high adsorption capacity for plant alkaloids but were poorly*

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absorptive for tannins. They were rich in clay minerals of proven buffering capacity. Geophagy at Arashiyama may improve the health of macaques via buffering gastric upset. We discuss the results from the viewpoint of several hypotheses on geophagy.

INTRODUCTION

Functional interpretations of geophagic behavior—the deliberate ingestion of soil—amongst nonhuman primates vary as it occurs across a wide taxonomic spectrum and at many geographic locations. Among the possible functions of geophagy, the following have been suggested: 1. taste enhancement (Mahaney *et al.*, 1995b); 2. mineral supplementation (Marriott *et al.*, 1993a; Mahaney *et al.*, 1990; Heymann and Hartmann, 1991; Ganzhorn, 1987); 3. an antidiarrheal mediator (Mahaney *et al.*, 1995a & b; Knezevich, 1998; Mahaney *et al.*, 1996); 4. adjustment of digestive disorders (Oates, 1978; Kraulen, 1985) 5. detoxification of deleterious chemicals in plant foods (Mahaney *et al.*, 1995b; Muller *et al.*, 1997; Hladik, 1977 a & b; Wiley and Katz, 1998; Julliot and Sabatier, 1993) and 6. a behavior with no apparent physiological benefits (Inoue, 1987). Davies and Baillie (1988) noted that geophagy possibly serves multiple roles concurrently or distinct roles at different times.

There have been few detailed attempts in primate studies to directly test these hypotheses, either singly or in combination. Indeed, the majority of reports on geophagy are difficult to compare directly due to methodological inconsistencies (Klaus and Schmid, 1998). Moreover, the predominant study design has been to compare eaten versus uneaten soils to detect the physical-chemical cues that may attract animals to ingest them rather than to show causal links between geophagy and diet. This traditional bias in research methodology has lead to a prevalent lack of empirical data on other features of geophagy. For instance, little is understood of the amount or frequency with which soils of known composition are habitually sought after and ingested by the respective primate species. In the absence of data as basic as the magnitude of geophagy, it remains difficult to comprehend its advocated benefit(s)—regardless of the actual content of the soils being ingested.

Geophagy is a common phenomenon in herbivorous animals (Kraulen, 1985); whereas there is yet little evidence for its occurrence in strict carnivores such as lions (Moe, 1993). The close link between geophagy and herbivory suggests that the intake of plant foods underscores the need to ingest soil. If this is so, the relevant question is: In which way(s) do(es) herbivory benefit from geophagy? Surely, blanket explanations for it remain unjustified

since benefits to the foragers are expected to vary according to the ecological context in which it is practiced (Klaus and Schmid, 1998). It follows therefore, that in order to gain further insight into the causes of geophagy, we need to gather systematic, relevant and simultaneous information on plant foods and soils as actually ingested by animals in specific locales. Such an approach will be necessary to narrow our scope of interpretation and to delineate explanations that are plausible for particular dietary conditions. This paper is a step towards fulfilling this goal. We quantified the year-round amounts of foods and soils ingested by individual Japanese macaques of known identity. We also carried out detailed chemical analyses of the soils eaten together with bioassays of their potential interaction with select bio-active plant food compounds. We report on the observed patterns in quantity and quality of their diet in relation to composition, quantities and frequency of soil ingestion and discuss the results from the viewpoint of several previously proposed hypotheses for the adaptive significance of geophagy.

STUDY METHODS

Study Area and Subjects

The study site is the Iwatayama Monkey Park, Arashiyama, located at an altitude of 330 m asl. on the western outskirts of Kyoto city, Japan at 35°00′N and 135°40′E. The soils are only briefly described as dry brown with a heavy clay texture dominated by shale, sandstone and chert (Mahaney et al., 1993). The macaques have been systematically provisioned and studied since 1954, though they also range and feed freely on the surrounding natural vegetation (Huffman, 1984, 1991). They typically consume soils from numerous spots scattered throughout their range that they variably excavate and abandon. Intensive habituation to human presence has made it possible for investigators to observe them at close proximity. Extensive research on them has yielded numerous publications (Fedigan and Asquith, 1991). The study group, Arashiyama E troop, had 158 members of which 107 were females and 51 males.

FIELD PROCEDURES

Behavioral Sample Size

Wakibara conducted behavioral sampling from 5 May 1998 to 30 June 1999 covering roughly 12 mo. We present data collected from 8 adult females between 13 and 24 years olds. Since social ranking adversely affects feeding

behavior in provisioned macaques (Soumah and Yokota, 1977), equal proportions of both high- and low-ranking individuals are included in the sample. Of the 123 total field visit days, Wakibara monitored the daily behavior of the subjects in full for a total of 100 days yielding an efficiency of about 81%. Complete observation days per subject fluctuated between 8 and 15 with a mean of 9 days. Wakibara observed each individual for between 8 and 40 hrs per season yielding a mean of 97.5 focal observation hrs/subject over the whole study period.

Quantification of Foods Eaten

To quantify the amount of foods eaten, we used the focal-animal observation method (Altmann, 1974) on the 8 adult female subjects. Normally Wakibara collected data from 0900 hr up to 1700 hr when the monkeys usually retired, except on a few occasions in late autumn when the daylight hours decreased and observations were terminated between 1600 and 1700 hr. Each day, Wakibara followed one individual for as long as possible, with a continuous record of the time spent in four behavioral categories: feeding; resting; social activity; and moving. He detailed records of feeding to include duration and number of ingestions of specific food units such as one fruit, one seed, one leaf, or one flower depending on the habitual intake of respective units by the monkeys. When the view of a subject became obscure, but it was apparently feeding, Wakibara estimated the amount of food units consumed from the time spent feeding, based on earlier or succeeding complete records for such units for that individual (Iwamoto, 1974, 1988; and Hladik, 1977a, b). The natural food categories are leaves, grass, seed, fruit, bark, flower, pith and others (animal matter and fungi). During intake of fixed provisioned foods (alternately wheat or soybeans depending upon supply in fixed amounts per feeding time), Wakibara timed and counted their ingestion until the subject seemed to be satiated or moved away from the feeding ground. Wakibara recorded additional foods fed to them by park visitors similarly. He counted food units via a hand-held tally counter, timed feeding with an electronic stop watch to .01 sec, and weighed items to 0.01 g via a portable electronic balance. It was thus possible to estimate the individual specific daily intake of food units by fresh weight. We include only incidences in which a focal subject was observed for >7 hr per focal day in the statistical analyses.

Quantification of Soils Eaten

For the same focal individuals, Wakibara recorded geophagy via a method similar to that of other feeding. He recorded the amount and

duration of soil intake for each geophagic event—defined to commence with the act of ingesting ≥1 morsel of soil in a particular spot and to end when the subject moved away to engage in activities other than geophagy. A brief switch (≤30 sec) to a nearby spot and resuming geophagy constituted a continuation of the preceding event. We tallied the number of events in a given day as the geophagic frequency for that day. During soil-eating, the monkeys would typically nibble on morsels and drop others in the immediate vicinity. We estimated mean diameter and weight of morsels from 300 bits of various sizes collected from three sites >10 m apart. We also recorded geophagic events from nonfocal subjects *ad libitum* (Altmann, 1974). Wakibara counted and weighed soil units like other ingested items.

LABORATORY PROCEDURES

Nutritional Quality of Sample Foods

We collected about 100 g of fresh sample material for each of the 17 regularly eaten natural leaf food species on a single day in June 1999. We also collected equivalent amounts of provisioned foods: wheat, soybeans, potatoes, peanuts. Shortly after collection, we sealed the samples in preweighed polyvinyl bags, labeled them by species, and weighed them via a portable electronic balance. We preserved them in an icenon cooled ice box while transporting them to the Section of Ecology laboratory at the Primate Research Institute (PRI), Kyoto University, where they were analyzed by Wakibara. In the laboratory, we oven-dried samples at 60°C and 76 cmHg for ≥20 hr until constant weights were attained and consider weight lost to be water content. We milled the dried samples for 30 sec into a fine powder and analyzed them in triplicate following standard analytical procedures derived from Allen (1989). We determined protein by the micro-Kjeldahl method using K_2SO_4 and $CuSO_4$. We collected the distillate in 0.1N sulfuric acid and titrated it with 0.1N NaOH. We calculated protein content using the standard conversion factor of 6.25 times total nitrogen. We extracted lipids via anhydrous ether in a micro-Soxhlet apparatus and determined total ash by ignition. We determined fiber content by igniting the remains of acid-alkali boiling. We estimated the amount of total nonstructural carbohydrates as the percent dry weight balance for fat, fiber, ash and protein. We used the crude fiber method to determine the amount of fiber in this analysis for logistical reasons, but it is considered outdated by the detergent system of fiber analysis (Robertson and Van Soest, 1980). Our crude fiber results fall within reasonable limits.

Analysis of Soil Composition

Huffman collected representative eaten and control soil samples in May 1997 from sites regularly utilized and from ones never utilized by Arashiyama macaques. As a positive control, he purchased Kaolinite, in August 1998 from the Bossou market in Guinea West Africa where it is sold as a traditional cure for acid stomach upset. He sent the samples to Canada and Germany where we analyzed them. For particle size and mineralogy at the Geomorphology and Pedology Laboratory of York University and for geochemistry using the SLOWPOKE Reactor Facility of the University of Toronto in Toronto. At Heidelberg, we conducted *in vitro* bioassays of adsorptive affinities for sample plant secondary compounds likely to occur in wild plant foods.

Particle Size and Mineralogy

Mahaney conducted particle size analysis via methods of Day (1965) and Mahaney (1990) with sands determined by wet washing the air-dried equivalent of 50 g of oven-dried soil through a 63 μm stainless steel sieve. He determined the fine fractions of silt and clay by sedimentation. He determined the mineralogy of the <2 μm fraction by centrifuging a subsample of the clay slurry onto ceramic tiles (1956), giving oriented mounts, and X-rayed the samples via CuK alpha radiation generated by a Toshiba ADG-301H X-ray diffractometer.

Geochemistry

The major, minor and trace elements were measured by Aufreiter and Hancock via instrumental neutron activation analysis (INAA). They weighed approximately 500-mg subsamples of the bulk materials into polyethylene vials and analyzed them per procedures of Hancock (1978; 1984). Two neutron irradiations and four counts were required to produce a database of 35 elements for each sample. They prepared extracts of selected samples by shaking approximately 1 g of soil in 10 mL of 2.0 pH ammonium oxalate solution for 30 min, followed by gravity filtration through Whatmann 42 ashless filter paper. The filtrates stood for 48 hr to allow very fine particles to settle; then they weighed approximately 1 mL volumes of the filtrates into polyethylene vials and heat-sealed them. They analyzed them by INAA for short-lived, isotope-producing elements and (with selected samples) also for long-lived isotope-producing elements (Hancock, 1978; 1984).

Sodhi used X-ray photoelectron microscopy (XPS) to estimate bioavailability of minerals on the surface layers of the soil samples. He obtained the

spectra on the Leybold MAX 200XPS system. He prepared samples for XPS analysis by pressing the dried powdery soils onto adhesive copper tape, removing loose particles with light gas pressure, and mounting the tapes on a sample holder. Un-monochromatized A1 K alpha X-ray radiation was the excitation source. He ran the source at 15 keV and 25 mA. He performed large area analysis (4×7 mm^2) to minimize collection time while maximizing signal. He obtained relative atomic percentages from survey spectra (1000–02V, step size 800 meV) note space between data and units run in a low resolution mode (pass energy = 192 eV). He normalized the data to unit transmission of the spectrometer via a routine provided by the manufacturer (Berresheim *et al.*, 1991). He obtained peak areas of the elemental core levels of interest from the survey spectra via ESCATOOLS (Surface Interface Inc. Mountain View, Ca) and converted them to relative atomic percentages using sensitivity factors empirically derived by the manufacturer. The surface specific nature of XPS limited depth analysis to 7–10 nm.

Food Toxicity and Detoxification by Soils

Wink and Reich tested directly the capacity of soils to adsorb alkaloids and tannins in two wild plant food species ingested by the monkeys: *Quercus glauca* (acorns) and *Styrax japonica* (seeds) collected by Huffman in September 1997 at Arashiyama. For comparison, we also tested the action of soils on selected standard alkaloid solutions, which is representative for toxic secondary metabolites. We purchased atropine, sparteine and quinine and isolated, lupanine from *Lupinus albus*. We suspended approximately 150 mg of dried and pulverized soil in 1.5 mL of phosphate buffer (pH 7), then, added 150 μl of an alkaloid solution (0.035 mol/l) and shook the mixture for 5 sec. on a vortex. We repeated shaking after 5, 10, 15 and 20 min, followed by centrifugation at 12,000 rot/min for 5 min. Then we made 0.7 mL of the supernatant alkaline (pH 12) with 1M KOH and subjected it to solid liquid extraction via ChemElute columns. We eluted the sample with methylene chloride (3×2 ml) and analyzed the crude alkaloid extract by capillary gas chromatography. We used external standards of the authentic alkaloids and blanks (samples without soil) to quantify the adsorbed part. We analyzed each sample in triplicate.

We prepared the tannin solution of *Quercus glauca* by heating 500 mg of pulverized acorns with 50 mL distilled water at 100°C for 10 min, cooling it down to room temperature and centrifuging it (4000 rot/min; 10 minutes). Quantification of amount of tannins in the acorns follows the method of Folin-Denis (Harborne and Turner, 1984). To quantify their adsorption by soils, we shook 1 mL of the supernatant, 1 mL of distilled water, and 250 mg

of dried and pulverized soil twice for 5 sec. on a vortex within a 3-min span. After centrifugation (4000 rot; 10 min; RT), we added 1 mL of the liquid to 5 mL of reagent (0.1% p-dimethylamino-cinnamaldehyde in methanol + hydrochloric acid 25% (3:1; v/v)) and shook it, for 5 sec. After 10 min, we measured the absorption at $\gamma = 640$; the blank being 1 ml H_2O +5 ml reagent.

We assessed the haemolytic effect of *Styrax japonica* seed case saponin on rabbit erythrocytes. We separated the blood cells from plasma by centrifugation at 2500 rot/min and washed them with 0.9% Na-Cl solution. We boiled 250 mg of pulverized seed cases for 1 min in 2.5 ml distilled water. After centrifugation (10 min; 4000 r.p.m. at 20°C) we took two samples of 0.5 mL from the supernatant and mixed them with blood cells. We equilibrated the first sample with charcoal for 1 min and then centrifuged it (10 min; 4000 rot/min at 20°C). We made dilution sequences (1:5 to 1:3125) for both samples. After 1 hr, we shook each sample for 5 sec and kept them for 2 hrs before we measured them via UV/VIS spectroscopy at 415 nm. We repeated the procedures for seeds and seed shells.

RESULTS

BEHAVIORAL ANALYSIS

Intake of Natural Plant Foods

The natural plant species that focal subjects ate during the behavioral study of 1998–1999 are in Table I. They ate parts of 31 species from at least 19 families, from which they obtained about 34% of their total food on a fresh weight basis. They consumed foliage in the greatest proportion by weight (73%), but *Ischne globosa* (Graminae) alone accounted for 75% of the ingesta, indicating that grass is a major leafy food item for them. Among non-grass foliage, the important species are *Eurya japonica* (20%); *Quercus glauca* (14%); and *Prunus grayana* (13%).

The proportion of non-foliage plant parts in the natural diet, except for seeds and acorns is very low: fruits and flowers (6.7%), bark (1.9%), seeds and acorns (16%) by fresh weight. However, there was an obvious seasonality in their intake (Fig. 1). The typical seasonal pattern by plant part is bark (winter), leaves/fruits/flowers (spring), grass (summer), seeds and acorns (autumn). An item that we bio-assayed for toxicity, *Quercus glauca* acorns was their nearly exclusive food item for autumn, accounting for 86% of the natural diet in that season by fresh weight. Low-ranking monkeys consumed significantly more natural foods throughout the study period (Mann-Whitney U = 749.00; N1 = 49; n2 = 51; p < 0.006).

Table I. List of natural foods eaten by plant part and percentage eaten by wet weight

Plant Species	Family	Japanese name	Total no. foraging days	LF	SE	FR	PT	BK	FL	% Total species wet weight eaten
Ischne globosa	GRAMINAE	Sasa	52	100	—	—	—	—	—	63.67
Eurya japonica	THEACEAE	Hisakaki	23	100	—	—	—	—	—	10.01
Quercus glauca	FAGACEAE	Arakashi	42	22.1	28	—	50.2	—	—	5.56
Rhabdosia inflexa	LABEATACEAE	Bekiokoshi	21	0.63	93	—	6.4	—	—	3.64
Prunus grayana	ROSACEAE	Uwamizakura	14	100	—	—	—	—	—	3.50
Aphananthe aspera	ULMACEAE	Mukunoki	21	3.4	97.6	—	—	—	—	3.08
Prunus yamazakura	ROSACEAE	Yamazakura	15	30.4	—	55	—	—	14.5	2.61
Aucuba japonica	STERCULIACEAE	Aoki	9	100	—	—	—	—	—	0.96
Osmanthus frangans	STYRACEAE	Mokusei	12	89.2	0.8	—	—	—	10	0.90
Cryptomeria japonica	TAXOCIACEAE	Kitayamasugi	8	14.8	—	—	—	85.2	—	0.84
Chamaecyparis obtusa	CUPRESSACEAE	Hinoki	7	—	—	—	—	100	—	0.78
Osmanthus fortunei	STYRACEAE	Hiragi	19	100	—	—	—	—	—	0.74
Camellia japonica	THEACEAE	Tsubaki	12	100	—	—	—	—	—	0.69
Rhododendron sp.	ERICACEAE	Tsutsuji	12	100	—	—	—	—	—	0.64
Quercus acuta	FAGACEAE	Akagashi	9	100	—	—	—	—	—	0.57
Rhamnus crenata	RHAMNACEAE	Isonoki	13	100	—	—	—	—	—	0.40
Acer palmatum	ACERACEAE	Oomomiji	8	—	—	—	—	—	—	0.33
Quercus acutissima	FAGACEAE	Kunugi	10	100	—	—	—	—	—	0.24
Quercus serrata	FAGACEAE	Konara	7	100	—	—	—	—	—	0.21
Castanopsis cuspidata	FAGACEAE	Sudaji	7	100	—	—	—	—	—	0.17
Diospyrous kaki	EBENACEAE	Kaki	10	100	—	—	—	—	—	0.16
Osmanthus heterophyllus	STYRACEAE	Hiragi	8	100	—	—	—	—	—	0.16
Ilex pendunculata	AQUIFOLIACEAE	Soyogo	10	100	—	—	—	—	—	0.13
Unidentified (1)			7	100	—	—	—	—	—	0.06
Unidentified (2)			6	100	—	—	—	—	—	0.02
Sedges	CYPERACEAE		8	100	—	—	—	—	—	0.01
Clethra barvinervis	CLETHRACEAE	Ryobu	9	100	—	—	—	—	—	—
Rhus trichocarpa	ANACARDIACEAE	Yamaurushi	7	100	—	—	—	—	—	—
Ilex chinensis	AQUIFOLICACEAE	Nanaminoki	7	100	—	—	—	—	—	—
Unidentified (3)			7	100	—	—	—	—	—	—
Stryrax japonica	STYRACEAE	Egonoki	2	—	100	—	—	—	—	—

Abbreviations: LF (leaf), SE (seed), FR (fruit), PT (pith), BK (bark), FL (flower).
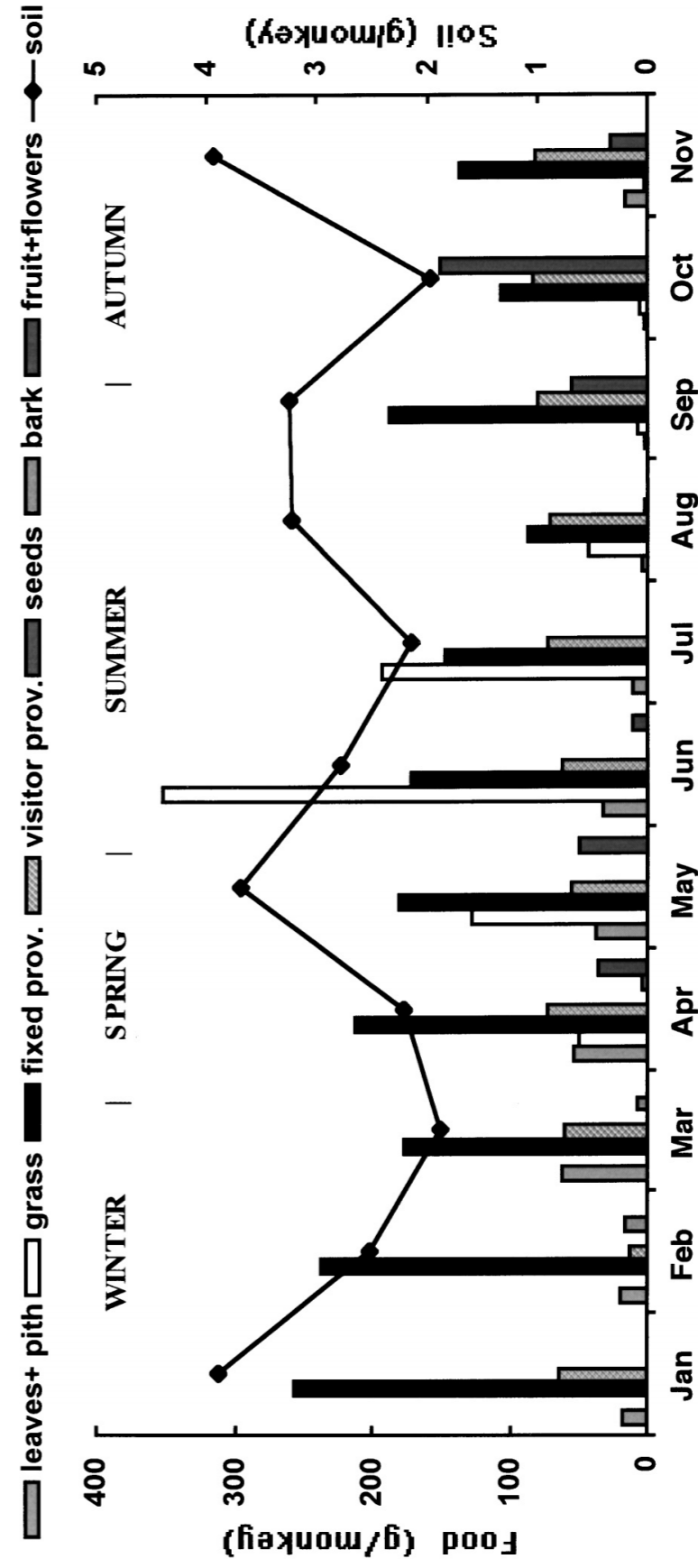

Fig. 1. Annual trend in intake of provisioned foods, natural foods and soils by Arashiyama macaques.

Intake of Provisioned Foods

Intake of provisioned diet versus natural diet is illustrated in Fig. 1. On average, focal subjects ate 153 g of provisioned grain per day, but there were wide individual differences (range 0-541.62; SD $\pm$ 124.93; N = 100). Since the total daily food consumption was 361.19 g/monkey/day by fresh weight, (range 0–1022.64; SD $\pm$ 187.60; N = 100) it is apparent that the fixed provisioning source alone—wheat and soy beans—provided about 46% of the daily mean weight of food intake. Inclusion of provisions—peanuts, sweet potatoes, and apples—from visitors (given from inside a fence windowed hut) increases the artificial dietary intake by a further 20% to about two thirds (66.32%) of daily mean intake. Clearly the monkeys subsist heavily on a provisioned diet (Fig. 1). However, much of the supplied food was eaten by the higher-ranking individuals during times of official park provisioning (Mann-Whitney U = 342.000; n2 = 51, n = 49; p < 0.001) and feeding by visitors (Mann-Whitney U = 444.000; n1 = 49; n2 = 51; p < 0.0001).

Intensity and Temporal Variation of Soil Consumption

Wakibara recorded a total of 69.6% of the troop, excluding infants (N = 158) *ad libitum* to eat soils ≥ 1 time during the study. A higher proportion of females ate soils, and young consumed soils much more frequently than adults did (Chi-square test: $\chi^2 = 19.950$; DF = 3; P = 0.0002). However, the picture of sex differences might be biased given that low-ranking adolescent males range further from the feeding area and are less represented in the *ad libitum* observations. We estimated that an individual ingested about 3 g (Table II) of soil on a daily basis. Soil was eaten during all diurnal hours, but there was a major peak in late afternoon (Fig. 2). There is no statistically significant interindividual difference in the frequency of geophagy (Fig. 3) or in the amount of soil consumed regardless of month, season or individual rank.

Table II. Quantitative summary of soil intake

Characteristic measure	Parameter			
	N	Mean	Range	SD ($\pm$)
No. observation days	100			
Geophagic events (no./day)	126	1.26	1–4	1
Soil units ingested (no./day)	1386	11	0–83	11
Geophagy duration (minutes/day)	174.28	1.38	0–5.26	1.43
Soil ingestion (g/day)	297	2.97	0–15.66	2.91
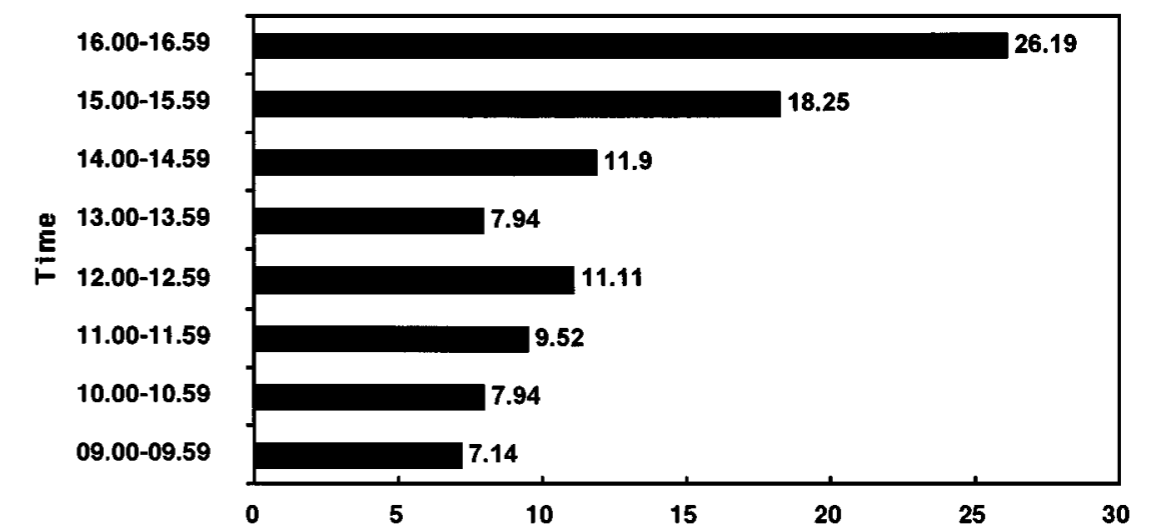
Fig. 2. Diurnal distribution of geophagic events. Data is expressed as the percent of observations during each daily period (N = 126).

For reasons of statistical convenience, we divided geophagic hours into two groups: 0900–1300 hr (morning) and 1300–1700 hr (afternoon). Of 126 geophagic events, 69% occurred in the afternoon; the tendency to consume soils more frequently after 1300 hr is statistically significant (Goodness of fit test for morning and afternoon hrs: $\chi^2 = 21.723$; G = 22.036; p < 0.0001). Accordingly, despite diurnal variation, the focal individuals consumed similar amounts of soil and at a rate that is more or less constant year-round.
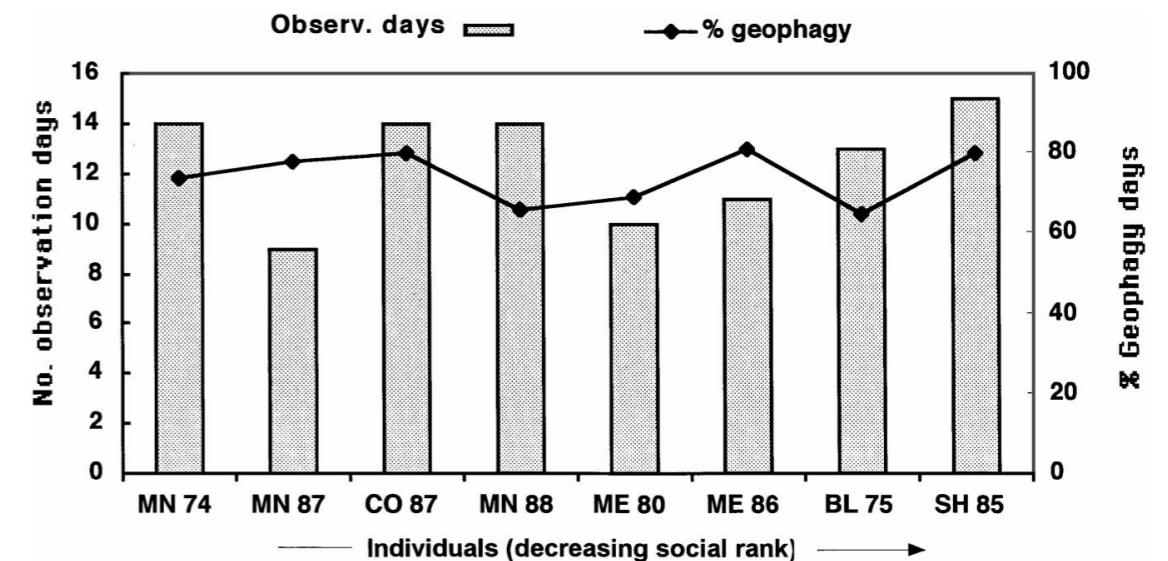
Fig. 3. Intake of soils by individual macaques. Names are abbreviated from the standard nomenclature via the matriline name and only the last two digets signifying the individual's year of birth (Mino 636974; Mino 637587; Copper 657187; Mino 63697488; Meme 628086; Blanche 596475; Shiro 62677985).

Table III. Nutritional profile of foliage relative to provisioned foods eaten

| Food	% Composition by dry weight (mean ±SE)							
	Water*	Protein (P)	Fiber (F)	Ash	Carbohydrates	Fat	P/F ratio	N
Grass	55.9 ± 4.9	14.9 ± 0.2	64.3 ± 2.9	8.9 ± 0.4	9.5 ± 3.1	2.4 ± 0.1	0.2 ± 0.25	3
Leaves	61.4 ± 1.3	16.6 ± 0.5	39.1 ± 2.2	5.3 ± 0.3	37.5 ± 4.9	3.6 ± 0.1	0.4 ± 0.21	51
Provisions	38.6	17.27	6.10	2.7	71.0	3.10	2.2 ± 1.69	5

*On total weight basis.

PLANT FOOD COMPOSITION

Nutritional Content

Table III is a summary of results of chemical analyses for the main foliage species by plant part and provisioned foods. The main natural food item, grass, is more fibrous (Unpaired t-test; $t = 2.717$; $DF = 49$; $n1 = 3$; $n2 = 48$; $P = 0.009$) and has more total ash, i.e. total minerals (Unpaired t-test; $t = 3.731$; $DF = 49$; $n1 = 3$; $n2 = 48$; $P = 0.0005$) than the other leafy foods. The rest are not statistically significant.

In general, the natural leaves contain higher amounts of water, total minerals (ash) and crude fiber (t-test; $DF = 54$; $n1 = 51$; $n2 = 5$; $0.0001 < P < 0.0037$). Conversely, the provided foods were superior in the amounts of protein and carbohydrates (t-test; $DF = 54$; $n1 = 51$; $n2 = 5$; $0.001 < P < 0.0028$) and presented a protein/fiber ratio about 9 times higher than the natural foods (Table III). The natural foods are particularly important as a source of fiber and various micronutrients expressed as total minerals, which are remarkably low in the larger amounts of provisioned foods.

Toxins and Their Adsorption by Soils

Figure 4 is a comparison of the adsorption potential of sample soils to 4 alkaloids—atropine, quinine, lupanine and sparteine—and to proanthocyanin-type tannins extracted from the acorns (*Quercus glauca*), a major wild food of the monkeys in fall. The amount of proanthocyanins is high in the acorns, reaching approximately 14% dry weight. The affinity of soils to bind alkaloids is high, ranging between 80 and 100% for both ingested and eschewed soils, except for one sample of the latter which adsorbed weakly to quinine and atropine. The positive control adsorbent, kaolinite, adsorbed to alkaloids at a similar level to that of soils. However, in contrast to the alkaloids, adsorption of tannins to soil is weak, ranging between 32 and 58%; with higher values in the eschewed versus ingested soils.
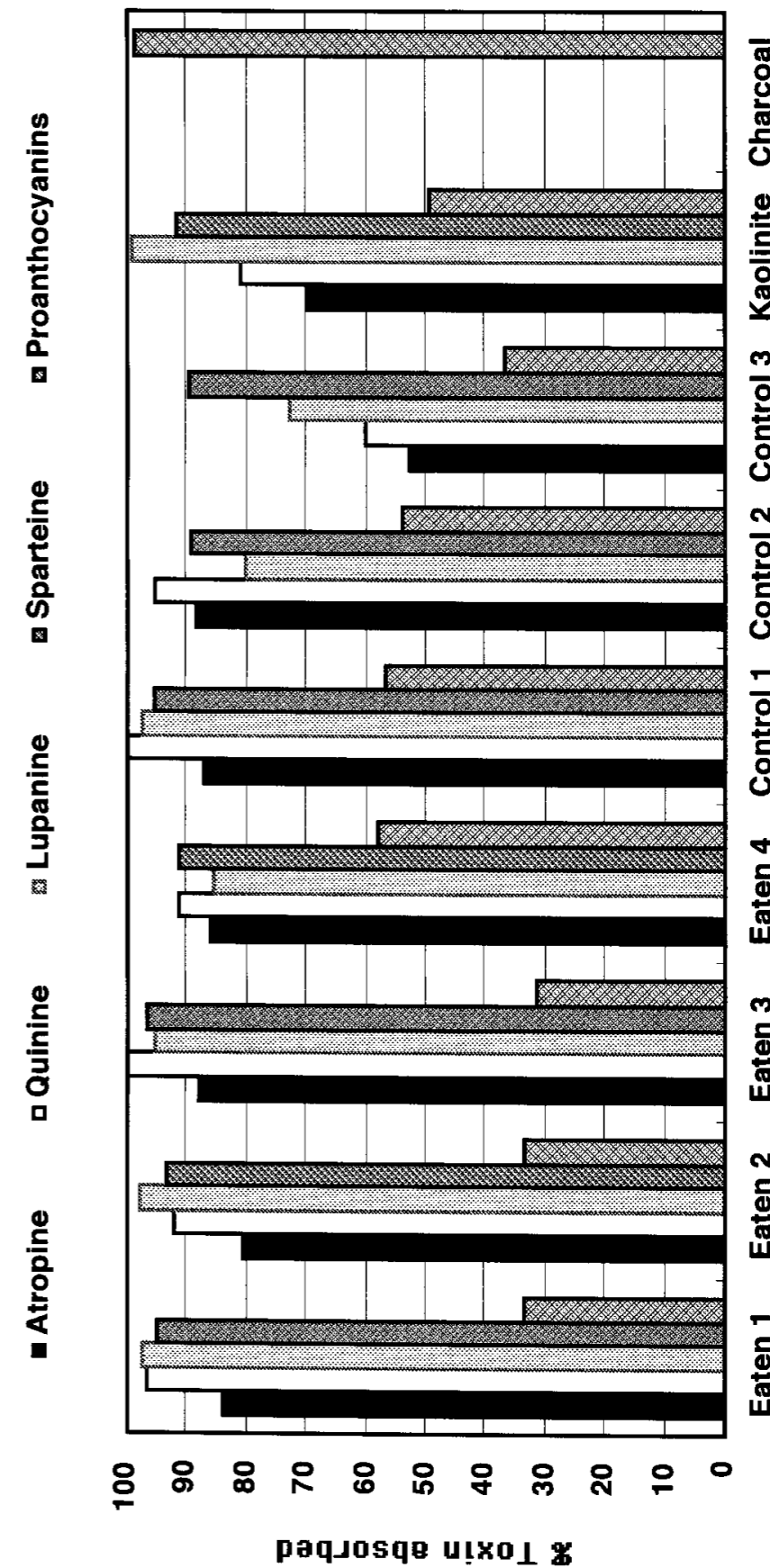
Fig. 4. Adsorption by geophagic and control soils, kaolinite, and charcoal of four plant alkaloids and proanthocyanin tannins from acorns. We used medicinal kaolinite from a traditional market in Guinea and charcoal as standard adsorbants.
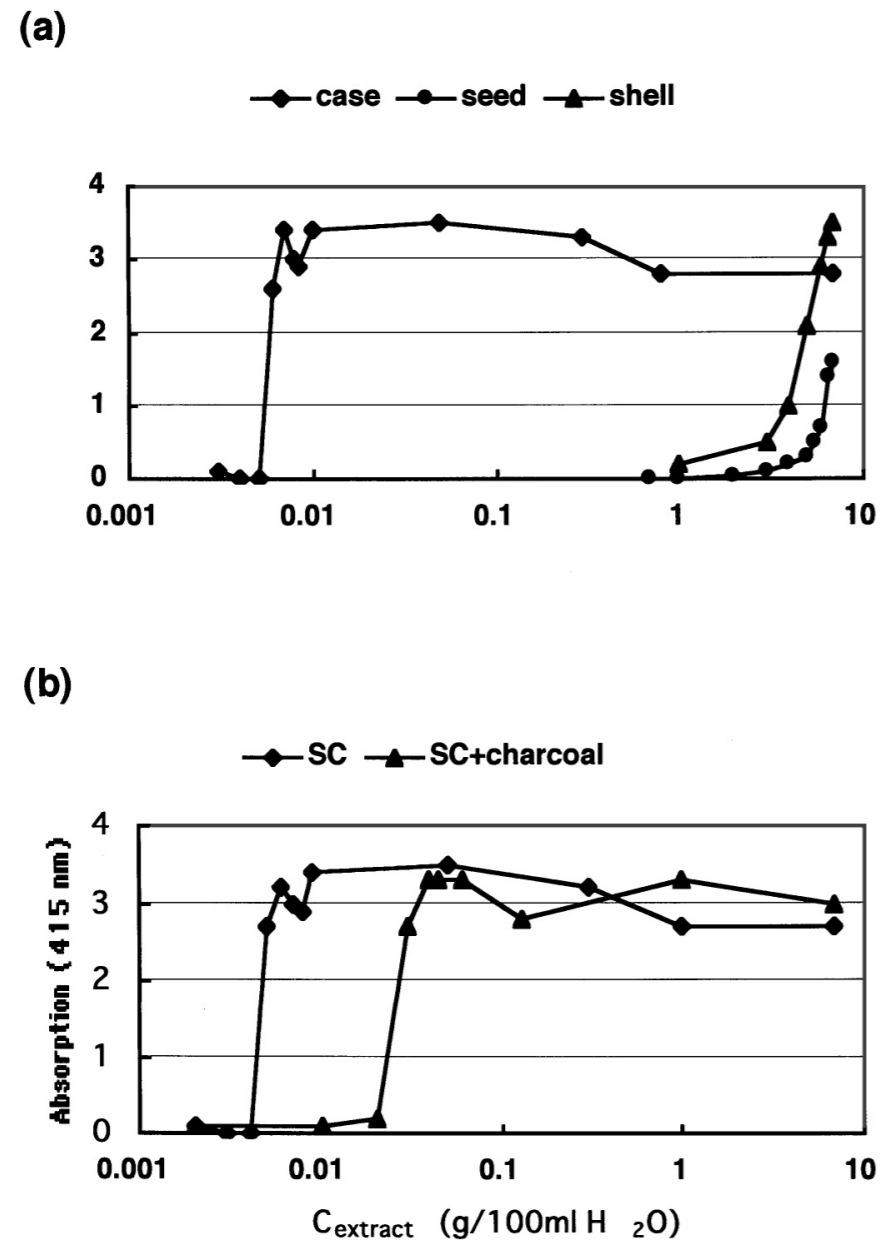
(a)

(b)

Fig. 5. (a) Haemolysis of erythrocytes by saponin extracts from different parts of seeds of *Styrax japonica*. (b) Detoxification effect of charcoal on saponin extracts from seed cases of *S. japonica*.

Seeds of *Styrax japonica* are rich in triterpene saponins, the concentration of which varies by seed part (Fig. 5a). Minute amounts of seed case extract lead to complete haemolysis of erythrocytes (ED 50 < 0.005 g/in 100 ml isotonic buffer) which is a typical biological activity of saponins, whereas extracts from seed shells and seeds are less haemolitic (EDca 5 g/100 ml or 8 g/100 ml). Like the tannins in *Quercus glauca*, saponins hardly bound to the standard adsorbent, charcoal, probably because of its amphilic proper-

Table IV. Particle size distribution and organic content of ingested and eschewed soils

Soil sample	Particle size %			Organic content[a]		
	Sand (2 mm–63 μm)	Silt (63–2 μm)	Clay (<2 μm)	C	N	H
Eaten 1	62.2	21.8	16.0	0.24	0.1	1.20
Eaten 2	39.3	42.7	18.0	0.09	0.07	1.30
Eaten 3	34.4	61.6	4.0	0.01	0.07	0.51
Eaten 4	36.2	57.4	6.0	0.08	0.06	1.50
Uneaten 1[b]	—	—	—	24.0	1.34	3.80
Uneaten 2	69.7	25.3	5.0	5.00	0.41	1.40

[a]Value to .01% measured by Leco Apparatus.
[b]Organically rich to the extent that particle size determination proved impossible.

ties (Fig. 5b). Since soil samples are always weaker in binding than charcoal, we can safely assume that the soils eaten by the monkeys do not prevent intoxification by saponins. The macaques had solved this problem by avoiding the most toxic parts of the plant via meticulously removing seed cases from the seeds before eating them.

SOIL COMPOSITION

Particle Size, Organic Content and Mineralogy

Data on particle size and organic content of the soil samples are in Table IV. The ingested soils are generally richer in clay and silt than control soils except for one overlapping value. In contrast, the control soils tend to

Table V. Concentration of minerals[a] in ingested and eschewed soils

Soil sample	Clay minerals								Primary minerals		
	1	2	3	4	5	6	7	8	9	10	11
Eaten (i)	XX	—	—	XX	X	X	X	XX	—	—	X
Eaten (ii)	XX	X	—	XX	tr	X	—	XX	—	—	X
Eaten (iii)	—	X	—	X	tr	X	—	X	X	tr	X
Eaten (iv)	XX	X	X	X	tr	tr	—	X	X	XX	XX
Uneaten (i)	tr	tr	—	0	—	—	—	X	X	—	?
Uneaten (ii)	X	X	tr	X	tr	tr	tr	XX	X	X	X

[a]Concentrations of minerals were determined from peak height where in the smallest amount is a trace (tr) followed by small (X), moderate (XX) and abundant (XXX). 1. kaolinite 2. metahalloysite 3. halloysite 4. illite 5. illite-smectite 6. vermiculite 7. chlorite 8. quartz 9. plagioclase 10. orthoclase 11. mica.

Table VI. Whole elemental concentrations in sample soils and their likelihood of bio-availability

Element	Whole soil elemental concentrations		(%) Conc. in 3 top atomic layers of soil particles	
	Eaten (mean ± SE; n = 4)	Control (mean; n = 2)	Eaten (mean ± SE; n = 4)	Control (mean; n = 2)
Aluminum %	12.6 ± 2.4	6.8	13.5 ± 1.4	8.3
Calcium	<2000	<1800	0.07 ± 0.05	0.1
Iron %	7.5 ± 5.2	6.6	1.5 ± 0.8	0.7
Potassium %	2.9 ± 0.90	1.9	0.5 ± 0.1	0.1
Sodium	9687.5 ± 18901	2575	0.6 ± 0.1	0.1
Manganese	1280 ± 1124	2160	0.1 ± 0.1	0.1
Nickel	<43.00	<34	0.2 ± 0.003	0.1
Titanium	9903 ± 9289	8960	0.08 ± 0.1	0.04
Magnesium %	1.4 ± 0.9	0.8		
Arsenic	296.5 ± 532.3	32.5		
Antimony	3.4 ± 4.7	2.5		
Barium	447 ± 4.7	385.0		
Bromine	3.2 ± 1.4	7.8		
Cerium	183 ± 35.4	89.1		
Chlorine	<340	<150		
Cobalt	27.6 ± 34.07	26.6		
Chromium	72.2 ± 82.6	107.5		
Cesium	24.3 ± 7.3	18.2		
Dysprosium	5.5 ± 1.2	2.4		
Europium	2.7 ± 0.5	1.3		
Gallium	59 ± 14.9	31.5		
Hafnium	11.7 ± 3.4	6.4		
Iodine	<23	<15.0		
Luthanum	110.6 ± 38.3	55.05		
Lufetium	0.6 ± 0.10	0.30		
Neodymium	45.7 ± 30.6	<6		
Rubidium	103 ± 34.3	92		
Scandium	15 ± 18.7	14.2		
Strontium	78.8 ± 41.7	<65.0		
Tantalum	9.3 ± 2.4	5.7		
Terbium	1.5 ± 0.4	0.90		
Thorium	21.03 ± 11.1	9.5		
Uranium	<1.8	1.5		
Vanadium	157 ± 162.1	163.5		
Ytterbium	4.5 ± 0.5	2.5		

contain more organic matter. Mineralogical analysis revealed consistently small-to-moderate levels of kaolinite, illite, and vermiculite in ingested soils (Table V). These minerals are present in only small-or trace amounts in the control-soils. Metahalloysite, halloysite, illite-smectite and the primary minerals, tend to occur in similar proportions in both the ingested and control soils.

Geochemistry and Chemical Bio-availability

The results of whole soil elemental concentrations and their likely bio-availability are in Table VI. Unfortunately, the breadth of data points does not permit statistical treatment. However, the concentrations of most elements are low and differences among control and ingested soils are not obvious. Values for Na, Ca, Fe, K, Mg and Mn overlap, while the amount of trace elements is either slightly higher in the eaten soils or overlap those of controls. Al and Br is particularly high in the ingested and control samples, respectively. The XPS results indicate that the likelyhood of bio-availability for most elements, except Al and Si, is similarly low and that differences between ingested soils and controls are not apparent. In fact, we detected no sodium in the 3 top layers of atoms on the surface of 3 of 4 ingested soil samples.

DISCUSSION

Our results highlight several features of plant feeding and soil intake by Japanese macaques that may illuminate the roles of geophagy. Accordingly, we integrate the separate results on food nutrition and soil chemistry, levels and patterns of soil and food ingestion, and the potential of the soils to adsorb wild plant food chemical constituents to explore the functional significance of geophagy by the provisioned Japanese macaques of Arashiyama.

Food Detoxification

The food detoxification hypothesis simply implies that the soils consumed together with other foods will help to adsorb the toxic (alkaloid, saponin) or antifeedant (tannins) constituents therein. Ideally, this hypothesis dictates that (1) the ingested soils are predominantly clay, (2) they are ingested regularly, (3) the major foods contain toxic or antifeedant properties and, (4) the soils can adsorb the agents responsible for these properties in the foods. That the soils ingested by the Arashiyama macaques is rich in clay is clearly confirmed by the particle-size analysis. Most primates, including humans, appear to select soils rich in clay for geophagy (Mahaney *et al.* 1996; Aufreiter *et al.* 1997). Unfortunately, comparable numerical estimates of masses of soil ingested by individual primates are extremely rare. The infrequently provisioned Japanese macaques on Koshima islet ingest soil at a rate of 0.43 g/indiv./day (range 0–45) (Iwamoto, 1982; Iwamoto, unpublished data), which represents a mere 16% of the level of geophagy on Arashiyama

(2.97 g/individual/day). Accordingly, Arashiyama monkeys consume soil in large amounts and at high frequency.

Whether Arashiyama foods are consistently toxic or contain antifeedant properties remains to be investigated and is open for further testing of the prediction that ingested soils alter these properties of the foods. The chemical component determination of every plant species and part consumed by them was not possible due to logistical limitations. However, the main finding that about two-thirds of the regularly ingested foods by weight are provisioned items—wheat, soybeans, apples, peanuts, potatoes, bananas—inlfuences our concerns of overt influences from food toxicity or antifeedant in the monkeys. Moreover, the relative toxicity of grass (Graminae), which comprises the bulk of their natural food, is low. However, some grass species are infested by fungi, which produce toxic alkaloids, e.g., pyrrolizidine or ergot alkaloids.

A closer look at the two food items that we analyzed for disruptive function, together with the actions of ingested soils to alter their capacity to act will serve as a model to further sharpen our interpretations. Acorns were almost the exclusive natural food for the monkeys during the autumn season, while they ate *Styrax japonica* not only very rarely (twice in the course of our study) but also only after careful manipulation. The finding that ingested soils have a weak binding affinity for the tannins in acorns and almost no effect on the saponins in seeds of *Styrax japonica* does not seem to favor the detoxification argument (Figure 5). The superiority in tannin-binding by the organically rich soil control samples reflects the high affinity of tannins for proteins (M. Wink, unpublished data). Conversely, the strong affinity of the ingested soils to standard alkaloids clearly demonstrates their superior detoxification potential for this class of toxins. Although we did not measure alkaloid contents in the monkey foods, tannins are the most ubiquitous class of secondary compounds in the plant kingdom (Freeland and Janzen, 1974). That the monkeys carefully manipulated the seeds of *styrax japonica* to get rid of the part most loaded with saponins (Huffman, 1984) evidences that they avoided intake of some toxic plant secondary compounds for which geophagy would be of no curative value. The argument for deliberate avoidance of toxic plants, or some parts thereof is well-founded in the primatological literature (Freeland and Janzen, 1974). For instance, Marriott *et al.* (1993b) reported a clear tendency for a similarly free-ranging provisioned macaque group to select the less toxic food items while foraging on natural food items.

Gilardi *et al.* (1999) furnished data *in vivo* on food detoxification by soils in parrots. They noted that the bulk of the parrot diet was dominated by highly toxic seeds typical of parrot foods elsewhere (Norconk *et al.* 1997). In contrast to the monophagous habit of parrots, intake of specific plant parts by the Arashiyama macaques is highly seasonal. Several other studies

on primates failed to reveal a close relationship between the intake of natural foods and soil ingestion (Davies and Baillie, 1988; Knezevich, 1998; Muller and Hartmann, 1997). For the Arashiyama macaques in particular, several lines of evidence emerge which tend to refute a consideration that the eaten soils served simply to detoxify their foods: (1) A high reliance (66.7%) on provisioned foods make food toxicity in general an unlikely problem at this site: (2) The bulk of natural food consumed was grass which is likely to have little or only low levels of toxic secondary plant compounds. (3) The adsorption of toxins by the ingested soils are inconsistent, showing weak affinity or failure to bind to tannins and saponins, but a high affinity to bind to alkaloids. Given this body of information, there is no clear indication that the soils eaten by Japanese macaques at Arashiyama served primarily to detoxify their foods, i.e., it could be a secondary trait. The advantages may be greater for primates ingesting a diet higher in alkaloids. However, until a chemical analysis of all the wild plant food species ingested by the group is performed, final rejection of the hypothesis is premature. Even if relatively small amounts of alkaloids are ingested, geophagy could provide important benefits.

Dietary Supplementation

Although several nutritional elements are necessarily gained when animals ingest soils, sodium is considered the most frequently limiting one in wild foraging situations; it is the most likely to be sought via alternative means such as geophagy (Kraulen, 1985). The amount of sodium was consistently lower in the ingested soils than in the controls except for one extreme ingested sample. The ingested soils at Arashiyama were known to be poor in dietetic elements, at least in comparison to soils eaten by other populations of Japanese macaques (Inoue, 1987; Mahaney *et al.* 1993). The extensive overlap in concentrations of most major, minor and trace elements between ingested and control soils (Table VI) suggests that similar mineral levels could equally be obtained by simply ingesting surface soils. However, of utmost importance is the extent to which the ingested soils might release nutritional elements for bodily absorption. Our X-ray photoelectron microscopic results (Table VI) clearly indicated that the likelihood of bioavailability, except for Al, is very low. That the Arashiyama macaques relied heavily on a provisioned diet makes it unlikely that they are nutritionally impoverished since provisioned macaques are nutritionally superior, which positive affects demographic parameters (Mori, 1979). Even in situations wherein animals are not provisioned, mineral concentrations in their natural foods often greatly exceed those of soils ingested at the same time (Hladik, 1977a, b; Gilardi *et al.* 1999).

Clark (1979) reported that the genetic siblings of the Arashiyama subjects, which were translocated to Texas, USA, continued to eat large amounts of soil only 10 days after arrival. However, unlike soils in the Japanese habitat, the unfamiliar soils were much richer by factors of 1.02, 2.3, 4.5 and 17.7 for Mg, P, K and Ca respectively. Thus, a considerable nutritional gain from eating soils, could occur in Texas versus Arashiyama. Apparently, the Arashiyama macaques are attracted to eating soils regardless of the concentration of mineral elements contained in them.

Antidiarrheal Mediator

The hypothesis on antidiarrheal effects hinges on the premise that ingested soils absorb excess water in the fecal matter, making it less watery and more consistent, thus alleviating part of the clinical symptoms of diarrhea. The mineral composition of the clay fraction of the ingested soils is a crucial factor to validate this idea. The tested soils have small-to-moderate amounts of illite, smectite, kaolinite and vermiculite; particularly so in the ingested versus eschewed soils. The presence of one or a combination of the clay minerals in sufficient proportions normally yields pharmaceutical properties similar to that of an industrial antidiarrheal formulation Kaopectate®. Kaolinite, in particular, consistently occurred at moderate levels in 3 of 4 ingested-soil samples. Given the high rate of geophagy at Arashiyama, it is hard to rule out considerable intake of these minerals and therefore an antidiarrheal function, as also proposed by Knezevich (1998) and Mahaney et al. (1990). Their hypothesis is supported by our results.

Buffering of Gastric Disorders

Geophagy seems to be associated with vegetal foods. The ways in which intake of vegetal foods bring about gastric upsets, and the several mechanisms by which geophagy can help to alleviate them has been a focal topic in veterinary medicine (Wheeler, 1980). Excessive intake of foods that are rich in soluble carbohydrates and proteins and low in fiber exacerbates the risk of digestive upsets. The ensuing clinical manifestations are complex, but include buffering of gastric disorders, accelerated fermentation, changes in the microbial population, increased volatile fatty acids and osmotic pressure, diminished salivation, and decrease in pH conditions (Kreulen, 1985). Reports on several wildlife species, indicate that geophagy is a highly seasonal phenomenon that is closely related to phenological changes by prevailing

in seasons of abundant young leaves and shoots (Kreulen, 1985; Moe, 1993; Fraser and Hristienko, 1981; Hebert and Cowan, 1971). Less fibrous material in the diet and sudden increases in intake of soluble carbohydrates and proteins may provoke gastric disorders, which, in turn, induce geophagic activity (Kreulen, 1985; Hebert and Cowan, 1971).

Were similar dietary conditions occurring in the macaque study population? The results of the nutritional analysis of foods indicate that the bulky and regularly eaten human-supplied foods were indeed very rich in proteins and carbohydrates, but low in fiber (Figure 2; Table III). Surprisingly, limited tests also revealed that sample 4 of ingested soils tested acidic (pH 4.54). Klaus and Schmid (1998) recommend a minimum of 10 samples before firm conclusion can be reached on geophagic soils. Nevertheless, other macaques in similarly provisioned situations tend to exhibit elevated levels of geophagy. Marriott (1988) reported that geophagy was pronounced in provisioned *Macaca mulatta* on Cayo Santiago, but it was practiced rarely by *M. mulatta* foraging naturally in Nepal. Indeed, on Cayo Santiago, geophagy is strongly associated both spatially and temporally with the ingestion of provisioned (Sultana and Mariott, 1982; Knezevich, 1998) but not natural foods (Knezevich, 1998). Knezevich (1998), noted that 64% of soil-eating episodes occurred while the monkeys still had commercial chow in their cheek pouches. Ganzhorn (1987) observed that when *Lemur fulvus* switched from provisioned foods to feeding extensively on spring flowers and buds, they reduced their geophagy level from ingestion of soil morsels to brief licking thus preventing further geophagic experiments. For Japanese macaques, the level of geophagy is lowest (29.0%) (Hanya, unpublished) in Yakushima where all their foods are wild; intermediate (66.6%) in Koshima with some history of provisioning (Iwamoto, 1982; Iwamoto, personal communication); and highest (73%) in Arashiyama with the highest, year-round level of provisioning, based on the year-round proportion of days in which focal individuals engaged in geophagy.

There is no macaque gut model for directly testing our physiological predictions. However, the occurrence of elevated geophagy at Arashiyama and in similarly provisioned macaques, suggests that even in macaques, provisioned foods probably exacerbate the digestive disturbances, which induces geophagy. The skewed intake of soils toward the afternoon hours (Figure 2) may be explained by the fact that peak digestive activities occur about 2–4 hours after a sizable meal (Wheeler, 1980). During our study, artificial provisioning occurred between 1030 and 1430 hr daily and the monkeys ate soil more frequently between 1300 and 1700 hr, which fits roughly into this prediction. Another common feature is the tendency for the younger monkeys to predominate in geophagy, which may reflect that younger animals are relatively more prone to digestive gastric disorders (Wheeler, 1980).

Enhancement of Food Taste

There is virtually no datum to test taste enhancement as a function of geophagy for the Arashiyama soils. However, taste enhancement is perhaps one of the most complicated phenomena to verify. Ganzhorn (1987) eliminated soil as a reliable modifier of mouth taste in *Lemur* spp. because minute fluctuations of the same mineral element may elicit different responses of taste buds. We propose a similar argument for the Arashiyama macaques and consider this redundant reasoning. The extremely complex taste, hormonal interactions, the ever slight changes in the composition of soils and the presence of modifying substances in both soils and the ingesta makes it hard to realize soil as a taste modifier in any consistent manner.

CONCLUSIONS

1. The Japanese macaques at Arashiyama ingest high amounts of soil, at a high rate throughout the year. Their annual intake of wild foods is remarkably low, which makes them less prone to the intoxication. Their heavy indulgence in high energy, low fiber human foods make them vulnerable to gastric upsets.

2. The soils they ingest are relatively poor in nutritional elements, the relative bio-availability of which is also low. However, the ingested soils have elevated amounts of several clay minerals, especially kaolin, which can buffer gastric related upsets.

3. The soils that they ingest can detoxify plant alkaloids quite well; but they can bind to tannins and saponins only weakly. Two of their tannin or saponin-loaded foods were only poorly detoxified by the soils they ate. Instead, the monkeys tended to deliberately avoid plant parts with elevated toxicity.

4. Data on amount, composition and rates of ingestion of foods and soils indicate that the most probable benefit of geophagy for the Arashiyama macaques is to improve the health of individuals via alleviating diarrhea or other gastric-related, upsets, possibly by gastric buffering.

5. Researchers should (a) analyze the mineral and toxicity spectrum of a larger sample of foods, (b) estimate the average mineral requirements for macaques, (c) experiment on the risk of acidosis and related upsets due to ingestion of provisioned or wild foods and the extent to which soil intake can buffer such risks, (d) experiment *in vivo* on the plant detoxification potential of ingested soils, and (e) compare

the trends reported here for provisioned Japanese macques with a similar study on totally wild populations.

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MINERALOGICAL AND CHEMICAL INTERACTIONS OF SOILS EATEN BY CHIMPANZEES OF THE MAHALE MOUNTAINS AND GOMBE STREAM NATIONAL PARKS, TANZANIA

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Abstract—Termite mound soils eaten by chimpanzees of the Mahale Mountains and Gombe National Parks, Tanzania, have mineralogical and geochemical compositions similar to many soils eaten by higher primates, but release very low levels of either toxic or nutritional inorganic elements to solution at acid pH. Comparison with control (uneaten) soils from the same areas showed lower levels of carbon and nitrogen in the eaten soils,

a relationship confirmed by surface analysis. Surface analysis also revealed lower levels of iron on particle surfaces versus interiors, and higher levels of iron on ingested versus control soil particle surfaces. The soils can adsorb dietary toxins, present in the plant diet or those produced by microorganisms. Taking the toxic alkaloids quinine, atropine, sparteine, and lupanine as examples, it is evident that soils from Mahale have a very good adsorptive capacity. A new adaptive advantage of geophagy is proposed, based on the prevention of iron uptake. The behavior of the soils in vitro is consistent with the theory that geophagy has a therapeutic value for these chimpanzees.

INTRODUCTION

Studies of soil consumption (geophagy) among nonhuman primates have focused on its potential value as a source of supplementary dietary minerals and as a vehicle for adsorption and hence removal of toxins with passage through the gut (Bolton et al., 1998; Muller et al., 1997; Mahaney et al., 1995; Izawa, 1993; Davies and Baillie, 1988; Fossey, 1974, 1983; Uehara, 1982; Oates, 1977, 1978; Hladik, 1977; Hladik and Gueguen, 1974). The possibility that geophagy is used by animals for seld-medication in disease and parasite infestation (as an adsorbent of harmful bacteria and toxins), as it is used by humans both traditionally (Johns, 1990) and in modern pharmaceuticals (Brouillard and Rateau, 1989), has also been suggested (Mahaney et al., 1996, 1997; Stambolic-Robb, 1997; Johns, 1990; Hladik and Gueguen, 1974).

Among higher primates, humans of many societies also have engaged in geophagy (Aufreiter et al., 1997; Johns and Duquette, 1991; Johns, 1990; Hunter, 1973; Laufer, 1930) for various reported reasons, e.g., to alleviate starvation in time of famine and to counter deficiencies of inorganic nutrients during pregnancy. A correlation was established between consumption of highly calcium-enriched termite-mount clay by pregnant women of various African ethnic groups and the absence of milk and other dairy products in their diet. Wiley and Katz (1998) found that soil consumption was absent in pasturalist societies with daily access to milk-producing livestock and used this to implicate geophagy soils as dietary calcium supplements. Geophagy has also been seen as a cultural tradition sponsored and/or advocated by religion. Clear reasons to justify the behavior are frequently unobtainable; possibly human geophagy was retained over evolutionary time due to survival benefits conferred by the behavior (Johns, 1990), and experimentation occurred with highly weathered natural earths in different environments (Mahaney, 1999).

Studies of geophagy soils from different geographical locations have shown that they are often similar in mineralogy and in chemical elemental content

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(Browman and Gunderson, 1993). These similarities lead to the inference that soils of a particular chemical environment are selected for ingestion, from the mounds of termites of the subfamily Macrotermitinae. Reports of the ingestion of bioturbation (insect-selected) soils of termites or ants by primates are not unusual (Julliot and Sabatier, 1993; Aufreiter et al., 1997), and ants and termites them-selves are often a source of food (Uehara, 1982; Watts, 1984). The chimpanzees of Mahale may be aware of key characteristics associated with termite mound soils. Uehara (1982) reported chimpanzees there sampling the soils of termite mounds through the year, apparently monitoring by taste the reproductive con-dition of the termites, for feeding on the termites.

The adaptive value of the soils is likely linked to their composition. Most of the analyzed soils have been found to contain a high proportion of $Al : Si = 1 : 1$ secondary clay minerals, with a lesser amount of $Al : Si = 2 : 1$ minerals, some quartz, and, often, high amounts of iron. The clay fraction of the soils is a physically and chemically active material that may absorb or adsorb (or conversely release) water, inorganic elements, or organic compounds to varying degrees, depending on the amounts of specific clay minerals and depending on variations of such conditions as pH. Many studies have considered a benefit of geophagy to be the adsorption of toxic organic components such as alkaloids in plant foods, as shown in vivo for parrots, by Gilardi et al. (1999), and for geese by Wink et al. (1993). Clay minerals are also capable of adsorbing bacterial or parasite-generated toxins (Said et al., 1980). This may be a key benefit of geophagy for the chimpanzees of the Mahale Mountains. Beneficial compounds or organisms could also be obtained from ingestion of the high-clay soils and provide sensory cues of smell and taste used by animals in choosing appropriate soils.

Behavioral research on chimpanzee groups in Mahale has been carried out since 1965 (Nishida, 1990). Investigation into the health and self-medicative behavior of chimpanzees at Mahale with a focus on plants has been carried out by one of the authors (M.A.H.) since 1985. Recently geophagy has also been stud-ied with the objective of determining if this behavior can be considered another form of self-medication (Huffman, 1997). Early behavioral observations have shown that chimpanzees of Mahale sometimes consume termite mound soils dur-ing periods of gastrointestinal distress and when beset by intestinal parasites, and thus a preliminary reconnaissance was made of such soils in 1994 (Mahaney et al., 1996). A larger set of samples of eaten soils and uneaten control soils col-lected in 1995 (Mahaney et al., 1999) confirmed that the chimpanzees chose soils characterized both by combinations of halloysite and kaolinite, similar to those in formulations used by humans to reduce gastrointestinal afflictions, and by high concentrations of iron, for which a nutrient role was considered. Further observations showed that the chimpanzees frequently consume small quantities of these soils (~3 cc per feeding) (Figure 1).

FIG. 1. A chimpanzee of Mahale Mountains National Park, Tanzania, sampling termite mound soil. Photo by Michael Huffman.

Here, a new, larger set of samples of ingested termite mound earths from the Mahale sites was examined along with controls chosen at several meters' greater distance from the mounds than for collection of previous control samples. Addi-tional analytical foci were placed on the potential of the soils to adsorb certain toxins and to release nutrients at acid pH and on an analysis of the soil particle surfaces. We also include information on two samples from the nearby Gombe

National Park research site where chimpanzees have similarly been observed engaging in geophagy. Results from a study of the microbiology of these soils by Ketch (1998) will be published separately. This paper concerns the chemistry, geochemistry, mineralogy, and adsorptive capacities of ingested soils, compared with control soils from the same areas, in order to isolate parameters that warrant further study.

METHODS AND MATERIALS

Behavioral Observations. Geophagy has been observed at Mahale and Gombe in both dry and rainy season months. In the course of ad libitum and focal-animal observations, the locations of chimpanzee geophagy were noted by researchers and field assistants at Mahale and Gombe. During the 15-day collection trip by Huffman in 1996, these sites were visited and collections made. No systematic observations covering all seasons have been made at Mahale, making systematic analysis of the seasonality of geophagy difficult. However, in 59 days of focal-animal observations made by Huffman in the 1991 rainy season, five incidences were recorded (Mahaney et al., 1996).

The Sites. The Mahale Mountains National Park is located on the eastern shore of Lake Tanganyika approximately 100 km south of the Gombe Stream National Park (6°S, 30°E; Figure 2). The climate of the area is influenced by north- and southeasterly (trade) winds. Local wind from the mountains has a drying effect, whereas wind from the lake brings moisture. The mountains range up to 2500 m asl, lake level is about 773 m asl. The underlying bedrock is primarily granite along with gneiss and schist (McConnell, 1950). Soils formed from this crystalline rock are relatively thin, stony, and porous (Collins and McGrew, 1988) with thin organic (Ah) horizons less than 18 cm thick. Soils were collected in mid-October 1996 over a period of five days during the end of the dry season at Mahale and during one day the same month at Gombe. Each termite mound was sampled by Huffman at those spots where chimpanzees were observed to consume the mound soil. All sites were located above ground at the top of a mound. Each control sample was collected approximately 4–6 m away from a mound, avoiding possible contamination down the slope of runoff mound material. Each sample came from the surrounding Ah horizons and had a darker tone than the mound soils.

Laboratory Analyses. Each sample was analyzed for water content and particle size of the <2 mm fraction. Water retention was determined by heating samples overnight at 110°C. The air-dried equivalent of 50 g oven dried soil was used to calculate the particle size. The sands (63–2000 μm) were separated by wet sieving. The silt and clay fractions were determined by pipet and hydrometer, (Day, 1965). The particle size curves were drawn from the dry weight of sand and from density measurements made by hydrometer. Particle size distributions
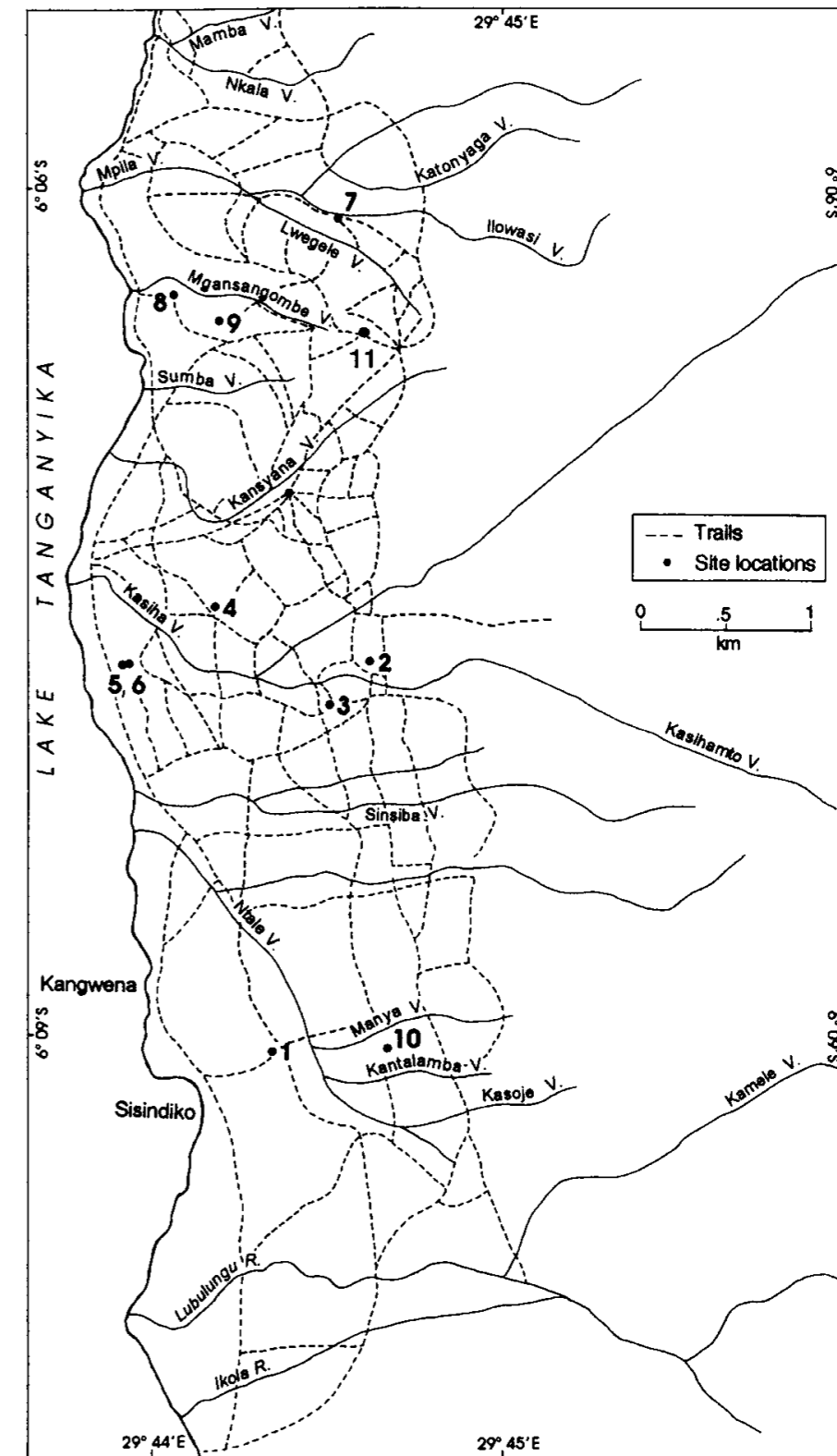

FIG. 2. Map showing the location of sample sites in Mahale Mountains National Park, Tanzania.

were analyzed as curves to determine the relative ratios of sand and silt to clay in each termite mound soil and each control soil.

Mineralogy was determined by X-ray diffraction (XRD) (Whittig, 1965). Major, minor, and trace elements were measured using instrumental neutron activation analysis (INAA). Five-hundred-milligram subsamples of the bulk materials were weighed into polyethylene vials and analyzed at the SLOWPOKE Reactor Facility of the University of Toronto, following procedures established by Hancock (1978, 1984). Two neutron irradiations and four counts were required to produce a database of 23 elements for each sample. Extracts of selected samples were prepared by shaking approximately 1 g of soil in 10 ml of pH 2.0 ammonium oxalate solution for 30 min, followed by gravity filtration through Whatman 42 ashless filter paper. The filtrates were allowed to stand for 48 hr to allow very fine particles to settle; approximately 1-ml volumes of the filtrates were weighed into polyethylene vials, which were then heat-sealed. The sealed samples were analyzed by INAA for short-lived, isotope-producing elements, and some selected samples were also analyzed for long-lived isotope-producing elements, following the procedures of Hancock (1978, 1984).

Selected soil samples were analyzed by scanning electron microscope (SEM) and energy dispersive spectrometry (EDS), including the light and heavy mineral fractions. For the light fraction, sands were randomly selected for analysis by SEM and EDS. All sands were analyzed for grain mineralogy and coatings that might reveal the chemistry of weathering products and coatings including clay minerals. The SEM-EDS analyses followed procedures outlined by Mahaney (1990) and Vortisch et al. (1987).

X-ray photoelectron spectroscopy (XPS) was used to analyze elements at the surface layers of individual particles of soil. The spectra were obtained on a Leybold MAX 200 XPS system based at the Institute for Biomaterials and Biomedical Engineering at the University of Toronto. Samples were prepared for XPS analysis by pressing the dried powdery <2-mm fraction of the soils onto adhesive copper tape, removing loose particles with light gas pressure, and mounting the tapes on a sample holder. Unmonochromatized Al K_α X-ray radiation was used as the excitation source. The source was run at 15 keV and 25 mA. Large-area analysis was performed (4×7 mm^2) to minimize collection time while maximizing signal. Relative atomic percentages were obtained from survey spectra (0–1000 eV, step size 800 meV) run in a low-resolution mode (pass energy = 192 eV). Details of the techniques are described in Sodhi et al. (1992). The data were normalized to unit transmission of the spectrometer by means of a routine provided by the manufacturer (Berresheim et al., 1991). The sensitivity factors (see Table 6 below) used to obtain these values were empirically derived by Leybold for the normalized spectra. Actual integration of the peaks was performed using Escatools (Surface Interface Inc., Mountainview, California). The surface specific nature of XPS limited depth analysis to 7–10 nm.

To measure the adsorptive capacity of soils, we employed the following design: About 500 mg of soil was added to 5 ml of water in 15-ml centrifugation tubes; 0.35 mmol of each of the alkaloids sparteine, lupanine, quinine, and atropine (adjusted to pH 6–7) were added. After 20 min of incubation, samples were vortexed and centrifuged for 20 min at 4000g. The separated supernatants were brought to pH 12 with 1 M KOH and subjected to solid–liquid extraction using ChemElute columns. Methylene chloride (3 × 15 ml) was used as eluent. The crude alkaloid extract obtained was analyzed by capillary gas–liquid chromatography, using a Carlo Erba gas chromatograph with a flame ionization detector (FID) and OV-1 (Ohio Valley) capillary column (15 m × 0.25 mm × 0.25 μm) (for details see Wink et al., 1993, 1995; Wink, 1993b). Authentic alkaloids were employed as external standards for quantification. Appropriate controls (samples with water and alkaloids) were used to define the amount of nonadsorbed alkaloids. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Particle Size. The data show little variation in sand + silt, but large variations in clay contents. Figure 3 shows results for ingested samples from sites in the Mahale Mountains. To read the data it is necessary to refer to the boundaries for sand (63–2000 μm), silt (2–63 μm) and clay (<2 μm). The y axis is cumulative, so the percentage at 4 phi is the sand and the percentage at 9 phi is the sand + silt. The percentage at 100 less 9 phi is the percent clay. The data for ingested soils show higher proportions of <2-μm material over control soils. There are clear textural differences between the ingested and control samples, showing that there is a preference for chimpanzees to select clay-rich material for ingestion.

Mineralogy. The X-ray diffraction data shown in Table 1 include both clay and primary minerals. Within the 1:1 group (Si:Al = 1:1), metahalloysite is predominant but is not confined to the ingested group. Kaolinite is dominant at site 3 and site 6 in moderate to abundant amounts and is present in eaten and control soils. Kaolinite and metahalloysite have similar chemistries (although metahalloysite has variable water content) and among the 1:1 sample group chimpanzees may be able to achieve the same benefit consuming both the control and ingested soils.

Within the 2:1 (Si:Al = 2:1) sample group, illite is present in both the ingested and control soils. Randomly interstratified illite–smectite is present in trace to moderate quantities in all sites; smectite is present in five sites including four where ingestion occurred. Smectite is often present in small amounts in geophagic soils and may have some value in geophagy, although the ingestion of Turkish soils high in smectite was shown in vivo in humans to pre-
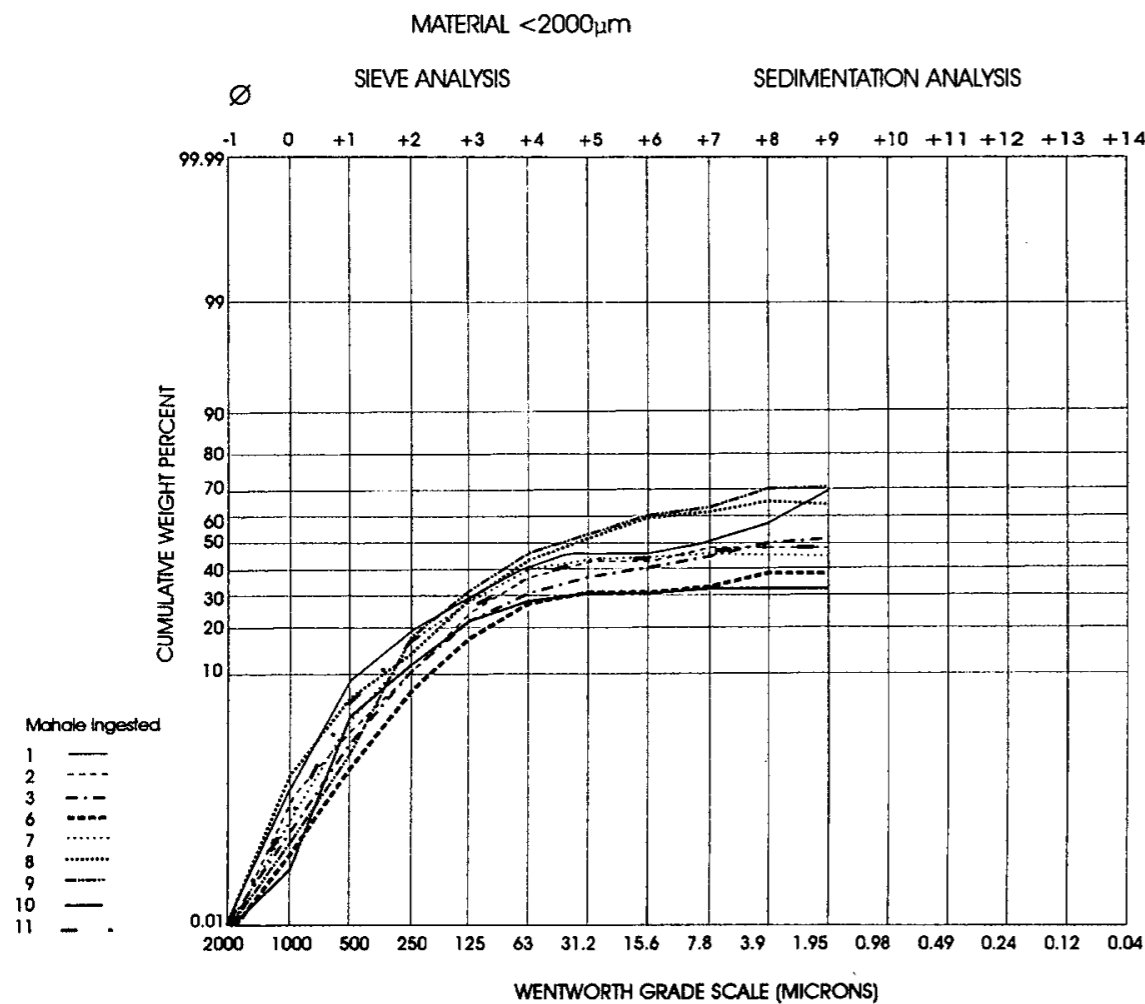
MATERIAL <2000μm

FIG. 3. Results for particle size analysis of soil samples ingested by chimpanzees.

vent uptake of Fe, whereas some soils of predominantly 1 : 1 clay mineral content, used by Native Americans, did not (Minnich et al., 1968). Vermiculite is absent in all soils; chlorite is also absent. Within the primary minerals, quartz, muscovite, orthoclase, biotite, and plagioclase feldspar are present in varying amounts. Quartz is somewhat more prevalent in the ingested samples. It is most abundant in weathered crystalline terrane and here may play a role in armoring the termite mound surface, by rain wash and surface hardening, against animal disturbance. With higher amounts of quartz, orthoclase and the micas are also seen to increase, indicating either younger soils (a sorting phenomenon) or a stronger granitic influence. Plagioclase occurred infrequently among the samples studied, and since it is generally one of the first minerals to weather by hydrolysis, this is not unexpected. Muscovite and its secondary cousins, on the other hand, are inert.

Metahalloysite and illite are common clay minerals in the Mahale soils. The

TABLE 1. MINERALOGY OF <2-μm FRACTION OF INGESTED AND CONTROL SAMPLES[a]

Sample	K	MH	I	I-S	S	C	V	Q	O	M	P
Mahale											
1E		X	XX	XX	tr			X	X	X	tr
1C		XX	XX	XX	tr			XX		XX	
7E		X	X	X				X			X
8E		XXX	XXX	X	XX			XXX	XXX	XX	
9E		XX	XXX	X	X			X	X	X	
11E		XX	X	X				X	X	X	
11C		XX	XX	XX				X	X	X	X
2E	X	XXX	X	X				X	X	X	
2C		XXX	X	X				X	X	X	
3E	XXX	X	XX	X	tr			XX	XX	X	
6E	X	XXX	X	tr				X	X	tr	
6C	XX	X	X	tr				X	X	X	
10E		XX	X	tr				tr	tr	tr	
10C		XXX	X	tr				tr	X	tr	
Gombe											
E		XX	XX	X				XX	X	X	X
C	X	X	XX	X				X			X

[a] The minerals identified include kaolinite (K), metahalloysite (MH), illite (I), illite-smectite (I-S), smectite (S), quartz (Q), orthoclase (O), mica (M), and plagioclase feldspar (P). Chlorite and vermiculite are not present even in trace quantities. Semiquantitative amounts of each mineral are given as trace (tr), small (X), moderate (XX) and abundant (XXX) quantities. E= eaten; C = control.

XRD traces show sharp, abundant reflections indicating a strong crystallinity and a mineralogy of a relatively high purity, similar to pharmaceutical clays marketed as digestive remedies. Data for sample 8E show that it contains somewhat more smectite than is common throughout the sample group. This profile is also similar to that from earth consumed by mountain gorillas in northwestern Rwanda (Mahaney et al., 1995). The chimpanzees do not always select soils with smectite present; the presence of chiefly kaolinite or metahalloysite is consistent.

Carbon and Nitrogen. The carbon and nitrogen contents (Table 2) of the control soils are consistently higher than those of eaten soils, as was also seen in the data from analysis of an earlier sample set from this area (Mahaney et al., 1999). This is probably a reflection of higher levels of organic matter in the surface soils of the area, as compared to the termite mounds that are derived from depths of up to 100 m. The lower levels of carbon and nitrogen of the termite mound soils are also seen in carbon values obtained for the XPS data (discussed below). This is likely due to the depth of acquisition as the insects build their mounds particle by soil particle from deep, wet soils. The resulting lower organic content may reflect lower levels of pathogens, a benefit of choos-

TABLE 2. TOTAL CARBON, HYDROGEN, NITROGEN, pH, ELECTRICAL CONDUCTIVITY, AND SOIL COLOR IN MAHALE SAMPLE GROUP[a]

Sample	Carbon (%)	Hydrogen (%)	Nitrogen (%)	pH	EC (1 : 5)	Color (1 : 5)
Mahale						
1E[b]	1.2	1.1	0.14	6.41	54	10YR 6/3
1C	2.1	0.79	0.20	5.36	52	10YR 5/3
7E	1.1	0.93	0.15	6.95	104	10YR 5/4
8E	0.9	0.72	0.12	7.49	97	10YR 6/3
9E	0.7	0.64	0.09	6.81	187	10YR 6/3
11E	0.9	0.97	0.11	7.52	138	7.5YR 6/4
11C	4.7	1.0	0.38	5.93	117	7.5YR 4/3
2E	1.0	0.87	0.14	6.72	161	10YR 6/6
2C	3.4	1.1	0.30	5.97	136	10YR 5/3
3E	1.0	1.1	0.14	7.41	130	10YR 7/4
6E	0.9	1.1	0.13	6.21	62	7.5YR 6/6
6C	4.1	1.1	0.38	5.01	137	10YR 4/4
10E	1.2	1.4	0.16	6.09	80	7.5YR 6/6
10C	2.5	1.2	0.26	5.25	98	7.5YR 5/4
Gombe						
E	1.0	0.70	0.14	4.66	62	7.5YR 5/6
C	2.6	0.59	0.24	4.44	48	7.5YR 4/4

[a]Color follows Oyama and Takehara (1970). E = eaten; C = control.

ing termite mound material for ingestion over the surrounding soils, although the composition is very similar in many other respects.

Acidity. pH measurements of the soils (Table 2) show that the eaten soils have consistently higher pH than the control soils, as did the data from Mahale geophagy soils of 1995 (Mahaney et al., 1999). An antacid function has been proposed for geophagy in some monkeys (Poirier, 1970; Oates, 1978; Davies and Baillie, 1988) to maintain a higher pH in the forestomach. Although primates such as chimpanzees and humans do not maintain a forestomach bacterial flora, these soils may play an analogous role. While the lower pH of the control soils may be related to their higher organic content, the higher pH of the termite mound soils may also be due to termite activities in creation and maintenance of suitable conditions for the cultivation of fungal gardens.

Electrical Conductivity. The electrical conductivities of pairs of eaten and control soils (Table 2) do not show any clear trends. Intersite variability is far greater than intrasite variability, likely a reflection of differences between the eaten and control soils seen in data presented here.

Soil Color and Texture. Soil colors of the eaten and control soils range from yellow–red hues of 10YR 5/4, 5/6 (bright to dull yellowish brown) to stronger

yellow–red 7.5YR 4/4, 4/6 (brown, bright brown to orange) hues (Oyama and Takehara, 1970). Control samples, collected approximately 6–8 m away from a mound, came from the surrounding Ah (organic) or subsurface (nonorganic) horizons, giving a mix of 10YR and 7.5YR colors (Oyama and Takehara, 1970) that were sometimes lighter and sometimes darker than the eaten soils (Table 2). In five of six cases in Table 2, the control soils are slightly darker by one color value and two chroma, indicating a slightly higher amount of organic matter. This is likely due to greater amounts of humus in the control soil compared to the termite mound soil. Of the eight eaten soils analyzed, four have 7.5YR colors, indicating higher amounts of iron. The change in yellow–reddish hues from 10YR to 7.5YR reflects increasing redness, and most probably reflects the form and relative amount of iron in these soils, either as goethite (yellow hues) or hematite (more reddish hues). Soil textures are clay to sandy clay loam (Mahaney et al., 1999), apparent as observed on well-worn animal paths in the area (M. A. Huffman, unpublished results). There are no dominant soil structures in the samples studied, but occasionally subangular blocks are present, which must play a minor role in soil aeration. The termite soil ingested by chimpanzees is considerably richer in clay-size material than the nearby control soils, which are closer in texture to those described by Collins and McGrew (1988).

Scanning Electron Microscopy. Sands in the eaten samples were studied to elicit information on their mineral composition, weathered state, and the composition of coatings they carry. Most grains are coated either with clay minerals or Fe oxides (Figure 4A) suggesting they serve as a source of fine particles or oxides which might play a role in nutrition or self-medication, or alternatively in increasing the adsorption of molecules on the soils. (Figures 4B, 4D). Quartz, which is present in all the samples studied, is either fairly fresh (10% of the samples studied; Figure 4C) or coated to varying degrees with 50% etching, sometimes as in Figure 4E. The remaining 40% are well etched, as in Figure 4E. The data suggest that sands carry coatings that could provide chemical elements important in nutrition. Iron is so prevalent in the suite of samples studied that we first assumed it would be available for absorption. The clay mineral coating on Figure 4F supports the clay mineralogy, which shows a preponderance of 1 : 1 clay minerals including halloysite and kaolinite. When the soils are ingested, these clays, similar to pharmaceutical clays, would be exposed to the gut surfaces as coatings on these sand grains. Organiogenic forms seen on some of the sands from local termite mounds may be related to the presence of micro-organisms.

Geochemistry. Data from analyses of whole soils (Table 3) show that they contain high amounts of iron (>2–6%) in combination with high aluminum (approximately 10%). Potassium occurs at 1–3%. Calcium values are low, with only one site (1C) showing >2%. Sodium levels are also low, at <1%, except for sites 8 and 9, which contain about 1.7%. The essential trace elements cobalt and
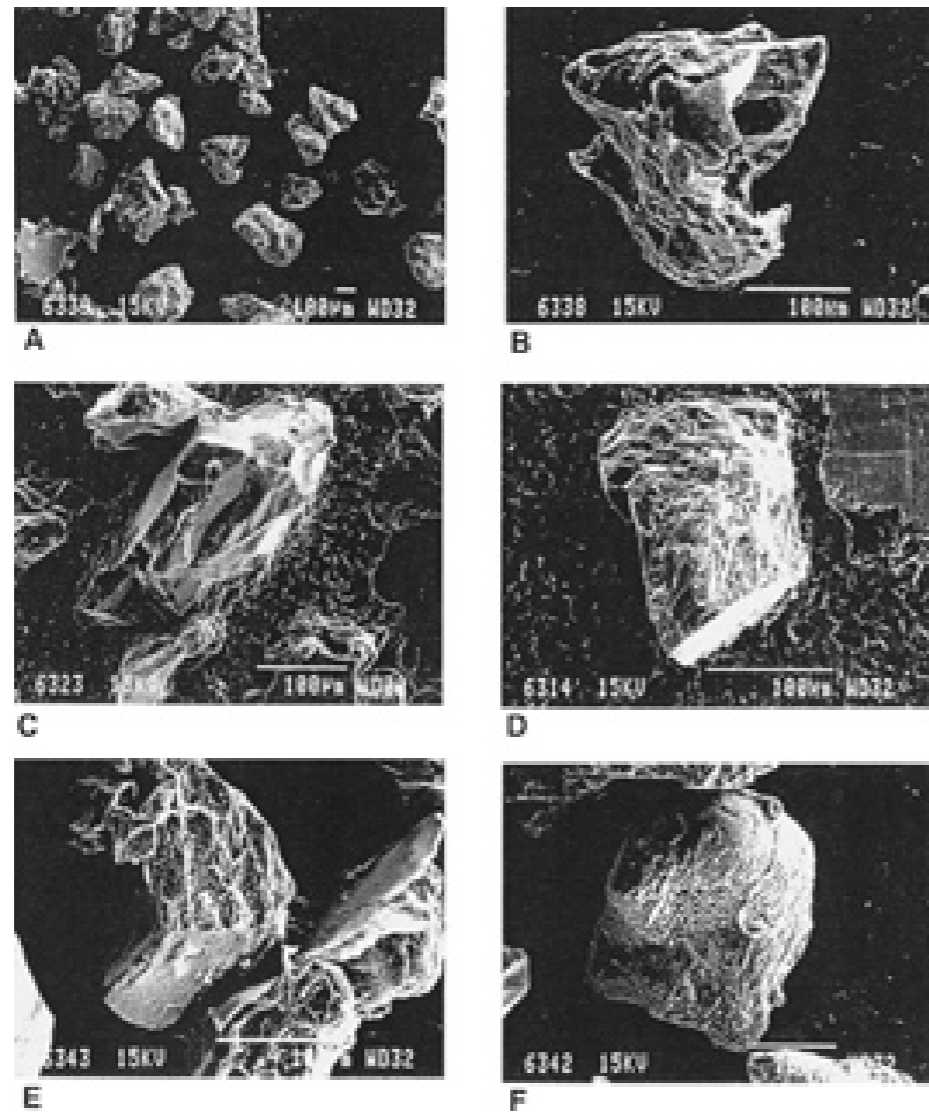
FIG. 4. Scanning electron microscopy from Mahale geophagy soils. (A) Group shot of mixed anhedral and euhedral grains of both lithic and mineral particles; most grains are coated with clay minerals or Fe oxides. Grain in center is enlarged in B. (B) Angular fragments from center of group. Flat, bent biotite mica on central surface, quartz above and amphibole (Fe, K, Mg, Al, Si) below. (C) Clean euhedral quartz showing polyhedral, grooved form with unworn, unetched surfaces—probably represents volcanic, pyroclastic particle. (D) Hornblende (Fe, Ca, Al, Si) showing compound morphology of etched, anhedral surfaces in contrast to cleavage-surface sides. Outcropping of amphibole cleavage on an anhedral surface form. (E) Quartz displaying a compound form with an organiogenic dendritic form on etched microstructural mineralic form, possibly a mineral other than quartz (calcite, feldspar or apatite), in contrast to fracture-surface sides. (F) Quartz displaying subround form, probably physically rounded. Etched clean upper surface is in sharp contrast to clay coating with Si : Al ratio of 1 : 1 indicating halloysite or kaolin group minerals. Linear and lobate-shaped clay ridges indicate downward movement of termite mount fluid on an overgrowth of possible crystalline, geopetal ornamentation.

TABLE 3. ELEMENT CONCENTRATIONS IN WHOLE SOILS DETERMINED BY INAA[a]

Sample	Al (%)	Ba	Ca (%)	Ce	Cl	Co	Cr	Cs	Eu	Fe (%)	Hf	I	K (%)	Lu	Mn	Na	Nd	Rb	Sc	Sr	Ta	Tb	Th	V	Yb
Mahale																									
1E	10.1	750	2.2	83	<120	12.6	18	1.8	2.5	5.77	7.3	<8	1.5	0.34	721	5050	53	72	19.6	260	0.4	0.83	10.3	135	3.2
1C	8.1	880	2.9	78	<150	8.4	16	1.1	2.2	4.44	12.0	<9	1.6	0.35	655	6360	39	58	23.0	360	1.1	0.65	16.2	144	3.0
7E	9.7	910	1.6	102	<160	11.7	19	1.7	2.0	4.14	8.4	<11	2.3	0.45	975	7360	44	113	15.9	270	1.0	0.76	14.2	95	3.7
8E	10.3	820	1.3	125	<200	10.1	16	1.5	2.6	4.28	8.5	<13	2.6	0.50	707	17500	69	110	13.6	320	0.8	1.01	14.5	83	4.1
9E	9.3	1280	1.4	98	<200	10.2	11	1.1	2.3	3.58	8.6	<10	2.5	0.49	798	17100	50	120	12.4	300	0.8	1.14	13.6	76	3.9
11E	11.6	580	0.5	112	<100	6.2	16	2.1	2.0	3.69	6.9	<8	2.9	0.72	438	5590	62	150	12.3	140	1.8	0.94	34.2	71	6.2
11C	7.9	510	0.6	80	<90	3.6	11	1.5	1.1	2.37	8.9	<4	3.4	0.40	362	7960	24	140	8.8	160	2.1	0.46	22.7	44	3.4
2E	9.6	680	0.4	92	<80	7.6	15	1.7	1.6	3.54	11.0	<8	2.6	0.36	617	2030	36	85	12.4	100	1.0	0.57	14.9	74	3.0
2C	9.0	680	0.4	77	<70	5.6	13	1.4	1.7	3.18	7.7	<7	2.7	0.34	519	2140	39	79	10.6	160	0.8	0.59	12.1	69	2.6
3E	10.9	700	0.5	114	<90	5.8	16	2.4	2.3	3.17	7.2	<8	2.8	0.52	464	1980	66	100	11.0	140	1.1	0.91	22.9	66	4.2
6E	12.0	680	0.1	108	<60	6.6	19	1.6	2.1	4.17	7.6	21	2.2	0.44	411	730	51	74	13.5	95	1.1	0.81	16.7	86	3.7
6C	8.7	680	0.2	94	<40	9.6	18	1.4	1.6	3.16	8.9	<4	2.3	0.36	413	920	40	79	10.6	160	1.1	0.57	14.0	62	2.7
10E	13.5	570	0.1	97	<60	5.8	20	2.4	1.6	5.64	7.9	22	1.5	0.37	254	700	37	65	17.5	51	1.0	0.63	17.4	121	3.3
10C	10.3	630	0.2	58	<40	4.9	17	2.1	0.9	4.15	12.0	16	1.7	0.27	220	1020	18	68	14.0	<45	1.0	0.30	13.9	92	2.2
Gombe																									
E	6.8	170	<0.1	72	190	3.6	136	3.4	1.5	4.15	6.9	15	1.7	0.29	304	330	31	80	13.0	120	1.8	0.50	12.5	155	2.5
C	3.4	91	<0.1	36	290	1.7	76	1.2	0.7	2.32	4.3	5	0.9	0.15	56	260	15	37	5.0	<32	1.0	0.20	5.9	99	1.3

[a] All data are in μg/g except those in marked %, as shown E = eaten; C = control.

chromium are present at measurable levels: cobalt is found in amounts of 2–13 μg/g and chromium at 10–20 μg/g, except for the Gombe site where roughly 100 μg/g was found. Several grams of soil are consumed daily.

Taking the north–south cline into consideration, calcium and manganese concentrations decrease in the sites from north to south, as does sodium (with some exceptions). Other chemical elements vary by up to a factor of 2 from site to site, indicating that we cannot use the whole data set as a coherent geochemical entity, but must restrict the geochemical evidence to a site-by-site (or microregion-to-microregion) basis. Electrical conductivity measurements (above) support this reservation.

In general, the levels of nutritionally important elements in these samples were similar to those reported by Hunter (1973) for soils consumed by humans in Africa. Hunter concluded they are a good source of mineral element supplementation. At Mahale, at each site for which we have both eaten and control material, the concentrations of most elements are highest in the eaten material. Enrichment of geophagy soils with essential elements has been noted previously. (Muller et al., 1997; Heymann and Hartmann, 1991; Mahaney et al., 1990; Davies and Baillie, 1988), and suggests that geophagy can be a strategy for obtaining mineral nutritional supplements. The presence of higher amounts of certain elements in soils is not, however, sufficient evidence of their availability for uptake, as shown below.

Acid Extractions. Acid extracts of the soils were prepared in order to simulate the dissolution of soil elements by the gastrointestinal tract. Although extraction using hydrochloric acid would have been a more realistic simulation of conditions in the stomach, the high chlorine levels thus produced would make analysis by INAA impossible for many elements. Thus, ammonium oxalate titrated to a pH of 1.99 was used to extract the soils. The elemental levels determined in these extracts are a measure of those available for absorption especially in the stomach. Changes of pH in the intestines may affect the element concentrations in solution at later points in passage of the soil through the digestive tract. Overall, low amounts of elements were found in the extracts. These levels were below those reported in a similar digestive experiment by Hunter (1973). The nutritionally important elements Ca, Co, Mn, Na, K, and V were determined in most of the samples analyzed. Cr, I, and Mg were below detection limits in many samples, although they were measured at site 10 (Tables 4 and 5).

Iron, an important nutritional element, although high in whole soils, was not detectable in extracts (Table 5). Aluminum, barium, and bromine, while generally not considered to be nutritionally important, may have some therapeutic effects and were present in most of the extracts tested. The enrichment, relative to control soils, of eaten samples with certain elements was not observed for these extracts. On the whole, the extracts contain only very low levels of inorganic nutrients, as shown by INAA. The results are not indicative of these soils as good sources of such nutrients.

TABLE 4. SHORT-LIVED ISOTOPE-PRODUCING ELEMENT CONTENT OF SOIL EXTRACTS[a]

Sample	Al	Ca	Cl	I	Mg	Mn	Na	V	K
Blank	0.02	—	0.25	—	—	—	0.49	<0.001	<0.4
Mahale									
1E	39	<16	59	<0.2	<12	0.51	25	0.11	24
1C	32	<17	71	<0.2	<10	0.18	35	0.07	110
7E	39	<16	59	<0.2	<12	0.57	25	0.11	17
8E	58	47	53	<0.2	<13	0.26	22	0.16	
9E	85	140	65	<0.2	<14	0.47	31	0.11	
11E	350	95	80	<0.4	<34	0.37	<26	<0.12	
11C	32	88	97	<0.1	<12	0.48	36	0.13	120
2E	26	95	76	<0.2	<11	0.27	34	0.09	88
2C	91	70	86	<0.2	<16	0.44	47	0.12	
3E	17	14	66	<0.1	<10	0.12	35	0.07	35
6E	54	<17	92	0.5	<13	0.76	37	0.13	41
6C	87	5	64	<0.2	<13	1.23	<13	0.15	65
10E	36	<19	62	0.9	28	0.54	25	0.13	54
10C	63	46	51	0.7	21	0.26	22	0.22	
Gombe									
E	61	<18	67	1.1	<13	1.82	26	0.17	34
C	130	54	98	0.2	13	0.55	36	0.17	54

[a] All data are in μg element/g dry soil extracted; blank values are in μg element/ml ammonium oxalate. E = eaten; C = control.

XPS Analyses. We hoped to obtain information on the surface characteristics of the soil particles by assaying inorganic elements on the soil surfaces using XPS analysis. These surfaces, the interface of soil particles and their environment, would, when ingested, interact directly with the digestive tract and its contents. The soil particles in these samples are likely to consist of mineral grains

TABLE 5. LONG-LIVED ISOTOPE-PRODUCING ELEMENT OF SOIL EXTRACTS[a]

Sample	Ba	Br	Co	Cr	Fe
Mahale					
7E	160	0.4	0.50	<2.3	<82
11C	350	3.1	0.75	<3.4	<92
10E	<50	<0.3	0.56	5.8	<65
Gombe					
E	330	0.5	1.0	<2.3	<130
C	<50	0.9	1.3	<2.0	<69

[a] Values are μg/g soil, determined in ammonium oxalate extracts of some geophagy. E = eaten; C = control.

TABLE 6. RESULTS OF ANALYSIS OF SOIL SAMPLES FROM MAHALE BY X-RAY PHOTOELECTRON SPECTROSCOPY REPRESENTING COMPOSITION OF APPROXIMATELY 3 SURFACE ATOMS DEPTH OF SAMPLE (RELATIVE ATOMIC PERCENT)

Sample	Fe	O	Ca	Mg	K	C	Si	Al	SiO_2/Al_2O_3
Mahale									
1	1.12	61.1	0.42	1.31	0.44	7.2	17.8	10.6	2.0
1C	0.67	56.8	0.45	1.30	0.43	13.9	16.7	9.8	2.1
7E	0.55	60.6	0.38	0.95	0.32	6.5	19.2	11.4	2.0
8E	1.05	58.6	1.08	0.65	0.23	10.1	18.9	9.5	2.4
9E	1.97	24.5	1.45	2.41	0.63	19.1	33.3	16.7	2.4
11E	0.57	30.9	0.19	0.45	0.08	7.7	39.0	21.1	2.2
11C	0.45	50.6	0.26	0.85	0.41	23.7	15.2	8.4	2.2
2E	1.62	60.6	0.22	0.51	0.33	7.3	18.4	11.7	1.9
2C	0.45	54.8	0.23	0.68	0.46	15.6	17.3	10.5	1.9
3E	0.63	60.3	0.24	0.54	0.29	8.4	17.9	11.7	1.8
6E	0.69	61.7	0.10	0.31	0.21	5.8	18.8	12.4	1.8
6C	0.46	56.1	0.13	0.36	0.25	14.9	16.9	11.0	1.8
10E	0.75	60.3	0.11	0.24	0.15	9.3	17.4	11.7	1.8
10C	0.64	56.4	0.10	0.32	0.18	13.3	17.2	11.7	1.8
Gombe[a]									
E	0.87	61.2	0.52	0.51	0.85	5.9	18.6	11.2	2.0
C	0.41	48.5	0.13	0.41	0.42	28.9	14.0	7.3	2.3

[a] E = eaten; C = control.

coated to varying degrees with clay minerals. The surfaces may thus represent the elemental composition of the clay minerals and molecules adsorbed onto the clay matrix, as well as some contribution from sharp or scraped-clean edges of hard mineral grain surfaces. The analytical technique allows for determination of all elements above a detection limit of about 1%. The results (Table 6) show that eight elements compose most of the soil particle surfaces.

Comparison with data from whole-soil analysis (Table 3) shows that Fe values, although relatively high (0.4–1.6%) in the surfaces, are more elevated in the interior of the particles. Although Fe is frequently found at high levels of several percent in soils ingested by primates, these soils do not seem to be good nutritional sources for the element, as Fe was not detectable in the extracts. However, without exception, the surfaces of the ingested soil particles were enriched with Fe compared to the controls. Whether iron plays a role in any physiological function of geophagy in this case is unknown, but charge relationships on the clay lattices could be altered by its presence, and hence adsorption properties of other molecules at these surfaces may be affected positively or negatively for different adsorbents. Fe may be present in a variety of forms in soils and clays, and further work would be useful to examine the form of iron in these soils which renders it unavailable for release at low pH. The interactions of iron in

aqueous media with clay surfaces are complex and alter the electrical properties of the clays (Henry et al., 1990), and the form of iron in these soils may play a role in their high adsorptive properties.

Magnesium was found to be enriched at the particle surfaces, relative to data for whole soil composition, but Ca was found at comparable levels in both the whole soils and at the soil particle surfaces, except in the Gombe samples, which were surface-enriched for Ca. Potassium was lower in the surfaces relative to whole-soil values. Aluminum was often slightly enriched, supporting the expectation that high-aluminum clay minerals coat the particles. Oxygen and silicon, however, were only measured by XPS. At all but one site, where both eaten and control samples were available, oxygen was found in higher concentrations in the eaten sample. This may be an indication of a higher oxide content and lower organic content in the eaten samples, as suggested by carbon concentration data for the whole soils (Table 2).

For each pair of samples, the carbon content was considerably higher in the controls, supporting the suggestion of Hladik and Gueguen (1974) that choosing insect-sorted soil reduces the ingestion of detrital organic material, including perhaps pathogens and parasites. The soil surfaces do not differ in carbon content between eaten and control samples except for the sample from Gombe, where the control soil is also enriched with carbon. Lower levels of carbon were found in the soil particle surface layers, compared to the whole soils. The surface carbon may include organic components or microorganisms peculiar to the chemistry of these soils and potentially beneficial to the chimpanzees. Organogenic forms on some surfaces were noted under SEM (see above).

Adsorption Capacity of Soils. Quinolizidine alkaloids (sparteine, lupanine), tropane alkaloids (atropine), and quinoline alkaloids (quinine) were employed as model compounds for toxic metabolites produced by plants (Wink, 1993a). The results, expressed as means ± SD, of triplicate analyses show that all the soil samples analyzed are capable of binding alkaloids, albeit to differing degrees (Table 7). Alkaloids remained unchanged in chemical terms, i.e., no degradation or conversion products were observed by GLC and GLC-MS. Soils from Mahale showed equally high adsorption rates for all four alkaloids. These binding properties are in general comparable to those of coal and charcoal, materials that are also used therapeutically to adsorb dietary and microbially produced toxins. However, usually the absolute capacities of soils were somewhat lower, depending on their chemical compositions. No differences were found between eaten and uneaten soils in most Mahale samples, an observation also made in earlier studies (Mahaney et al., 1999). However, in the Gombe samples, and in Mahale samples from sites 1 and 11, adsorption was lower in controls as compared to the eaten soils.

The chimpanzees of Mahale and Gombe National Parks eat soil with characteristic properties. Our results indicate certain features of the soils that should

TABLE 7. ALKALOID ADSORPTION OF SOILS FROM MAHALE AND GOMBE, TANZANIA[a]

Sample	Alkaloid adsorption (%)			
	Atropine	Lupanene	Quinine	Sparteine
Mahale				
1E	68.1 ± 0.1	49.6 ± 1.4	83.8 ± 0.6	54.7 ± 1.2
1C	26.7 ± 6.3	36.0 ± 2.5	60.3 ± 2.3	46.0 ± 1.8
7E	56.2 ± 4.8	32.5 ± 6.1	57.3 ± 1.1	34.4 ± 0.8
8E	84.4 ± 2.7	58.0 ± 1.1	88.8 ± 1.1	73.7 ± 3.1
9E	74.3 ± 0.0	445.9 ± 0.7	74.0 ± 1.0	60.8 ± 4.8
11E	59.6 ± 11.8	32.4 ± 2.9	74.4 ± 1.0	45.1 ± 2.8
11C	55.7 ± 12.3	22.3 ± 4.2	54.2 ± 1.3	43.1 ± 5.8
2E	56.8 ± 5.6	29.2 ± 2.3	54.1 ± 0.6	41.1 ± 6.9
2C	57.4 ± 6.3	38.1 ± 1.8	62.0 ± 3.2	47.5 ± 4.1
3E	65.2 ± 16.5	37.1 ± 0.0	76.9 ± 0.8	48.9 ± 2.9
6E	50.9 ± 2.8	21.4 ± 2.1	65.2 ± 2.8	34.9 ± 3.9
6C	54.1 ± 9.7	32.3 ± 2.9	50.3 ± 3.9	48.9 ± 3.7
10E	70.1 ± 0.2	39.3 ± 6.0	67.5 ± 5.5	46.6 ± 3.2
10C	63.1 ± 7.6	36.0 ± 3.1	54.3 ± 4.9	49.2 ± 1.1
Gombe				
E	57.9 ± 2.5	44.0 ± 5.5	46.8 ± 0.6	65.8 ± 3.6
C	46.0 ± 8.9	20.0 ± 1.0	6.5 ± 7.0	42.8 ± 5.4

[a]E = eaten; C = control.

be investigated further. One benefit of the ingestion of highly adsorptive high-clay soils has been considered to be the uptake by the soils of plant alkaloids and antinutrients ingested with a wild plant diet, shown to occur in vivo in birds (Wink et al., 1993; Gilardi et al., 1999), thereby preventing absorption of deleterious plant constituents via the gut. Such adsorptive properties are also known to be useful in preventing the absorption of bacterial toxins and in immobilizing and isolating pathogenic organisms in the gut by means of the adsorption of clay particles to surround the harmful organisms, to be eliminated with the feces (Said et al., 1980; Gardiner et al., 1993). Another possibility is the formation by clay particles of a mucoprotective barrier against intestinal pathogens, as shown in the lumen of *E. coli*-infected sections of rabbit ileum in vitro by Rateau et al. (1982). Knezevich (1998) cited low levels of diarrhea in geophagous rhesus macaques of Puerto Rico, in spite of high parasite loads, and thus geophagy may be a technique for tolerating the presence of some pathogens in the intestinal tract by blocking their action. Traditional human use of insect sorted soils in Zimbabwe for digestive upsets may be a parallel behavior to the use of termite mound soils by chimpanzees of Tanzania (Aufreiter et al., 1997). The mineral compositions of the Zimbabwean soils were comparable to that used in the pharmaceutical Kaopectate. However, such soils were reported to be recommended for collec-

tion from areas of the mound that had recently been disturbed by the insects. This suggests awareness of an influence on the soil resulting from insect contact, which could be the release of a functional compound or of beneficial microorganisms of insect origin applied as a remedy. Currie et al. (1999) demonstrated a mutualism between fungus-growing ants and a filamentous bacterium (an actinomycete) that produces antibiotics specific to a parasite of the insect-cultivated fungus. The ants studied by Currie et al. (1999) carry the bacterium on their ventral surfaces. The possibility that the chimpanzees ingesting termite mound soil are exploiting a similar insect-microbial relationship of termites is intriguing. Possibly some geophagy soils may also be a source of symbiotic organisms: Kortland (1984) pointed out the possible benefit to the health of zoo captives of a ciliate first observed and recognized by Reichenau (1920) as a potential symbiont of higher primates. The cellulose-digesting ciliate organisms, *Troglodytella abrasarti* and *T. gorillae*, were found attached to the mucous membranes of the cecum and large intestine, and although abundant in just-captured chimpanzees and gorillas, they disappeared soon from the gut of the captive animals, who also developed diarrhea in captivity. The source of these ciliates was not determined, and they were later dismissed as parasites, a conclusion disputed by Kortland (1984). These examples suggest the possibility of a relationship between intestinal microorganisms and the good health of higher primates that is maintained by geophagy. The soil is a possible source of beneficial gut microflora as well as of pathogens, and the gut flora of wild primates necessarily reflects their behavior and environment.

The role of geophagy among humans affected by iron depletion and anemia has long been a matter of debate. Geophagy in humans frequently has been associated with anemia and increased parasite loads, but it remains unclear whether geophagy of some soils is a causative factor or whether iron depletion or anemia may lead to geophagy. Geissler et al. (1997, 1998) found soils eaten by Kenyan children having a high prevalence of malnutrition to release iron at low pH sufficient to supplement their diet with 4.7 mg iron per day. They found, however, that there was more anemia and low serum ferritin among geophagous children, relative to those who did not eat soil. The soil eaten by chimpanzees of Tanzania did not release detectable iron at low pH. Iron depletion and anemia were associated with geophagy in Kenyan children (Geissler et al., 1998), whereas serum ferritin concentrations were found statistically to be dependent on geophagy, although no inference could be made on causality.

The possible prevention of iron absorption by geophagy of soils containing high iron concentrations may depend on the form of the iron. This has not been adequately investigated in geophagy studies but may help to predict the effect of iron on clay adsorptive and aggregation properties and on iron uptake or release by the clays. These are likely to be important in determining the consequences of geophagy. The effects of ingestion of several geophagy soils on iron uptake

in humans was tested by Minnich et al. (1968), who found that some soils, high in smectite, inhibited iron absorption while others, high in 1 : 1 clay minerals, did not. Iron may be associated with clay minerals in several forms. Ferric iron surface-complexed on mineral matter can be detrimental to health, in that it has been shown to play a role in increased lung inflammation related to inhalation of silicate dusts (Ghio et al., 1992), and silicate dusts can complex iron from biological sources. Structural iron, if in the form of Fe(III) on the other hand, may be reduced by soil bacteria to Fe(II). This reduction has also been shown to alter the swelling properties of smectites and also to affect particle and pore size (Gates et al., 1998). Acid may to some extent dissolve the edges of clay lattices and release such iron. Ferrous iron salts [Fe(II)] are readily absorbable, while ferric iron [Fe(III)], the rust-colored, oxidized form often found in reddish tropical and semitropical soils, is not considered bioavailable as a salt. However, it may be bioavailable but slowly released when complexed [for example, as in a polymaltose complex, as discussed in Tuomainen et al. (1999)]. The proportions of iron in different forms, the clay mineralogy of the soils, and the gut contents likely interact to confer particular properties on different soils when eaten. It is unknown if animals practicing geophagy can distinguish soils of different properties. The termite mound soils analyzed here, although shown to be high in iron, with slightly lower concentrations on the particle surfaces than the soil particle interiors (Tables 3 and 6) were found not to release detectable iron at low pH. The iron in these samples may be surface-complexed iron, with a limited availability for release from the clay, or it may be structural iron, since it was in a lower concentration at the surface of the soils particles. More extensive investigation would be necessary to clearly define the forms of iron present and the conditions for its bioavailability.

Minnich et al. (1968) described that high clay soils rich in iron and in smectites are habitually snacked on by women in Turkey, and they showed that their ingestion prevents iron uptake by the gut. The practice was associated with the occurrence of anemia among the women. The Turkish soils were also high in smectite. The termite mound soils of western Tanzania, however, contain significant amounts of illite and smectite–illite, 2 : 1 clay minerals comparable to smectite. Although they are rich in iron, they do not release it as low pH. The possible prevention of absorption of significant amounts of iron may have benefits for higher primates afflicted with pathogens: a wide spectrum of bacteria and fungi pathogenic to humans thrive in the presence of iron. Individuals with hyperferremia are more susceptible to pathogens: children with sickle cell disease commonly die of bacterial infection related to iron overload (Weinberg, 1974). Release of iron by the destruction of red blood cells in sickle cell anemia and thalassemia, and from the destruction of liver cells in malaria, increases susceptibility to pathogens as well as being damaging in its own right. Populations of humans practicing geophagy overlap with populations suffering from these diseases, and it would be valuable to determine whether ingestion of high clay soils may be helpful in this respect.

Episodes of rapid absorption of iron, such as may be obtained from modern mineral supplements, may temporarily saturate the body's system for trapping free radical chemical species, leading to higher rates of lipid peroxidation (Tuomainen et al., 1999). Clay slows the movement of food through the gut. If very low levels of iron were continually released from some clays as they slowly move through the gastrointestinal tract, some iron could be provided for absorption, but the occurrence of peaks of iron absorption could be prevented. The already high concentration of iron in the soils may lend the soils particular, possibly useful aggregative and adsorptive properties, but, depending on its form, may also be a safety factor in limiting the rate of iron uptake by the gut.

However, an increased oxidative capacity in red blood cells has also been suggested to be beneficial in suppressing malarial infection. The Hausa of northern Nigeria, among whom geophagy is common (Wiley and Katz, 1998), utilize a variety of plants with antimalarial properties in their diet. The efficacy of these plants is related to rendering erythrocytes more sensitive to oxidative damage (Etkin and Ross, 1983). Excess oxidation is controlled by the erythrocyte by catalase, an enzyme that uses ferric iron as a cofactor. Sufficient iron in this case is therefore also necessary in controlling excessive oxidation.

In relation to the chimpanzees of Mahale and Gombe, the relationship between pathogenicity of *Entamoeba histolytica* and serum iron in humans, described by Meerovitch (1982), is particularly suggestive. Dysentery due to *E. histolytica* is more severe on an iron-rich diet, while cultures such as the Masai show a much lower incidence of this disease. Meerovitch (1982) related this to their drinking of milk, which supplies lactoferrin and transferrin, to compete with the bacteria for iron uptake in the gut. If adsorbent clay-rich soil also acts to take up iron in the gut, thereby inhibiting the pathogenicity of such bacteria, it would have considerable value in a semitropical environment.

Observations of ingestion of termite mound soils by chimpanzees apparently suffering from intestinal parasites and gastrointestinal symptoms were the impetus to this study (Mahaney et al., 1996, 1999), and ingestion of these soils may represent for the chimpanzees a means to limit the virulence of dysentery. Meerovitch (1982) noted that a high-starch diet, such as in herbivores, promotes the encystment of *E. histolytica*, so that such individuals may have high rates of infection, but high iron must be available for pathogenicity and virulence to rise. A change to meat diets would therefore promote virulent dysentery in such populations. Iron deficiency in human infants and young children is, however, associated with (negative) changes in cognitive function (Pollitt, 1993), and maintenance by geophagy of low iron stores to control pathogens may bring developmental risks. If humans use geophagy at a cost to cognitive abilities in order obtain resistance to endemic pathogens, geophagy is less likely

to have been part of the human behavioral repertoire throughout the course of evolution. The survival of geophagy as a prevalent behavior among pregnant women of nonindustrial cultures argues for its adaptive value (Geissler et al., 1999). We suggest that an adaptive benefit of geophagy of some soils is restriction or control of iron uptake, which helps to limit deleterious effects of some pathogens present in semitropical environments. An important area of investigation in understanding the effects of geophagy would be the routine application of methods that characterize iron chemistry and bioavailability in relation to clay mineralogy in geophagy soils and determination of the effects of iron speciation on clay aggregative behavior, on bioavailability of inorganics including iron, on microbial populations in the soil and in the gastrointestinal tract of geophagous creatures, and on adsorption of organic molecules onto the soils in physiological conditions.

In this context, the results of our analyses of termite mound soils eaten by wild chimpanzees of Mahale and Gombe are consistent with several mechanisms by which ingestion of high-clay soils help in maintaining health in a semitropical environment. In general, geophagy can be described as use of specific soil types, having particular clay components, which form in semitropical environments, as agents for the possible transfer of functional microorganisms and of molecules between the environment and the animals of that environment. They filter toxins and influence the movement of inorganic elements among plants, microorganisms, intestinal parasites, and the digestive tracts of animals.

Some geophagy soils have been shown to release significant amounts of nutrient elements at physiological pHs and are thought to contribute supplementary elements to the diet. It is unknown if geophagy soils with differing properties can be distinguished by sensory means. Although cultural memory may help primates to choose a location for obtaining appropriate soils, the mineralogy and possibly the compounds adsorbed by the clay minerals may also be a basis for sensing which soils are suitable for ingestion among those available in a complex environment. In relation to the particle surfaces, behavioral observations included that the chimpanzees often sucked on the soils, "like hard candy" (M. A. Huffman, field observations) and thus they were wetted in the mouth with saliva and likely broken down into small pieces before swallowing. Adsorbed material on the surface could be released by this behavior, which could be due to liking the taste of the soils, possibly because of the taste of kaolin materials or the taste of organics left by termite activity or of microorganisms. Here, it may be associated with chimpanzees monitoring the mounds for the presence of termites (Uehara, 1982). Occasionally, the chimps were observed to chew the soils, which would expose fresh mineral surfaces to the gut environment. Kaolinite, a 1 : 1 mineral often in high abundance in geophagic soils, is said to have a characteristic odor (and hence taste), and primates have been observed sniffing at soils before ingestion or rejection (Bolton et al., 1998; Uehara, 1982). Local children

at Mahale have said that they eat termite mound soil or clay from the walls of their homes in the rainy season because of the good smell. Women of Kenya distinguished between soils on the basis of taste (Geissler et al., 1999). Animals experienced in geophagy may also recognize the smell and taste of appropriate soils depending on their composition.

In summary, termite mound soils eaten by wild chimpanzees of the Mahale Mountains are rich in clay, particularly 1 : 1 clay minerals, with significant amounts of 2 : 1 clay minerals present. Soils selected for geophagy are relatively high in pH as compared to surrounding (control) soils. They contain elevated iron, which may affect binding affinities for other molecules. They release markedly low levels of elements to solution at low pH, show a relatively high capacity to adsorb alkaloids such as are found in the plants forming the diet of wild chimpanzees, and have a lower organic content than surrounding surface soils. We have described the health benefits of ingesting these soils that are consistent with behavioral observations of the wild chimpanzees of Tanzania, which may be relevant to human populations practicing geophagy.

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Comparative microbial analysis and clay mineralogy of soils eaten by chimpanzees (*Pan troglodytes schweinfurthii*) in Tanzania

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Abstract

The abundance of culturable microorganisms in Tanzanian termite mound soils similar to those eaten by chimpanzees (*Pan troglodytes schweinfurthii*) was compared with corresponding non-eaten soils using a serial dilution plating method. Additionally, the particle size distribution and clay mineralogy of the soils were examined. The microbiology of these soils was examined as a preliminary step to investigate the hypothesis that this behaviour relates to self-medication with antibiotics. Significantly higher ($P = 0.037$) numbers of filamentous bacteria (actinomycetes), were detected in termite mound soils relative to control soils, but numbers of non-filamentous bacteria and fungi did not differ between the two groups. The clay content was significantly higher ($P = 0.007$) in eaten soils, but the mineralogy of the less than 2 μm fraction was similar for all soils. This study suggests that if chimpanzees selectively consume soils based on their content characteristics, the importance of the type of clay minerals may be secondary to the overall clay content. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Chimpanzees; Geophagy; Gombe; Mahale; Microorganisms; Termites

1. Introduction

Geophagy, or earth-eating, occurs worldwide in a range of ethnic, religious, and social groups and has been documented as early as 1398 (Parry-Jones and Parry-Jones, 1992). Throughout history, humans have eaten soil for a variety of reasons that include medicinal purposes, use during pregnancy, as a famine food, as a food additive or condiment, and for religious or ceremonial purposes (Laufer, 1930; Anell and Lagercrantz, 1958; O'Rourke et al., 1967). Geophagy in non-human primates has received increasing attention in the past two decades, and it has been documented in various species, including gorillas (*Gorilla gorilla*), chimpanzees (*Pan troglodytes*), and macaques (*Macaca mulatta*) (Hladik and Gueguen, 1974; Mahaney, 1993; Mahaney et al., 1993).

Given the long history and widespread occurrence of geophagy in both animals and humans, it is reasonable to hypothesize that the practice confers selective benefits. However, a conclusive biological explanation for the practice is lacking. Mason (1833) postulated that the consumption of soil might be a method of self-medication, and not a manifestation of disease, because of the presence of useful elements in soil, such as iron and alkalis. Similarly, mineral supplementation has been suggested as a reason for geophagy in red leaf monkeys (*Presbytis rubicunda*) and moustached tamarins (*Saguinus mystax*) (Davies and Baillie, 1988; Heymann and Hartmann, 1991), as well as a means by which to adsorb ingested dietary toxins (Oates, 1978; Gilardi et al., 1999). Vermeer and Ferrell (1985), Mahaney (1993) and Knezevich (1998) have all called attention to mineralogical similarities between eaten soils and pharmaceutical preparations commonly used for the treatment or prophylaxis of gastrointestinal upset or diarrhea in humans. Although these comparisons must be interpreted cautiously, they present a potential area for further research.

Termite mounds are a common source of soils eaten by animals and humans. Termites belonging to the subfamily Macrotermitinae are widespread throughout Africa and their mounds form a conspicuous part of the African landscape. These termites have evolved a symbiosis with a basidiomycetous fungus that they cultivate and eat (Darlington, 1994). The insects play an important role in the soil

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ecosystem by moving and rotating large quantities of soil. A small number of studies have investigated the microorganisms present in these mounds. Most work has focused on microorganisms present in the fungal garden, or those occurring in the gut or on the exoskeleton of the insect. Few studies have investigated the microorganisms present in the outer casing of termite mounds, which are the areas most often ingested by primates.

Chimpanzees (*Pan troglodytes schweinfurthii*) in the Mahale Mountains National Park and Gombe National Park, Tanzania, have been observed to eat soil, as have chimpanzees in Gabon and Uganda (Hladik and Gueguen, 1974; Mahaney et al., 1996, 1997; Goodall, personal communication). In Mahale and Gombe, chimpanzees eat soil from termite mounds, but this behaviour appears unrelated to the "fishing" and eating of termites by chimpanzees. Chimpanzees of all ages engage in geophagy; a piece of soil approximately 2.5 cm^3 is broken off from the top of the mound and is often rolled around in the mouth for a few minutes before swallowing.

Many soil microorganisms produce compounds with antimicrobial and/or antiparasitic activity. Among the prokaryotes in soil, filamentous bacteria (actinomycetes) are responsible for about three-quarters of all known medicinal antibiotic compounds (Kutzner, 1981). The present study used a serial dilution plating method to enumerate culturable microorganisms in termite mound soils eaten by chimpanzees and non-eaten, non-termite mound control soils. The microbiology of these soils was examined as a preliminary step to investigating the hypothesis that this behaviour relates to self-medication with antibiotics. Additionally, the particle size distribution and clay mineralogy of the soils were examined. The hypotheses were that termite mound soils would differ quantitatively in microbial content from control soils, and that the total clay content and mineralogy of the soils would be different between termite mound and control soils.

2. Materials and methods

2.1. Study sites

The Mahale Mountains National Park in western Tanzania (6°7′S, 29°55′E) and Gombe National Park (4°40′S, 29°38′E) are two field stations in East Africa where long-term study of chimpanzees has been conducted (Nishida et al., 1983; Goodall, 1986). Both parks contain groups of the same chimpanzee subspecies, *Pan troglodytes schweinfurthii*, and border Lake Tanganyika, one of Africa's three largest lakes. Both areas have a single rainy season from November to April, and average temperatures range from a minimum of 18.4 to 21°C to a maximum of 25 to 30.5°C (Goodall, 1986; Collins and McGrew, 1988). Soils in both parks tend to be relatively young, stony and porous, and in areas containing an organic horizon it is usually 1–2 cm deep (Collins and McGrew, 1988).

Mahale has numerous tall peaks and Nkungwe, the tallest, is 2462 m above sea level and composed of granitic gneisses and schists (Collins and McGrew, 1988). Mist and cloud development often occur in Mahale as a result of damp air blowing from the lake (Nishida, 1990). This dampness sustains a concentration of gallery forests which can be found at the north-western foot of the mountains from 780 to 1300 m, providing food and shelter necessary for the survival of the chimpanzees (Nishida, 1990). In Gombe the highest land points are about 1500 m, and composed of hard quartzite and some gneiss and sandstone (Collins and McGrew, 1988).

2.2. Sample collection

Soil samples were collected from the outer parts of Macrotermitinae mounds in Mahale Mountains National Park and Gombe National Park in October, 1996, using an alcohol-flamed spade. At each site, a sample of 200 to 600 g of soil was collected in a Ziploc bag. Soil samples were also collected approximately 5 m from each termite mound to serve as controls. All samples were double bagged and shipped in an insulated box to protect against temperature extremes during shipment. All samples were received in Toronto 10 days after collection.

Five termitarium soils (TM) and their corresponding control soils (C) were analysed: four samples were from Mahale Mountains National Park (1, 2, 6 and 10), and the fifth was from Gombe National Park ("G"). An additional sample from Mahale (11) was analysed for particle size distribution.

2.3. Soil pH and particle size distribution

The pH of the soil samples was determined by glass electrode, using a soil:water ratio of 1:5. For determination of particle size distribution, sand (63–2000 μm) was separated by wet sieving, and silt (2–63 μm) and clay (< 2 μm) were determined by hydrometer (Gee and Bauder, 1982). Mineralogical analyses of the clay fraction were on oriented mounts prepared by centrifugation, and X-rayed with a Toshiba ADG-301H XRD using CuK$_\alpha$ radiation and a scan rate of 1°2θ per minute (Whittig, 1965).

2.4. Serial dilution plating

A serial dilution plate method (Waksman, 1927; Johnson et al., 1959) was used to enumerate microorganisms in the soils. Dilution series were prepared for five subsamples of each soil. For the enumeration of fungi, 1 ml aliquots of dilution series were transferred individually into sterile petri plates and mixed with molten dextrose–peptone yeast-extract agar (Papavizas and Davey, 1959) supplemented with streptomycin, chlorotetracycline, and penicillin G (50 μg/ml of each). Starch–casein agar (Küster and Williams, 1964) was used to enumerate actinomycetes, and tryptic-soy agar (Martin, 1975) was used to enumerate

non-filamentous bacteria. Both media were supplemented with cycloheximide and nystatin (50 μg/ml of each). Plates were incubated at 20°C, and colonies were counted 4 days after inoculation.

2.5. Statistical analyses

Colony forming units per gram of dry weight soil (CFU/g) were determined for actinomycetes, non-filamentous bacteria, and fungi for each of the five subsamples of five termite mounds and their five corresponding control soils. Statistical analyses of microbiological data were performed on log$_{10}$ transformed data to correct for heteroscedasticity, and on pooled samples to obtain statistically valid replicates. The mean counts of microorganisms in termite mound and control soils and the percent clay in these soils were compared using a one-tailed pair-wise *t*-test (Rosner, 1990).

3. Results

Termite mound soils had significantly higher numbers of actinomycetes than control soils (Table 1; $t = 2.42$, df $= 4$, $P = 0.037$). There was no significant difference in the number of non-filamentous bacteria detected in termite mound and control soils (Table 1; $t = 1.40$, df $= 4$, $P = 0.12$), although the number of non-filamentous bacteria enumerated from termite mounds 1 and 10 was higher by an order of magnitude than control soils. Levels of culturable fungi were not statistically different between termite mound and control soils (Table 1; $t = 1.84$, df $= 4$, $P = 0.070$).

Termite mound soils were generally more alkaline (pH 4.7–6.7) than corresponding control soils (pH 4.4–5.4) (Table 2). The clay content of termite mound soils ranged from 30 to 68% and was significantly higher (Table 3;

Table 1
Concentration (CFU/g) of actinomycetes, non-filamentous bacteria and fungi in termite mound and control soils with standard errors of the means

Group	Termite mound		Control	
Actinomycetes (× 10^6)	11	6.5	1.7	0.42
Non-filamentous Bacteria (× 10^6)	15	7.9	5.3	1.2
Fungi (× 10^4)	14	2.6	32	9.2

Table 2
pH of soils

Sample	Termite mound	Control
1	6.4	5.4
2	6.7	ND
6	6.2	5.0
10	6.1	5.3
G	4.7	4.4

Table 3
Distribution of sand, silt, and clay in termite mound and control soils

	Sample										
	Termite mound						Control				
	1	2	6	10	11	G	1	6	10	11	G
Sand (63–2000 μm)	41	36	28	28	38	40	59	45	34	74	68
Silt (1.95–63 μm)	29	12	10	4	13	15	15	21	18	14	13
Clay (< 1.95 μm)	30	52	62	68	49	45	26	34	48	12	19

$t = 4.12$, df $= 4$, $P = 0.007$) than that of control soils. The mineralogy of the less than 2 μm fraction of all soils was dominated by metahalloysite, illite, and illite-smectite (Table 4). The relative amounts of 1:1 (Si:Al) clay minerals, such as kaolinite, halloysite, and metahalloysite, and 2:1 clay minerals such as illite, illite-smectite, and smectite, were similar between termite mound soils and control soils.

4. Discussion

Our findings suggest that termite mounds soils are notable in the numbers of actinomycetes they contain. Boyer (1955) and Meiklejohn (1965) reported higher numbers of certain types of bacteria in Macrotermitinae mounds compared with control soils in the Ivory Coast and Zimbabwe, respectively. Similarly, higher levels of bacteria, actinomycetes, and fungi as compared with control soils were reported from a mound of *Macrotermes bellicosus* in Nigeria (Amund et al., 1988). However, the numbers of actinomycetes in termite mound and control soils were lower than those detected in the present study (3000 and 220 CFU/g respectively). Zoberi (1979) found numbers of bacteria ranging from 3×10^6 to 1×10^7 CFU/g and numbers of fungi ranging from 4.5×10^4 to 2.03×10^5 CFU/g in the upper layer of a mound of *Macrotermes natalensis* in Nigeria. However, filamentous and non-filamentous bacteria were considered together in this study, and the surrounding soil was not analysed for comparison.

The percentage of clay-sized particles in termite mound soils was higher than in control soils, supporting our preliminary hypothesis that similarity in content of clay-sized minerals would be different between termite mound and control soils. This finding is comparable with other studies. Nye (1955) suggested an upper limit to the size of particles used to construct mounds of *Macrotermes* in the Ibadan area of Nigeria: termites preferred particles less than 2 mm, and Nye (1955) suggested an upper limit to the size of particle that the termites could carry of 4 mm. Arshad et al. (1982), investigating mounds of *Macrotermes* in Kenya, found the outer casing of a mound of *M. subhyalinus* to be 36% clay and the outer casing of a mound of *M. michaelseni* to be 43% clay, whereas the adjacent soil contained from 22 to 49% clay, depending on depth. *Macrotermes* mounds in Zimbabwe were also found to have a higher clay content

Table 4
Mineralogy of the <2 μm fraction of termite mound (TM) and control (C) soils. Semi-quantitative amounts of each mineral are given as trace (tr), small (⁻), moderate (⁻ ⁻), abundant (⁻ ⁻ ⁻) or not detected (0)

Sample	Kaolinite	Metahalloysite	Halloysite	Illite	Illite-smectite	Smectite	Quartz	Orthoclase	Mica	Plagioclase feldspar
1TM	0	–	–	– –	– –	tr	–		–	tr
1C	0	– –	0	– –	– –	tr	– –	0	– –	0
2TM	–	– – –	0	–		0	–	–		0
6TM	– –	– – –	0	–	tr	0	–		tr	0
6C	– –	–	0	–	tr	0	–		–	0
10TM	0	– –	0	–	tr	0	tr	tr	tr	0
10C	0	– – –	0	–	tr	0	tr	–	tr	0
GTM	0	–	0	– –	–	0	–	–	–	–
GC	–	–	0	– –	–	0	0	–	0	–

relative to adjacent soils, but only when the mounds occurred in areas with minimal rainfall (Watson, 1975). McComie and Dhanarajan (1993) reported a significantly larger percentage of clay ($P < 0.05$) compared with adjacent soil in a mound of *M. carbonarius* in Malaysia, and Stoops (1964) reported a higher clay content in mounds constructed by *Cubitermes* (Termitidae: Termitinae: *Cubitermes*) compared with subsoil in Congo.

The types of clay minerals present in termite mound and control soils were similar. All soils were dominated by metahalloysite, illite, and illite-smectite. If chimpanzees selectively consume soils based on their content characteristics, our findings suggest that the importance of the type of clay minerals may be secondary to the overall clay content. The presence of certain clay minerals may indirectly influence the microbial composition of soils (Stotzky, 1986). However, regardless of the actual mechanisms and extent of this effect, it may be of secondary importance to the hypothesis that the high clay content and actinomycete numbers in termite mound soils are sufficient to reinforce geophagy.

There is growing evidence that chimpanzees selectively consume certain plant species for their medicinal value. Evidence in support of this hypothesis includes: (1) ingestion does not confer nutritional benefits (Wrangham and Nishida, 1983); (2) ingestion of certain plant taxa is correlated to illness at the time of consumption (Huffman and Seifu, 1989); (3) certain plant species are preferentially ingested during periods of high risk of parasitic infection (Kawabata and Nishida, 1991; Huffman et al., 1997); and (4) the relative amount of consumption of these plant species is considerably lower than other plant taxa common to the chimpanzee diet (Wrangham and Nishida, 1983; Huffman and Seifu, 1989). Moreover, antimicrobial properties have been demonstrated in some of these plants (Huffman, 1997). If chimpanzees are selectively consuming these plants for their medicinal value, then it can be hypothesized that they engage in geophagy for similar reasons. Data on the relation between health and geophagy in chimpanzees are limited. However, Mahaney and colleagues (1996) reported five episodes of

geophagy by three chimpanzees, with four episodes being clearly linked to severe diarrhea resulting from parasitic infection.

The data presented in this study indicate that soils selected for ingestion differ quantitatively in microbial and clay content from other soils present in the chimpanzees' habitat. Further studies are required to determine the therapeutic impact of the ingestion of these soils and the relation between health and geophagy in chimpanzees.

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Self-Medicative Behavior in the African Great Apes: An Evolutionary Perspective into the Origins of Human Traditional Medicine

MICHAEL A. HUFFMAN

Close to a century ago a Tanzanian medicine man, Babu Kalunde, discovered an important treatment that saved the lives of many people in his village, who were suffering an epidemic of a dysentery-like illness. He learned about the potential medicinal value of a plant known to the WaTongwe as *mulengelele* by observing a similarly sick young porcupine ingest the roots of the plant. Before these opportune observations, Babu Kalunde and the people of his village had avoided this plant, which they knew to be highly poisonous. After telling the villagers his story of the porcupine, however—and taking small doses of the plant himself—he persuaded them to use the plant on the sick. To this day, the WaTongwe use the roots of mulengelele as medicine. Babu's grandson, Mohamedi Seifu Kalunde, now a respected elder and healer himself, uses this plant to also treat gonorrhea and syphilis.

By comparison with Babu, scientists are latecomers to the study of animal self-medication and its possible applications for modern medicine. In recent years, however, a growing body of scientific evidence has been gathered in support of animal self-medication, or zoopharmacognosy (Huffman 1997). Starting with chance observations of a sick chimpanzee in 1987 (Huffman and Seifu 1989), Mohamedi and I have worked together with a growing group of collaborators to learn how chimpanzees in the wild deal with parasites and what their behavior can tell us about treating other diseases.

Unnecessary for nutrition, growth, or reproduction, the secondary compounds in a plant have evolved to give protection from insect and mammalian predators. Whereas ecologists who study animal foraging behavior focus on how animals cope with these secondary compounds in their diet (Freeland and Janzen 1974, Glander 1975, 1982, Hladik 1977, Janzen 1978, Wrangham and Waterman 1981), the basic premise of zoopharmacognosy is that animals utilize these secondary compounds to medicate themselves. Taking a broader per-

IN ADDITION TO GIVING US A DEEPER UNDERSTANDING OF OUR CLOSEST LIVING RELATIVES, THE STUDY OF GREAT APE SELF-MEDICATION PROVIDES A WINDOW INTO THE ORIGINS OF HERBAL MEDICINE USE BY HUMANS AND PROMISES TO PROVIDE NEW INSIGHTS INTO WAYS OF TREATING PARASITE INFECTIONS AND OTHER SERIOUS DISEASES

spective, we are interested in putting these lessons of evolutionary medicine to practical use for humans (Huffman and Seifu 1989, Ohigashi et al. 1994, Plotkin 2000).

Much of the plant material that is consumed by animals in the wild contains an array of secondary compounds. Johns (1990) argues that the herbal medicines and modern pharmaceuticals used by humans today have replaced the nonnutritive chemicals commonly present in our primate ancestors' diets. In this light, the nonnutritive components of items ingested by African great apes—and, indeed, all

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primates—are worth investigating in greater detail for clues to the medicinal advantages that such a diet may afford.

In traditional human societies, the difference between food and medicine may not always be clear. This idea is expressed in a Japanese saying, "ishoku dougen," which directly translated means "medicine and food are of the same origin." It is perhaps no coincidence, then, that traditional spices, condiments, and vegetables used around the world are also important sources of antitumor agents or possess antioxidant, antibacterial, antiviral, and antiparasitic qualities (Ohigashi et al. 1992, Billing and Sherman 1998, Murakami et al. 1994, 1996, Sherman and Billing 1999). The concept of food as medicine goes a step further. Etkin (1996) found that 30% of the plant species identified as food among the agricultural Hausa of Nigeria were also used as medicine. Furthermore, 89% of species used to treat symptoms of malaria were also used in a dietary context (Etkin and Ross 1983). Etkin and Ross (1994) propose that many Hausa foods were originally acquired from noncultivated plants first used as medicine.

One of the difficulties of interpreting animal self-medication is distinguishing between (a) possible indirect medicinal benefits derived from secondary compound-rich plants ingested, presumably, for their nutritional value and (b) limited, situation-specific ingestion of items that are processed solely for their medicinal properties. Observations of the great apes provide the clearest scientific evidence to date for direct forms of self-medication in animals. The hypothesis I am currently developing is that these behaviors aid in the control of intestinal nematodes and tapeworms or provide relief from related gastrointestinal upset, or both. Perhaps because of their phylogenetic closeness and common neural pathways of chemosensory perception, humans and chimpanzees, when displaying similar symptoms of illness, learn to associate and select for similar properties in medicinal plants (Huffman et al. 1996a).

Unquestionably, the evolution of medicinal habits from the great apes to early hominids and modern humans has important implications for modern medicine. Thus, this article reviews the evidence for self-medication as a form of parasite control in the African great apes, relates that information to the evolution of medicinal plant use in traditional human societies, and suggests how natural plant products might play a role in modern health care.

Great ape self-medicative behavior and parasite infection

Most of the details about two types of self-medicative behavior in the great apes—namely, bitter-pith chewing and leaf swallowing—come from three study sites, Mahale and Gombe in Tanzania and Kibale in Uganda), although these behaviors have been documented from 10 additional sites across Africa (Figure 1). The geographical, ecological, and climatic variation of these sites is great, ranging from low-elevation, moist tropical forest and woodland to montane forest. Such wide variation in geography, ecology, and climate where leaf swallowing and bitter-pith chewing are known to occur suggests

that great ape populations elsewhere on the continent might also engage in these behaviors.

Parasites can cause a variety of diseases that have an impact on the overall behavior and reproductive fitness of an individual. The effect of parasitosis on the host and the host's response to infection are undoubtedly the product of a long evolutionary process (Anderson and May 1982, Futuyma and Slatkin 1983, Barnard and Behnke 1990). It would be extremely surprising, therefore, if all animals had not evolved at least one means of defense against parasites.

A longitudinal investigation showed that Mahale chimpanzees (Huffman et al. 1997) are naturally infected by numerous parasite species. The species found were three nematodes, *Strongyloides fueleborni* (thread worm), *Trichuris trichiura* (whip worm), and *Oesophagostomum stephanostomum* (nodular worm); one trematode, *Dicrocelium lanceatum* (lancet fluke); three protozoan, *Entamoeba coli*, *Endolimax nana*, and *Iodamoeba buetschlii* (all of which are amoebas); and one flagellate, *Giardia lamblia*.

Oesophagostomum stephanostomum infections were associated significantly more frequently with bitter-pith chewing and leaf swallowing (in 14 out of 15 cases, or 93%) than any of the other parasite species (Huffman et al. 1997). The evidence from Mahale points to this parasite as the stimulus for, and the only parasite directly affected by, self-medication. The nematode parasites of the genus *Oesophagostomum* (Strongyloidea, Oesphagostominae)—called nodular worms, because they encapsulate themselves in nodules in the intestinal wall during their development—are common parasites in the proximal hindgut of rodents, pigs, ruminants, nonhuman primates, and humans (Figure 2). Several species are found in gorillas and chimpanzees and occasionally in humans (Brack 1987, Polderman and Blotkamp 1995). Some of these nodular worm species are significant pathogens in domestic livestock and in primates. Symptoms of moderate to heavy infections of *O. stephanostomum*, *O. bifurcum*, and *O. aculeatum* found in apes range from anorexia, weight loss, enteritis, diarrhea, anemia, and lethargy to intense pain that simulates appendicitis. The direct economic loss caused by *Oesophagostomum* species in animal husbandry and the hazards it presents to human health have stimulated much research on their biology and control (Roepstorff et al. 1987, Polderman and Blotkamp 1995, Varady et al. 1996).

The behavioral ecology of bitter-pith chewing

Detailed plant chemistry, behavioral observations, and parasitological surveys of patently ill chimpanzees at Mahale led to the hypothesis that bitter-pith chewing has medicinal value (Huffman and Seifu 1989, Huffman et al. 1993, 1997). Very much a collaboration of scientific method and traditional knowledge, these were the first reported observations to verify illness and obvious improvements in health after chimpanzees ingested *Vernonia amygdalina* Del. (Compositae), a putative medicinal plant.
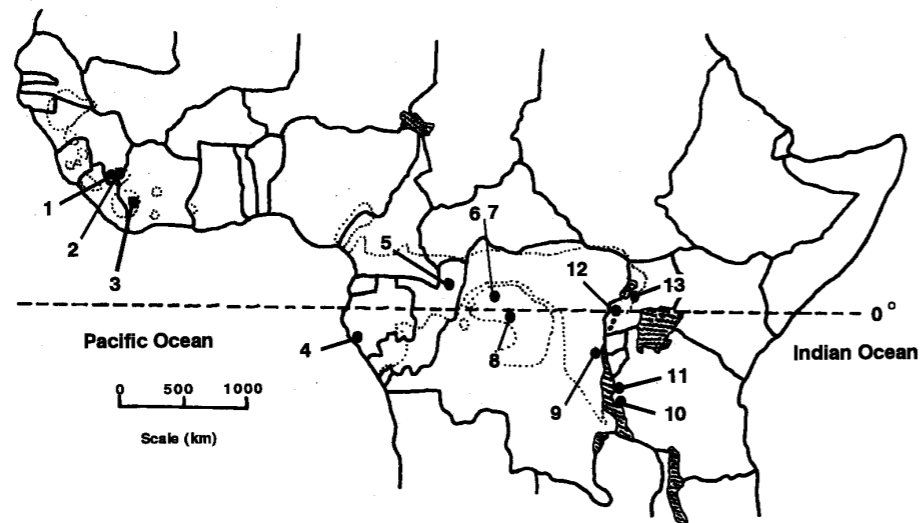
Figure 1. African great ape study sites where leaf swallowing and bitter-pith chewing behavior has been reported. The dotted areas show the current overall distribution of the African great apes: chimpanzee (Pan troglodytes verus, sites 1, 2, 3; P. t. troglodytes, sites 4, 5; P. t. schweinfurthi, sites 9, 10, 11, 12, 13); bonobo (P. paniscus, sites 6, 7, 8); and lowland gorilla (Gorilla gorilla graueri, site 9). From Huffman (1997), with the addition of new data from sites 4, 6, and 7 provided by Y. Takenoshita, J. Dupain, B. Fruth, and G. Hohmann.

1 Bossou
2 Nimba
3 Tai
4 Petit Loango
5 Ndoki
6 Lomako
7 Lyema
8 Wamba
9 Kahuzi-Biega
10 Mahale
11 Gombe
12 Kibale
13 Budongo

Vernonia amygdalina occurs throughout tropical sub-Saharan Africa (Watt and Breyer-Brandwijk 1962). Chewing of the pith of other *Vernonia* species has been observed at Gombe (*V. colorata* [Willde.] Drake [Huffman and Wrangham 1994]), Hilali (Janette Wallis [Department of Psychiatry & Behavioral Sciences, University of Oklahoma Health Sciences Center], personal communication, 1999), and Kahuzi-Biega (*V. hochstetteri* Schi-Bip., *V. kirungae* Rob. E. Fries; Yumoto et al. 1994; Augi.K. Basabose [Laboratoire de Primatologie CRSN, Lwiro, Congo], personal communication, 1997). At Tai, the bitter pith of *Paliosota hirsuta* (Thunb.) K. Schum. (Commelinacea) and *Eremospath macrocarpa* (Mann

Figure 2. General life cycle of Oeophagostomum spp., as exemplified in the chimpanzee (Huffman and Caton 2001). Eggs are laid at the 16–32 cell stage. While in the feces, the eggs rapidly develop into L1 rhabditiform larvae, hatching in as few as 24 hours under optimal conditions. The larvae feed on bacteria and molt to the L2 stage within 24 hours of hatching. Within 3–4 days after hatching, the L2 molt to become infective L3. L3 retain the protective cuticle of the L2 stage and are capable of surviving long periods of adverse environmental conditions (e.g., the hot, dry conditions of the dry season) in a state of dormancy. Infection occurs via ingestion of filariform L3 larvae that make their way onto vegetation eaten by the host. After ingestion, L3 larvae pass to the cecum, where they exsheath within approximately 3 days after ingestion. They then invade the tunica mucosa, stimulating the formation of separate cysts around individual larvae in the gut wall. In these cysts the larvae develop to the L4 stage, eventually returning to the lumen of the hindgut as immature adults. Once in the lumen, the larvae molt and reach the adult stage. Females begin to reproduce and deposit eggs about 1 month after infection.
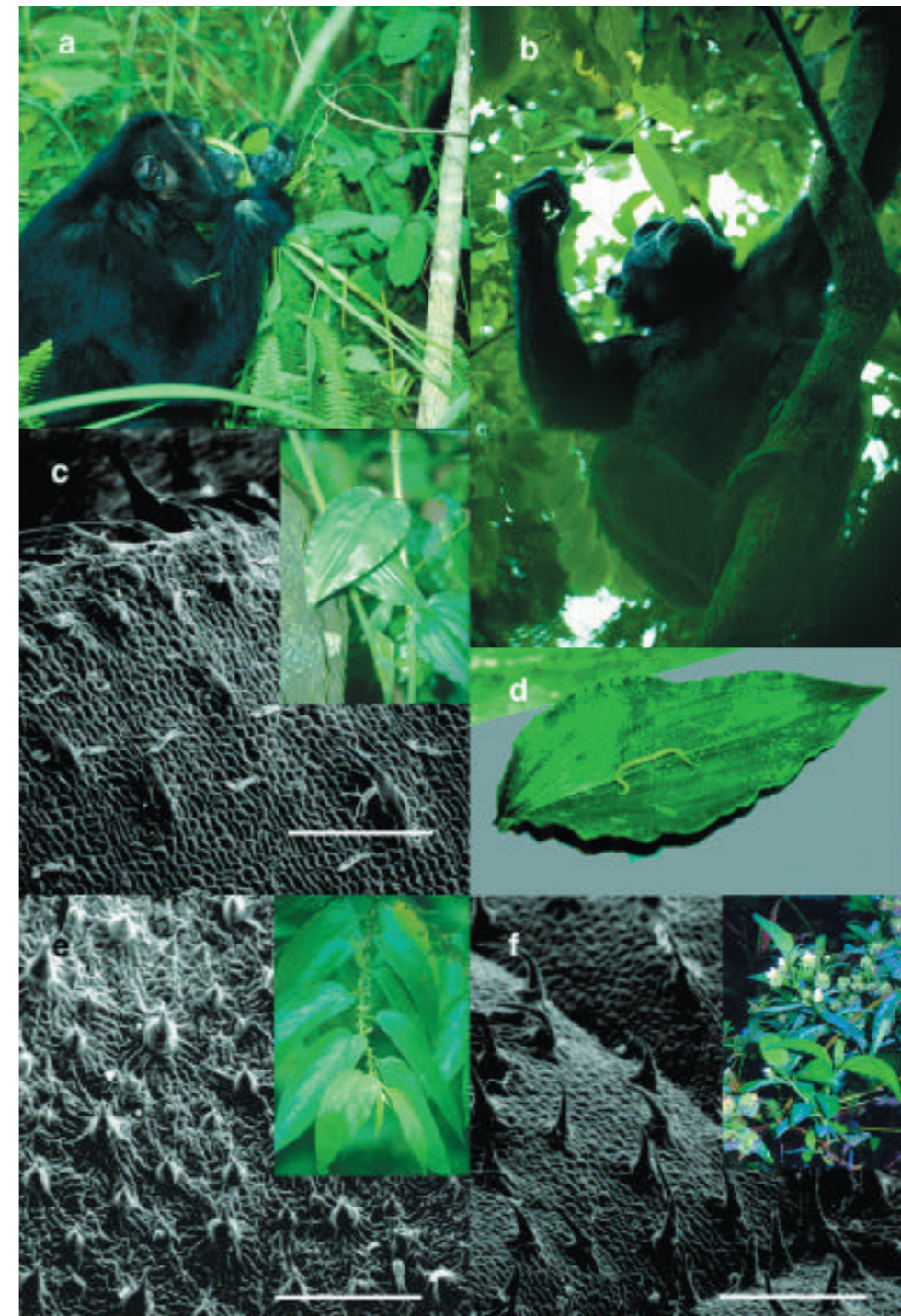

Figure 3. Self-medicative behaviors. (a) Adult male JI with an Oesophagotsomum stephanostomum infection chews on the bitter pith of Vernonia amygdalina. (b) Adult female LD is swallowing leaves of Aspilia mossambicensis. (c, e, f) Scanning electron micrographs (SEM) showing the characteristic bristly surface of the leaves swallowed whole by apes species, accompanied by pictures of each species (Aneilema aquinoctiale, Trema orientalis, and Lippia plicata, respectively). (d) This adult O. stephanostomum worm (approximately 2.5 cm) was expelled in the dung along with 20 others, together with 50 folded leaves of A. aquinoctiale, shown here. Photos: Michael A. Huffman. SEM: Thushara Chandrasiri.

and Wendl.) Wendl.(Palmae) are chewed (Christophe Boesch [Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany], personal communication,1996).

When ingesting the pith from young shoots of *V. amyg - dalina*, chimpanzees meticulously remove the outer bark and leaves to chew on the exposed pith, from which they extract extremely bitter juice and residual amounts of fiber (Figures 3a,4a,4b). The amount of pith ingested in a single bout is relatively small, ranging from portions 5–120 cm long by 1 cm in width. The entire process, depending on the amount ingested,takes anywhere from less than 1 to as long as 8 minutes. Mature conspecifics in proximity to individuals chewing *Vernonia*bitter pith (or leaf swallowing, described below) show no interest in ingesting the pith. Infants,however, have on occasion been observed to taste the pith discarded by their ill mothers. Thus,from a very young age individuals in a group are exposed to the plant-eating behaviors and to the plants and their context of use.

At Mahale, use of *V. amygdalina* has been recorded in all months except June and October in the late dry season (Nishida and Uehara 1983). Despite such broad availability, use of *V. amygdalina* by chimpanzees is highly seasonal, occurring mainly during the rainy season months of November, December, and January.

Impact of bitter-pith chewing on parasite load

In general,when an individual chews the bitter pith of *V. amyg - dalina*, that individual is in ill health, as evidenced by diarrhea, lethargy, weight loss, and nematode infection. In two cases recorded in detail, recovery from such symptoms was evident 20–24 hours after the individuals chewed the bitter pith (Huffman and Seifu 1989, Huffman et al. 1993). In one of these cases, the eggs per gram (EPG) of feces level of an *O. stephanostomum*infection could be measured; it was found to have dropped from 130 to 15 in 20 hours. Seven other individuals,monitored over the same period,had *O. stephanos - tomum*infections but were not observed chewing bitter pith; these individuals did not register such a dramatic drop in EPG. In these seven control cases, *O. stephanostomum*EPG levels actually increased over time. The rise in EPG levels represents the overall trend for increased reinfection by *O. stephanosto - mum* at the beginning of the rainy season (Figure 5). The ingestion of the bitter pith of *Vernonia*appears to affect nodular worm reproductive output and provide relief from symptoms of related gastrointestinal upset.

Ethnomedicinal and phytochemical evidence for the pharmacological effectiveness of bitter-pith chewing

The similarities between chimpanzees and humans in their use of *V. amygdalina*strengthen support for the effectiveness of bitter-pith chewing on parasite control and offer interesting insight into the common criteria for plant selection (Huffman et al. 1996a). For several African ethnic groups, a concoction made from *V. amygdalina*is prescribed treatment for
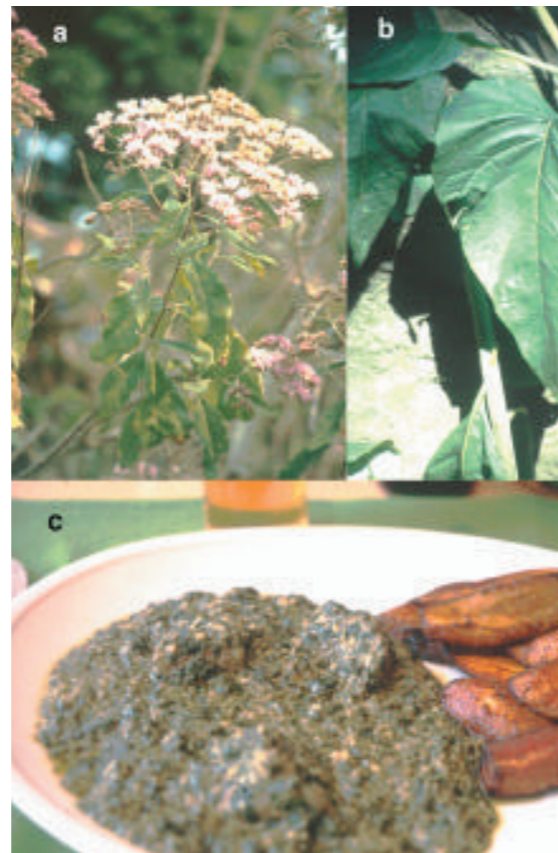

Figure 4. (a) Vernonia amygdalina, *or bitter leaf (b),is used in a traditional West African dish known in Cameroon as N'dole (c), which is prepared from the leaves of the plant. Photos: (a) and (b), Michael A. Huffman; (c), Koichi Koshimizu.*

malarial fever, schistosomiasis, amoebic dysentery, several other intestinal parasites, and stomachaches (Dalziel 1937,Watt and Breyer-Brandwijk 1962, Burkill 1985, Huffman et al. 1996a). The WaTongwe of Mahale use this plant as a treatment for intestinal parasites, diarrhea, and stomach upset. Ugandan farmers feed their pigs young branches and leaves of *V. amygdalina* to rid them of intestinal parasites. A number of bitter *Vernonia* species found across Africa, the Americas, and Asia are known both for their wide ethnomedicinal use and pharmacological effectiveness against gastrointestinal-related ailments,including parasite infections. As the name implies, *V. anthelmintica* is a clinically important traditional treatment in India for parasite infection.

Phytochemical analysis of *V. amygdalina*samples collected at Mahale from individual plants known to be used by chimpanzees revealed the presence of two major classes of bioactive compounds (Figure 6). A number of known sesquiterpene lactones,and 13 new stigmastane-type steroid glucosides and their freely occurring aglycones, have been isolated (Ohigashi et al. 1991, Jisaka et al. 1992a,1992b, 1993a,1993b). The

Figure 5. Seasonal variation in the infection levels ofstrongyle nematode Oesophagostomum stephanotomum *in Mahale chimpanzees. While infections are carried year round, reinfection in chimpanzees of western Tanzania occurs mainly during the rainy season. Reinfection,noted by a significant elevation in individual eggs per gram (EPG) counts, occurs approximately 1–2 months after the onset of the rainy season, regardless of which month the rains first begin (Huffman et al.1997). This lapse in time corresponds with the 1-month prepatent period (period between when the parasite enters the host and when it begins to reproduce) of* Oesophagostomum spp. *(Anderson 1992). Arrested larval development occurs in* Oesophagostomum spp. *(Armour and Duncan 1987, Krepel et al. 1994), and at Mahale,shortly after the onset of the rainy season, the external environmental conditions of rising humidity and temperature become optimal for rapid development of the eggs and larvae. The markedly higher individual EPG levels at the beginning of the rainy season are thought to be caused by the increase in transmission ofinfective stage L3 larvae into the host from the moist environment.*

sesquiterpene lactones present in *V. amygdalina* are also found in *V. colorata* and in a number of other *Vernonia* species. They are well known for their anthelmintic, antiamoebic,antitumor, and antibiotic properties (Toubiana and Gaudemer 1967, Kupchan et al. 1969, Asaka et al. 1977,Gasquet et al. 1985, Jisaka et al. 1992a,1993b). Crude methanol extracts of the leaves exhibited immunosuppressive activity and inhibition of the process that initiates the first stage of tumor cell growth (Koshimizu et al. 1993). The cytotoxic sesquiterpene lactones were found to be most abundant in the leaves and bark, the parts that chimpanzees at Mahale have always avoided. This is quite interesting, given that the leaves from wild growing plants can be lethal if ingested raw and in large amounts, as is sometimes observed among domestic goats in West Africa. That chimpanzees avoid these parts, yet domestic animals seem unable to do so, suggests a higher level of sophistication in chimpanzees' knowledge of plant secondary compounds and their beneficial use. People across western Africa soak leaves from the less toxic cultivated plant in water several times to reduce their bitterness and toxicity

so they may be cooked with meat and eaten as a tonic food called N'dole in Cameroon (Figure 4c). The woody branches, which have noteworthy antibacterial properties, are used widely as chew sticks—the famous African "toothbrush."

In vitro tests on the antischistosomal activity of the plant's most abundant steroid glucoside (vernonioside B1) and sesquiterpene lactone (vernodaline) showed significant inhibition of movement of the adult parasites and of the adult parasite females' egg-laying capacity (Jisaka et al. 1992b). These findings are consistent with the observed decline in egg output of *O. stephanostomum*over a 20-hour period in one adult female chimpanzee at Mahale after her ingestion of *V. amygdalina* pith (Huffman et al. 1993). The sesquiterpene lactones showed significant in vitro plasmodicidal activity, while that of the steroid glucosides was weaker (Ohigashi et al. 1994).

Some of the species whose bitter pith is ingested by chimpanzees at Gombe, Kahuzi-Biega, and Tai also have a number of ethnomedicinal and pharmacological properties. *Ver - nonia colorata* and *V. amygdalina*are not distinguished from
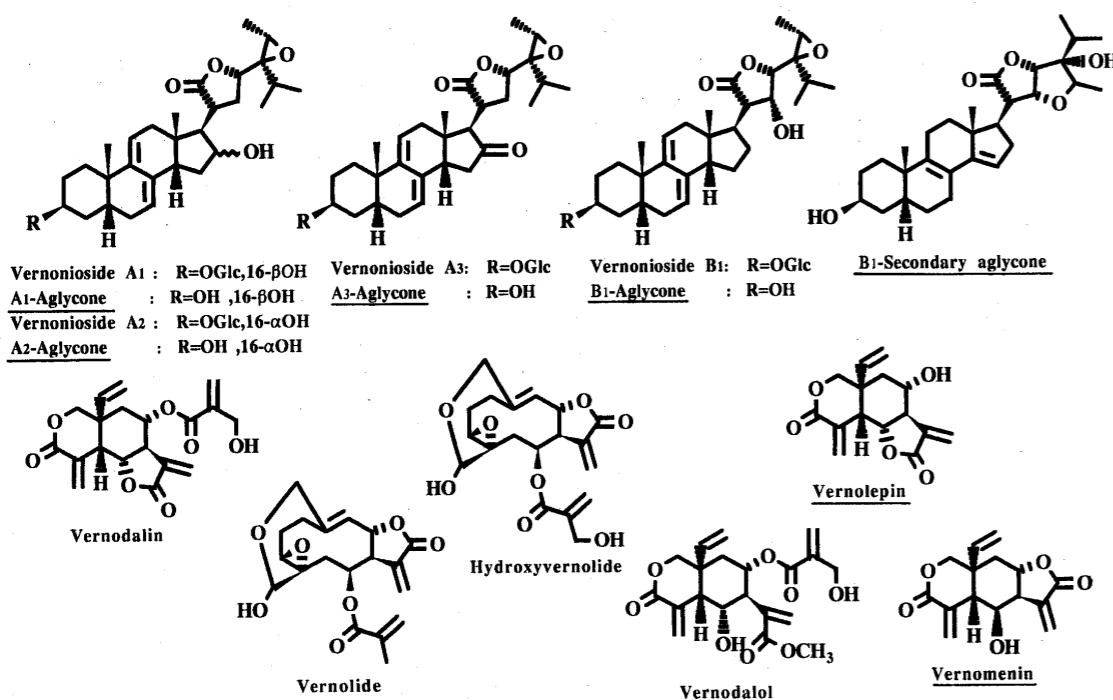
Figure 6. The steroid glucoside (upper row) and sesquiterpene lactone (lower row) compounds were isolated from the leaf, stem, pith, and root parts of Vernonia amygdalina *specimens collected from Mahale National Park.*

each other ethnomedicinally with regard to their medicinal properties and folk classification (Burkill 1985). Alkaloids occur in the pith as well as in the flower and leaf of *V. hochstetteri* (Smolenski et al. 1974). *Palisota hirsuta* and *E. macrocarpa* are used in West Africa as a treatment for upset stomach, colic, and venereal disease and as an antiseptic and analgesic (Abbiw 1990, Neuwinger 1996). Molluscicidal activity has also been reported for *P. hirsuta* (Okunji and Iwu 1988).

Leaf-swallowing behavioral ecology

Leaf-swallowing behavior was first recorded for chimpanzees at Gombe and Mahale (Wrangham and Nishida 1983). Observers at both sites found the folded, undigested leaves of *Aspilia mossambicensis* (Oliv.) (Compositae), *A. pluriseta* (O. Hoffm.) Wild, and *A. rudis* Oliv. & Hiern in the dung of the chimpanzees, suggesting that leaf swallowing was unlikely to have any nutritional value and that chimpanzees may possess a sophisticated pharmacopoeia (Rodriguez et al. 1985). Other field researchers began to look for similar anomalous feeding habits among apes at their study sites. Currently, leaf-swallowing behavior involving more than 34 different plant species has been observed at 13 great ape study sites across Africa (Huffman 1997). The plant species used vary in life form (herb, vine, shrub, and tree), but the common property functionally linking all of these plants is their bristly, rough-surfaced leaves (Figure 3c, 3e, 3f). The distal half of leaves are selected one at a time, folded by the tongue and palate as they are slowly pulled into the mouth, and swallowed whole (Figure 3b). An individual may swallow anywhere from one to 56 leaves in one bout.

Longitudinal data on the temporal occurrence of leaf swallowing from both direct observations and the presence of leaves in the dung for *Aspilia* species at Mahale and Gombe demonstrate that leaf swallowing is an extremely rare behavior. Researchers have reported an average rate of use ranging from once every 69.0 hours ($n = 18$ bouts over 1242 hr) to once every 102.6 hours ($n = 10$ bouts during 1026 hours) for *Aspilia* species (Wrangham and Nishida 1983, Huffman 1997).

The species appropriate for leaf swallowing are available year-round at both Gombe and Mahale. Nonetheless, use is most common at Mahale after the beginning of the rainy season (November through May), with peak frequencies in January and February 10–12 times greater than those of other months (Wrangham and Nishida 1983). At Gombe, peak frequencies of use have also been observed in January, February, March, and May, but also once in July (Wrangham and Goodall 1989).

During a 4-month study at Mahale, nematode infection was demonstrated in 83% of all cases of leaf swallowing (10 out of 12 instances; Huffman et al. 1996b). Multiple-species infections were common, but *O. stephanostomum* species (78%) were most commonly associated with leaf-swallowing behavior, followed by *S. fuelleborni* (56%) and *T. trichiuria* (33%) species. Symptoms associated with infections by these nematodes (diarrhea, malaise, abdominal pain) were verified

from direct observation in seven of the eight chimpanzees at the time they swallowed the leaves (Huffman et al. 1996b, 1997).

Impact of leaf swallowing on parasite load and the proposed mechanism of expulsion

Oesophagostomum stephanostomum worms were found in only 4% of the 254 dung samples collected from individuals and observed in detail (Figure 3d). Their occurrence in the dung was limited to chimpanzees that displayed symptoms of malaise and diarrhea.

In 1993–1994, six of the nine dung samples found to contain worms also contained whole undigested leaves of *A. mossambiensis, Trema orientalis* (L) Blume (Ulmaceae), or *Aneilema aequinociale* (P. Beauve.) Loudon (Commelinaceae). The relationship between the presence of both leaves and nodular worms in the dung was highly significant (Fisher's exact test, two-sided, $p = 0.0001$; Huffman et al. 1996b). On average, 10 worms were recovered with 20 leaves per stool, at a rate of 0.54 worms per leaf expelled by leaf swallowing (Huffman and Caton 2001), indicating a strong relationship between leaf swallowing and the expulsion of nodular worms (Huffman et al. 1996b). All worms were alive and motile at the time of expulsion; therefore, no chemical nematocidal activity in these plants is suspected.

No data exist for total worm burdens of *O. stephanostomum* in wild chimpanzees, though reports on *O. bifurcum* in humans living in rural Togo and Ghana indicate that an individual has an average burden of 96 worms (standard deviation 89.06, range 12–300, $n = 12$) (Krepel and Polderman 1992). Estimating from the rate of worm-to-leaf expulsion calculated above, a chimpanzee would have to pass at least 176 leaves or engage in leaf swallowing on average 10 times over the rainy season to rid itself of a comparable worm burden. The actual number of leaves observed to be swallowed by an individual in a single case ranges from 5 to 55. This scenario strongly suggests that repeated swallowing of leaves by chimpanzees over consecutive days or weeks can have a significant impact on its overall worm burden, which may account in part for the observed decline in the *O. stephanostomum* egg burden of many individuals later in the rainy season (Figure 5).

The proposed physiological response of the gut was to expel whole leaves approximately 6 hours after swallowing (Huffman and Caton 2001). Considering the details of the life cycle of *Oesophagostomum* (Figure 2), leaf swallowing most probably controls nematode infection in at least three ways: By (1) causing adult worm detachment and expulsion, (2) flushing out some of the incoming infective L3 larvae before they are able to exsheath and penetrate the mucosa, and (3) decreasing pathology of nodular cysts (and reducing discomfort) by inducing the emergence of juvenile L4 larvae into the lumen (Huffman and Caton 2001).

At Mahale, leaf swallowing and bitter-pith chewing are sometimes displayed by the same individual on the same day; they are likely to act synergistically in lowering *O.*

stephanostomum infection levels. Like bitter-pith chewing, leafswallowing at Mahale appears to affect only *O. stephanostomum*. *O. stephanostomum* adults inhabit the large intestine, where they attach themselves to the mucosal wall through the suction of the buccal capsule. This attachment is not permanent, and worms migrate around the large intestine in search of food and mates. *Strongyloides fuelleborni* (2 mm) and *T. trichium* (30–40 mm), however, are smaller than *O. stephanostomum* and burrow into the mucosa of the small intestine and cecum, respectively, where they embed themselves firmly. They are not susceptible to mechanical removal by the leaves.

The hypothesis about control of nematode infection predicts that because nodular worm infections are self-limiting (reproductive adults do not reinfect the host), the infection may be controllable if a chimpanzee periodically reduces parasite numbers during the most likely period of reinfection. At Mahale, the peak period of reinfection by *O. stephanostomum* (after the onset of the rainy season, around December or January) was found to closely correspond to the time when chimpanzees most frequently swallow leaves and chew bitter pith.

At Kibale, Wrangham (1995) found that whole leaves in the gut also increase the probability that tapeworm proglottids will be shed. Whole leaves were found in dung during the 21 months of the survey, whereas leaves and tapeworms were found together in only 33% of these months. The greater overall occurrence of leaves in dung without tapeworms, however, suggests that the expulsion of proglottid fragments may not be a direct function of leafswallowing. Wrangham (1995) proposes that the proximate stimuli for leaf swallowing as observed at Kibale could be abdominal pain caused by tapeworm infections, and thus leaf swallowing may function to alleviate pain. In this light, it is possible that chimpanzees were responding to discomfort caused by the presence of tapeworms in the intestines regardless of whether the fragments were being expelled. The relief of pain from parasite infection appears to be a significant stimulus for both bitter-pith chewing and leaf swallowing.

Chimpanzees' acquisition of self-medicative behaviors and the evolution of medicine in traditional human societies

The way in which proposed self-medicative behaviors are individually acquired by the African great apes is a challenging topic for investigation. To suggest that self-medication is a behavioral tradition leaves open questions about how the behavior started and how individuals become predisposed to ingest medicinal plants. At one extreme, animals may have an innate tendency to select appropriate plants when ill, so that the role of tradition is reduced to local enhancement (i.e., naive individuals have their attention drawn to plant species used by others; Huffman and Wrangham 1994). However, with leaf swallowing and bitter-pith chewing, the species being ingested is not the only question to be resolved; what parts of the plant are ingested and how must also be learned for the

behavior to be effective. Given the high degree of conservatism in chimpanzee feeding habits (Nishida 1987), random sampling of novel food items, especially when ill, does not occur frequently. Perhaps the traditional behavior began during a period of extreme food scarcity, when ill and hungry apes who were forced to try new foods recovered their health and associated their recovery with the new food item.

Selective association between taste and gastrointestinal illness is a widely accepted principle of taste-aversion learning among mammals (Revusky 1984). The learning mechanism of food aversion in response to induced sickness has been well documented in several animal species (Zahorik and Albro Houpt 1977, Rozin and Vollmecke 1986, Letarte et al. 1997). Although the highly adaptive significance of the reversed process—that is, being able to associate improved health with the ingestion of novel plants with medicinal properties—seems self-evident, such learning mechanisms have received little attention (Zahorik 1977). Obviously, this area greatly needs further investigation.

In nonhuman primates, important benefits come from social learning, which allows naive individuals to acquire information through the experience of others and over time to perfect the behavior themselves (Galef 1977, Fragaszy and Visalberghi 1996). If the effectiveness of a behavior in bringing about a positive change in health is recognized, then perhaps it will spread through the group, at first slowly but then more rapidly as it is passed on to the youngest members. At this stage, it may be just one more part of the mother's foraging and behavioral repertoire to be acquired. At Mahale and presumably elsewhere, initial exposure to self-medicative behaviors take place by individuals at an early age, not when they are ill but when they observe the behavior of those that are ill, usually their mothers (Huffman and Wrangham 1994). Young chimpanzees have been observed on several occasions to closely watch these behaviors and immediately thereafter to attempt to perform them on their own (Huffman and Seifu 1989, Huffman and Wrangham 1994). These biological and psychological processes make up the core of nonhuman primate behavioral tradition and, as Johns (1990) argues, they are the biological seeds of the human cultural practice of medicine.

From apes to humans

The strong similarities in plant selection criteria among the African great apes in response to parasite infection and gastrointestinal upset, and the common use of some plants by chimpanzees and humans to treat such illnesses, are tantalizing evidence for the evolution of medicine. Our earliest hominid ancestors may have exhibited some similarities in plant selection criteria with both extant apes and modern humans. Although the fossil record provides no direct evidence concerning the subtleties of feeding behavior and diet, it seems reasonable to hypothesize that early hominids would have displayed at least the range of extant ape self-medicative behaviors.

It appears that the fundamentals of perceiving the medicinal properties of a plant by its taste, smell, and texture have their roots deep in our primate history. A major turning point in the evolution of medicine is likely to have been the advent of language in early humans, which enabled people to share and pass on detailed experiences about plant properties and their effects against disease. Another major event in human history is the adoption of food preparation and detoxification technologies, which allowed humans to exploit a wider range of plant life as food. Johns (1990) argues that it was at this turning point that humans' dependence on plant secondary compounds increased, because those compounds disappeared from the daily diet when detoxification technology was employed. Simply put, removal of the disease-fighting prophylactic properties of some of these secondary plant compounds from the diet may have contributed to a rise in certain illnesses that otherwise would have been kept in check. This development may have driven humans toward the greater use of some plants specifically as medicine and the use of others as "food–medicine." Furthermore, with the skilled use of fire to boil, steam, vaporize, condense, or otherwise extract useful secondary compounds from plants, these compounds could be used in a greater variety of ways.

The current level of sophisticated medicinal practices in traditional human societies may be the product of the greater variety of diseases and stress brought about by the change in subsistence methods from hunting and gathering to dependence on domestic crops and livestock in a sedentary setting. In this light, our early modern human ancestors may have had a smaller pharmacopoeia, but not for lack of technical sophistication. More likely, there were simply fewer diseases and less stress. Furthermore, a worldwide increase in human population and population density enables diseases to pass from person to person more easily, perhaps selecting for greater virulence. Thanks to the technological advancements of modern medicine, the lives of millions of people are saved or prolonged every year. Yet it is also technological advancement that introduces changes in our diets and exacerbates stress in our lives, which in turn boost rates of the modern diseases that nations spend fortunes trying to cure.

Future directions

As was true for Babu Kalunde almost a century ago, the study of animal self-medication and ethnomedicinal practices may provide important leads to future sources of medicine. A closer look into the manner in which animals use natural plant products may, for example, provide novel insights into viable new strategies for suppressing or slowing down the rate of acquisition of chemoresistance by parasites that infect livestock and humans.

A strong movement is under way in many African nations to evaluate and integrate those traditional medicines shown to be effective into modern health care programs. My collaborators and I in Africa and elsewhere realize the importance and urgency of such efforts. Our multidisciplinary approach to this research, wherein the biological activity of novel,

plant-derived compounds is assessed against parasite species found across a wide range of hosts, maximizes the chances of success. At the same time we recognize the importance of preserving the intellectual property rights of individuals, regions, and countries to any new discoveries derived from indigenous plant material. One objective of this research is to integrate our results into local health care and livestock management systems so that locally available plants can be properly used to the benefit of all.

Recently, Mohamedi said that he had heard of a traditional healer located east of Mahale who was using mulengelele to treat AIDS patients. He cautioned that he had not tried it on patients himself, so he could not verify its effectiveness. My colleagues and I were investigating the properties of mulengelele root in the laboratory as this article was being prepared.

We all may have much to gain from the evolutionary wisdom of our primate ancestors and the wealth of traditional medicine. Africa, the birthplace of humankind, may also have been the starting point for the evolution of modern medicine. That continent too has an important role to play in the world's future.

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VIVAMUS

New Evidence for Leaf Swallowing and *Oesophagostomum* Infection in Bonobos (*Pan paniscus*)

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We collected data on parasitic prevalence and leaf-swallowing behavior of bonobos (Pan paniscus*) between August 1998 and April 1999 at the Iyema research site, Lomako Forest, Democratic Republic of Congo. We report the first detailed observations of leaf-swallowing among bonobos and the first record of the behavior at Iyema-Lomako. Bonobo leaf-swallowing closely fits the description of the behavior among chimpanzees. Bonobos ingested leaves of* Manniophyton fulvum, *as occurs in two chimpanzee populations in Central and Western Africa and among bonobos at Wamba, about 200 km from Iyema-Lomako. All leaf-swallowing occured in the rainy season. In conformity with patterns among Mahale chimpanzees, the prevalence of* Oesophagostomum sp. *infection in bonobos increased after the onset of the rainy season.*

INTRODUCTION

Since Janzen (1978) suggested the possible use of plant secondary compounds as medicine by animals, the evidence for self-medication in

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African great apes has grown (Huffman, 1997; Huffman *et al.*, 1993, 1996; Huffman and Seifu, 1989; Huffman and Wrangham, 1994; Wrangham, 1995; Wrangham and Goodall, 1989; Wrangham and Nishida, 1983). Self-medication in the great apes is best documented for the well habituated chimpanzee communities at Mahale, Gombe and Kibale. The two main patterns distinguished are bitter pith chewing and whole leaf-swallowing, which are antiparasitic. The benefits of bitter pith chewing is pharmacologically based on the activity of steroid glucosides (Jisaka *et al.*, 1993; Ohigashi *et al.*, 1994).

The hypothesized nematocidal action of leaf-swallowing (Page *et al.*, 1992; Rodriguez *et al.*, 1985; Rodriguez and Wrangham, 1993) has not been supported by evidence (Huffman *et al.*, 1996; Page *et al.*, 1997). Instead the mode of action of leaf-swallowing is physical, based on the rough hispid surface of the leaves (Huffman *et al.*, 1997, Huffman and Caton, 2001). Leaf-swallowing facilitates expulsion of tapeworm fragments by Kibale and Budongo chimpanzees (Huffman, in prep.; Wrangham, 1995) and of the adult nematodes (*Oesophagostomum stephanostomum*) by Mahale chimpanzees (Huffman *et al.*, 1996). An important fact demonstrating the role of the behavior in parasite control is synchrony of leaf-swallowing and a rise in *Oesophagostomum* infections (Huffman *et al.*, 1990, 1997; Kawabata and Nishida, 1991) with onset of the rainy season. Huffman *et al.* (1997) found that the increase in infection became notable approximately 3–6 weeks after the onset of the rainy season. This again dropped to a low, undetectable level by the following dry season.

Knowledge of intestinal parasites in wild bonobos (*Pan paniscus*) is limited to two surveys (Dupain *et al.*, 1999; Hasegawa *et al.*, 1983). Both studies demonstrated the presence of *Oesophagostomum sp.* infections. In addition, Wamba bonobos swallow whole leaves of *Manniophyton fulvum* (Huffman, 1997).

In 1998, we started a study on the prevalence of intestinal parasites in sympatric bonobos and humans in conjunction with the use of medicinal plants in the Lomako Forest, Equateur Province, Democratic Republic of Congo (DRC). We conducted the study at the Iyema site maintained by the Centre for Research and Conservation of the Royal Zoological Society of Antwerp, Belgium. During the first phase of this study, we obtained both direct and indirect evidence of bonobos swallowing leaves of *Manniophyton fulvum*.

The preliminary evidence allows us to consider the possible role of leaf-swallowing as a form of self-medication in bonobos. We aim first to describe the leaf-swallowing evidence and secondly to present the results of a survey on parasitic prevalence and leaf-remains in bonobo dung. We will discuss the results in light of the control of parasitic infection hypothesis first proposed by Huffman *et al.* (1996, 1997) for chimpanzees. Conditions

supporting the hypothesis would be an increase in the prevalence of *Oesophagostomum* infections at the onset of the rainy season and evidence for increased leaf swallowing behavior then.

MATERIALS AND METHODS

Study Site

The Iyema study site, is in the Lomako Forest, Equateur Province, Democratic Republic of Congo (Dupain *et al.*, 2000). The study group is partly habituated. We individually recognized 12 of *ca.* 50 members of the community. Nell and Dupain collected data between August 1998 and April 1999. Data collection was discontinuous because of local warfare, and two of the researchers were held captive by soldiers. We observed the bonobos for 10 h in August and 27 h in October 1998.

Seasonality

Climatological data were only partly available for October and November 1998. Based on our records of weather conditions during our stay in Mbandaka and Basankusu (150 and 300 km respectively west of Iyema-Lomako) in September, and the information from local collaborators about rain conditions at the study site in September, the onset of the heavy rainy season—first month with >200 mm rainfall—occurred in October (4–30 October: 202 mm). The dry season (<100 mm/month) started in January.

Dung Collection and Analysis

We collected all dung samples freshly in the morning under bonobo night nests. Accordingly, on any given day we could measure the random group estimates of parasitic prevalence as individuals were only sampled once. For parasitological analysis, we used the 86 faecal samples collected in October–November 1998, rainy months. They were immediately diluted 1:3 in formalin and transported for analysis to the Department of Parasitology, Fac. Pharmacy, Universidad Complutence de Madrid, Spain. Ponce and Garcia performed the analysis (Ritchie, 1948). We observed the samples under 16–64x magnification after resuspension in PSB, and under light/phase contrast microscopy at 100–400x magnification. We examined the samples for intestinal parasites. From the results, we assessed the increase in the

prevalence of *Oesophagostomum*, *Troglodytella* and *Strongyloides* infection after the onset of the rainy season in two ways. Via regression analysis we tested for a correlation between time and rate of infection. We used the two-tailed Fisher's exact test to test for a significant increase of parasitic prevalence between October and November.

RESULTS

Leaf-Swallowing

Direct Evidence

We observed leaf-swallowing on 20 October:

0750 h We relocate a party of 8 individuals *ca.* 60 m from the previous night nest site. In the previous hour, we had observed foraging on *Gambeya lacourtiana* and foraging by an adult female (Tsjoep) with infant in a liana (*Manniophyton fulvum*). She sits 15 m up in a tree. An adult male (Mobali) sits in the same tree at 10 m *ca.* 5 m from Tsjoep.

0755 h Tsjoep is sitting in the same place. Mobali climbs into the liana to a height of approximately 13 m. He takes two leaves, moves up the branch 2 more m and sits down. With his right hand, he slowly puts it into his mouth and swallows it without any apparent sign of chewing. Meanwhile he holds the second leaf in his left hand.

0800 h Mobali puts the second leaf into his mouth in the same slow way and swallows it without chewing.

0801 Mobali starts to travel; Tsjoep and the others follow him.

Dupain climbed into the liana and collected leaves. L. Pauwels (National Botanical Garden of Belgium) confirmed them to be *Manniophyton fulvum* (Euphorbiaceae).

Indirect Evidence

We analysed a total of 131 faecal samples to examine for the presence of hispid leaf-remains; 98 during the rainy and 33 during the dry season (Table I). We found unchewed leaf remains of *Manniophyton fulvum*, which varied in size ($>10 \, \text{cm}^2$, $<5 \, \text{cm}^2$) in two different samples in the rainy season. We also recorded the remains of two *Cola spp.* in 9 dung samples, 8 of which were from the rainy season. None of them remains was $>5 \, \text{cm}^2$.

Table I. Presence of unchewed leaf portions in fecal samples of Lomako bonobos

|	Rainy season ($n = 98$)	Dry season ($n = 33$)
Manniophyton fulvum	2	0
Cola sp.	6	0
Cola bruneeli	2	1

Parasitic Prevalence and Seasonality

We found a significant increase in the prevalence of *Oesophagostomum* infection during the first weeks of the rainy season. There is a significant positive correlation between time and rate of infection (r=0.731; F1,12=13.8, p<0,05) (Fig. 1), but not for *Troglodytella* (r=−0,13, F1,12=0,2, p>0,5) or *Strongyloides* (r=0,288, F1,12=1,1, p>0,5). Furthermore, only *Oesophagostomum* infection rates differ significantly between October and November (Fisher's exact test, two-tailed p value=0.05), versus infection rates of *Troglodytella* and *Strongyloides* (Fisher's exact test, two-tailed p value>0.1,>0.1 resp.) (Table II).

DISCUSSION

This is the first detailed published record on leaf-swallowing behavior by bonobos and the first record of this behavior at Iyema-Lomako. We confirmed leaf-swallowing by direct observation and indirectly by dung sample analysis.

An important element of the indirect evidence demonstrating the role of leaf-swallowing in parasitic control by chimpanzees is the synchrony of *Oesophagostomum* (tapeworm) infection and the occurence of leaf-swallowing (Huffman *et al.*, 1996, 1997; Wrangham, 1995). Wrangham (1995) focused on an outbreak of *Bertiella studeri* proglottid expulsion and Huffman *et al.* (1996) focused on the seasonal increase of *Oesophagostomum stephanostomum* infection and expulsion of adult worms with leaves. The significant increase in infection rates of *Oesophagostomum* in chimpanzees during the rainy season was first described by Huffman *et al.* (1990). As *Oesophagostomum* is listed as a common parasite for bonobos (Dupain *et al.*, 1999; Hasegawa *et al.*, 1983), and we observed them swallowing of *Manniophyton fulvum* leaves, we wanted to compare their incidences at Iyema-Lomako for similarity with observations in Mahale chimpanzees. Due to the life cycle of *Oesophagostomum*, we expected an increase of infection by during the rainy season (Huffman *et al.*, 1997).
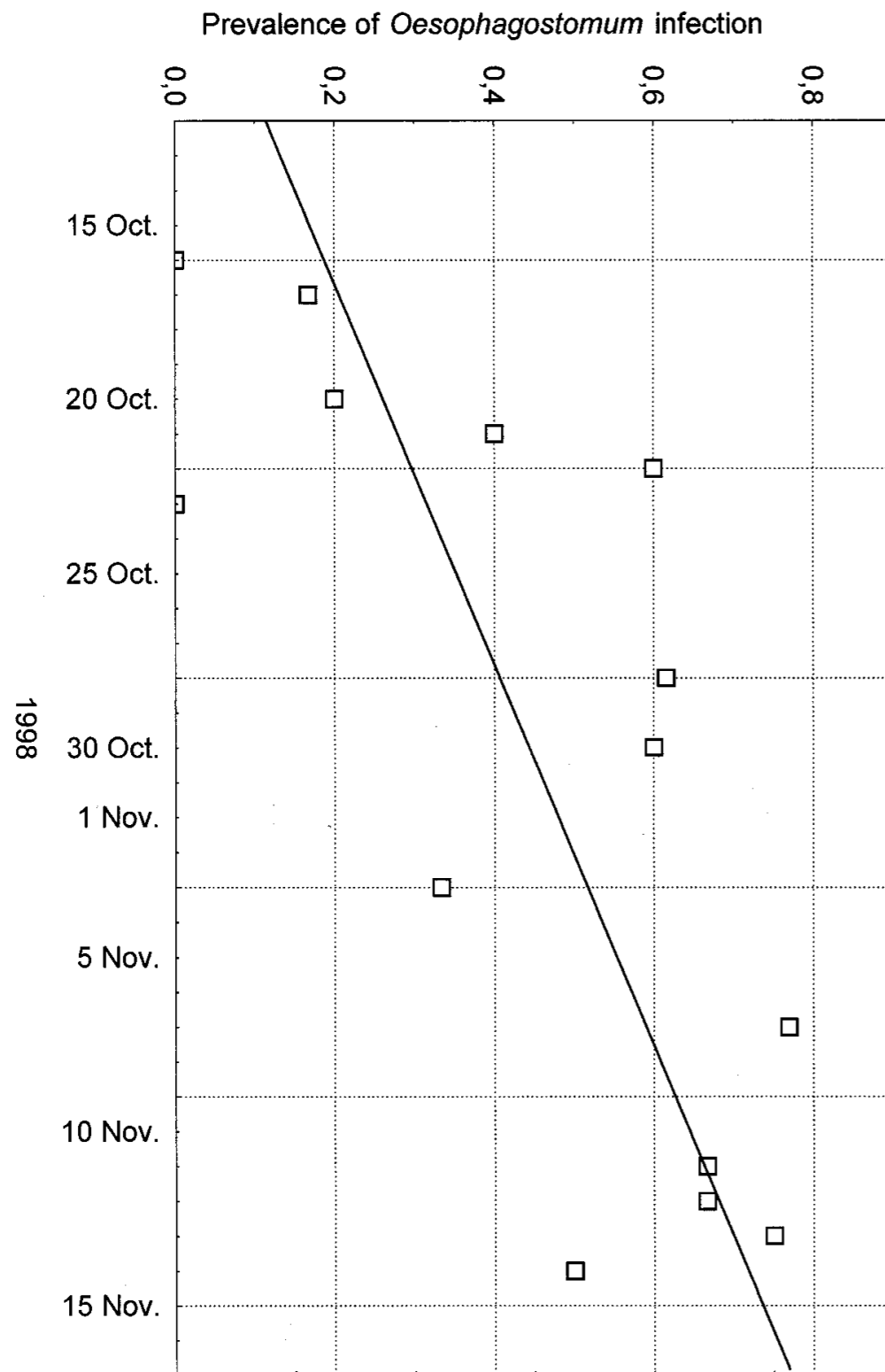
Fig. 1. Regression analysis of time vs. prevalence of occurence of *Oesophagostomum* infection in Lomako bonobo dung samples collected at the onset of the rainy season ($R = 0.73$, $F = 13.8$, $p < 0.005$).

Table II. Prevalence of *Oesophagostomum*, *Troglodytella* and *Strongyloides* infection in dung samples of Lomako bonobos collected in October–November 1998

Month (No. of samples)	*Oesophagostomum*[*]	*Troglodytella*	*Strongyloides*
October (48)	19 (40%)	39 (81%)	16 (33%)
November (38)	24 (63%)	25 (71%)	15 (39%)
Total (86)	43 (50%)	64 (74%)	31 (36%)

[*]$p = 0.05$, Fisher exact test: two-tailed.

The rainy season started at the end of September/beginning of October. Heavy rains continued until December. The dry season started in January. We collected samples for parasitological analysis in the rainy season. Infection by *Oesophagostomum* has a minimum prepatent period of 3 weeks (Anderson, 1992). Therefore, we expected a gradual measurable increase in the prevalence of parasitic infection from the second half of October on as the infective larvae mature to adulthood and they begin to lay eggs, with an increasingly higher rate of infection in the second month after the onset of the rainy season. We analyzed simultaneously for the prevalence of infection of two other chimpanzee intestinal parasites, *Strongyloides* and *Troglodytella*, known for their lack of seasonality (Ashford *et al.*, 2000; Huffman *et al.*, 1997; Kawabata and Nishida, 1991) as control measures for sample bias.

We confirmed a significant increase in the prevalence of *Oesophagostomum* infection in Iyema-Lomako bonobos via a significant correlation between time and the rate of infection during the study period and by the significantly higher prevalence of infection in November versus October. The increase is further confirmed by the lack of correlation with time and by the even distribution over the rainy season of *Troglodytella* and *Strongyloides* infection. As expected, evidence for leaf-swallowing was predominant in the rainy season.

The observation of bonobo swallowing leaves of *Manniophyton fulvum* closely resembles the description of leaf-swallowing in *Pan troglodytes* (Huffman and Caton, 2001; Wrangham and Nishida, 1983). The leaves were selected in the early morning, (0755 h) within about 1–2 h after leaving the night nest, and they were swallowed singly and slowly. The presence of leaves of *Manniophyton fulvum* in dung samples on two separate occasions confirms the probably habitual nature of leaf-swallowing. We also found remains of other hispid leaves (*Cola bruneeli* and *Cola sp.*) in dung samples and found remains from feeding on leaves of *Cola sp.* on one occasion early in the morning. Idani *et al.* (1994) observed bonobos eating leaves of *Cola bruneeli* and *Cola chlamydantha* but provided no information on whether they were swallowed or chewed. Given the rough hispid characteristic of the leaves, *Cola spp.* are possible candidates for leaf-swallowing by bonobos.

Leaf-swallowing and regular chewing are not mutually exclusive. Mahale and Bossou (Guinea) chimpanzees both chew and swallow the rough leaves of *Ficus exasperata* at different times of the day, demonstrating a dual but strictly seperated nutritional versus self-medicative use of the same species of leaf.

There is a tendency for apes in neighboring regions to select the same plant species for leaf-swallowing. For example, in eastern Africa, Mahale, Kibale and Gombe chimpanzees swallow leaves of *Aneilema aequinoctiale* (*Commelinaceae*), and Mahale and Gombe chimpanzees swallow leaves of *Hibiscus aponeurus* (*Malvaceae*) (Huffman, 1997). Our observations of bonobos swallowing the leaves of *Manniophyton fulvum* further support the reports from three other sites in Central and West Africa where apes also swallow leaves of *Manniophyton fulvum*. (Boesch, 1995; Huffman and Wrangham, 1994).

Leaf-swallowing by Lomako bonobos closely resembles leaf-swallowing in Kibale, Mahale and Gombe chimpanzees. Furthermore, our data support the increase in prevalence of *Oesophagostomum* infections in bonobos during the rainy season, as reported for the Mahale chimpanzees. These common elements in the behavior of Lomako bonobos and leaf-swallowing by apes elsewhere provide strong support for the hypothesis that leaf-swallowing might be wide spread in bonobos.

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