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Leaf-Swallowing by Chimpanzees: A Behavioral Adaptation for the Control of Strongyle Nematode Infections

Michael A. Huffman,[1,2,7] Jonathan E. Page,[3] Michael V. K. Sukhdeo,[4] Shunji Gotoh,[5] Mohamedi S. Kalunde,[6] Thushara Chandrasiri,[4] and G. H. Neil Towers[3]

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Swallowing whole leaves by chimpanzees and other African apes has been hypothesized to have an antiparasitic or medicinal function, but detailed studies demonstrating this were lacking. We correlate for the first time quantifiable measures of the health of chimpanzees with observations of leaf-swallowing in Mahale Mountains National Park, Tanzania. We obtained a total of 27 cases involving the use of Aspilia mossambicensis *(63%),* Lippia plicata *(7%),* Hibiscus *sp. (15%),* Trema orientalis *(4%), and* Aneilema aequinoctiale *(11%), 15 cases by direct observation of 12 individuals of the Mahale M group. At the time of use, we noted behavioral symptoms of illness in the 8 closely observed cases, and detected single or multiple parasitic infections (*Strongyloides fulleborni, Trichuris trichiura, Oesophagostomum stephanostomum*) in 10 of the 12 individuals. There is a significant relationship between the presence of whole leaves (range, 1-51) and worms of adult* O. stephanostomum *(range, 2-21) in the dung. HPLC analysis of leaf*

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[1] Section of Ecology, Kyoto University, Primate Research Institute, 41 Kanrin, Inuyama 484 Aichi, Japan.

[2] Department of Anthropology, University of Colorado at Denver, Denver, Colorado 80217-3364.

[3] Botany Department, University of British Columbia, Vancouver, B.C., Canada.

[4] Department of Animal Sciences, Cook College, Rutgers University, New Brunswick, New Jersey 08903.

[5] Laboratory Primate Center, Kyoto University Primate Research Institute, 41 Kanrin, Inuyama, Japan.

[6] Mahale Mountains National Park, Box 1374, Kigoma, Tanzania.

[7] To whom correspondence should be addressed to Section of Ecology, Kyoto University, Primate Research Institute, 41 Kanrin, Inuyama 484 Aichi, Japan.

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samples collected after use showed that thiarubrine A, a compound proposed to act as a potent nematocide in swallowing Aspilia *spp., was not present in leaves of* A. mossambicensis *or the three other species analyzed. Alternative nematocidal or egg-laying inhibition activity was not evident. Worms of* O. stephanostomum *were recovered live and motile from chimpanzee dung, trapped within the folded leaves and attached to leaf surfaces by trichomes, though some were moving freely within the fecal matter, suggesting that the physical properties of leaves may contribute to the expulsion of parasites. We review previous hypotheses concerning leaf-swallowing and propose an alternative hypothesis based on physical action.*

KEY WORDS: chimpanzees; antiparasite behavior; physical expulsion; *Oesophagostomum stephanostomum*; trichomes.

INTRODUCTION

Pathogens and parasites can cause a variety of diseases that affect the overall behavior and reproductive fitness of an individual (Hart, 1990; Holmes and Zohar, 1990). Indeed, the impact of parasitism on the host is considered to play an important role in the evolution of behavior throughout the animal kingdom (Anderson and May, 1982; Bernard and Behnke, 1990; Futuyma and Slatkin, 1983; Hamilton, 1974a, b; Hamilton and Zuk, 1982; Price, 1980). Coevolution between host and parasite has developed mechanisms by which the host can limit parasitic infection (Allison, 1982; Toft *et al.*, 1991). Chimpanzees, which are susceptible to infection by a wide range of parasite species (Myers and Kuntz, 1972), may ingest certain plants to control infection. While still limited, evidence of such antiparasitic adaptation in chimpanzees is being generated from investigations of two types of medicinal plant use: bitter pith chewing and whole leaf-swallowing (Huffman and Wrangham, 1994; Rodriguez and Wrangham, 1993).

With regard to the use of the bitter pith of *Vernonia amygdalina* Del., an ongoing study at Mahale Mountains National Park, Tanzania (Huffman, 1994), has documented cases of the use of this plant followed by recovery in chimpanzees suffering from the effects of gastrointestinal upset and nematode infection (Huffman and Seifu, 1989; Huffman *et al.*, 1993a). In the only case documented in detail, an unusual drop in parasitic load—eggs per gram feces—of an ill chimpanzee was detected ≤20 hr after ingestion of this pith (Huffman *et al.*, 1993a). This activity is consistent with pharmacological studies demonstrating significant *in vitro* inhibition of egg-laying activity and mobility in schistosomes by chemical constitutents, including the steroid glucoside vernonioside B_1, found in the pith of *V. amygdalina* (Jisaka *et al.*, 1992; Ohigashi *et al.*, 1994).

Leaf-swallowing in chimpanzees at Gombe and Mahale was reported in detail by Wrangham (1975, 1977) and Wrangham and Nishida (1983), who observed that this habit provided little or no nutritional value after they found the folded, undigested leaves of *Aspilia mossambicensis*, *A. pluriseta* Wild, and *A. rudis* in the dung. Usually chimpanzees select the hispid, rough-surfaced leaves one at a time, place them in the mouth, and swallow them whole.

Currently, there are two hypotheses based on the presence of biologically active secondary chemicals to explain swallowing leaves of *Aspilia*. A powerful antibiotic, antifungal, and anthelminthic diathiacyclohexadiene polyyne, thiarubrine A (Towers *et al.*, 1985), was reported by Rodriguez *et al.* (1985) to be in the leaves of *A. mossambicensis* and *A. pluriseta*. Based on the presence of this compound, Wrangham and Goodall (1989) speculated that chimpanzees may ingest the three *Aspilia* spp. to control intestinal parasites. This hypothesis was expanded by Rodriguez and Wrangham (1993), who proposed that 100 μl of thiarubrine A per leaf [Rodriguez *et al.* (1985) report 5 mg present per leaf] of *A. mossambicensis* could act as a nematocide. They calculated that 50 leaves contain more than enough thiarubrine A to kill all of a chimpanzee's intestinal nematodes without harming the host (Rodriguez and Wrangham, 1993).

Confounding the thiarubrine A hypothesis is the fact that others have been unable to replicate the original findings. In a survey of the general biological activity of African plants, Ohigashi *et al.* (1991a) found no significant insecticidal, herbicidal or fungicidal activity in the leaf extracts of *A. mossambicensis* from Mahale. More directly, Page *et al.* (1992) were unable to detect thiarubrine A in any sample of *Aspilia* spp. that they have collected from Mahale or Gombe. The compound occurred in small amounts only in the roots.

Alteratively, Page *et al.* (1992) suggested that the uterostimulatory effect of two diterpenes, kaurenoic acid and grandiflorenic acid, may be exploited by female chimpanzees as a regulator of fertility. Both compounds can be isolated from Mahale *A. mossambicensis*, and kaurenoic acid, which shows antimicrobial and antihepatotoxic activities, is present in *A. pluriseta* from Kenya (Lwande *et al.*, 1985; Yang *et al.*, 1986).

Wrangham (1996) proposed that leaf-swallowing may be stimulated by, and might function to relieve, abdominal pain. He did not discuss chemical activity.

The use of *Aspilia* and the possibility that chimpanzees may possess a sophisticated pharmacopeia prompted other researchers to look for anomalous feeding habits in other primate populations. Since 1985, the widespread nature of leaf-swallowing has become apparent; 16 other species have been observed to be swallowed whole at nine sites across Africa including Mahale, by chimpanzees, bonobos, and western gorillas (Huffman, 1993; Huffman and Wrangham, 1994; Matsuzawa and Yamakoshi, 1996; Takasaki and Hunt, 1987; V. Reynolds, personal communication).

Attempts to explain leaf-swallowing and other medicinal plant use by chimpanzees have focused mainly on the role that the biologically active secondary chemicals found in swallowed leaves may play in mediating this behavior. In the case of *V. amygdalina* pith ingestion, the presence of such chemicals and direct observations of changes in levels of parasitic infection and ill health at the time of use seem to support such a hypothesis of antiparasitic function. However, direct detailed observation or evidence of ill health at the time of swallowing the leaves of *Aspilia* or other species is lacking. However, peaks in leaf-swallowing have been correlated with seasonal increases in parasitic infection, in particular, those of *Oesophagostomum stephanostomum* at Mahale (Huffman *et al.*, 1990; Kawabata and Nishida, 1991; Huffman *et al.*, in preparation) and an unusual outbreak of tapeworm infections at Kibale (Wrangham, 1996).

In addition to uncertainty concerning the presence of thiarubrine A, the utility of chemical hypotheses for leaf-swallowing is brought into question by the geographic and taxonomic diversity for this type of plant use. Thus plant secondary chemicals, which are taxonomically restricted in their distribution, may not provide a common feature uniting all species that are swallowed. But this does not eliminate the possibility that such plants may share some biological activity sought by leave-swallowing chimpanzees.

When one considers all species swallowed, the characteristic shared by all such species is not chemical but physical: the hispid surfaces of their leaves. Huffman and Wrangham (1994) suggested that hispidity is important in the use of these plants.

While there are several hypotheses to explain the function of whole leaf-swallowing behavior, field studies designed specifically to test them have not been conducted. Ideally, one should examine plant specimens collected concurrently with observations of their use for the presence or absence of biologically active chemicals as well as physical characteristics that may be exploited by chimpanzees. This data would be correlated with quantifiable measures of the health of the user (Huffman and Seifu, 1989; Huffman *et al.*, 1993a, b).

As part of an ongoing investigation into the use of medicinal plants as an adaptation for the control of parasite-related disease (Huffman, 1994), we present results from a study designed to correlate the behavioral and health-related aspects of leaf-swallowing with the phytochemical and physical properties of leaves swallowed whole by chimpanzees at Mahale.

MATERIALS AND METHODS

Study Site

We observed the M group of chimpanzees in the Mahale Mountains National Park, Tanzania, between 23 November 1993 and 25 February 1994.

Situated on the eastern shore of Lake Tanganyika, the Mahale climate is influenced by weather from the lake and the mountainous terrain, which ranges from 772 to 2500 m above sea level. Chimpanzees are supported mainly by the semideciduous gallery forests between 780 and 1300 m in elevation. The year can be divided into two distinct seasons, with the rainy season lasting from around mid-October to mid-May (Nishida, 1990, Takasaki *et al.*, 1990).

We selected the early rainy season months for this study because of seasonal peaks in the use of *V. amygdalina* and *A. mossambicensis* at Mahale between December and February (Huffman *et al.*, 1990; Wrangham and Nishida, 1983) and the correlation between the rainy season and rise in parasitic infections (Huffman *et al.*, 1993b), most notably by *Oesophagostomum stephanostomum*.

Behavioral Data Collection

We recorded 199 hr of focal-animal observations for 14 individuals during 43 observation sessions. Observations were conducted by Huffman, with the assistance of Kalunde and L. I. Ramadhani. Visible cues of an individual's state of health were noted, paying specific attention to activity patterns and stool type. Because of frequent group fission and fusion, some focal individuals could not be found daily with a high degree of predictability. The decreasing availability of fruit during the study period caused individuals to travel alone or to form smaller groups, which further confounded this problem. In spite of this, we endeavored to locate and to collect consecutive-day data on as many focal individuals as possible.

Dung Collection

We collected dung concurrently with behavioral observations immediately after discharge and stored the samples individually in 5.0-ml Corning sterile vials. At camp, we weighed the vials and contents and fixed 1-g dung samples via a 10% neutral formalin solution. We thoroughly mixed contents of the vial before sealing and storing them in a cool dark room.

During focal-animal and ad libitum observations, we inspected all fresh dung for the presence of whole leaves and adult parasitic worms whenever possible. We collected dung with whole leaves or worms and stored it in plastic bags for close inspection at camp. We noted the number of leaves and worms and stored the worms in formalin.

Gotoh examined all 1-g samples microscopically ≤6 months after collection for the presence of parasitic eggs and their identification. R. C. Anderson identified O. *stephanostomum* from adult worms. We previously reported them as a *Ternidens* sp. based on egg size alone (Huffman, 1993; Huffman *et al.,* 1993a, b). We measured parasitic load via the MGL and McMaster techniques (expressed as eggs/g feces; EPG). We conducted three egg counts per sample and calculated the EPG for each sample as the mean value derived from those trials.

Plant Collection

During focal-animal and ad libitum observations, we sampled plant items that chimpanzees ingested in a peculiar manner (bitter pith chewing, whole leaf-swallowing). We placed ≤3-g portions of them into small amber glass specimen bottles containing methanol. Most "plant-food" species utilized by chimpanzees at Mahale had been identified by Nishida and Uehara (1983). S. G. Mathenge and E. Knox verified the key species that we collected. We deposited voucher specimens in their herbariums in Nairobi. Recently, Robinson (1992) sank *Aspilia* into *Wedelia*, and it seems likely that the *Aspilia* spp. ingested by chimpanzees will also be renamed *Wedelia*.

We monitored areas in M group's home range, where stands of *V. amygdalina, A. Mossambicensis*, and *Lippia plicata* Baker are located and visited by chimpanzees. We checked them in the mornings or early afternoons, when chimpanzees were expected to pass through the area, or on days after chimpanzees had been observed in this part of their range.

Indirect evidence for leaf-swallowing consisted of disturbed stands or freshly discarded stalks and small branches on the ground nearby with missing leaves in areas where chimpanzees had recently passed through as determined by direct sighting, fresh dung, fresh tracks, and vocalization. Stands of *A. mossambicensis* and *L. plicata* utilized by chimpanzees are usually distinguishable from those disturbed by herbivores. Tracks, fresh dung, and the presence of woody shoots broken and bent to the ground by chimpanzees to bring the young leaves into reach versus the lower-standing stems cleanly nipped off by herbivores were key distinguishing signs. We collected no sample from ambiguous sites.

Nishida (1990) reported that Mahale chimpanzee chew large quantities of leaves of *A. mossambicensis* in the early evening, instead of swallowing them as is common during the morning. It is possible that during this study some samples collected as indirect evidence of leaf-swallowing from plant remains could have been chewed instead of swallowed. This does not appear very likely because samples were collected in the morning (0840–1120 hr) from areas visited earlier that day by chimpanzees in all but two cases, both of which were leavings of *Aspilia*. In these two cases, few of the available leaves had been removed from the tips of the stalks. This differs from the method used when chewing large quantities of leaves in which the entire stalk is often stripped entirely of its leaves with one swift movement of the tightly flexed hand (Nishida, personal communication).

When we noted signs of disturbance by chimpanzees, we recorded the number of missing leaves and collected similarly positioned distal leaves (first four to six rows) from the remaining undisturbed branches, as well as stem and root material, which were prepared as described above for phytochemical analysis. All samples (Table I) were sent to Page for analysis at the end of the field study.

Phytochemical Analysis

Sample Preparation. To minimize the photodegradation of dithiacyclohexadiene polyynes, we performed all procedures and workup of samples in dim light and stored samples and extracts in a freezer at -10°C before analysis. We decanted the methanol extract of each 2-, 2.6-, or 3-g sample from the vial and washed the remaining plant material with 5 ml of methanol. We combined the extract and washing and passed them through a C_{18} cartridge. We adjusted the final volume of each extract to 25 ml with additional methanol to produce a solution with an effective concentration of 0.12 g fresh weight of plant material/ml. We analyzed these final solutions for the presence of thiarubrine A by direct injection onto the reversed-phase HPLC column. To check the efficacy of the sample preparation method in extracting thiarubrine A from plant material, we analyzed samples of *Ambrosia chamissonis* (Less.) Greene, a plant that contains thiarubrine A in its leaves (Ellis, 1993) and roots (Balza *et al.*, 1989; Freeman *et al.*, 1993), in a fashion identical to the Mahale samples of *Aspilia*. HPLC analysis showed that the method effectively extracts the range of thiarubrine A concentration present in the leaves and roots of *A. chamissonis*.

To check for the presence of kaurenoic acid, we purified the methanol solutions by removing 1-ml aliquots and drying them under a stream of N_2

Table I. Evidence of Leaf-Swallowing in Mahale M-Group During 1993–1994[a]

	Plant species					
Type of evidence	*Aspilia mossambicensis*	*Lippia plicata*	*Hibiscus* sp.	*Trema orientalis*	*Aneilema aequinoctiale*	Total
Direct observation	8 (8)	0	2	0	0	10 (8)
Dung samples	1	0	0	1	3	5
Plant remains	8 (7)	2 (2)	2 (1)	0	0 (1)	12 (11)
Total	17 (15)	2 (2)	4 (1)	1	3 (1)[b]	27 (19)

[a] The number of observations in which leaf samples were collected for HPLC analysis is in parentheses.

[b] Sample collected for HPLC analysis not from specific plant after it was used.

and low heat. We recovered the residue in 2 × 1 ml hexane and dried it with N_2. We analyzed the residue in 1 ml of acetonitrile via HPLC.

Isolation of Standards. We isolated thiarubrine A from a crude thiarubrine mixture extracted from the roots of *A. chamissonis* via semipreparative HPLC per Balza and Towers (1993) and determined the concentration of thiarubrine A solutions spectrophotometrically ($\varepsilon = 3000$ at 490 nm; Pie Uicam uv/vis scanning spectrophotometer). We isolated kaurenoic acid from the leaves of greenhouse *A. mossambicensis* per Page *et al.* (1992).

HPLC Assay. We conducted reversed-phase HPLC analysis of leaf extracts on a Waters 600E System Controller and a Waters 994 Programmable Photodiode Array Detector (PDA). For detection of thiarubrine A in leaf extracts, we applied injections corresponding to 2.4 mg fresh weight of sample (20, 23.1, or 30 μl) to a 3.9 × 150-mm (4-μm) C_{18} column (Waters NovaPak) eluting with a mobile phase of acetonitrile–water (76:24) at a flow rate of 0.5 ml/min, with detection at 340 nm. Under these conditions, thiarubrine A elutes at 15.4 min (SD, 0.02 min; $n = 14$). Thiarubrine A exhibits a distinct uv/vis spectrum (Fig. 1A, inset) and our detection of it in extracts is based on retention time and uv/vis spectroscopy via PDA detection over the wavelength range 200–600 nm. The lower limit of detection is <16 ng on column. To establish the practical detection limit for thiarubrine A by the use of this assay, we spiked a leaf extract of *A. mossambicensis* that did not contain thiarubrine A with 0.1% of fresh weight of this compound. Serial dilution and HPLC analysis of the spiked solution showed that thiarubrine A is detectable to ca. 0.002% of fresh weight.

For the detection of kaurenoic acid, we applied injections corresponding to 6 mg fresh weight of sample (50, 57.6, or 75 μl) to the column and eluted them with a mobile phase of acetonitrile–water (80:20) at a flow rate of 1.0 ml/min, with detection at 205 nm. Under these conditions, kaurenoic acid elutes at 7.7 min (SD, 0.12 min; $n = 20$); the detection limit is lower than 0.15 μg on the column. We used retention time and uv/vis spectroscopy via PDA detection over the wavelength range 200–400 nm to identify kaurenoic acid in leaf extracts.

Electron Microscopy of Leaf and Parasite Material

Huffman and Kalunde collected from plant and fecal specimens leaf samples of seven species known to be swallowed whole at Mahale. We fixed them in 2.5% glutaldehyde for transportation. We also sent a few adult

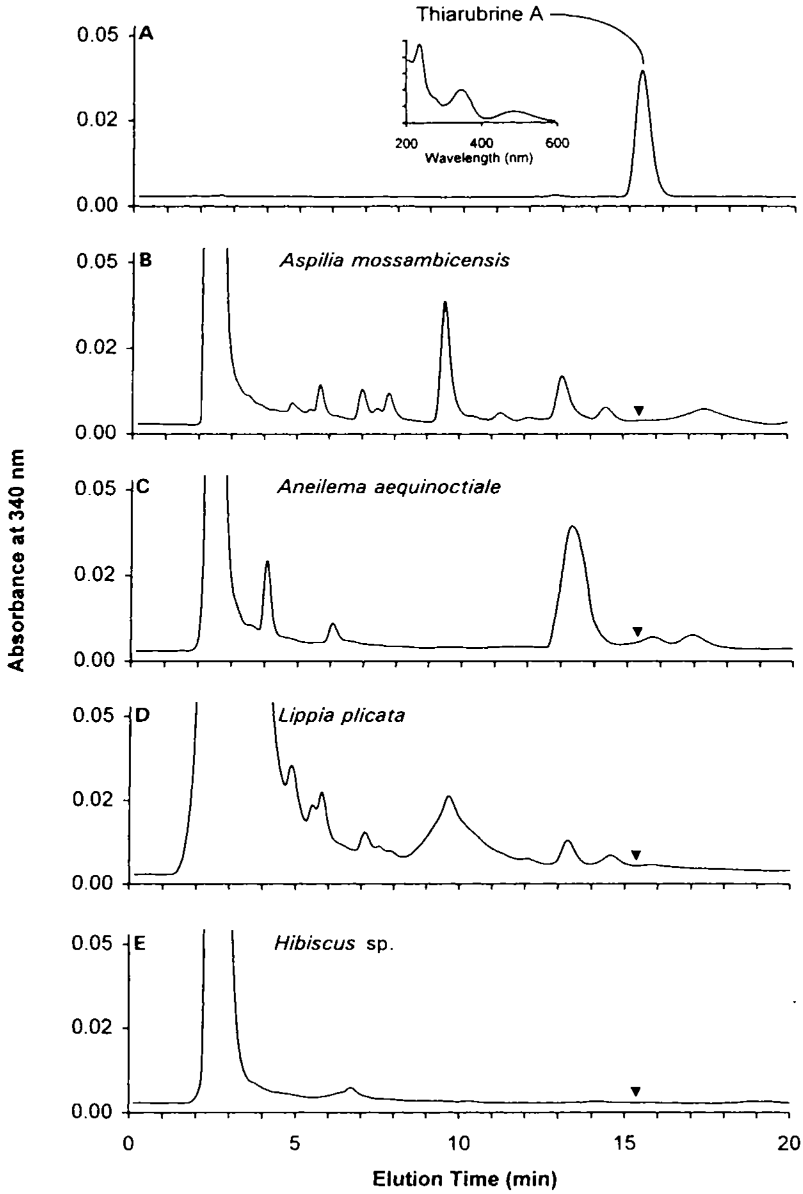

Fig. 1. Reversed-phase HPLC of methanolic extracts of leaves swallowed by chimpanzees: thiarubrine A analysis. (A) Standard solution of thiarubrine A (retention time, 15.4 min). Peak represents injection of 0.25 μg (inset). Ultraviolet/visible spectrum of thiarubrine A. (B) *A. mossambicensis*: sample collected from plant used by adolescent male BB; 1035, 7 February 1994 (Table II). Injection volume was 20 μl. (C) *A. aequinoctiale*. (D) *Lippia plicata*. (E) *Hibiscus sp.* Arrowheads indicate the retention time of thiarubrine A.

parasites retrieved from the dung and preserved in formalin with the plant samples to Sukhedo and Chandrasiri for electron microscopic examination.

Our preparative and operative procedures follow those of Bozzola and Russell (1991) using a Baltec (CPD 020) critical-point drier and Hitachi (S450) scanning electron microscope.

RESULTS

Direct and Indirect Evidence of Leaf-Swallowing

We documented 27 cases of leaf-swallowing of *A. mossambicensis, L. plicata, Hibiscus* sp., *Trema orientalis* (L.) Blume, and *Aneilema aequinoctiale* (P. Beauve.) Loudon (Table I). *A. mossambicensis* accounts for 63% (n = 17) of the data set. The swallowed leaves of *Hibiscus* sp. and *A. aequinoctiale* are new records for M-group chimpanzees. Identification of *Hibiscus* to the specific level could not be made due to the absence of flowers during collection. The specimen was morphologically identical in gross structure to the Gombe Research Centre Herbarium specimens identified as *H. aponeurus* Sprague & Hutch, which is swallowed whole by Gombe chimpanzees (Wrangham and Goodall, 1989). [This species was positively identified as *H. aponeurus* in 1996.]

Details of the 15 cases of leaf-swallowing obtained from direct observation or the dung of identified individuals are in Table II. They occurred on 6 days between 25 November and 19 February. The number of leaves swallowed during an observation ranged from 5 to 55 ($\bar{x}$ = 13.36, SD = 14.61; n = 10) and the number of leaves recovered from dung samples ranged from 1 to 55 ($\bar{x}$ = 19.75, SD = 25.28; n = 4). Neither count necessarily represents the total number of leaves swallowed or egested by an individual in 1 day, but the upper range shows that many leaves can be swallowed in a single bout. The mean fresh weight per distal leaf is 0.69 g (SD = 0.32 g; n = 94) for *A. mossambicensis* and 0.49 g (SD = 0.11 g; n = 20) for *L. plicata*.

Direct observations involved encounters on 2 days with groups of chimpanzees visiting specific areas in their range that are particularly abundant in *A. mossambicensis*. The following is a brief account of these observations.

> January 10, 1994:
>
> At 8:37 a group of 18 chimpanzees including the alpha male, other adults, adolescents, and juveniles of both sexes is located. Most of the group is feeding on *Ficus* sp. fruits ≤10 m from fresh beds, suggesting that they may have slept here the previous night. Between 0900 and 1030 three of them (one adolescent male, two adult females) swallow the leaves of *A. mossambicensis* (Table II, cases 3–5).
>
> February 7, 1994:

> At 0944, a group of 12 chimpanzees including the alpha male, other adults, juveniles, and adolescents of both sexes approach a patch of *A. mossambicensis* that Huffman and Kalunde are inspecting. One particular plant appears to have been utilized earlier that morning; 30 leaves had been removed. Five individuals in the group (three adult males, one adult female, two adolescent males) swallow leaves of *A. mossambicensis* and one swallows leaves of *Hibiscus* sp. (Table II, cases 6–12). The other group members remain nearby and wait for the alpha male to move on. Later that afternoon a group of 11 chimpanzees, most of the same members followed since morning, are moving in procession along the trail. At 1720, an adolescent female suddenly separates from the group and heads directly to a small lone shrub of *Hibiscus* sp. 5 m off the trail. She breaks off one branch and brings it back to the trail where she swallows 9 leaves (Table II, case 13) before moving on with the group.

On both, there is no statistically significant age–sex class difference with regard to the number of individuals in each group that swallowed leaves versus those that did not.

The times of leaf-swallowing range between 0912 and 1720, with a strong tendency toward the late morning hours between 1000 and 1100 (Table II). After heavy rains in the night and/or on rainy or dark overcast mornings, chimpanzees tended to stay in their sleeping nests longer, sometimes until midmorning or as late as midday. This may account for the tendency toward late-morning occurrence of this normally early-morning activity in our direct observations of leaf-swallowing. On the morning of 10 January it was dark and overcast and heavy rains had fallen during the night. The morning of 7 February was cloudy and overcast. Heavy rains fell between 0800 and 0900 just before our observations. In these two instances it was very likely that these plants were among the first things that the chimpanzees ate. The case of late-afternoon leaf-swallowing (case 13) is an interesting exception.

State of Health at the Time of Use

Table II also shows behavioral and parasitological profiles of the 12 individuals (13 cases) for which we obtained evidence of leaf-swallowing.

Behavioral symptoms of illness were available for eight of them. In seven cases, individuals showed symptoms of malaise or diarrhea or both. Malaise is characterized by frequent stops to rest, prolonged sleeping while others fed, or lagging behind the group. Adult worms of *O. stephanostomum* (2–3 cm in length) were in the dung of three of the individuals that we followed most closely (cases 1, 6, 12) on the day of leaf-swallowing.

According to parasitological analysis, 83% (n = 10/12 cases; 11 individuals) of the subjects sampled on the day or on the day after leaf-swallowing was observed were infected by parasites. Individuals were infected by single and multiple species (Table II), with infections of *O.*

Table II. Behavior and Parasitological Profiles of Indiviuals at the Time of Medicinal Plant Use[a]

Case No.	Date, time of use	Plant species: amount used, observational source	Symptoms of illness at the time of use	Parasitic infections detected on the day of use
1	25 Nov. 1993	*Aspilia:* in dung, not counted	Diarrhea, malaise, passed *O. stephnostomum* adult worms	*T. trichiura* (45) *O. stephanostomum* (+)
2	27 Dec. 1993	*Trema:* 18 in dung	Vomiting, severe malaise cough, digestive problems	Nematode sp. larva (+) *O. stephanostomum* (+)
3	10 Jan. 1994, 0912–	*Aspilia:* 9 swallowed	No data	*S. fuelleborni* (+)
4	10 Jan. 1994, 0939–	*Aspilia:* 14 swallowed	No data	*S. fuelleborni* (+)
5	10 Jan. 1994, 1030	*Aspilia:* 6 swallowed	Deep cough, runny nose	*O. stephanostomum* (20)
6	7 Feb. 1994, 0955–	*Hibiscus* sp.: 5 swallowed	Diarrhea, malaise, passed *O. stephanostomum* adult worms	*S. fuelleborni* (+) *O. stephanostomum* (37) *T. trichiura* (7)
7	Same day	*Aneilema:* 51 in dung		
15	19 Feb. 1994	*Aneilema:* 1 in dung	Diarrhea, malaise	*S. fuelleborni* (+) *O stephanostomum* (43)
8	7 Feb. 1994, 1000	*Aspilia:* 6 swallowed	Malaise, frequent diarrhea	*S. fuelleborni* (++) *O. stephanostomum* (50)
		V. amygdalina: 70 cm pith chewed		
14	8 Feb. 1994	*Aneilema:* 4 in dung	Diarrhea	

Table II. Continued.

Case No.	Date, time of use	Plant species: amount used, observational source	Symptoms of illness at the time of use	Parasitic infections detected on the day of use
9	7 Feb. 1994, 1020	*Aspilia:* 9 swallowed	No data	Nothing detected
10	7 Feb. 1994, 1032	*Aspilia*: 20 swallowed *V. amygdalina:* 84 cm pith chewed	No data	*O. stephanostomum* (985)
11	7 Feb. 1994, 1033	*Aspilia:* 6 swallowed	No data	*O. stephanostomum*
12	7 Feb. 1994, 1035–1055	*Aspilia*: 55 swallowed	Diarrhea, passed *O. stephanostomum* adult worms	*S. fuelleborni* (+) *O. stephanostomum* (97) *T. trichiura* (217)
13	7 Feb. 1994, 1720	*Hibiscus* sp.: 13 swallowed	No data	Nothing detected

[a] Parasitological measures: degree of infection estimation (+, 1–9; ++, 10–99; +++, 100 or more eggs perg by MGL method; EPG (eggs per gram dung) by McMaster's technique.

stephanostomum being the most common (78%), followed by *Strongyloides fulleborni* (56%) and *Trichuris trichiura* (33%). The mean individual degree of infection, based on counts of eggs per gram (EPG) of feces, is 166 (SD = 313; $n = 9$; Table II). Symptoms caused by infections from these species include diarrhea, malaise, and abdominal pain (Brack, 1987; Anderson, 1992).

The cumulative effect of multispecific infections may have intensified these symptoms, consequently affecting the intensity (number of leaves swallowed) or frequency of medicinal plant use. Although analysis is limited by this small sample, three individuals that used at least two or three species of medicinal plants on the same day or used them over 2 consecutive days also had some of the highest EPG counts (Table II: cases 6–8, 14, 10).

Possible Effects of Leaf-Swallowing on Health

Seven leaf samples were available for microscopic parasitological analyses within 1–14 days after subjects had swallowed them (Table III; cases 1–4, 6–15). Immediate clinical signs of improved health were not apparent. There was no evidence for the suppression of adult worm egg-laying activity in any of the three nematode species that are associated with swallowing leaves of *A. mossambicensis, T. orientalis, A. aequinoctiale*, or *Hibiscus* sp. EPG counts remained stable, with no striking difference between the day of leaf-swallowing and the 1–14 days thereafter. One important change was the presence of worms of *O. stephanostomum* in the dung with these undigested leaves.

The Presence of Leaves and O. stephanostomum in the Dung

We inspected 245 dung samples for the presence of leaves or adult parasitic worms or both (Table IV). Parasitic worms, all *O. stephanostomum*, were in 3.7% ($n = 9/245$) of them. The occurrence of worms in these dung samples was a rare event limited to individuals that displayed symptoms of malaise and diarrhea.

Six of the nine dung samples containing worms also contained the whole undigested leaves of *A. mossambicensis, T. orientalis*, or *A. aequinoctiale* (Table III; cases 1, 2, 7, 14, 15). The remaining three cases are from three of these individuals (Table II: cases 1, 6, 12) later in the days when they had swallowed leaves. This relationship between the presence of both leaves and worms in the dung is highly significant (Fisher's exact test, p 0.0001, two-sided) and suggests a relationship between the expulsion of worms (*O. stephanostomum*) and leaf-swallowing.

Table III. Parasitological Observations Following Medicinal Plant Use[a]

Case No.	Observations
1	Several adult worms of *O. stephanostomum* passed with *Aspilia* leaves, *T. trichiura* EPG remains stable (45 on day of use; 35, 65 thereafter) over the next 14 days, dies 1 month later, between December 14 and 25, passed worms and blood from urethra while trying to urinate on 13, 14 Dec.
2	18 adult worms of *O. stephanostomum* passed with 18 leaves of *Trema*; *O. stephnostomum* (+) EPG remains low
3	No change in level of *S. fulleborni* (+; +) infection next day
4	Level of infection of *S. fulleborni* (+; +) remains stable within next 1–10 days
6, 7	21 adult worms of *O. stephanostomum* passed with 51 leaves of *Aneilema*; *O. stephanostomum* (37; 43) and *S. fulleborni* (+; +) EPG remain stable within next 10 days
8, 14	1 leaf of *Aneilema* passed without worms; *S. fulleborni* (++; +) and *O. stephanostomum* (50; 20) EPG remain stable
15	2 adult worms of *O. stephanostomum* passed with 1 leaf of *Aneilema*

[a] Parasitological measures: degree of infection estimation (+, 1–9; ++, 10–99; +++, 100 or more eggs per g by MGL method; EPG (eggs per gram dung) by McMaster's technique.

Possible Modes of Parasite Expulsion

A nematocidal property to leaf-swallowing was not evident because all visible worms were alive and motile at the time of egestion. Furthermore, in the three cases for which we collected dung containing whole leaves (one case each of *A. mossambicensis*, *T. orientalis*, or *A. aequinoctiale*) in their entirety, worms were alive and motile *in situ* 4 days later, when the dung samples were washed and the number of leaves and worms counted. At this time, one to five worms were found between the folds in a leaf along with worms (*O. stephanostomum*) moving freely within the liquid dung matter. In one instance, the entire length of two worms (*O. stephanostomum*) (body, ca. 2.5 cm) were firmly attached to the upper surface of a leaf (*A. aequinoctiale*) as if by Velcro. Electron microscopy revealed no sign of puncture or surface damage by trichomes to the tough cuticle. We used a subset of 81 dung samples classified as normal or diarrheal to test the relationship between stool type and the presence of worms in the dung (Table III). There is no statistically significant relationship between the presence of parasites and stool type alone (Fisher's exact test, $p = 0.0577$, two-tailed), but there is a significant relationship between the presence of leaves and stool type (Fisher's exact test, $p = 0.0172$). This suggests that while diarrhea alone may not be directly related to the process of purging worms, whole undigested leaves may induce diarrhea. When combined with the physical expelling action of leaves, this may assist the purging process.

Table IV. Frequency of Whole Leaves and Adult *O. stepanostomum* in Dung and the Relationship Between Leaves and Stool Type[a]

	Leaves present	Leaves absent	
Worms present	5	4	$n = 245$ $p < 0.0001$
Worms absent	1	235	
Diarrhea	5	23	$n = 81$ $p = 0.0172$
Normal	1	52	
	Diarrhea	**Normal**	
Worms present	6	3	$n = 81$ $p = 0.0577$
Worms absent	22	50	

[a] Fisher's exact test; two-sided.

Although data are too few to conduct statistical tests, we noted a relationship between the number of leaves swallowed and the number of worms expelled: 18 leaves:18 worms, 51:21, 1:2, and 1:0 (Table II: cases 2, 7, 14, 15).

Phytochemical Analysis for the Presence of Thiarubrine A and Kaurenoic Acid in the Leaves Swallowed

Analytical HPLC tests on the methanolic extracts of 13 *A. mossambicensis* leaf samples, 8 of which were collected after direct observations of leaf-swallowing (Table I) and 5 from indirect evidence, failed to detect the presence of thiarubrine A in any of the samples (Fig. 1B). Methanolic leaf extracts of three other species that chimpanzees swallowed (*A. aequinoctiale, L. plicata*, and *Hibiscus* sp.) also did not contain detectable levels of thiarubrine A (Figs. 1C–E). Results of spiking experiments showed that the detection limit of the HPLC assay is ca. 0.002% thiarubrine A of fresh leaf. Small amounts of both thiarubrine A and its corresponding thiophene are detectable in roots of *A. mossambicensis* from Mahale.

Via a separate HPLC assay, we found kaurenoic acid at low concentrations in all 13 of the leaf samples of *A. mossambicensis* (Fig. 2B) but none in *A. aequinoctiale,* L. plicata, or *Hibiscus* sp (Figs. 2C–E).

The absence of detectable amount of thiarubrine A in the leaves of *A. mossambicensis*, including samples of plants utilized by chimpanzees, rules out a role for it in mediating leaf-swallowing behavior in our subjects. Likewise, the presence of kaurenoic acid in only one of the four species indicates that it is not common to all plant species swallowed.

Page *et al.* (in prep.) will present a quantitative analysis and comparison of polyynes and kaurenoic acid in leaf, stem and root samples of *A. mossambicensis* from Mahale elsewhere.

Microscopic Characteristics of Leaf Surface, a Common Physical Property for Parasite Expulsion

Figs. 3A–F show a microscopic view of the spiny characteristics of the leaf surface of five of the seven species that are swallowed whole by chimpanzees at Mahale: *A. mossambicensis, T. orientalis, A. aequinoctiale, Hibiscus* sp., *L. plicata, Ficus exasperata* Vahl, and *Melastomastrum capitatum* Vahl A. and R. Fernandes. The same *Hibiscus* sp. is also known to be swallowed by chimpanzees at Gombe, and *A. aequinoctiale* is swallowed by chimpanzees at Kibale (Wrangham, 1996).

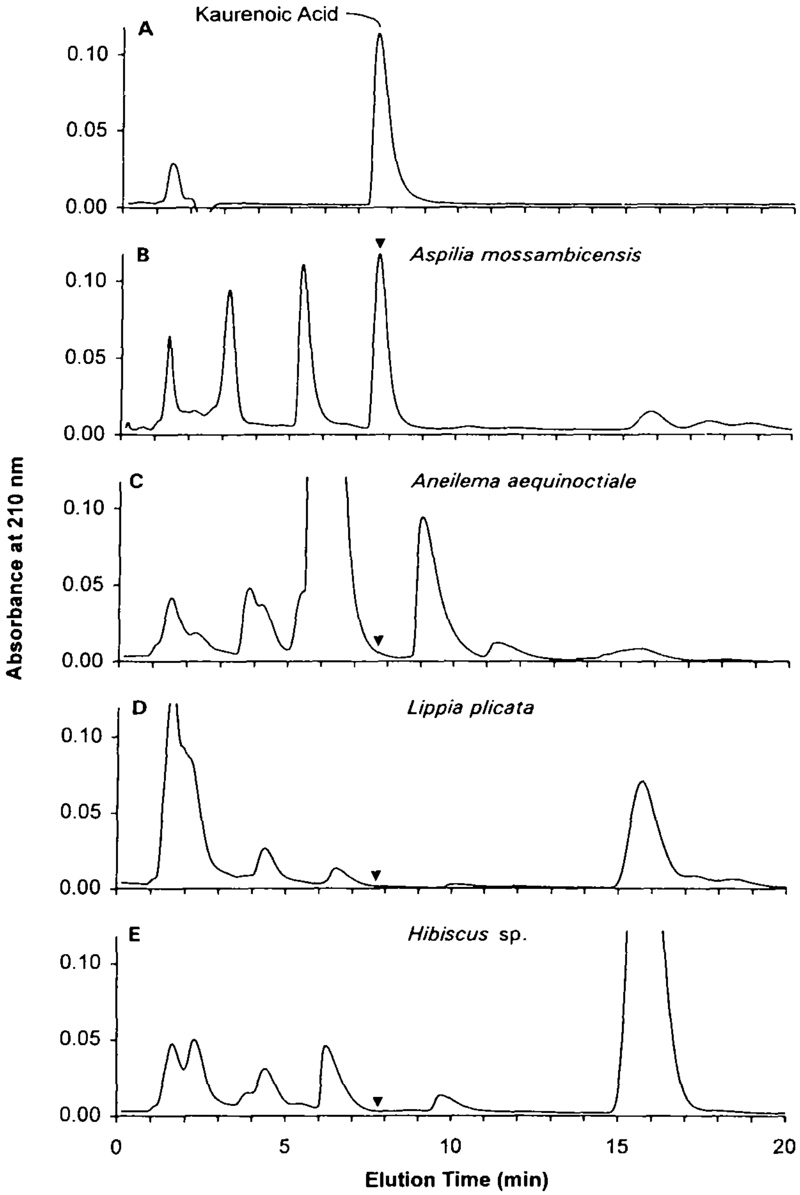

Fig. 2. Reversed-phase HPLC of methanolic extracts of leaves swallowed by chimpanzees: kaurenoic acid analysis. (A) Standard solution of kaurenoic acid (retention time, 7.7 min). Peak represents injection of 2.4 μg. (B) *A. mossambicensis*: sample collected from plant used by adult female CY; adult male BE and JI between 1005 and 1045, 7 February 1994 (see Table II). Injection volume was 20 μl. (C) *A. aequinoctiale*. (D) *Lippia plicata*. (E) *Hibiscus* sp. Arrowheads indicate the retention time of kaurenoic acid.

The hispid characteristics of these leaves take on various shapes and forms at the microscopic level, but all seven species have trichomes on their upper surfaces. The texture and abundance of trichomes are distinctively different from those of leaves chewed before being swallowed by Mahale chimpanzees. Some trichomes are long and narrow (*A. mossambicensis, M. capitatum, Hibiscus* sp., and *A. aequinoctiale*), while others are short and more spike-like (*F. exasperata, T. orientalis, L. plicata*).

We found the leaves of three species—*A. mossambicensis, T. orientalis*, and *A. aequinoctiale*—whole and folded in the dung with *O. stephanostomum* either attached to the surface and or trapped within the folds. Despite differences in trichome structure between species, the outcome was the same.

DISCUSSION

Illness and Parasitic Infection at the Time of Leaf-Swallowing

In intestinal nematode infections of any vertebrate population, most hosts tend to be infected with low numbers of parasites, which produce little pathology (Anderson and May, 1978; Crofton, 1971), but high infection levels may produce severe gastrointestinal disease characterized by malabsorption, malaise, and diarrhea (Sukhdeo and Mettrick, 1984; Georgi, 1985).

Huffman and Wrangham (1994) hypothesized that leaf-swallowing controls intestinal parasites, and Wrangham (1996) suggests that it eases pain. The former hypothesis is based on a recognized trend at Mahale for leaf-swallowing to occur most frequently during the rainy season months (Wrangham and Nishida, 1983), a period when the number of chimpanzees infected with nematodes and the number of different nematode infections per individual significantly increases (Huffman *et al.*, 1993b; Kawabata and Nishida, 1991). Recently, the finding by Wrangham (1996) of a significant relationship between the presence of whole *A. aequinoctiale* and *Rubia cordifolia* L. leaves with *Bertiella studeri* (tapeworm) proglottid fragments in the dung of chimpanzees during a 6-month period at Kibale provides further support for the association of leaf-swallowing with parasitic infection. However, he found no evidence for direct effect of the behavior in controlling these infections.

No report of leaf-swallowing has provided direct detailed evidence of illness or discomfort (Wrangham and Nishida, 1983; Wrangham and Goodall, 1989; Wrangham, 1996). Our subjects that showed symptoms consistent with severe parasitic infections, most notably that of *O.*

stephanostomum, and other symptoms of ill health and discomfort swallowed leaves. All species of *Oesophagostomum* known to infect African monkeys and apes are noted for their severe pathology resulting in abdominal pain, diarrhea, malaise, weakness, and high mortality (Brack, 1987). Interestingly, the most frequently occurring genera of parasite in Kibale and Gombe chimpanzees are *Oesophagostomum* spp. At Kibale 62% (Table II) (Rodriguez and Wrangham, 1993), and at Gombe 91%, of the samples were infected with *Oesophagostomum* spp. (File *et al.,* 1976).

O. stephanostomum is described by Brack (1987) as being perhaps the most hazardous species of the genus for great apes. These results support the hypotheses that leaf-swallowing is a response to parasitic infection and pain. The significant relationship between the presence of whole leaves (*A. mossambicensis, A. aequinoctiale, T. orientalis*) and adult worms (*O. stephanostomum*) in the dung underscores the finding that chimpanzees that swallow leaves are parasitized.

Immediate clinical signs—drop in EPG—are not apparent after single bouts of leaf-swallowing. However, the compound effect of repeated removal of *O. stephanostomum* by successive leaf-swallowing bouts may be the long-term function of this behavior. Some preliminary findings show that elevated EPG levels decline after extended periods of leaf-swallowing and tentatively support this hypothesis (Huffman *et al.,* in prep.).

As the list of plants known to be swallowed by chimpanzees and other African apes has grown to encompass at least 19 species representing 12 families and 15 genera, many with entirely different secondary chemical characteristics, the likelihood that a common chemical agent mediates leaf-swallowing has decreased. Huffman's (1993) literature search for the ethnomedicinal and pharmacological activities of these plants revealed evidence for some kind of known physiological activity in only 10 of them.

Several researchers suggested that thiarubrine A and kaurenoic acid might be important in the use of *Aspilia* spp. by chimpanzees (Newton and Nishida, 1990; Page *et al.,* 1992; Rodriguez *et al.,* 1985; Rodriguez and Wrangham, 1993). We detected thiarubrine A in no sample of *A. mossambicensis*, and neither compound was common to a subset of four species whose leaves are swallowed whole by chimpanzees of at least three sites in East Africa (Mahale, Gombe, Kibale). The hypotheses for leaf-swallowing based solely on the acquisition of thiarubrine A or kaurenoic acid for their therapeutic value do not adequately explain this phenomenon.

On the basis of our phytochemical analysis and an absence of evidence for the inhibition of egg-laying activity in any of the three nematode species in the dung (Table II), together with observations of live, motile adult worms (*O. stephanostomum*) being expelled with leaves, we reject the chemical hypothesis for leaf-swallowing as a nematocidal (sic Rodriguez and Wrangham, 1993) or mobility-inhibiting agent [Jisaka *et al.* (1992) for *V. amygdalina*].

This does not rule out some role for chemicals in the ingestion of leaves. For example, some as yet unidentified compound(s) may act as a signal(s)—bitter taste—to change the taste preferences of ill individuals (Koshimizu *et al.,* 1994) or may act as a chemical attractant to draw worms to the leaves or aid in some way in the expulsion of worms by decreasing their ability to adhere to the intestinal wall.

We cannot disregard the possibility that secondary compounds in the swallowed leaves can also have an indirect effect on these immunosuppressed individuals. Further bioactivity tests to evaluate this possibility are under way.

A New Hypothesis for the Mechanism of Whole Leaf-Swallowing

A trait common to all plant species known to be swallowed whole throughout Africa is the presence of trichomes on the leaf surface (Fig. 3). Their leaves are rough and hispid to the touch, which may be an important factor in their selection by chimpanzees. The presence of leaf trichomes on all swallowed species and the significant relationship between the presence of leaves in the dung and the expulsion of adult *O. stephanostomum*, some of which are trapped by the trichomes and folds of the swallowed leaves, leads us to propose a new hypothesis for this behavior. That is, the structures of leaves swallowed whole by chimpanzees function to trap and to expel parasites such as the strongyle nematodes (*Oesophagostomum*).

The exact details of the mechanism by which leaves remove the nematodes are unclear. However, during passage through the large intestine, the trichomes on the leaves may scour off adult worms or tightly attach to them without puncturing the cuticle. Our observations suggest that pockets created by the leaves when being folded at the time of ingestion were partly responsible for transporting worms outside the body, presumably after being dislodged. Also, a bolus of ≤55 hispid leaves with angular folds moving through the intestinal tract could contribute to the expulsion of parasites.

O. stephanostomum inhabits the large intestine, where it attaches by its buccal capsule (Fig. 3F) to the mucosa (Beaver *et al.,* 1984; Brack, 1987; Anderson, 1992). This habitat localization is predictable and constant (Sukhdeo, 1990; Sukhdeo and Sukhdeo, 1994), and the worms are always recovered from the mucosa (Anderson, 1992).

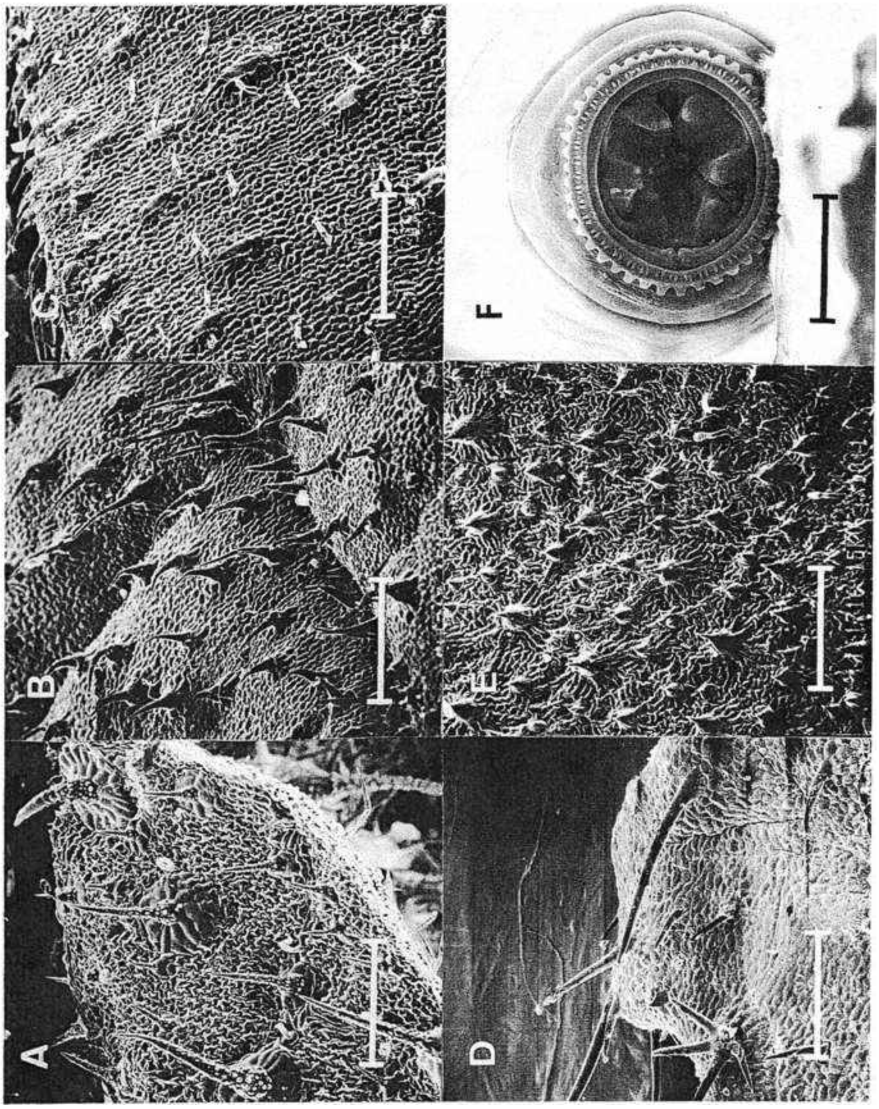

Fig. 3. Electron micrographs of the upper leaf surface of five species swallowed whole by chimpanzees. (A) *A. mossambicensis*; (B) *L. plicata*; (C) *A. aequinoctiale*; (D) *Hibiscus* sp.; (E) *T. orientalis* (scale bar = 3000 μm); the mouth part of the intestinal nematode (F) *O. stephanostomum* (scale bar = 500 μm).

The mechanical properties of the vertebrate gut are also predictable and constant within species (Diamant and Bortoff, 1969; Grundy, 1985), and within the large intestine there is considerable mixing effect that separates the ingesta by particle size (van Soest, 1982). This effect can be likened to stirring a bowl of differently sized pebbles; the large pebbles move to the periphery. Similarly, whole leaves, because of their large size, would tend to be assorted to the mucosal regions containing the worms where the probability of contact with nematodes is high. Adult worms of *S. fulleborni* and *T. trichiura* are smaller and burrow into the mucosa of the small intestine and caecum, respectively, where they embed themselves firmly (Anderson, 1992) and become less susceptible to mechanical removal by the leaves.

The folded leaves may also provide some kind of chemical attractant or a structurally attractive substrate or orifice for newly emerged *O. stephanostomum* not yet attached to the mucosa. In either case, such worms may actively move between the folds and become trapped.

Wrangham (1996) reports that whole leaves also tend to cause proglottids of *B. studeri* to be shed. While he proposed no mechanism, this is consistent with the hypothesis that physical expulsion is an important function of leaf-swallowing behavior. A mechanism similar to that which we describe is unlikely because adult tapeworms attach themselves to the mucosa in the small intestine. Furthermore, the proglottides, instead of the head and body, are found with leaves in the dung, which shows that leaf-swallowing may be performed by chimpanzees in other populations in response to different parasites and or for different reasons.

Chimpanzees at Gombe, Mahale, Kibale, and the Tai Forest, Ivory Coast, tend to swallow the leaves of many different species, with some exceptions, early in the morning within the first few hours after leaving their sleeping nests (Boesch, 1996; Wrangham and Nishida, 1983; Wrangham and Goodall, 1989; Wrangham, 1996; this study) and, thus, on a relatively empty stomach. This might enhance the physical removal of worms by limiting the amount of matter in the digestive tract. The immediate stimulus for chimpanzees to swallow leaves early after leaving the sleeping nest, however, is unknown.

Possible Stimulus for Leaf-Swallowing and Control of Infection

O. stephanostomum produces abdominal pain along with bowel irritation and diarrhea (Brack, 1987), which may provide a stimulus for leaf-swallowing and bitter-pith chewing (Huffman and Seifu, 1989; Huffman *et al.*, 1993a) by chimpanzees at Mahale infected with this strongyle nematode. In the life cycle of *O. stephanostomum*, the infective larvae penetrate the wall of the gut, develop, and molt twice to become adults. The adults leave the mucosa to migrate back to the lumen of the bowel. The larvae become encapsulated by excessive reactive inflammation (Georgi, 1985) and are responsible for the acute inflammatory reaction that leads to clinical symptoms characterized by diarrhea, abdominal pain, and in severe cases, simulated appendicitis (Beaver *et al.,* 1984). Adult worms in the lumen rarely cause pathology. Yet leaf-swallowing removes only the adult worms attached to the tissue, and not the larvae in the tissue. Why? The answer may lie in premunition—a state wherein the presence of a stable population of adults in the lumen inhibits the maturation of larvae within the tissue (Georgi, 1985). It is only when adults are removed, as occurs via drugs in humans, that the larvae will develop; no anthelmintic is known to be effective against larvae in the tissue (Beaver *et al.,* 1984).

Thus, in chimpanzees, the removal of adult worms via leaf-swallowing may in the same way stimulate the maturation of the larvae, which then migrate out of the tissue, thereby alleviating symptoms and reducing discomfort. It is also possible that newly emerging adult worms move into the folds of the leaves in search of a place to attach for reproduction. Both scenarios explains why egg counts of *O. stephanostomum*—a function of adult worm numbers—do not change before and after leaf swallowing (Table II): because the removed adults are replaced by newly matured adults migrating from the tissue or because newly emerging adults are expelled before they have a chance to start laying eggs or a combination of both factors.

Since *Oesophagostomum* infections are usually self-limiting (Beaver *et al.,* 1984), the total infection may be controllable if a chimpanzee periodically swallows leaves during the most likely period of reinfection. Reinfection occurs with the ingestion, and possibly penetration through the skin, of infective-stage larvae, which develop from eggs excreted into the environment (Anderson, 1992). According to recent data from Mahale, the peak period of reinfection of *O. stephanostomum* occurs 1 to 2 months after the onset of the rainy season and closely correlates with the seasonality of leaf-swallowing (Huffman *et al.,* in prep).

The purging of adult worms via physical entrapment or expulsion or both is a unique and hitherto unknown antiparasitic adaptation. This approach may be considered a behavioral adaptation for life in an environment in which reinfection with nematodes is inevitable.

In the case of *B. studeri*, the expulsion of the proglottids found with whole leaves in dung at Kibale is a normal part of the organism's reproductive strategy. In this respect, it seems quite different from the expulsion of whole adult worms of *O. stephanostomum*, and appears to deny the hypothesis of parasitic control. However, if leaf-swallowing causes the expulsion of immature or nongravid proglottids, this would clearly select against the worms and might suggest a beneficial effect for the host. Wrangham (1996) concluded that the shedding of proglottids by leaf-swallowing may act to relieve or to control pain but how this might work is not clear. This species apparently produces no lesion, and infections are tolerated without recognizable clinical signs (Beaver *et al.,* 1984; Brack, 1987). In this case, while evidence of discomfort was only indirectly inferred, the idea that leaf-swallowing may control and is induced by pain is supported by our findings and the mechanism that we propose for parasitic expulsion. This may help to explain the similarities between Mahale and Kibale chimpanzees despite reported differences of parasites associated with leaf-swallowing.

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