American Journal of Primatology 76:757–773 (2014)

RESEARCH ARTICLE

Chimpanzee Diet: Phytolithic Analysis of Feces

CAROLINE PHILLIPS[1]* AND CARLA LANCELOTTI[2,3]
[1]Department of Archaeology and Anthropology, University of Cambridge, Cambridge CB2 1QH, England
[2]CASEs, IMF—Spanish National Research Council (CSIC), Barcelona E-08001, Spain
[3]Department of Communication and Information Technologies, Universitat Pompeu Fabra, Barcelona 08018, Spain

Most primate populations remain unobservable; therefore, researchers depend on the analyses of indirect evidence encountered at a study-site in order to understand their behavioral ecology. Diet can be determined through the analyses of scats or feeding remains encountered on-site. This allows aspects of their dietary repertoire to be established, which has implications both for conservation efforts (by locating food resources), and for understanding the evolution of hominin diet (if used as referential models). Macroscopic inspection of fecal samples is a common method applied to ascertain a primate population's diet. However, new approaches are required to identify food-items unrecognizable at this level. We applied a dry ash extraction method to fecal samples ($N = 50$) collected from 10 adult chimpanzees in Kanyawara, Kibale National Park, Uganda and also to plant parts ($N = 66$) from 34 species known to be included in the diet of this community of apes. We recovered phytoliths in 26 of the 34 plant species. Fifteen phytolith morphotypes were only detected in 14 plant species (termed "distinct" phytoliths). We used these distinct phytoliths to identify plant foods (i.e., that they were associated with) in fecal samples. We then validated findings by checking if the 10 chimpanzees had eaten parts of these plants ~24 hr prior to fecal sample collection; six plant species associated with five distinct phytoliths had been eaten. Finally, we compared plant foods identified in fecal samples from phytolith analyses with plants that had been identified from macroscopic inspection of the same fecal samples. Findings from phytolith analyses corroborate with those from macroscopic inspection by expanding the total number of plant species identified per fecal sample (i.e., we identified certain plant parts that remained unrecognizable at macroscopic level). This study highlights the potential of phytolith analyses of feces to increase our knowledgebase of the dietary repertoire of primate populations. Am. J. Primatol. 76:757–773, 2014. © 2014 Wiley Periodicals, Inc.

Key words: ape; fecal; phytolith; plant food-items

INTRODUCTION

Ascertaining the full range of dietary constituents of a primate population allows further understanding of their feeding ecology. Furthermore, such work can assist in the identification of habitat types utilized for food resources within their home range, which may then assist conservation efforts. Moreover, increased knowledge of the omnivorous diet of our closest living relatives (*Pan troglodytes* and *P. paniscus*), who range and forage in different habitats may provide a greater understanding of hominin diet and its evolution from our last common ancestor (when used as referential models to determine both similarities and differences in plant availability and food-intake [Copeland, 2009; Hohmann, 2009]). Direct observation of feeding by primates reveals crucial aspects of their diet. However, for chimpanzees, such behavioral data are available at fewer than 25% of current study-sites, where populations tolerate human observers at close range (i.e., they are habituated [McGrew, 2007]).

It is likely that most primate populations will remain unobservable and there are good reasons for this. First, a long-term commitment by researchers is required to accomplish habituation, due to the subjects' initial wariness. Indeed, full habituation may take years to achieve [Bertolani & Boesch,

Contract grant sponsor: Murray Edwards College of Cambridge; contract grant sponsor: Board of Graduate Studies of University of Cambridge; contract grant sponsor: Ridgeway-Venn Travel Fund; contract grant sponsor: Sir Richard Stapely Education Trust; contract grant sponsor: Leverhulme Trust

*Correspondence to: Evolutionary Studies Institute, University of Witwatersrand, Wits 2050, South Africa.
E-mail: caroline.phillips@wits.ac.za

Received 5 August 2013; revised 28 January 2014; revision accepted 30 January 2014

DOI: 10.1002/ajp.22267
Published online 23 February 2014 in Wiley Online Library (wileyonlinelibrary.com).

© 2014 Wiley Periodicals, Inc.

2008; Doran-Sheehy et al., 2007]. Also, some researchers choose not to habituate chimpanzees in unprotected areas to maintain their caution towards human hunters and farmers [Deblauwe & Janssens, 2008]. Furthermore, there is a rising concern of the risk of fatal disease transfer from human observer to subject [Köndgen et al., 2008; Woodford et al., 2002].

For unhabituated populations, dietary information can be obtained from recovered food remains and macroscopic inspection of fecal samples. The latter is commonly used [Doran et al., 2002; Julliot & Sabatier, 1993; Tutin & Fernandez, 1993] and entails weighing and sluicing samples through fine-meshed sieves to remove fecal matrix [McGrew et al., 2009; Moreno-Black, 1979]. This process leaves residual food components visible to the naked eye, which then can be separated, identified, and counted [McGrew et al., 1988]. However, food-items that have been thoroughly masticated or digested, (e.g., leaves and "soft-bodied" insects) may be unrecognizable at this level of inspection [Phillips & McGrew, 2013] compared to indigestible food-items (e.g., fruit seeds and chitinous exoskeleton). Consequently, if researchers apply this method alone to fecal samples, the full dietary repertoire of species ingested by a primate population may remain unknown.

Phytoliths are opal silica microfossils, or "plant stones" (*phyto* meaning plant and *lith* meaning stone [Baker, 1959]). They are formed when soluble silica enters the plant during water, nutrient and other mineral uptake and is then deposited within cell lumina, in the cells' wall or in between cells. With evapotranspiration, the deposited silica solidifies producing silica plant-cell-structure formations, which can be distinctive due to their size and morphology [Piperno, 2006]. These formations are used to identify plants, where possible, to family or genus level [Piperno, 1988; Rovner, 1971].

As a plant decomposes, phytoliths are normally deposited locally into sediments. Deposited phytoliths can dissolve [Cabanes et al., 2011], which has been shown to maintain equilibrium in concentrations of silica in soils [Farmer et al., 2005], but not all phytoliths undergo dissolution. As a consequence, preserved phytoliths that have remained predominantly undisturbed [Cabanes et al., 2011; Runge & Fimbel, 2001] have been found in excavated sediments worldwide. This, along with the ability to diagnose phytoliths to plant type (e.g., grass), and potentially to taxon makes phytoliths a powerful tool to reconstruct paleoenvironments, in particular to investigate landscape use by human ancestors [Barboni et al., 2010; Madella et al., 2002; Wang et al., 2003] and modern humans [Jiang, 1995; Pearsall, 1994; Piperno, 1994].

By surviving both mastication and digestion, phytoliths have also been used to study diet. Phytoliths successfully extracted from dental calculus [Henry et al., 2010; Lalueza & Pérez-Pérez, 1994], including calculus from the extinct primate *Gigantopithecus blacki* [Ciochon et al., 1990], coprolites [Horrocks & Irwin, 2003; Prasad et al., 2005], and more recently, from fresh feces [Lancelotti, 2010; Lancelotti & Madella, 2012] have all provided valuable insights into dietary intake of various extinct and extant species. A preliminary study of phytoliths detected in mantled howler monkey (*Alouatta palliate*) feces is provided by Teaford and Glander [1996], and the potential of analyzing plant microfossils (including phytoliths) in feces of extant primates to infer diet is discussed further in Henry [2012].

The objective of this study was to investigate phytolith content of feces from 10 adult chimpanzees of the Kanyawara community in Kibale National Park, Uganda to assess what can be gleaned about the diet of a wild ape population using these proxies. We applied a dry ash extraction method [Lancelotti, 2010; Parr et al., 2001] to fecal samples collected from the 10 focal individuals. Plant samples collected in the home range of this ape community were also analyzed using this method. Firstly, we sought to establish if phytolith production occurs in the separate plant parts, and if so, if these parts could then be differentiated from each other by their phytolith content and morphology. We then compared phytoliths from fecal samples collected from the 10 focal individuals to the reference library created to investigate diet. Finally, we review the potential of this technique to corroborate with findings from alternative fecal analysis, in this case macroscopic inspection. A particular aim was to detect those parts of plant species seen to be eaten by the 10 focal individuals (e.g., leaves or piths) that had not been identified at macroscopic level in the same fecal samples [Phillips & McGrew, 2013].

METHODS

Study-Site

Kanyawara is located in the northwestern part of Kibale National Park (0°13′–0°41′N, 30°19′–30°32′E) in Uganda, at about 1,500 m elevation [Wrangham et al., 1994]. This mature, mid-altitude semi-deciduous and evergreen ecotype has swamp, primary, and regenerating forest that was logged pre-1992 [O'Driscoll Worman & Chapman, 2004]. Dominant trees in unlogged areas are *Parinari excelsa*, *Celtis gomphophylla*, and *Markhamia lutea* [Sekercioglu, 2002], while the woody shrub *Acanthus polystachius* dominates previously logged areas [Osborne et al., 2001]. Dominant grasses within the national park include *Pennisetum purpureum*, *Hyparrhenia* spp., and *Cymbopogon nardus* [Zanne & Chapman, 2005]. There are distinct wet and dry seasons: May–August

and December–February are drier than other months [Chapman et al., 2001]. We collected data over 162 days between June and December 2008 (excluding August). Thus, data covered two of the four months of one dry season (D_1), all of one wet season (W) (September–November) and 1 of 4 months of a second dry season (D_2). During the study period, mean monthly rainfall during the dry seasons combined was $52.7 \pm$ SE 6.6 mm and in the wet season was $212.1 \pm$ SE 65.9 mm. Mean maximum and minimum monthly temperatures in the dry and wet seasons were $27.9 \pm$ SE 0.4°C (range: 18.3–38.0°C; $N = 78$ days) and $14.1 \pm$ SE 0.1°C (range: 0.0–17.0°C); and $28.3 \pm$ SE 0.5°C (range: 14.1–43.3°C; $N = 84$ days) and $14.1 \pm$ SE 0.1°C (range: 11.9–17.5°C) (C. Chapman, personal communication).

Data Collection

Focal individuals

The Kanyawara community numbered *ca*. 50 individuals at the time of the study. We followed 10 fully-habituated adult chimpanzees (five males and five females) individually from the time that they arose out of their arboreal bed in the morning until they made another arboreal bed in the evening (one focal sample, Martin & Bateson, 2007). Each focal-sample ($N = 19$) lasted up to three consecutive days, where we followed nine individuals twice (for test and re-test purposes). The research adhered to guidelines as set down by the Division of Biological Anthropology, University of Cambridge, and the American Society of Primatologists' principles for the ethical treatment of non human primates. The Uganda Wildlife Authority and Uganda National Council for Science and Technology permitted data collection on the Kanyawara chimpanzee community in Kibale National Park.

Sample Collection

We collected 34 plant species (13 herbaceous dicotyledons, 18 woody dicotyledons and three herbaceous monocotyledons) representing 25 families to create a reference library. We identified plant specimens with the aid of permanent staff of the Kibale Chimpanzee Project. All species have been observed to be eaten by members of the Kanyawara chimpanzee community within the last 21 years. We collected plant parts seen to be eaten as well as parts not eaten by the Kanyawara community to compare their phytolith productivity. We saw the 10 focal individuals eat parts of 30 of the 34 plant species collected. Leaves, fruit and pith of 12 of these 30 plant species had not been identified during macroscopic inspection of fecal samples collected from the same 10 focal individuals [Phillips & McGrew, 2013]. We sun-dried all collected plant matter, separated them into plastic bags, and stored them in airtight containers with silica gel.

We collected most fecal samples ~24 hr after the first encounter (78% of samples) with each focal individual. This allowed time for digestion and the passing of food items we saw the focal individuals eat, which would then appear in subsequent fecal samples. We collected all samples in 50 ml centrifuge tubes and desiccated them with silica gel (to make sample processing off-site easier), however, as phytoliths survive plant decomposition, mastication, and digestion [Piperno, 2006], degradation of feces is unlikely to affect phytolithic analysis.

Sample Preparation and Extraction

We separated collected plant parts ($N = 66$) accordingly. There were: 34 leaves; 20 fruits (whole); 10 pith or stem sections, and 2 blossoms. We prepared samples and extracted phytoliths from ashed plant and fecal samples using Lancelotti's [2010] dry ash extraction method (Fig. 1). We did not use hydrogen peroxide during the extraction process as most organic matter was burnt away when we ashed samples at 550°C. Furthermore, this chemical may be very aggressive on phytoliths where organic matter is not abundant, which in turn can affect the total number of identifiable phytoliths [Cabanes et al., 2011].

We observed extracted phytoliths from both plant and fecal samples under a transmitted light microscope at 400×. We counted phytoliths along seven random vertical transects per slide, resulting in a total of 371 view fields (×53 view fields per transect). This count was based on Van der Veen and Fieller's [1982] minimum count of 350 for valid sample material representation per slide. Using a set number of view fields per slide provided a general overview of the relative abundance of phytoliths per plant part analyzed.

In each sample we only counted phytoliths judged to be complete (i.e., not broken), meaning that the shape, margin, texture and where possible, the location within the plant part could be identified (e.g., epidermal layer). We counted phytoliths within silica skeletons (phytoliths of plant cells that are still articulated) individually, as complete silica skeletons are less frequently found in sediments due to their breaking up post-plant decomposition [Tsartsidou et al., 2007]. We expected that after mastication and digestion, fewer complete silica skeletons would be present in fresh fecal samples compared to those found in the plant reference samples.

We determined phytolith concentrations using Albert and Weiner's [2001] calculation, which provides a total for the number of phytoliths in 1 g of the acid insoluble fraction (AIF). This fraction denotes the remaining dried matter that was not dissolved by acids during the extraction process. Listing the total number of phytoliths per gram of AIF (phytoliths/gAIF) for each plant part allowed intra- and inter-species comparison (Table I).

Preparation of desiccated plant parts

1) Submerge desiccated plant parts and place into an ultra-sonic bath for two 15-min bouts to clean them. Drain and replace distilled water in between bouts. Remove each plant part from their glass beaker and oven-dry overnight.
2) Place each dried sample into pre-weighed 50ml centrifuge tubes. Reweigh each tube to determine the weight of dry plant matter (g).
3) Deposit each plant sample into 25ml crucibles. Cover with a crucible lid, (leaving a small gap for air circulation), and place into a Carbolite muffle furnace at 550ºC for 4 to 15 hr, until each sample has burnt predominantly to a white ash.
4) Cool sample and place ash back into their original 50ml centrifuge tube. Reweigh to determine the weight of ash (g).

Preparation of feces

1) Place ~50g of feces onto a pre-weighed glass petri dish. Oven dry at 60 ºC until remaining moisture has been removed. Deposit dried samples into 25ml crucibles, and follow ashing procedure as for plant samples above.

Dry ash extraction method for plant and fecal samples

a) Add up to 10ml of hydrochloric acid (10% HCl) to each 50ml centrifuge tube to dissolve all carbonates in the sample. If necessary, continue to add a few more drops of the hydrochloric acid until the reaction stops.
b) Add distilled water to each tube up to the 50ml mark and centrifuge for 5 mins at 2000 rotations per minute (rpm) in order to 'wash' and rinse the remaining matter. Immediately afterwards, pour away most of the supernatant in one continuous motion, ensuring all visible matter in remaining liquid is retained. Repeat this twice.
c) Swirl remaining liquid and matter in each tube and then immediately pour each into a pre-weighed (g) glass vial (10ml). Oven-dry each liquid-filled vial until all liquid has evaporated.
d) Reweigh each vial in order to determine the weight (g) of the dry matter containing phytoliths.

Slide preparation for plant and fecal samples

1) Mount approximately 5mg of dried matter onto a pre-weighed (g) glass slide (25 x 55mm) per sub-sample. Reweigh each slide in order to determine the weight (mg) of mounted dry matter.
2) Add 5 drops of Entellan® (solution used to disperse and set sample on the slide). Place a glass cover-slip over the top. If necessary, add more drops of Entellan® along edges of the glass cover-slip to remove air-bubbles. Leave slide(s) to dry for four days in a fume cupboard prior to use. Entellan® does not allow rotation of phytoliths during microscopy. See Piperno [2006] for alternative mounting medium.

Fig. 1. Protocol for phytolith extraction from plant and fecal samples.

TABLE I. List of Plant Species Analyzed and Total Number of Phytoliths Counted Per Slide and Per Gram of Acid Insoluble Infraction in Each Plant Part (N = 66)

Species, Plant authority	Family	Form	Group	Part	Eat	Slide count	Phy/gAIF (10^6)
Acalypha ornata Hochst. ex A.Rich.	Euphorbiaceae	THV	D	**Leaf**	Y	38	7.5
Acanthus polystachius Delile	Acanthaceae	THV	D	Leaf	N	14	2.8
***Aframomum* spp.**	Zingiberaceae	THV	M	**Leaf**	Y	1,584	156.5
				Stem	Y	0	—
Aneilema aequinoctiale (P.Beauv.) Loudon	Commelinaceae	THV	M	Leaf	Y	74	7.3
				Stem	N	0	—
Cordia africana Lam.	Boraginaceae	TRE	D	Leaf	N	859	169.8
				Fruit	Y	103	6.8
				Stem	N	322	63.6
Crassocephalum vitellinum (Benth.) S.Moore	Compositae	THV	D	Leaf	Y	186	3.3
				Blossom	N	0	—
Chaetacme aristata Planch.	Ulmaceae	THV	D	**Leaf**	Y	1,163	76.6
				Fruit	N	50	1.6
Dovyalis macrocarpa Bamps	Flacourtiaceae	SHB	D	Leaf	N	0	—
				Fruit	Y	0	—
Ficus asperifolia Miq.	Moraceae	SHB	D	Leaf	Y	2,040	201.6
				Fruit	Y	24	2.4
Ficus exasperata Vahl	Moraceae	TRE	D	Leaf	Y	2,251	444.9
				Fruit	Y	132	4.3
Ficus ottoniifolia subsp. *lucanda* (Ficalho) C.C.Berg	Moraceae	TRE	D	Leaf	N	2,164	85.5
				Fruit	Y	178	7.0
Ficus sansibarica subsp. *macrosperma* (Warb. ex Mildbr. & Burret) C.C.Berg	Moraceae	TRE	D	Leaf	N	229	2.8
				Fruit	Y	196	38.7
Ficus sur Forssk.	Moraceae	TRE	D	Leaf	N	288	28.4
				Fruit	Y	24	<0.5
Hoslundia opposita Vahl	Lamiaceae	THV	D	Leaf	N	0	—
				Fruit	Y	0	—
***Jasminum* spp.**	Oleaceae	CLI	D	**Leaf**	Y	23	4.6
				Stem	N	0	—
Lepistemon owariense (P. Beauv.) Hallier f.	Convolvulaceae	THV	D	Leaf	Y	0	—
Maesa lanceolata Forssk.	Primulaceae	THV	D	**Leaf**	Y	232	45.9
				Fruit	Y	53	3.5
Marantochloa leucantha (K.Schum.) Milne-Redh.	Marantaceae	THV	M	**Leaf**	Y	1,868	123.1
				Fruit	Y	3,466	685.1
				Pith	Y	5,894	233.0
Mimusops bagshawei S.Moore	Sapotaceae	TRE	D	Leaf	N	5	<0.5
				Fruit	Y	0	—
				Twig	N	0	—
Monodora myristica (Gaertn.) Dunal	Annonaceae	TRE	D	Leaf	N	461	22.8
				Fruit	Y	0	–
				Twig	N	0	—
Neoboutonia macrocalyx Pax	Euphorbiaceae	TRE	D	Leaf	N	1,264	41.6
Piper capense L.f.	Piperaceae	THV	D	Leaf	N	244	9.6
				Stem	Y	0	—
Phytolacca dodecandra L'Hér.	Phytolaccaceae	THV	D	Leaf	N	0	—
				Fruit	Y	0	—
				Stem	N	0	—
Pseudospondias microcarpa (A.Rich.) Engl.	Anacardiaceae	TRE	D	**Leaf**	Y	843	166.6
				Twig	N	0	—
Psychotria mahonii C.H. Wright	Rubiaceae	TRE	D	Leaf	N	0	—
				Fruit	Y	0	—
Rubus apetalus Poir	Rosaceae	THV	D	Leaf	N	0	—
				Fruit	Y	0	—
Secamone africana (Oliv.) Bullock	Apocynaceae	CLI	D	Leaf	N	739	48.7

TABLE I. (*Continued*)

Species, Plant authority	Family	Form	Group	Part	Eat	Slide count	Phy/gAIF (10^6)
				Fruit	Y	0	—
Tabernaemontana pachysiphon Stapf	Apocynaceae	TRE	D	Leaf	N	0	—
				Fruit	Y	0	—
Tarenna pavettoides (Harv.) Sim	Rubiaceae	SHB	D	Leaf	N	0	—
				Fruit	Y	0	—
Toddalia asiatica (L.) Lam.	Rutaceae	SHB	D	Leaf	N	600	59.3
				Fruit	Y	89	17.6
Trichilia splendida A.Chev.	Meliaceae	TRE	D	**Leaf**	Y	288	56.9
Triumfetta tomentosa Bojer ex Bouton	Malvaceae	THV	D	**Leaf**	Y	113	5.6
Urtica massaica Mildbr.	Urticaceae	THV	D	Leaf	N	447	88.4
				Blos	Y	239	47.2
Uvariopsis congensis Robyns & Ghesq.	Annonaceae	TRE	D	Leaf	N	17,798	502.6

Plant form: terrestrial herbaceous vegetation [THV]; tree [TRE]; shrub [SHB]; and climber [CLB]. Plant group: dicotyledon [D], monocotyledon [M]. Part eaten by chimpanzee: yes [Y], no [N]. Total number of phytoliths counted per slide, and phytolith concentration—total number of phytoliths found per gram of acid insoluble fraction (Phy/gAIF) for each plant part analyzed. Plant species ($N = 10$) and part ($N = 11$) in bold were not identified during macroscopic inspection of fecal samples collected from the 10 focal chimpanzees.

We compared findings from phytolith analyses of 20 samples with sub-samples from the same faecal sample that had been macroscopically analyzed. Such a comparison would determine if our micro-remains composition corroborated with the macroscopic inspection data by increasing the total number of plant species identified per fecal sample. For cross-validation of phytolith findings in fecal samples with food-intake of each focal individual, we compared plant species that we had identified from phytolithic analysis in fecal samples collected from the focal individual in question against the plant species that we had directly seen them eat during their focal sample. We provide a total percentage of positive validation-checks (i.e., plant species in feces had been eaten by the focal individual at least 22 hr prior to defecation).

Statistical Analyses

We performed canonical correspondence analysis (CCA; linear covariance matrix) on JMP version 10 to reduce dimensions and provide a smaller set of derived variables, and to see if phytoliths detected in particular plant parts could discriminate plant type as opposed to redundant phytolith morphotypes we encountered across multiple plant parts. The first CCA explored if the phytoliths commonly encountered across multiple plant species in our reference library (redundant morphotypes) could be assigned to a particular plant type: herbaceous monocotyledon (HM); herbaceous dicotyledon (HD); and woody dicotyledon (WD). A second CCA explored if less common phytolith morphotypes (found only in selected plant species analyzed) assigned to a particular plant type; and the third CCA combined redundant and less common phytolith morphotypes encountered across the reference library to ascertain how they all assigned to the three plant types. We used weighted averages of phytolith/gAIF of each phytolith type detected in each plant part analyzed for CCA [Ter Braak, 1987]. We did not include phytolith morphotypes ($N = 11$) that were only encountered in parts of a single plant species to minimize zero loading.

We used a Wilcoxon's signed-rank test (one-tailed) to compare findings of phytolith totals in silica skeletons between plant and fecal samples to determine if mastication and digestion affect the level of phytoliths articulation (i.e., presence of silica skeletons). As sample size differed across the three seasonal periods, we used a Kruskall–Wallis test (two-tailed) to determine if change in total number of phytoliths per gram of AIF extracted from the fecal samples occurred which may reflect seasonal change in diet. Statistical analyses were completed on MINITAB Release 14 where alpha was set at 0.05.

RESULTS

Plant Reference Samples

We observed phytoliths in 26 plant species (76%), and 39 plant parts (25 leaves; 10 fruit; 2 stems, 1 pith; and 1 blossom) from 19 families. The 10 focal individuals had been observed to eat parts from 19 (73%) of the 26 plant species in which phytoliths were present. Table I lists these plant species. Ten species and their parts that had phytoliths which we saw the 10 focal individuals eat, but which were not identified during macroscopic inspection of their scats, are highlighted in bold. The remaining eight plant species in which we did not recover any phytoliths were all dicotyledonous (Table I) and

represented 16 of the 66 plant parts analyzed. We observed the 10 focal individuals eat parts of all of these taxa.

We detected phytoliths in the leaves of all three monocotyledons, and for *Marantochloa leucantha*, the fruit had more than double the abundance of phytoliths (measured in phytolith/gAIF) than the leaf and pith combined. We saw the 10 focal individuals eat the leaves of all three species, as well as the pith and fruit of *M. leucantha* and *Aframomum* spp.

Of the herbaceous dicotyledonous species analyzed, we detected phytoliths in all leaf parts (Table I). Abundance of phytoliths was highest for *Chaetacme aristata* and *Urtica massaica* leaves, although for the latter, the focal individuals ate only the blossom.

For all woody dicotyledonous species in which phytoliths were found in multiple plant parts, abundance of phytoliths was higher in leaves than other parts, with the exception of *Ficus sansibarica macrosperma*, in which the phytolith count/gAIF was approximately 14 times greater in its fruit than leaves. Chimpanzees ate leaves as well as fruit of both *F. exasperata* and *F. asperifolia*. Leaves of both species had a substantially higher abundance of phytoliths compared to fruits, with *F. exasperata* having a phytolith concentration nearly 100 times higher and *F. asperifolia* about 84 times higher. No species had phytoliths only in the fruit parts, however, this is common finding in other species in which multiple plant parts have been analyzed [Piperno, 1988].

Phytolith Morphotypes

We detected 32 phytolith morphotypes in the 26 plant species analyzed; their occurrence across our reference library is shown in Figure 2. The nomenclature classifications used for phytolith identification are from Madella et al. [2005], Mercader et al. [2009], Piperno [1985, 1988, 2006], and Runge [1999].

Eight phytolith morphotypes occurred frequently across plant parts of trees, shrubs, terrestrial herbs (or "THV", Malenky et al., 1994) and climbers in our reference library (SI, Supplementary Material). These redundant phytoliths were: two epidermal phytoliths (polyhedral and jigsaw of varied textures); stellate (star-shaped) hair-bases; psilate (smooth) non-segmented hairs; and stomatae found in tissue of upper and lower epidermal layers of leaves and fruit skins [Mercader et al., 2009; Piperno, 1988, 2006]; mesophyll phytoliths from tissue located below the epidermis; and tracheids and vascular cells, both found in the vascular tissue of xylem tracheary elements [Piperno, 2006]. Redundant morphotypes made up >60% of the total assemblage of phytoliths counted in our plant reference library and are shown in Figure 3a–f.

Redundant phytoliths accounted for $80.9 \pm$ SE 6.1% of total number of phytolith morphotypes identified for each herbaceous dicotyledon species. We detected fewer redundant phytoliths in the three herbaceous monocotyledon THV species; <30% of phytoliths in *Aframomum* spp. and *Marantochloa leucantha* parts were redundant. Polyhedral epidermal phytoliths, stomatae and non-segmented, psilate hairs however, were the only phytolith morphotypes detected in *Aneilema aequinoctiale* leaves. Redundant phytoliths accounted for $94.3 \pm$ SE 3.3% of total number of phytolith morphotypes identified in each of the 14 woody dicotyledon species. For all fruit (except *Ficus asperifolia*), and leaves of each tree species (except *Ficus* spp. and *Cordia africana* leaf and stem), polyhedral epidermal phytoliths, mesophyll phytoliths, tracheids and vascular cells were the only phytolith morphotypes we identified.

We found 15 other phytolith morphotypes in parts of 14 of the 26 plant species (Figure 3g–s, SII, Supplementary Material). These phytolith morphotypes are not univocally produced by these species and can occur in other species of their genus and across the multiple families represented [Barboni et al., 2007; Piperno, 1988; Runge & Fimbel, 2001]. However, as one of our aims was to assess whether the phytolith content of fecal samples reflected the diet of the 10 focal individuals, we discuss these morphotypes in the context of our plant reference collection. They are referred to below as "distinct" phytoliths for this study (i.e., they are not unique, but they are distinguishable from other phytolith morphotypes recovered within the plant reference library).

Forty-seven per cent of these distinct phytoliths occurred in plant parts of THV species that we saw the 10 focal individuals eat. We commonly found a biconical convex (termed "hourglass-shaped" below), segmented hair that had a striated texture ("*elongated elements in a parallel line*" Madella et al., 2005) in the leaves of *Maesa lanceolata*. We found four types of globular-shaped phytoliths of differing textures (nomenclature of each in SII, Supplementary Material) in the leaves of various terrestrial herbs (both monocotyledon and dicotyledon) and in the leaf of *Cordia africana*. We also detected them in the pith and fruit of *M. leucantha*. Hat-shaped phytoliths with a sinuated ("*alternating but uneven concavities and convexities*" Madella et al., 2005) margin and verrucate ("*irregularly shaped, wart-like*" Madella et al., 2005) texture were both common in the leaves of *Aframomum* spp. and all parts of *M. leucantha*. Hat-shaped phytoliths have been detected in various families including genera of the Zingiberaceae and Marantaceae [Chen & Smith, 2013; de Albuquerque et al., 2013; Piperno, 1985, 1988; Runge, 1999]. Elongate cystoliths (outgrowths of cell walls in epidermal layers) occurred in the leaf of *M. leucantha* and in the leaf and blossom of *U. massaica*. These morphotypes have been observed in other species of

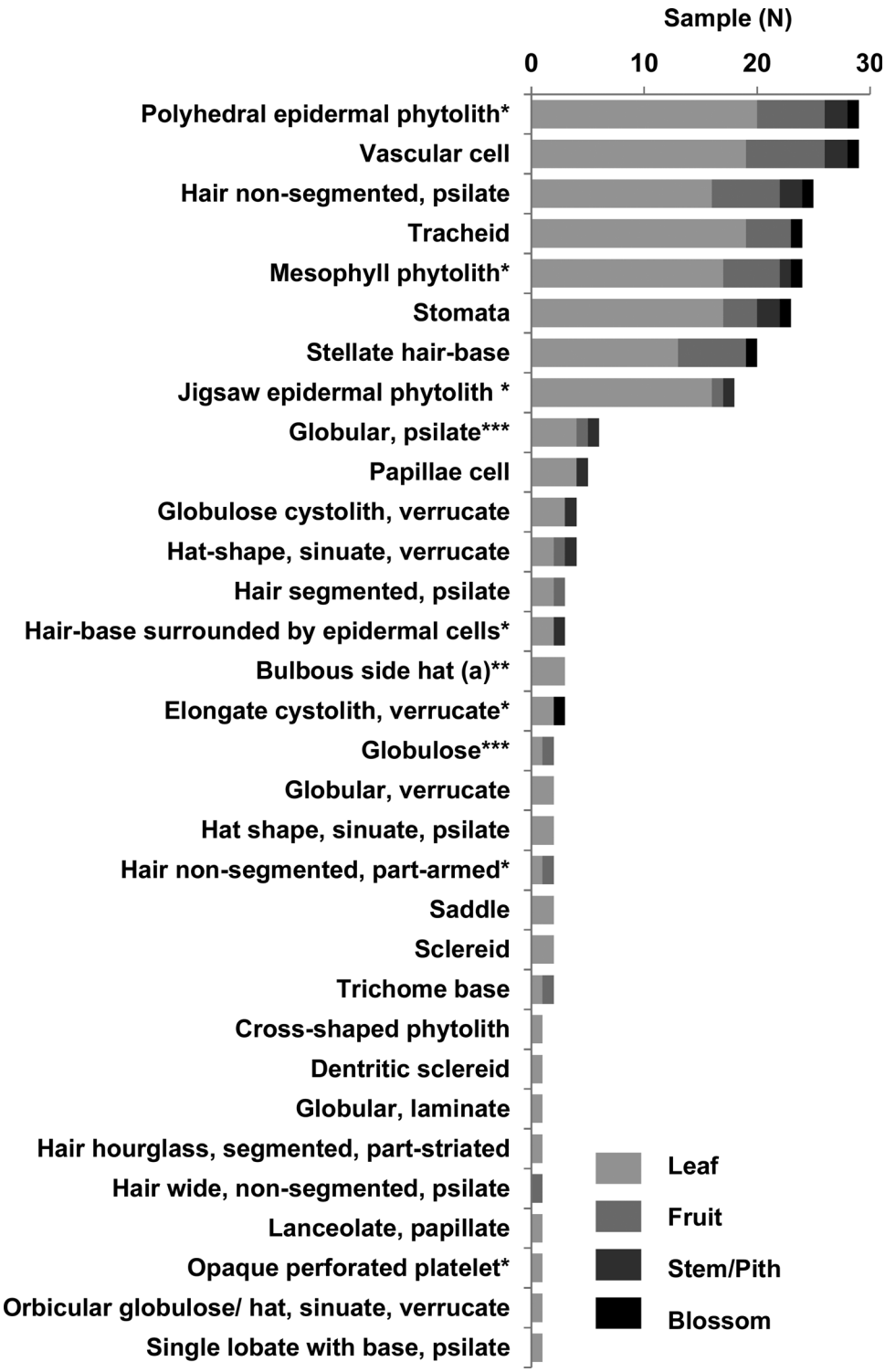

Fig. 2. Total number of samples and plant parts (leaf, fruit, stem/pith, blossom) in which each phytolith morphotype ($N = 32$) was observed (e.g., polyhedral epidermal phytoliths were observed in 20 leaves, 6 fruits, 2 stem/piths and 1 blossom in the 26 plant reference samples analyzed). Phytolith morphotype, listed by its shape, margin (edges of phytolith), and texture. Nomenclature used from: Madella et al. [2005]; Mercader et al. [2009] (*); Piperno [1988, 2006] (**); Runge [1999] (***).

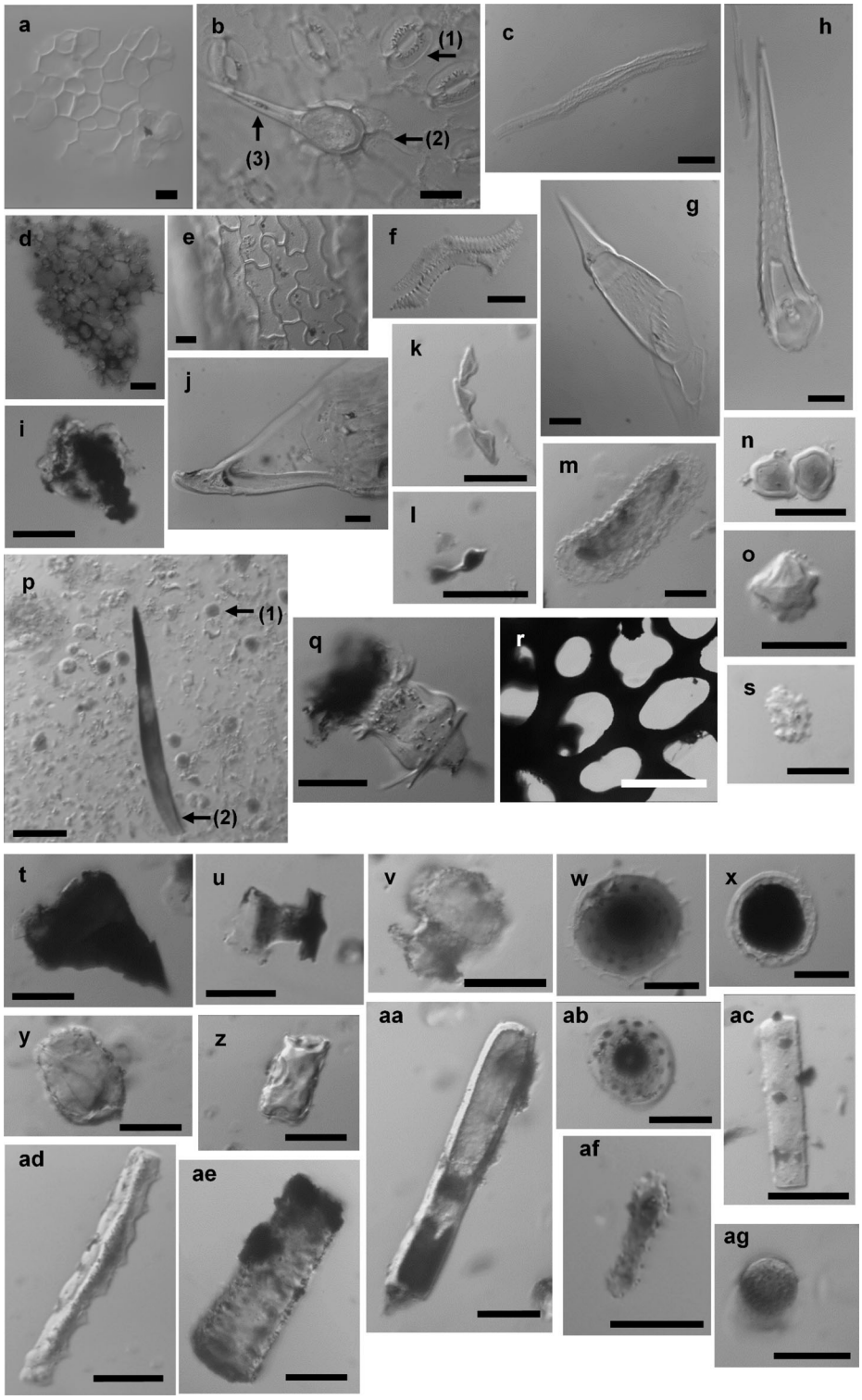

Fig. 3. Redundant and distinct phytolith morphotypes in plant and fecal samples (Scale 20 μm). Redundant phytoliths: (**a**) polyhedral epidermal phytoliths in leaf of *Toddalia asiatica*; (**b**) in fruit of *Ficus exasperata* (1) stomatae (2) stellate hair-base (3) psilate non-segmented hair; (**c**) vascular cell in the leaf of *Crassocephalum vitellinum*; (**d**) mesophyll phytoliths in fruit of *Toddalia asiatica*; (**e**) jigsaw epidermal phytoliths in leaf of *Crassocephalum vitellinum*; (**f**) tracheid in leaf of *Pseudospondias microcarpa*. Distinct phytoliths: (**g**) segmented hourglass hair with striate patterning in leaf of *Maesa lanceolata*; (**h**) part-armed, non segmented hair in leaf of *Ficus asperifolia*; (**i**) lanceolate phytolith with a papillate texture in leaf of *Acanthus polystachius*; (**j**) non-segmented (wide) psilate hair in leaf of *Ficus exasperata*; (**k**) hat-shape, sinuate verrucate sideways view in pith of *Marantochloa leucantha*; (**l**) hat-shape, sinuate psilate sideways view in leaf of *Piper capense*; (**m**) elongate cystolith in blossom of *Urtica massaica*; (**n**) globulose in leaf of *Marantochloa leucantha*; (**o**) orbicular globulose/hat-shaped phytolith, with sinuate margin and *verrucate* texture in the leaf of *Marantochloa leucantha*; (**p**) in leaf of *Aframomum* spp. (1) globular, with a psilate texture (2) psilate, non-segmented hair; (**q**) bulbous shaped side-hat type (a) phytolith in leaf of *Ficus sur*; (**r**) opaque perforated platelet in leaf of *Acanthus polystachius*; (**s**) elongate cystolith in fruit of *Marantochloa leucantha*; Phytoliths in fecal samples not found in plant reference samples: (**t**) bulbous lanceolate hat; (**u**) bulbous side hat type (b); (**v**) bulbous side hat type (b); (**w**) armed hair-base; (**x**) lacunose, hair-base; (**y**) ovate blocky; (**z**) rectangular, elongate blocky; (**aa**) cylindroid with ridge; (**ab**) armed hair-base; (**ac**) elongate tenius lacunose; (**ad**) elongate, echinate with central ridge; (**ae**) rectangular, elongate blocky; (**af**) clavate granulate; (**ag**) globular granulate.

the Urticaceae family [Piperno, 1988, 2006]. We detected an orbicular globular/hat-shaped phytolith with a sinuated margin and verrucate texture only in the leaf of *M. leucantha*. Although we recovered psilate, non-segmented hairs in 68% of the 26 plant species, in *F. exasperata* fruit they were particularly wide (25–140 μm). Also, we commonly detected a non-segmented hair, which was part-armed ("*having a granular surface, composed of fine knobs or knots*" Madella et al., 2005) from the base to half-way up the hair only in the leaves and fruit of *F. asperifolia*,. A bulbous-shaped side-hat was associated with only the leaves *F. sansibarica macrosperma, F. sur* and *F. exasperata*. Similarly, we only observed a single-lobate phytolith with base in the leaf of *Jasminum* spp. Leaves of two species of plants that we did not see the focal individuals eat presented distinct phytoliths: an elongated, lanceolate-shaped (triangular) phytolith with papillate (*has protuberances which are rounded or pointed*) texture as well as opaque perforated platelets which had a "Swiss cheese-like" appearance in the leaf of *Acanthus polystachius*; and psilate, hat-shaped phytoliths with sinuated margin in *Piper capense* leaves. We also found the latter were in the leaf of *M. leucantha*.

In the first CCA performed, the first canonical component (CC1) accounted for 91.1% of variance; the second (CC2), 8.9% (SIII, Supplementary Material). For redundant phytoliths, the three plant groups (herbaceous monocotyledon (HM), herbaceous dicotyledon (HD) and woody dicotyledon (WD)) overlapped, but tracheid phytoliths separated WD from HD plant type along CC1 (standardized score coefficients SIV, Supplementary Material). Plant types were not clearly separated along CC2; however, coefficients reveal a positive loading of redundant phytoliths toward WD. In the second CCA performed using non-redundant phytoliths, CC1 accounted for 89.7% of variance where HM was separated from WD and HD. Along CC2 which accounted for 10.3% of variation; saddles and segmented hairs with a psilate texture were more negatively loaded by HM plant type. With the inclusion of both redundant and non-redundant phytoliths in the third CCA performed, the first CC accounted for 75.9% of variance; CC2 for 24.1%. The inclusion of redundant phytoliths did not modify plant type separation; as with the second CCA, HM was separated from HD and WD plant types. Saddles and segmented hairs with a psilate texture were again negatively loaded by HM plant type. Misclassification of plant species into plant type was highest for redundant phytoliths in the first CCA performed (SIII, Supplementary Material).

Fecal samples

We analyzed fecal samples "blindly" (i.e., diet was assessed from phytoliths observed in fecal samples prior to cross-validating with data on directly observed food-intake by the 10 focal individuals). In order to test if mastication and digestion affect the silica skeletons present in ingested plant parts (i.e., mechanical taphonomy—Madella & Lancelotti, 2012), we compared the total number of polyhedral epidermal phytoliths present per silica skeleton in plant parts analyzed with those counted in each silica skeleton present in fecal samples. We chose this morphotype as it was one of the most frequent in plant parts analyzed, and was most often observed in silica skeletons (i.e., were still articulated). No difference in total number of polyhedral epidermal phytoliths per silica skeleton in plant samples and in fecal samples occurred (Wilcoxon's signed-rank test: $T = 45.2$; $P = 0.20$, $N = 15$; median number of polyhedral epidermal phytoliths per silica skeleton = 9 for plants, 6 for fecal samples; range: 1–133 in plants; 1–315 in feces). Therefore, mastication and digestion did not necessarily break down silica skeletons in plant parts ingested.

Total number of phytoliths counted/gAIF for fecal samples ranged from 56,000 to 166 million. No seasonal change occurred for the abundance of phytoliths in samples across the late part of the first dry season (D_1), wet season (W) and early part of the second dry season (D_2) (Kruskal–Wallis H-test: $H = 0.8$, $P = 0.67$, $N_{D1} = 10$, $N_W = 31$, $N_D = 9$). Phytoliths were present in all 50 fecal samples with a sample mean of $8.7 \pm$ SE 0.6 phytolith morphotypes (range: 1–17). We identified 39 phytolith morphotypes, 27 (69%) of which had been identified in the plant reference samples (Fig. 4, also Fig. 3). The 12 remaining morphotypes are illustrated in Figure 3; of these, armed hair-bases and elongate, rectangular blocky phytoliths were most frequent (in $\leq$38% fecal samples). We detected redundant phytoliths in 94% of fecal samples with a mean of $4.2 \pm$ SE 0.3 types per sample, giving a mean proportion of $55 \pm$ SE 0.7% of total number of phytolith morphotypes detected per sample (range:0–100). Three redundant phytoliths that had been detected in multiple plant parts were the most frequently recovered ($\leq$78% of fecal samples); non-segmented psilate hairs; stellate hair-bases; and polyhedral epidermal phytoliths.

We excluded 18 samples from further analyses either because they had no distinct phytoliths or they had distinct phytoliths, but had been collected on the first day of each two to three consecutive-day focal sample, and so data comparison with directly observed plant food-intake was not possible. Of the 32 remaining fecal samples, we detected 13 of the 15 distinct phytoliths from the reference library. Seven of these distinct phytoliths were rarely encountered in the fecal samples (each occurred in $\leq$5 samples); only the lanceolate papillate phytolith we recovered in 14 samples. We did not see the focal individuals in question eat the parts of plant reference samples in which we had detected them. The five remaining distinct phytolith types were frequently recovered in

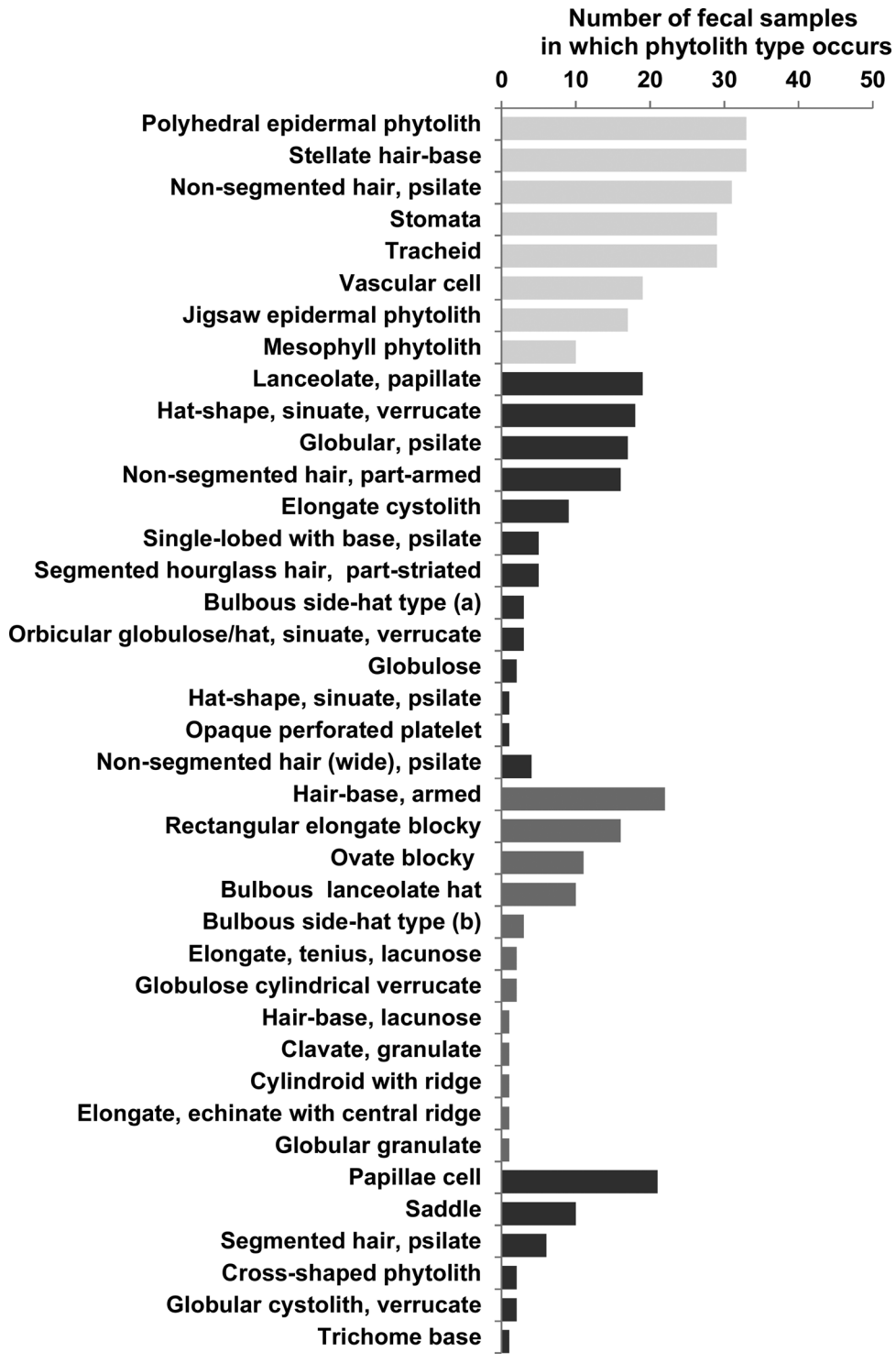

Fig. 4. Total number of phytolith morphotypes ($N = 39$) found in fecal samples analyzed ($N = 50$). Phytolith morphotypes categorized into: redundant (light grey); distinct (charcoal grey); not found in plant reference samples (mid grey); uncommon, but non-distinct phytoliths (black).

fecal samples, and came from plants which we had observed the focal individuals eat. Of these the non-segmented, part-armed hair from *F. asperifolia* was the most frequently detected (18 samples), where we saw the focal individuals eat the leaf or fruit up to 42 hr prior to defecation (75% of cross-validation checks). We identified the non-segmented, wide, psilate hair from *F. exasperata* fruit in five samples, which was directly observed to be fed upon up to 29 hr prior to defecation (60% of cross-validation checks).

Hat-shaped phytoliths with a sinuated margin and verrucate texture we recovered in 12 samples for *M. leucantha* pith, which the focal individuals ate for 43% of cross-validation checks and up to 51 hr previously. We found elongate cystoliths in six samples where *Urtica massaica* blossom had been eaten up to 22 hr prior to defection for 33% of cross-validation checks. In 16 samples, we found globular psilate phytoliths in leaves of *Aframomum* spp., *Chaetacme aristata*, and also in multiple parts of *M. leucantha*. All of these plant foods had been eaten up to 51 hr 44% of cross-validation checks.

Combining Phytolith and Macroscopic Data

We compared phytolith findings from 20 fecal samples with species identified during macroscopic inspection of paired samples. As a conservative measure, we had seen the focal chimpanzees eat all plant species identified 51 hr prior to defecation. Distinct phytoliths identified added up to two more plant species per fecal sample (sample mean = 38% of species identified; SV, Supplementary Material). These were phytoliths that had been detected in the leaves, fruit, pith or blossom of five species and one genus. Of these, blossom of *U. massaica*, along with the fruit and leaves of *F. asperifolia* and leaves of *F. exasperata* had been recognized during macroscopic inspection. The leaves of three THV plant foods (*M. leucantha*, *Aframomum* spp., and *Maesa lanceolata*), pith of *M. leucantha*, and the fruit from *F. exasperata* had not.

DISCUSSION

The eight redundant phytolith morphotypes represented a high proportion of those identified in the plant reference samples, in particular in dicotyledons, which is to be expected [Piperno, 2006]. Redundant morphotypes were shared across parts of several plant taxa. The overlap of plant type in CCA for these phytoliths appears to reflect their redundancy in diagnostic ability. Plant type separation, in particular herbaceous monocotyledon from the two dicotyledon plant types occurred when we subjected non-redundant phytoliths to CCA. Few distinct phytoliths were discriminated by plant type; however, neutral loading of distinct phytoliths also occurred, as well as large loading weights of non-distinct phytolith morphotypes. These factors could explain why some phytolith types were not assigned to plant type. Furthermore, small sample size of species ($N = 3$) categorized into HM, and multiple zero values present for non-redundant phytoliths may have further constrained our findings from CCA. Introducing phytolith morphotypes encountered in other monocotyledon species to our current reference library for additional CCA, or using an alternative analytical tool such as cluster analysis or probability modeling may provide further insights in future for phytolith and plant type association in the diet of this ape community.

Of the 15 distinct phytolith morphotypes that we found in select plant species analyzed, eight of them occur in parts of *Aframomum* spp. or *M. leucantha*. These monocotyledons were also two of the highest phytolith-producers in this study. Studied genera of their families have shown high phytolith production [Piperno, 1988, 2006; Runge & Fimbel, 2001], in particular in their reproductive structures [Piperno, 1989], but this is not a general feature for all monocotyledons [Hodson et al., 2005]. We found the globular-shaped, psilate textured phytolith in the leaves of various species, but also in the pith and fruit of *M. leucantha* plus the pith of *Aframomum* spp. Chen and Smith [2013] noted that globular phytoliths were small in species of Zingiberaceae, as was observed in this study for *Aframomum* spp. when compared to *M. leucantha* plant parts. This phytolith was abundant in samples from East-African tropical forests [Barboni et al., 2007], and along with the other described globular phytoliths, occurs in various tropical dicotyledon leaves and seeds, but in fewer tropical herbaceous monocots [Piperno, 1988]; however, they occur in the reproductive structure of most families of Zingiberales [Chen & Smith, 2013].

Finding hat-shaped phytoliths in *M. leucantha* was expected as they have been detected in other species of the Marantaceae family [Chen & Smith, 2013; Piperno, 1989], but they are also noted in other plant families [Kealhofer & Piperno, 1998]. Identifying their texture and size may further assist in taxonomic diagnosis. Hat-shaped phytoliths with a verrucate texture have been detected in external fibers of vascular bundle sheaths in species of *Maranta* (de Albuquerque et al., 2013). We found hat-shapes with a verrucate texture were of similar size in *M. leucantha* and *Aframomum* spp., but those with a psilate texture were smaller in *P. capense* vs. *M. leucantha* (6 μm vs. 11 μm).

Having compared phytolith morphotypes in the leaves and fruit of five *Ficus* species, hair-bases and psilate hairs occurred in both parts for most. These redundant phytoliths have been found in other fig plants [Piperno, 1985; Tsartsidou et al., 2007], but not the bulbous side-hat type (a) detected in the leaf of three of the five *Ficus* species; it may be that it is specific to regions of Africa. The particularly wide psilate hair found only in the fruit of *Ficus exasperata* is also not recorded for other fig species. Nodular-textured phytoliths, which have a similar appearance to the non-segmented armed hairs we found in *F. asperifolia* fruit and leaf, have been recorded for *F. opposita* in Australia [Wallis, 2003]. Armed hairs have also been recovered in *Ficus* species of Southeast Asia [Kealhofer & Piperno, 1998]. Although only recovered in one of the *Ficus* species analyzed, armed hairs occur in other genera of the Moraceae family [Kealhofer & Piperno, 1998].

Phytoliths studied in parts of *Aneilema* spp. have revealed platelets and psilate flake phytoliths in West African species [Eichhorn et al., 2010]. It is yet to be determined if the hourglass-shaped hair with a striated texture found in *Aneilema aequinoctiale* in this study are found in other species of this genus. In general, species of Commelinaceae are high phytolith producers, in which "*taxonomically significant morphotypes*" [Eichhorn et al., 2010] can occur.

Phytoliths are not observed or are uncommon in the families of the plant species analyzed in this study in which phytoliths were not detected [Piperno, 1988, 2006]. However, silica concentration, which is used as an indicator of phytolith productivity, can be wide-ranging for members of these families [Hodson et al., 2005], therefore, other genera in these families may be high producers, and the low phytolith productivity in species analyzed may not necessarily reflect a general pattern.

Distinct Phytoliths Versus Cross-Validation Checks

Certain phytoliths associated with plant parts in the reference library that we did not see the focal individuals eat during the focal sample in question were detected in a small number of fecal samples. There may be a number of reasons for this: First, some of the plant species we observed them eat may have been misidentified. Second, certain phytolith morphotypes may have been misidentified due to: a "weathering" effect caused by partial dissolution during the extraction process [Cabanes et al., 2011]; the fact that we were unable to rotate phytoliths on the slide (Fig. 1); or some morphotypes having a similarity to other phytolith morphotypes. Third, the focal individuals ate the plant part in question when they were out of view, which was sample mean = 4.06 ± SE 0:36 hr ($N = 19$ focal samples) prior to collection of the 50 fecal samples. Finally, these phytoliths occurred in other plant species or parts that were not included in the plant reference library analyzed.

We saw the 10 focal individuals eat parts from eight non-analyzed plant species during focal samples. However, they account for only 23% of cross-validation checks of food-items eaten during each focal sample period ($N = 19$ focal samples). Misidentification is possible, but it is highly probable that unobserved feeding bouts occurred in the time the focal individuals were out of view. These gaps could account for the presence of these distinct phytoliths. All three distinct phytoliths found in either the leaves of *Piper capense* or *Acanthus polystachius* could: (a) reflect non-observed consumption of their leaves, (b) signal the inclusion of pith for *A. polystachius* observed to be eaten in four feeding bouts by four of the chimpanzees 13 to 43 hr previously; or (c) come from other non-analyzed plant species known to be included in the diet of the Kanyawara chimpanzee community. Further analyses of all of these plant species and their parts should confirm this.

Direct geophagy (seeking and eating soil fragments) or indirect geophagy (soil particles attached to plant parts eaten) by focal individuals can account for any recovered phytoliths from plant species that are not included in our reference library. We witnessed seven bouts of geophagy (soil consumption) by four males and four females 13 to 51 hr prior to fecal sample collection. This may have contributed to the phytoliths found in fecal samples, having potentially been present in soil fragments ingested. We cross-validated observed bouts of geophagy with phytoliths detected in fecal samples. Of the 12 phytolith morphotypes that were not identified in the plant reference samples, only the lacunose hair-base was present in a fecal sample that had been deposited after geophagy. We also detected three other morphotypes that were not identified in the plant reference samples. These were: (1) rectangular elongate blocky; (2) armed hair-base; and (3) bulbous lanceolate hat. Each of these occurred in fecal samples ($N = 13$) collected either before geophagy, or during focal samples in which we did not see geophagy. Therefore, geophagy could not account solely for the sourcing of 92% of the additional non-plant reference sample phytolith morphotypes detected.

In terms of frequency, blocky phytoliths were one of the highest occurring phytolith morphotypes in fecal samples. We found none in plant parts from our reference library. They have been found in the stem tissue and leaf tissue of various miombo woodland plants in Mozambique, which include members of families in which our select plants analyzed also belong to (e.g., Annonaceae, Euphorbiaceae, Rubiaceae in the stem tissue and Flacourtiaceae in the leaf tissue) [Mercader et al., 2009] and are associated with shrubs and forbs [Blinnikov et al., 2001]. This may indicate that they occur in plants or parts that were eaten but not yet analyzed.

Of the five distinct phytolith morphotypes that we found in fecal samples, and which occur in plant species that we saw the focal individuals eat, two are associated with leaves, pith or fruit of species in both Marantaceae and Zingiberaceae; one with another terrestrial herb; and two were hair phytoliths recovered in the leaves of two different fig species. This result is encouraging as the identification of foods such as pith and leaf of THV that are not easily detected during macroscopic inspection of feces [Phillips & McGrew, 2013]. Such foods may therefore be indicated with the study of phytoliths. Using phytolith morphotypes to differentiate between *Ficus* species eaten and potentially their parts also highlights the value of analyzing this microfossil, as this can be difficult to achieve during macroscopic inspection.

A possible contamination issue affected two plant samples (*Aframomum* spp. and *Urtica massaica* leaf parts) in which cross-shaped and saddle phytoliths were recovered. As these morphotypes are normally found in grasses this discrepancy may indicate that extraneous matter containing grass-associated phytoliths was still present on these two samples [Albert et al., 2007], or that the phytoliths detected had a similar morphology, but were non-grass phytoliths and were misidentified. We also found saddle phytoliths in 10 fecal samples (range: 1–5 per sample) which may be the result of geophagy, or the consumption of grass by the focal individuals. Within Kibale National Park, shrubs and THV included in the diet of the Kanyawara chimpanzees have been recorded at grassland sites, and in open areas which also contain grass species (usually disturbed sites resulting from human activity) [Duncan & Duncan, 2000; Zanne & Chapman, 2005]. Dominant grasses in Kibale National Park are from the Panicoideae sub-family [Lang et al., 1962]. Phytolith work on Panicoideae grass species indicate cross-shaped phytoliths occur in high proportions, but saddles more so in species of the Chloridoideae sub-family [Bremond et al., 2007; Twiss et al., 1969]. Grass plant parts may have been harvested and simultaneously eaten by a chimpanzee feeding on other terrestrial plants. Parts of *Pennisetum purpureum* and *Zea mays* have been observed to be eaten by members of the Kanyawara chimpanzee community. We did not see focal individuals eat grass, except one adult female who ate the pith of *Zea mays* during a crop-raiding bout. This grass-intake was on the third day of the focal sample, and so subsequent fecal samples were not collected for analysis. No grass parts were visible to the naked eye in any of the fecal samples analyzed.

Applying the dry ash extraction method to plant samples revealed phytoliths in 10 of the 12 plant species that were part of the observed diet, but that had not been identified at macroscopic level. Nine of the 10 distinct phytoliths were associated with leaves or pith from seven terrestrial herbs (SI, Supplementary Material). The pith or leaf of four of these plant species had been seen to be eaten by the focal chimpanzee in question within the previous 51 hr of fecal sample collection, and made up 3% of the total feeding observed for the 10 adult chimpanzees. Having been observed in fecal samples, distinct phytoliths added up to two species to the list of those identified per fecal sample during macroscopic inspection. Phytolith analysis therefore corroborated macroscopic inspection. The financial cost of applying the dry ash extraction method to all plant and fecal samples analyzed was also relatively low compared to wet oxidation. This study illustrates that a multiple-method approach to fecal analyses need not be expensive in order to establish plant foods included in the diet of an ape population.

If most primate populations remain unhabituated, and also unprotected from human hunters, they are potentially exposed to fatal zoonotic diseases, and can experience habitat encroachment by local human populations. For this reason, the high level of precision in understanding their dietary habits offered by such a multiple-method approach could assist conservation efforts for these populations. Analyses of phytoliths in feces can be used to illustrate and to monitor, for example, the impact of habitat encroachment by humans on primate populations. As the loss of habitat reduces wild-food plant availability to a population, they may incorporate non-wild food-items into their dietary repertoire [Campbell-Smith et al., 2010; Hockings et al., 2009]. Phytoliths have been identified from various crops such as *Musa* spp., *Oryza* spp., *Saccharum* spp., *Triticum* spp., *Hordeum* spp., and *Zea mays* [Ball et al., 1999; Harvey & Fuller, 2005; Piperno, 1984, 2006] and have been shown to resist digestion processes [Baker et al., 1961; Brochier et al., 1992; Shahack-Gross, 2011]. Therefore, their associated phytolith morphotypes may be detected in feces.

Furthermore, habitat use can be reconstructed from phytoliths identified in feces in order to assist in the identification of important food resources. Distinct phytoliths associated with *Aframomum* spp., *Acanthus polystachius*, *Marantochloa leucantha*, *Maesa lanceolata, Piper capense* and *Urtica massaica* detected in feces in this study indicate the 10 focal chimpanzees utilized patches of terrestrial herbaceous vegetation (patch size unknown) from areas with dicotyledonous trees. By reconstructing the habitats used by extant chimpanzee populations we can infer information on plant food availability and diet, thus providing a valuable perspective (i.e., highlight similarities and differences) to then interpret potential plant foods that would have been available to hominin ancestors [Copeland, 2009].

Following on from this preliminary study, efforts should focus on the expansion of the phytolith reference library for Kanyawara flora, in particular, parts of all species known to be included in the diet of this chimpanzee community, but also plants that are dominant in the various habitat types found within their home-range (including grasses). This extension should increase current understanding of phytolith morphotype-plant association for Kanyawara, and also highlight any potential misidentification of phytolith morphotypes. Furthermore, the expansion of our reference library may reveal which plant taxon the 12 phytolith morphotypes detected only in fecal samples come from. Particular attention should be paid to plant species in which either the leaf, pith or stem are eaten. These are predicted to appear either pulverised or fully-digested in feces at macroscopic level.

Conclusion

Phytolith research corroborates with findings of primate feces using macroscopic inspection and this preliminary study advocates for the use of phytoliths in feces as a diagnostic tool to help determine the dietary repertoire of primates. Such analyses can therefore, be done to investigate diet of unhabituated populations. The building of a plant reference library is possible through collection of plant foods that have been observed to be eaten; have been identified from macroscopic inspection of scats or the study of feeding-remains encountered on-site; or from data available on plant foods eaten by another population of the study species. Adding non-food plants, such as dominant species present in different habitat types is advantageous as they provide perspective into which redundant and distinct phytolith morphotypes occur across both foods and non-foods within the reference library; their addition can also clarify phytolith morphotypes not included in the diet of the study population. Analyses of soil samples may help to identify phytoliths recovered in feces that are not found in plants in the reference library (as a result of direct or indirect geophagy), and give information on phytolith productivity in soils across the home range. By applying this bioarchaeological method, and using extant primates as proxy for human ancestor dietary practices, this line of research also falls under primate archaeology [Haslam et al., 2009]. Of course, identification of phytoliths recovered in any sample material may not necessarily lead to identification of plants to specific plant taxa, but still, as shown from this study, they can provide insight into habitat utilized for food resources by extant primates and expand dietary knowledge from feces of a study population that is not attainable from other methods of analysis.

ACKNOWLEDGENTS

The authors thank: Uganda Wildlife Authority, Uganda Council for Science and Technology, and Makerere University Biological Field Station for permission to live and work in Kibale National Park; Richard Wrangham and Martin Muller of the Kibale Chimpanzee Project for permission to work under the project and generous logistical support; all staff of the Kibale Chimpanzee Project for help in data collection and for identification of plant samples; Louise Butterworth, Catherine Kneale, Tamsin O'Connell, Chris Rolfe and Steve Boreham, University of Cambridge for assistance during laboratory analysis; and William McGrew for guidance and helpful comments during the research. We thank Tea Jashashvili, Mike Huffman and four anonymous reviewers for helpful comments on the manuscript.

REFERENCES

Albert RM, Weiner S. 2001. Study of phytoliths in prehistoric ash layers from Kebara and Tabun caves using a quantitative approach. In: Meunier JD, Colin F, editors. Phytoliths: applications in earth sciences and human history. Lisse, South Holland, Netherlands: A Balkema Publishers. p 51–266.

Albert RM, Bar-Yosef O, Weiner S. 2007. Use of plant remains in Kebara Cave: phytoliths and mineralogical analysis. In: Bar-Yosef O, Meignen L, editors. Kebara Cave, Mt. Carmel, Israel: the middle and upper paleolithic archaeology, Part 1 (American School of Prehistoric Research Bulletin 49). Cambridge, MA: Peabody Museum of Archaeology and Ethnology, Harvard University. p 147–164.

Baker G. 1959. Fossil opal-phytoliths and phytolith nomenclature. Australian Journal of Science 21:305–306.

Baker G, Jones LHP, Wardrop ID. 1961. Opal phytoliths and mineral particles in the rumen of the sheep. Australian Journal of Agricultural Research 12:462–472.

Ball T, Gardner JS, Anderson N. 1999. Identifying Inflorescence phytoliths from selected species of wheat (*Triticum monococcum*, *T. dicoccon*, *T dicoccoides* and *T. aestivum*) and barley (*Hordeum vulgare* and *H. spontaneum*) (Gramineae). American Journal of Botany 86:1615–1623.

Barboni D, Bremond L, Bonnefille R. 2007. Comparative study of modern phytolith assemblages from inter-tropical Africa. Palaeogeography, Palaeoclimatology, Palaeoecology 246: 454–470.

Barboni D, Ashley GM, Dominguez-Rodrigo M, et al. 2010. Phytoliths infer locally dense and heterogeneous paleovegetation at FLK North and surrounding localities during upper bed I time, Olduvai Gorge, Tanzania. Quaternary Research 74:344–354.

Bertolani P, Boesch C. 2008. Habituation of wild chimpanzees (*Pan troglodytes*) of the south group at Taï Forest, Côte d'Ivoire: empirical measure of progress. Folia Primatologica 79:162–171.

Blinnikov MS, Busacca AJ, Whitlock C. 2001. A new 100,000-year record from the Columbia Basin, Washington, USA. In: Meunier JD, Colin F, editors. Phytoliths: applications in earth sciences and human history. Lisse, South Holland, Netherlands: A Balkema Publishers. p 27–55.

Bremond L, Alexandre A, Wooller MJ, et al. 2007. Phytolith indices as proxies of grass subfamilies on East African tropical mountains. Global and Planetary Change 61:209–224.

Brochier JE, Villa P, Giacomarra M. 1992. Shepherds and sediments: geo-ethnoarchaeology of pastoral sites. Journal of Archaeology and Anthropology 11:47–102.

Cabanes D, Weiner S, Shahack-Gross R. 2011. Stability of phytoliths in the archaeological record: A dissolution study of modern and fossil phytoliths. Journal of Archaeological Science 38:2480–2490.

Campbell-Smith G, Simanjorang HVP, Leader-Williams N, Linkie M. 2010. Local attitudes and perceptions toward crop-raiding by orangutans (*Pongo abelii)* and other nonhuman primates in northern Sumatra, Indonesia. American Journal of Primatology 72:866–876.

Chapman CA, White FJ, Wrangham RW. 2001. Party size in chimpanzees and bonobos: A re-evaluation of theory based on two similarly forested sites. In: Wrangham RW, McGrew WC, de Waal FBM, Heltne PG, editors. Chimpanzee cultures. Cambridge, MA: Harvard University Press. 41–57.

Chen ST, Smith SY. 2013. Phytolith variability in Zingiberales: A tool for the reconstruction of past tropical vegetation. Palaeogeography, Palaeoclimatology, Palaeoecology 370: 1–12.

Ciochon RL, Piperno DR, Thompson RG. 1990. Opal phytoliths found on the teeth of the extinct ape *Gigantopithecus blacki*: Implications for paleodietary studies. Proceedings of the National Academy Sciences of the United States of America 87:8120–8124.

Copeland SR. 2009. Potential hominin plant foods in northern Tanzania: Semi-arid savannas versus savanna chimpanzee sites. Journal of Human Evolution 57:365–378.

de Albuquerque ESB, Braga JMB, Vieira RC. 2013. Morphological characterisation of silica phytoliths in Neotropical Marantaceae leaves. Plant Systematics Evolution 299:1659–1670.
Deblauwe I, Janssens GJP. 2008. New insights in insect prey choice by chimpanzees and gorillas in southeast Cameroon: the role of nutritional value. American Journal of Physical Anthropology 135:42–55.
Doran D, McNeilage A, Greer D, et al. 2002. Western lowland gorilla diet and resource availability: new evidence, cross-site comparisons, and reflections on indirect sampling methods. American Journal of Primatology 58:91–116.
Doran-Sheehy D, Derby AM, Greer D, Mongo P. 2007. Habituation of western gorillas: the processes and factors that influence it. American Journal of Primatology 69:1354–1369.
Duncan RS, Duncan EV. 2000. Forest succession and distance from forest edge in an afro-tropical grassland. Biotropica 32:33–41.
Eichhorn B, Neumann K, Garnier A. 2010. Seed phytoliths in West African Commelinaceae and their potential for palaeoecological studies. Palaeogeography, Palaeoclimatology, Palaeoecology 298:300–310.
Farmer VC, Delbos E, Miller JD. 2005. The role of phytolith formation and dissolution in controlling concentrations of silica in soil solutions and streams. Geoderma 127:71–79.
Harvey EL, Fuller DQ. 2005. Investigating crop processing using phytolith analysis: The example of rice and millets. Journal of Archaeological Science 32:739–752.
Haslam M, Hernandez-Aguilar A, Ling V, et al. 2009. Primate archaeology. Nature 460:339–344.
Henry AG. 2012. Recovering dietary information from extant and extinct primates using plant microremains. International Journal of Primatology 33:702–715.
Henry AG, Brooks AS, Piperno DR. 2010. Microfossils in calculus demonstrate consumption of plants and cooked foods in Neanderthal diets (Shanidar III, Iraq; Spy I and II, Belgium. Proceedings of the National Academy Sciences of the United States of America 108:486–491.
Hockings KJ, Anderson JR, Matsuzawa T. 2009. Use of wild and cultivated foods by chimpanzees at Bossou, Republic of Guinea: feeding dynamics in a human-influenced environment. American Journal of Primatology 71:636–646.
Hodson MJ, White PJ, Mead A, Broadley MR. 2005. Phylogenetic variation in the silicon composition of plants. Annals of Botany 96:1027–1046.
Hohmann G. 2009. The diets of non-human primates: Frugivory, food processing and food sharing. In: Hublin J, Richards MP, editors. The evolution of hominin diets: integrating approaches to study palaeolithic subsistence. Berlin, Germany: Springer. p 1–14.
Horrocks M, Irwin GJ. 2003. Pollen, phytoliths and diatoms in prehistoric coprolites from Kohika, Bay of Plenty, New Zealand. Journal of Archaeological Science 30:13–20.
Jiang Q. 1995. Searching for evidence of early rice agriculture at prehistoric sites in China through phytolith analysis: an example from central China. Review of Palaeobotany and Palynology 89:481–485.
Julliot C, Sabatier D. 1993. Diet of the red howler monkey (*Alouatta seniculus*) in French Guiana. International Journal of Primatology 14:527–550.
Kealhofer L, Piperno DR. 1998. Opal phytoliths in southeast Asian flora. Smithsonian Contributions to Botany 88:1–39.
Köndgen S, Kühl H, N'Goran PK, et al. 2008. Pandemic human viruses cause decline of endangered great apes. Current Biology 18:260–264.
Lancelotti C. 2010. Fuelling Harappan hearths: human-environment relations as revealed by fuel exploitation and use [PhD thesis]. Cambridge: University of Cambridge.
Lancelotti C, Madella M. 2012. The invisible product: developing markers to identifying dung in archaeological contexts. Journal of Archaeological Science 39:953–963.
Lang Brown JR, Harrop JF. 1962. The ecology of soils of the Kibale Grasslands, Uganda. East African Agricultural and Forestry Journal 27:264–272.
Lalueza Fox C, Pérez-Pérez A. 1994. Dietary information through the examination of plant phytoliths on the enamel surface of human dentition. Journal of Archaeological Science 21:29–34.
Madella M, Jones MK, Goldberg P, et al. 2002. The exploitation of plant resources by Neanderthals in Amud Cave (Israel): the evidence from phytolith studies. Journal of Archaeological Science 29:703–719.
Madella M, Alexandre A, Ball T. 2005. International code for phytolith nomenclature 1.0. Annals of Botany 96:253–260.
Madella M, Lancelotti C. 2012. Taphonomy and phytoliths: a user manual. Quaternary International 275:76–83.
Malenky RK, Kuroda S, Vineburg EO, Wrangham RW. 1994. The significance of terrestrial herbaceous foods for bonobos, chimpanzees and gorillas. In: Wrangham RW, McGrew WC, de Waal FBM, Heltne PG, editors. Chimpanzee cultures. USA: Harvard University Press. p 59–76.
Martin P, Bateson P. 2007. Measuring behaviour. An introductory guide. Cambridge, UK: Cambridge University Press.
McGrew WC. 2007. Savannah chimpanzees dig for food. Proceedings of the National Academy Sciences of the United States of America 104:19167–19168.
McGrew WC, Baldwin PJ, Tutin CEG. 1988. Diet of wild chimpanzees (*Pan troglodytes verus*) at Mt. Assirik, Senegal: I. Composition. American Journal of Primatology 16:213–226.
McGrew WC, Marchant LF, Phillips CA. 2009. Standardised protocol for fecal analysis. Primates 50:363–366.
Mercader J, Bennett T, Esselmont C, et al. 2009. Phytoliths in woody plants from Miombo woodlands of Mozambique. Annals of Botany 104:91–113.
Moreno-Black G. 1979. The use of scat samples in primate diet analysis. Primates 19:215–221.
O'Driscoll Worman C, Chapman C. 2004. Seasonal variation in the quality of a tropical ripe fruit and the response of three frugivores. Journal of Tropical Ecology 21:689–697.
Osborne TZ, Chapman LJ, Chapman CA, et al. 2001. Invertebrate community structure and oxygen availability in an intermittent stream/wetland/river system of the Ugandan highlands. Verhandlungen des Internationalen Verein Limnologie 27:3599–3603.
Parr JF, Lentfer CJ, Boyd WE. 2001. A comparative analysis of wet and dry ashing techniques for the extraction of phytoliths from modern plant material. Journal of Archaeological Science 28:875–886.
Pearsall D. 1994. Investigating: New World tropical agriculture: contributions from phytolith analysis. In: Hather G, editor. Tropical archaeobotany: applications and new developments. New York, NY: Routledge. p 115–135.
Phillips CA, McGrew WC. 2013. Identifying species in chimpanzee (*Pan troglodytes*) faeces: A methodological lost cause? International Journal of Primatology 34:792–807.
Piperno DR. 1984. A comparison and differentiation of phytoliths from maize and wild grasses: use of morphological criteria. American Antiquity 49:361–383.
Piperno DR. 1985. Phytolith analysis and tropical paleoecology: production and taxonomic significance of siliceous forms in new world plant domestics and wild species. Review of Palaeobotany and Palynology 45:185–228.
Piperno DR. 1988. Phytolithic analysis: an archaeological and geological perspective. San Diego, CA: Academic Press.
Piperno D. 1989. The occurrence of phytoliths in the reproductive structures of selected tropical angiosperms and their significance in tropical paleoecology, paleoethnobotany and

systematic. Review of Palaeobotany and Palynology 61: 147–173.
Piperno DR. 1994. Phytolith and charcoal evidence for prehistoric slash-and-burn agriculture in the Darien rain forest of Panama. Holocene 4:321–325.
Piperno DR. 2006. Phytoliths: a comprehensive guide for archaeologists and paleoecologists. Oxford, UK: Altamira Press.
Prasad V, Strömberg CAE, Alimohammadian H, Sahni A. 2005. Dinosaur coprolites and the early evolution of grasses and grazers. Science 310:1177.
Rovner I. 1971. Potential of opal phytoliths for use in paleoecological reconstruction. Quarternary Research 1: 343–359.
Runge F. 1999. The opal phytolith inventory of soils in central Africa—quantities, shapes, classification and spectra. Review of Palaeobotany and Palynology 107:23–53.
Runge F, Fimbel RA. 2001. Opal phytoliths as evidence for the formation of savannah islands in the rain forest of Southeast Cameroon. In: Heine K, editor. International Union for Quarternary Research, Congress (15th: 1999: Durban, South Africa). Lisse, South Holland, Netherlands: Swets and Zeitlinger B. V. p 171–185.
Sekercioglu CH. 2002. Effects of forestry practices on vegetation structure and bird community of Kibale National Park, Uganda. Biological Conservation 107:229–240.
Shahack-Gross R. 2011. Herbivorous livestock dung: formation, taphonomy, methods for identification, and archaeological significance. Journal of Archaeological Science 38:205–218.
Teaford MF, Glander KE. 1996. Dental microwear and diet in a wild population of mantled howling monkeys (*Alouatta palliata*). In: Norconk MA, Rosenberger AL, Garber PA, editors. Adaptive radiation of neotropical primates. New York, NY: Plenum Press. p 433–450.
Ter Braak CJF. 1987. The analysis of vegetation-environment relationships by canonical correspondence analysis. Vegetatio 69:66–97.
Tsartsidou G, Lev-Yadun S, Albert R, et al. 2007. The phytolith archaeological record: strengths and weaknesses evaluated based on a quantitative modern reference collection from Greece. Journal of Archaeological Science 34:1262–1275.
Tutin CEG, Fernandez M. 1993. Fecal analysis as a method of describing diets of apes: examples from sympatric gorillas and chimpanzees at Lopé, Gabon. Tropics 2:189–197.
Twiss PC, Suess E, Smith RM. 1969. Morphological classification of grass phytoliths. Proceedings—Soil Science Society of America 33:109–115.
van der Veen M, Fieller N. 1982. Sampling seeds. Journal of Archaeological Science 9:287–298.
Wallis L. 2003. An overview of leaf phytolith production patterns in selected northwest Australian flora. Review of Palaeobotany and Palynology 125:201–248.
Wang W, Lui J, Zhou X. 2003. Climate indexes of phytoliths from *Homo erectus*' cave deposits in Nanjing. Chinese Science Bulletin 48:2005–2009.
Woodford MH, Butynski TM, Karesh WB. 2002. Habituating the great apes: the disease risks. Oryx 36:153–160.
Wrangham RW, Chapman CA, Chapman LJ. 1994. Seed dispersal by forest chimpanzees in Uganda. Journal of Tropical Ecology 10:355–368.
Zanne AE, Chapman CA. 2005. Diversity of woody species in forest, treefall gaps, and edge in Kibale National Park, Uganda. Plant Ecology 178:121–139.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.