Applying wet sieving fecal particle size measurement to frugivores: a case study of the Eastern chimpanzee (Pan troglodytes schweinfurthii)

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Number of pages: 25
Number of figures: 2
Number of tables: 6
Abbreviated title: Chimpanzee diet and fecal particle size
Key words: nutritional ecology, digestive physiology, frugivory, fallback foods, mastication

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Grant sponsorship: Herchel Smith-Harvard Undergraduate Science Research Fellowship
ABSTRACT

Objectives: Fecal particle size (FPS) as quantified by wet sieving analysis is a measure of chewing efficiency relevant for the understanding of physiological adaptations and constraints in herbivores. FPS has not been investigated systematically in frugivores, and important methodological problems remain. In particular, food items that are not chewed may skew estimates of FPS. We address such methodological issues and also assess the influence of diet type and age on FPS in wild chimpanzees.

Materials and Methods: 130 fecal samples of 38 individual chimpanzees (aged from 1.3 to ~55 years) from the Kanyawara community of Kibale National Park (Uganda) were collected during three fruit seasons and analysed using standardized wet sieves (pores from 16 mm to 0.025 mm). The effects of using different sieve series and excluding large seeds were investigated. We also assessed the relationship between FPS and sex, age and fruit season.

Results: The treatment of seeds during the sieving process had a large influence on the results. FPS was not influenced by chimpanzee sex or age, but was smaller during a fig season (0.88 ±0.31 mm) than during two drupe-fruit seasons (1.68 ±0.37 mm) (0.025-4 mm sieves, excluding seeds).

Discussion: The absence of an age effect on FPS suggests that dental senescence might be less critical in chimpanzees, or in frugivores in general, than in folivorous herbivores. To increase the value of FPS studies for understanding frugivore and hominoid dietary evolution we propose modifications to prior herbivore protocols.
Chewing efficiency is a critical variable influencing energy acquisition from food in herbivores, because the size of a particle influences the speed at which it can be digested or fermented (Dehority and Johnson, 1961; Bjorndal et al., 1990). This effect is explained by smaller particles having a larger surface-to-volume ratio, therefore offering enzymes or microbes a larger attack area for their digestive action. Strictly speaking, chewing efficiency (or chewing effectiveness) is the rate at which a defined amount of a defined food is reduced to a certain mean particle size, and this rate is influenced by factors like oral anatomy, dental design and wear stage, number, direction and intensity of chewing movements as well as the force applied during chewing (Pérez-Barbería and Gordon, 1998). Because this number of factors can only be observed under very controlled experimental settings, a convenient proxy for chewing efficiency is the particle size of the digesta, measured as fecal particle size (FPS). In terrestrial mammalian herbivores, chewing is the main determinant of digesta particle size, with comparatively little change due to gastrointestinal digestion (Poppi et al., 1980; McLeod and Minson, 1988; Spalinger and Robbins, 1992). Therefore, fecal particle size has been used repeatedly as a proxy for ingesta particle size reduction, mainly in non-primate herbivores (Fritz et al., 2009; Steuer et al., 2010; Clauss et al., 2015) and to some extent in primates (Dunbar and Bose, 1991; Matsuda et al., 2014; Venkataraman et al., 2014). With the exception of a study on geladas (*Theropithecus gelada*) (Venkataraman et al., 2014), however, the cited investigations were mainly focused on comparisons among species, and rarely investigated causes of variation within a species.
Here we consider the value of investigating fecal particle size for assessing chewing efficiency in the Eastern chimpanzee (*Pan troglodytes schweinfurthii*), a frugivorous primate. Our study population was the Kanyawara community in Kibale National Park, Uganda. Ripe fruits dominate the Kanyawara chimpanzee diet (yearly average of feeding time spent eating ripe fruits = 65-75%, varying across months from ~35% to 95%). Ripe fruits are complemented principally by pith and young leaves, which together average about 25% of feeding time, ranging from ~20-45% (Wrangham et al., 1998; Potts et al., 2011).

The fact that FPS measurement is non-invasive makes it especially useful for studying the chimpanzee, an endangered species for which most wild populations remain unhabituated (Phillips & McGrew, 2014).

Two hypotheses guided our approach. First, we expected tooth wear, and hence chewing efficiency, to be related to age. An age effect was previously demonstrated in geladas (*Theropithecus gelada*), a graminivore specialized in eating tough diets of grass or sedge leaves: in the dry season, old geladas produced larger fecal particles than younger individuals (Venkataraman, et al., 2014). This suggests that chewing efficiency was reduced in old gelada, presumably due to molar wear. In contrast, Edward's sifakas (*Propithecus edwardsi*) (King, et al., 2005) and mountain gorillas (*Gorilla beringei beringei*) (Glowacka, et al., 2016) maintain their molar morphology related to shearing function despite increased age-related occlusal wear. However whether FPS increases in old individuals of these species is unknown. We hypothesized that chimpanzee FPS would increase with age, particularly in past-prime individuals.
Second, again prompted by findings in geladas (Venkataraman, et al., 2014), we tested the hypothesis that fecal particle size would rise during a season of reduced availability of preferred ripe fruits, because we expected preferred diet items to have structural properties that would be advantageous for digestion (i.e., properties that lead to smaller particles at similar chewing investment compared to less-preferred diet items).

In contrast to most previous studies on fecal particle size in herbivorous mammals, we anticipated methodological complications due to the inclusion of large seeds from fruits in the feces of chimpanzees. Seeds of ripe fruits are often swallowed unchewed (Wrangham et al., 1994). Therefore, they will be retrieved in sieve analysis of feces and skew the resulting measurement of FPS upwards. Additionally, chimpanzees sometimes egest large plant particles, including complete leaves that function in self-medication by being swallowed unchewed (Wrangham, 1995). Such material would similarly affect fecal particle size measurements. Finally, previous studies have used a range of sieve pore-sizes (Fritz et al., 2009; Matsuda et al., 2014). Whereas variation in the number of sieves within a sieve column (i.e., the 'middle' sieves) has little effect on the resulting fecal particle size measurement (Fritz et al., 2012), expanding the sieve column at either end (i.e., adding sieves with smaller or larger pores) can strongly influence the calculated mean particle size.

In order to explore these effects we included the most comprehensive set of sieves, and the largest number of intra-specific samples, used in primate studies.
so far, and compared fecal particle size measurements calculated based on different sieve series and exclusion protocols.

METHODS

Field site

The Kanyawara community of wild chimpanzees (*Pan troglodytes schweinfurthii*) has been studied continuously by the Kibale Chimpanzee Project (KCP) since 1987. Their home range consists of about 37.8 km² of forest (Wilson et al., 2001) in Kibale National Park (0° 34’ N, 30° 21’ E) in western Uganda. The habitat is mainly evergreen forest transitional between lowland and montane rainforest, interspersed with secondary forest, grassland, and swamp, at an average elevation of ~1,500 meters above sea level (Chapman and Wrangham, 1993). Trained Ugandan field assistants conduct all-day behavioral observations of the chimpanzees. During the study period, individuals were well habituated and individual identification was reliable. The Kanyawara community has never been provisioned. In 2015 it included ~ 55 individuals.

[Table 1 here]

Over a total of six weeks during the months of January, July and early August 2015, we collected 130 fecal samples opportunistically from 38 chimpanzees aged 1.3 to ~55 years old. Age categories are shown in Table 1. Ages of the chimpanzees were known to within less than a week for most individuals younger than 25 years of age, and estimated to within five years for those older than 25 years.
The three sample collection months corresponded approximately to three fruit 'seasons' in each of which the chimpanzees’ diet was dominated by a different species of ripe fruit (Table 2). Figs (*Ficus natalensis*) were the most frequently eaten fruit species in January (50% feeding time), and drupe fruits were the main fruit types in the second and third seasons (77% feeding time on *Pseudospondias microcarpa*, and 73% feeding time on *Linociera johnsonii*, respectively). For Kanyawara chimpanzees, seasons when the diet is dominated by figs are typically those when drupes are less available (Wrangham et al., 1996), and they are associated with poorer energy harvesting (Thompson and Wrangham, 2008). The fact that diets in the July and August seasons were both dominated by drupes therefore suggests that the quality of food would be higher than in January. Terrestrial herbaceous vegetation ('foliage'), representing the stems, piths, and leaves of a number of species of terrestrial herbs and shrubs, is a major class of fallback foods for the Kanyawara community and is typically eaten in inverse proportion to the availability of preferred foods such as ripe fruits (Wrangham et al., 1991; Wrangham et al., 1998).

No more than one fecal sample was taken per day from any individual. Fecal samples were placed in water-tight plastic bags in the field immediately after defecation to prevent loss of moisture, and were weighed at the end of the day. About ten grams of each sample were stored in 50 mL plastic test tubes with just enough laboratory grade ethanol (70% solution for January and 96% for July-August) to fully immerse it for preservation. An equivalent portion of each
sample was dried to constant weight in a food dehydrator at 71°C to determine dry matter concentration. These data, however, proved unreliable in later calculations, most likely due to heterogeneity between fecal subsamples (see Discussion).

Laboratory analysis

Fecal samples were analyzed at the Clinic for Zoo Animals, Exotic Pets and Wildlife of the University of Zurich using a standardized wet sieving method (Fritz et al., 2012). The sieve cascade (Retsch, Haan, Germany) contained 11 sieves with pore sizes (linear dimension of holes) of 0.025 mm, 0.040 mm, 0.063 mm, 0.125 mm, 0.25 mm, 0.5 mm, 1.0 mm, 2.0 mm, 4.0 mm, 8.0 mm, and 16.0 mm. In contrast to previous FPS studies, the two finest sieves were added to further differentiate the very small particles. Fecal samples were left in beakers of water overnight with magnetic stirrers to disintegrate the sample without changing particle sizes. Subsequently the sample was poured onto the sieve cascade on a sieving machine (Retsch AS 200 digit, Haan, Germany) set to a vibration amplitude of approximately 2 mm, with a water throughput of 2 liters per minute, and sieved for 10 minutes. The size of the largest particles on the largest sieve was noted as the length of that particle (maximum particle length, or MPL). The remains on each sieve were transferred onto pre-weighed petri dishes, dried at 103°C for at least 15 h, and weighed after cooling to room temperature in a desiccator using an analysis balance with measuring accuracy of 1 mg (Kern AEJ 220-4M, Kern, Balingen, Germany). Larger seeds passed intact in the feces were
removed manually from the three largest sieves (16 mm, 8 mm, 4 mm), and their amount weighed individually for each sieve and subtracted. Thus, the results contained, for these three sieves, the dry matter weight of the complete fecal sample, and of the fecal sample without seeds. The total weight of the removed seeds (as a proportion of the total dry matter retained on all sieves) was used as a proxy for the amount of large-seeded fruit contributing to the fecal sample. Fig seeds were evident on smaller sieves, but were too numerous to be sorted out reliably.

Calculations

We used the dry-matter weights on each sieve to calculate fecal particle size (FPS) according to a variety of scenarios. FPS was calculated by first expressing the dry weight on each sieve as a proportion of the total dry mass retained on all sieves. Note that if the FPS is calculated for the whole cascade (FPS\_0.025-16), then the total dry mass is the sum of that retained on all these sieves, whereas if the FPS is calculated from a subset of the cascade (e.g., FPS\_0.125-4), then the total dry mass is only the sum of that retained on that subset of sieves (i.e., ignoring the material on the other sieves), and the proportions for each sieve change accordingly. The FPS was calculated according to the dMEAN procedure of Fritz et al. (2012) as

\[
FPS = \sum_{i=1}^{n} p(i) \times \frac{S(i+1) + S(i)}{2}
\]

where \( i \) is the number of sieves in the respective cascade (with 1 as the number of the smallest sieve), \( p(i) \) the proportion of dry matter on sieve \( i \), and \( S(i) \) the pore
size of the sieve. For the largest sieve $S(i_{\text{max}})$, no 'higher' sieve $S(i_{\text{max}}+1)$ exists by definition. In this case, the manually measured maximum particle length MPL is used to calculate the size of particles on that sieve, and $S(i+1)$ corresponds to MPL. Alternatively, particles on the largest sieve can simply be ascribed the size of that sieve, without considering MPL. In this way, FPS was calculated for various sieve cascades and scenarios with and without excluding large seeds. The different FPS are indicated by subscripts of the range of the sieve cascade (e.g. FPS$_{0.025-16}$), whether or not MPL was included (e.g. FPS$_{0.025-16MPL}$ vs. FPS$_{0.025-16}$), and whether or not seeds were included (e.g. FPS$_{0.025-16MPL(s)}$ vs. FPS$_{0.025-16MPL}$).

**Statistical analysis**

Because most data were not distributed normally we used nonparametric tests. Differences in the proportion of large fruit seeds and fecal dry matter concentration between the seasons were compared by U-test, correcting for multiple comparison by Sidak adjustment. We used the complete dataset (all 130 samples) to test for differences between the 13 calculated FPS measurements; these differences were assessed by related-samples Wilcoxon signed rank test between all pairs of measurements, again with Sidak adjustment. Next, means for each individual were calculated per season, to compare measurements for the two sexes (individuals > 14 years of age; U-test) and test for a correlation with age (Spearman's $\rho$) and with the proportion of large seeds within each season.

Subsequently, means for each individual were calculated for each fruit type (figs or drupes). Again, differences between sexes and correlations with age and
proportion of large seeds were evaluated, and the difference between the two fruit
types was assessed using the related-samples Wilcoxon signed rank test in those
14 individuals (males and females, aged six to 34 years) for which data were
available from periods of both fruit types. Finally, the data from these 14
individuals was used in a General Linear Model (GLM), using ranked data due to
a lack of normal distribution of the original data, with FPS as the dependent
variable, age as covariable and both sex and fruit type as cofactors. Statistical
analyses were performed using SPSS (IBM®, Version 22), with the significance
level generally set to 0.05.

RESULTS

Figure 1 shows a view of a typical fecal sample fractionated by sieve
analysis. The 16 mm sieve often contained large fibrous strands of stems or even
whole leaves as in Figure 1; large seeds (mainly from drupe fruits) were evident
on sieves of 4, 8 and 16 mm.

The proportion of such seeds as well as the fecal dry matter concentration
was significantly higher during the two drupe fruit seasons than during the fig
season (Table 2). Across all fecal samples, there was a highly significant positive
correlation between the proportion of large seeds and the fecal dry matter
concentration ($r = 0.58, P < 0.001$). The calculated proportion of particles
escaping the finest sieve averaged below 0.2, but showed high variation and
included negative values, indicating that the dry matter concentration measured in
the subsample for dry matter determination was most likely not representative of
the subsample used for sieve analysis; the proportion of escaped dry matter was
also significantly correlated with the fecal dry matter concentration \( r = 0.44, P < 0.001 \).

As expected, FPS calculated while excluding large seeds was invariably
smaller than those that included the weight of the seeds, and FPS calculated
including the MPL for the largest sieve were larger than those that did not use
MPL (Table 3). With the exception of two pair wise-comparisons, differences
between all 13 FPS calculated were significant. Excluding seeds and ignoring
MPL led to reductions in standard deviations (Table 3).

Within seasons, there were no differences in FPS between the sexes, and
there were no significant correlations with age or with the proportion of large
fruits in the fecal sample (Table 4). Rather, there was substantial variation across
all age-classes and for all fruit types (Fig. 2).

For those 14 individuals for which FPS measurements existed for both the
fig and the drupe fruit period (see above, “Statistical Analysis”), the FPS for the
fig period was always smaller. Most differences were statistically significant
(Table 5).
In the GLM using ranked data, FPS varied significantly among fruit
periods, but was not significantly associated with age and did not differ
significantly between the sexes (Table 6).

DISCUSSION

Chewing efficiency as measured by digesta or fecal particle size is a
crucial component of herbivore digestive physiology; for example, ungulate
herbivores can be ranked according to their chewing efficiency, their chewing
efficiency is linked to other physiological measurements such as digesta retention
times or digestibility, and scenarios of increasing chewing efficiency during
mammalian ungulate evolution have been invoked (Clauss et al. 2009, Fritz et al.
2009, Clauss et al. 2015). By contrast, chewing efficiency in frugivores has not
been studied in detail. The present study investigated individual and seasonal
variation in fecal particle sizes in wild chimpanzees. Apart from typical
methodological aspects of sieve analysis of feces, such as the choice of the sieve
sizes used in the sieve cascade, additional considerations apply when dealing with
frugivorous or omnivorous species (such as chimpanzees) as compared to
herbivorous ungulates. With an improved and standardized methodology for
frugivores, the study of FPS can provide informative comparisons of digestive
strategies and nutritional ecology beyond ungulate herbivores. For instance,
comparisons between humans and apes will be particularly instructive, given that
humans are adapted to external processing of their foods (Wrangham and
We here discuss methodology first, and then our specific results.

**Methodological aspects**

Previous work has shown the importance of using similar sieve cascades, and especially of using the same maximum and minimum sieve sizes, in comparative studies (Fritz et al., 2012); in particular, it is important that the smallest and the largest sieve size is similar. Our calculations using different cut-off sieve sizes at the lower end (Table 3) underline this fact. Additionally, a standardized method is required for dealing with those parts of the fecal matter that do not represent chewed material. In material that contains relevant proportions of sand from the ingestion of soil, for example, the amount of dry matter retained on a sieve must be corrected for its ash content (Schwarm et al., 2013). For frugivorous primates, the problem of including both large (cherry-kernel size) and small (fig-seed size) seeds in sieve analyses of fecal material was mentioned briefly by Matsuda et al. (2014), but it was not investigated how their inclusion or exclusion affects sieve results. Evidently, the inclusion of large seeds in the material retained on sieves used for the calculation of FPS will lead to larger values (Table 3) that do not appropriately reflect the chewing efficiency of the species, because chewing this material is mostly avoided. Manually removing seeds from the material retained on a sieve, and weighing them individually, as in the present study, allows for an assessment of this effect.

However, manual removal of seeds at an earlier instance might yield even
more reliable results. An important limitation of the present study was the large variation in the calculated amount of particulate matter passing through the finest sieve. If physical collection of this material is logistically not feasible under the respective laboratory conditions, as in the present study, then this fraction is calculated as the difference between the assumed total dry matter in the subsample submitted to sieve analysis and the sum of the dry matter actually retained on the sieves. The assumed dry matter is derived from drying another subsample of the same defecation, typically performed directly in the field. In the present study, both subsamples—the one submitted to drying, and the one submitted to sieve analysis—often contained large seeds. Large seeds contain less water than other fecal matter, and the dry matter content of fecal samples hence increased with an increasing proportion of large seeds contained in them. If the proportion of large seeds in both subsamples of a defecation is not identical—a likely possibility, given their large size and the comparatively small amount of total material defecated—then these discrepancies may easily lead to an over- or under-estimation of the actual non-seed dry matter contained in the subsample submitted to sieve analysis. This source of error could only be avoided if either the particles escaping the smallest sieve were also caught and quantified (e.g. via the use of filter paper or centrifugation of the eluent), or by removing (and quantifying) large seeds prior to dry matter analysis.

Additionally, we observed that the tough skins of drupe fruits such as *Pseudospondias microcarpa* and *Linociera johnsonii* were expelled almost entirely intact, and therefore should be treated as indigestible fruit material in the
same way as seeds (as done in FPS calculations that excluded material on 16 and
8 mm sieves). Similarly, some long, folded strands of foliage or stems, as well as
unchewed whole leaves and hair from mammals consumed in occasional
predation events, should be excluded from FPS calculations. Similar exclusions of
long, tangled matter have been recommended previously in a macroscopic study
of chimpanzee feces, albeit for a different particle weighing technique (Phillips
and McGrew, 2014).

Another methodological issue concerns a possible change in digesta
particle size during digestion. Although it has been shown that digestion has only
a small effect on particle size in ungulate herbivores (Poppi et al., 1980; McLeod
and Minson, 1988; Spalinger and Robbins, 1992), reduction in digesta particle
size along the digestive tract has been demonstrated for animals feeding on less
rigid plant material, such as dugongs (*Dugong dugon*) feeding on sea grass
(Lanyon and Sanson, 2006), or captive collared peccaries (*Pecari tajacu*) and a
captive sloth (*Choloepus didactylus*) fed diets consisting mainly of domesticated
fruits and/or vegetables (Schwarm et al., 2013). To what extent the wild fruits
ingested by free-ranging chimpanzees are subjected to particle size reduction
during digestion (as opposed to during ingestive mastication) is currently
unknown. Likewise, studies also remain to be performed on the degree to which
smaller seeds, such as fig seeds, are actually swallowed whole, or are deformed or
reduced in size by mastication.

Chimpanzee fecal particle size: effect of diet
Within each of the studied seasons there was no significant correlation between the proportion of seeds in the fecal sample and its FPS. However, there was a distinct and surprising difference among the fruit periods: FPS was larger when drupe fruits rather than figs were the main diet item. The opposite relationship was expected, since in Kanyawara drupes are generally preferred foods, whereas figs tend to be fallback foods (Wrangham et al., 1996; Marshall and Wrangham, 2007). In line with these expectations, the pulp of *F. natalensis* fruits has been found to contain less metabolizable energy (134-236 kilocalories/gram of organic matter) than the pulp of *P. microcarpa* (164-254 kcal/g OM) or *L. johnsonii* (207-284 kcal/g OM) (Conklin-Brittain et al., 2006). Consumption of drupes (including *Pseudospondias microcarpa*) has also been shown to correlate with reproductive timing for adult females in Kanyawara (Emery Thompson and Wrangham, 2008), again indicating their high energetic value. Drupes are therefore expected to be a higher-quality food. In theory the low FPS during the fig season could be explicable by chimpanzees ‘wadging’ a significant proportion of their fig meals, i.e. if they chewed the fruits and sucked the solubles but did not swallow the solids, leaving them as a ‘wadge’ that combined seeds and other low-value parts (skins, fibrous strands, and large particles of pulp). ‘Wadging’ could allow larger food particles to be over-represented in the unswallowed portions of the chewed food, leaving swallowed portions to have disproportionately low FPS. However, the Kanyawara chimpanzees do not ‘wadge’ figs of *F. natalensis*. Thus while wadging could
contribute to the reduction of FPS for some foods, it did not do so for *F. natalensis* fruits.

Alternatively, the presence of larger seeds in drupes (that are not spat out but swallowed whole) may negatively affect chewing efficiency, forcing chimpanzees to exert fewer and/or more restrained masticatory movements, in contrast to the small seeds in figs that can be masticated without difficulty. Additionally, during the fig season, THV was a larger proportion of the Kanyawara diet than during the drupe season. Fibrous vegetation, while a lower quality food option, does not contain these large seeds that may hinder chewing efficiency.

The reasons for lower FPS during the fig season are thus uncertain. Whatever the reason, however, the finding of low FPS during the fig season supports the hypothesis that in contrast to typical herbivores, particle size may be less critical for frugivores, because the less-preferred diet items apparently do not exhibit structural properties that render them less prone to particle size reduction.

**Comparison to other FPS studies in chimpanzees**

The FPS measured in this study was substantially higher than in two previous studies. FPS\textsubscript{0.063-16} Kanyawara was $4.15 \pm 3.33$ mm. In contrast, Matsuda et al. (2014) reported a mean FPS of $1.90 \pm 0.96$ mm for *P. t. schweinfurthii* in the Kalinzu Forest, Uganda, and recalculation of values for captive chimpanzees (probably mostly *P. t. verus*) given in Fritz et al. (2009) yields a mean FPS\textsubscript{0.063-16} value of $2.02$ mm. The comparison indicates a larger proportion of large (and hence potentially less well chewed) material in the chimpanzees of the present
study in general. However, when using the FPS<sub>0.063-8</sub> of the fig period (1.99 ± 1.29 mm) of the present study (and thus excluding large unchewed particles), the data are very similar. These results emphasize the importance for comparative studies not only of defining the diet items consumed by different populations, but also of defining how to handle material that was evidently not subjected to mastication.

**Chimpanzee fecal particle size: no relationship with sex or age**

Chimpanzees are moderately sexually dimorphic, with males averaging 33% higher body mass than females (Plavcan and van Schaik, 1992). However, there was no influence of sex on FPS. This finding corresponds to results from several other mammalian herbivores (sheep, equids, rhinoceroses, and elephants) where no intra-specific differences in FPS with body mass were evident (Pérez-Barbería et al., 2008; Clauss et al., 2015).

Similarly, but contrary to data from geladas (Venkataraman et al., 2014), FPS in chimpanzees showed no relationship to age. This result suggests that chimpanzee diets tend to have fewer mechanical demands on masticatory function than gelada diets. Folivores such as geladas consume foods that are both tough and hard, which necessitates more average daily chewing cycles and larger average bite forces compared to frugivorous primates (Taylor, 2002). To cope with this, geladas have a highly specialized hypsodont dentition with shearing crests that are formed from repeated use, similar to a mechanism described for Edward's sifakas (King et al., 2005) and mountain gorillas (Glowacka, et al., 2016), but that wear down substantially in old age. This wear pattern is apparently
responsible for prime adults having greater chewing, and thus digestive, efficiency than infants and past-prime adults (Venkataraman et al., 2014).

Irrespective of measures of particle size, associations between tooth wear and senescence have been demonstrated in primate species (e.g., King et al., 2005 for *Propithecus edwardsi*).

Our data indicate that chimpanzees, on the other hand, have consistent chewing efficiency throughout life. The youngest chimpanzees that we sampled were 16 to 45 months old. Those ages compare to 0-6 years as the period during which suckling occurs (Machanda et al., 2015). The oldest-aged samples came from 42- to 55-year-old individuals, sufficiently old for their teeth to be visibly worn (R. Wrangham, pers. obs.). Unfortunately, although wear in chimpanzee teeth has been demonstrated (Klukkert et al., 2012), a study that systematically links wear to age is still lacking. The fact that no age-related trends in FPS were detectable indicates that chewing efficiency remained stable both during the weaning process and even when molars become worn with age. This observation indicates that for a mainly frugivorous diet, particle size reduction may be less critical than for a folivorous diet—because there is no discernable optimization in FPS across the lifespan as evident by an age- or wear-induced loss of chewing efficiency. It also suggests that fruit pulp is less resistant to mastication, and requires less wear-susceptible adaptations, than leaves or grasses. The former hypothesis could be tested using *in vitro* digestion assays with natural foods in different stages of particle size reduction (cf. Bjorndal et al., 1990). The latter hypothesis has been confirmed repeatedly in physical analyses of primate
feedstuffs (Lucas et al., 2012), and leads to the hypothesis that frugivores are less constrained, in their longevity, by dental functionality than folivores. Some support for this proposal could come from other observations. Ring-tailed lemurs (Lemur catta) are frugivores that can survive in the wild even with substantial tooth loss (Sauther et al., 2002; Cuozzo and Sauther, 2004). Similarly, edentulous chimpanzees have been recorded surviving in the wild for years (Miles and Grigson, 1990). The absence of an effect of age on FPS could thus be representative for other primates, including hominins, where tooth wear, tooth loss, and/or reduced chewing efficiency may represent less of a longevity constraint than in those mammalian species that rely on their teeth for the killing of live prey or the grinding of tough plant diets.

ACKNOWLEDGEMENTS

Research was funded through the Herchel Smith-Harvard Undergraduate Science Research Program. The Uganda Wildlife Authority, the Uganda National Council of Science and Technology, and the Makerere University Biological Field Station kindly provided permission and logistical help. For field assistance we thank Martin Muller, Melissa Emery Thompson, Zarin Machanda, Emily Otali and staff members of the Kibale Chimpanzee Project, where long-term support was funded by the Leakey Foundation, NSF grants 1355014 and 0849380, and NIH National Institute on Aging award R01AG049395. Research was non-invasive and adhered to the legal requirements of Uganda. We thank David Watts
and two anonymous reviewers for their helpful comments. The authors declare no conflicts of interest.

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Wrangham RW. 1995. Relationship of chimpanzee leaf-swallowing to a


**Figure 1** Wet sieved material from a single chimpanzee fecal sample. Sample collected from AT, an adult male, during the fig period when he was 15.2 years old. Large, undigested particles are apparent in the largest sieves.

**Figure 2** Mean fecal particle size for each individual (n=38) compared with age.

**Table 1** Demographic composition of the chimpanzee (*Pan troglodytes schweinfurthii*) sample population (numbers of individuals in each sex-age class).

<table>
<thead>
<tr>
<th></th>
<th>Infant (0-4 years)</th>
<th>Juvenile (5-9 years)</th>
<th>Adolescent (10-14 years)</th>
<th>Prime Adult (15-41 years)</th>
<th>Old Adult (42+ years)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>12</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>18</td>
<td>3</td>
<td>38</td>
</tr>
</tbody>
</table>
Table 2 Chimpanzee (Pan troglodytes schweinfurthii) fecal samples by season and dietary composition.

<table>
<thead>
<tr>
<th>Dates</th>
<th>Fecal samples/individuals (n)</th>
<th>Fruit species eaten most frequently</th>
<th>Number of food scans (% of scans with preferred fruit)</th>
<th>Proportion of large seeds mean ± SD (minimum-maximum)</th>
<th>Dry matter concentration (% wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 6-11</td>
<td>17/17</td>
<td>Ficus natalensis</td>
<td>330 (50)</td>
<td>0.02 ± 0.05a (0-0.19)</td>
<td>26.0 ± 2.3a (21.3-29.3)</td>
</tr>
<tr>
<td>July 5-19</td>
<td>67/33</td>
<td>Pseudospondias microcarpa</td>
<td>439 (77)</td>
<td>0.42 ± 0.46b (0-0.96)</td>
<td>35.4 ± 11.8b (17.2-63.3)</td>
</tr>
<tr>
<td>July 20-August 3</td>
<td>46/23</td>
<td>Linociera johnsonii</td>
<td>278 (73)</td>
<td>0.53 ± 0.14b (0.27-0.75)</td>
<td>31.7 ± 8.7b (16.3-53.9)</td>
</tr>
</tbody>
</table>

Food scans are the times recorded in KCP’s archive of 15-minute interval behavior scans in which the chimpanzees were observed feeding. For the proportion of large seeds and fecal dry matter concentration, different superscripts indicate significant differences between the seasons.

Table 3 Mean ± SD (minimum-maximum) fecal particle size (FPS) in 130 fecal samples of 38 individual chimpanzees (Pan troglodytes schweinfurthii) measured using different cascades of sieves (indicated by the sieve size of the smallest and largest sieve), with large seeds (s) or without them, and with or without using the maximum particle length (MPL) when including the largest sieve.

<table>
<thead>
<tr>
<th>Method</th>
<th>FPS (mm)</th>
<th>Proportion of particles passing the finest sieve</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025-16 MPL</td>
<td>9.03 ±6.59 (0.70-42.36)</td>
<td>0.17 ± 0.23 (-0.78-0.72)</td>
</tr>
<tr>
<td>0.025-16 MPL</td>
<td>5.17 ±6.58 (0.67-42.36)</td>
<td>b</td>
</tr>
<tr>
<td>0.025-16 (s)</td>
<td>7.54 ±3.55 (0.70-15.60)</td>
<td>c</td>
</tr>
<tr>
<td>0.025-16</td>
<td>3.95 ±3.30 (0.67-14.76)</td>
<td>d</td>
</tr>
<tr>
<td>0.025-8 (s)</td>
<td>6.45 ±3.17 (0.70-11.71)</td>
<td>e</td>
</tr>
<tr>
<td>0.025-8</td>
<td>3.10 ±2.39 (0.61-10.49)</td>
<td>f</td>
</tr>
<tr>
<td>0.025-4 (s)</td>
<td>1.75 ±0.89 (0.50-5.74)</td>
<td>g</td>
</tr>
<tr>
<td>0.025-4</td>
<td>1.62 ±0.84 (0.50-5.74)</td>
<td>h</td>
</tr>
<tr>
<td>0.063-16 MPL</td>
<td>9.28 ±6.65 (0.90-42.89)</td>
<td>i</td>
</tr>
<tr>
<td>0.063-16 MPL</td>
<td>5.41 ±6.71 (0.84-42.89)</td>
<td>j</td>
</tr>
<tr>
<td>0.063-16</td>
<td>4.15 ±3.33 (0.84-14.80)</td>
<td>k</td>
</tr>
<tr>
<td>0.063-8</td>
<td>3.29 ±2.41 (0.74-10.54)</td>
<td>bd</td>
</tr>
<tr>
<td>0.125-4</td>
<td>2.24 ±0.91 (0.59-5.78)</td>
<td>l</td>
</tr>
</tbody>
</table>

Different superscripts indicate significant differences between FPS measures.
Table 4 Results of statistical analyses (P values) testing for differences between the sexes (in adult animals) or for correlations with age and the proportion of large seeds in feces of chimpanzees (*Pan troglodytes schweinfurthii*) in the three different seasons of this study.

<table>
<thead>
<tr>
<th>FPS Method</th>
<th>Sex</th>
<th>Age</th>
<th>Proportion seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>0.025-16 MPL (s)</td>
<td>0.485</td>
<td>0.773</td>
<td>0.456</td>
</tr>
<tr>
<td>0.025-16 MPL</td>
<td>0.699</td>
<td>0.100</td>
<td>0.383</td>
</tr>
<tr>
<td>0.025-16 (s)</td>
<td>0.394</td>
<td>1.000</td>
<td>0.902</td>
</tr>
<tr>
<td>0.025-16</td>
<td>0.485</td>
<td>0.167</td>
<td>1.000</td>
</tr>
<tr>
<td>0.025-8 (s)</td>
<td>0.589</td>
<td>0.384</td>
<td>0.259</td>
</tr>
<tr>
<td>0.025-8</td>
<td>0.699</td>
<td>0.482</td>
<td>0.073</td>
</tr>
<tr>
<td>0.025-4 (s)</td>
<td>0.818</td>
<td>0.650</td>
<td>0.383</td>
</tr>
<tr>
<td>0.025-4</td>
<td>0.818</td>
<td>0.592</td>
<td>0.383</td>
</tr>
<tr>
<td>0.063-16 MPL (s)</td>
<td>0.485</td>
<td>0.711</td>
<td>0.383</td>
</tr>
<tr>
<td>0.063-16 MPL</td>
<td>0.699</td>
<td>0.100</td>
<td>0.383</td>
</tr>
<tr>
<td>0.063-16</td>
<td>0.485</td>
<td>0.167</td>
<td>1.000</td>
</tr>
<tr>
<td>0.063-8</td>
<td>0.699</td>
<td>0.482</td>
<td>0.073</td>
</tr>
<tr>
<td>0.125-4</td>
<td>0.818</td>
<td>0.536</td>
<td>0.710</td>
</tr>
</tbody>
</table>

Table 5 Mean SD (minimum-maximum) fecal particle size (FPS, in mm) in 14 chimpanzees (*Pan troglodytes schweinfurthii*) measured using different cascades of sieves (indicated by the sieve size of the smallest and largest sieve), with large seeds (s) or without them, and with or without using the maximum particle length (MPL) when including the largest sieve, measured both during the fig and the drupe fruit period, and the P values from pair-wise comparisons.

<table>
<thead>
<tr>
<th>FPS</th>
<th>Fig period</th>
<th>Drupe period</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025-16 MPL (s)</td>
<td>4.53 ±7.50 (0.99-30.04)</td>
<td>11.57 ±3.43 (6.88-19.52)</td>
<td>0.016</td>
</tr>
<tr>
<td>0.025-16 MPL</td>
<td>4.43 ±7.53 (0.99-30.04)</td>
<td>6.58 ±3.40 (2.45-13.32)</td>
<td>0.056</td>
</tr>
<tr>
<td>0.025-16 (s)</td>
<td>2.96 ±2.44 (0.92-10.00)</td>
<td>9.29 ±1.83 (6.79-12.51)</td>
<td>0.001</td>
</tr>
<tr>
<td>0.025-16</td>
<td>2.86 ±2.47 (0.92-10.00)</td>
<td>4.75 ±2.23 (2.45-8.81)</td>
<td>0.064</td>
</tr>
<tr>
<td>0.025-8 (s)</td>
<td>1.98 ±1.26 (0.80-4.34)</td>
<td>7.52 ±1.58 (5.10-10.63)</td>
<td>0.001</td>
</tr>
<tr>
<td>0.025-8</td>
<td>1.89 ±1.24 (0.80-4.34)</td>
<td>3.35 ±1.60 (1.50-7.02)</td>
<td>0.084</td>
</tr>
<tr>
<td>0.025-4 (s)</td>
<td>0.92 ±0.42 (0.54-2.12)</td>
<td>1.80 ±0.38 (1.17-2.46)</td>
<td>0.005</td>
</tr>
<tr>
<td>0.025-4</td>
<td>0.88 ±0.31 (0.54-1.61)</td>
<td>1.68 ±0.37 (1.17-2.46)</td>
<td>0.002</td>
</tr>
<tr>
<td>0.063-16 MPL (s)</td>
<td>4.71 ±7.73 (1.06-31.01)</td>
<td>11.80 ±3.44 (7.07-19.80)</td>
<td>0.016</td>
</tr>
<tr>
<td>0.063-16 MPL</td>
<td>4.62 ±7.76 (1.06-31.01)</td>
<td>6.83 ±3.42 (2.61-13.54)</td>
<td>0.056</td>
</tr>
<tr>
<td>0.063-16</td>
<td>3.00 ±2.55 (0.98-10.32)</td>
<td>4.95 ±2.23 (2.61-8.94)</td>
<td>0.064</td>
</tr>
<tr>
<td>0.063-8</td>
<td>1.99 ±1.29 (0.85-4.56)</td>
<td>3.54 ±1.61 (1.64-7.19)</td>
<td>0.084</td>
</tr>
<tr>
<td>0.125-4</td>
<td>1.07 ±0.32 (0.69-1.77)</td>
<td>2.38 ±0.41 (2.01-3.60)</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table 6 Results of General Linear Models (using ranked data) with fecal particle size (FPS) as the dependent variable, age as a covariable, and sex and fruit period (figs vs. drupes) as cofactors for 14 chimpanzees (*Pan troglodytes schweinfurthii*) sampled during both fruit periods.

<table>
<thead>
<tr>
<th>FPS</th>
<th>Age</th>
<th>Sex</th>
<th>Fruit type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F_{1,24}$</td>
<td>$P$</td>
<td>$F_{1,24}$</td>
</tr>
<tr>
<td>0.025-8</td>
<td>0.822</td>
<td>0.373</td>
<td>0.000</td>
</tr>
<tr>
<td>0.025-4</td>
<td>0.311</td>
<td>0.582</td>
<td>1.709</td>
</tr>
<tr>
<td>0.063-8</td>
<td>0.797</td>
<td>0.381</td>
<td>0.010</td>
</tr>
<tr>
<td>0.125-4</td>
<td>0.078</td>
<td>0.782</td>
<td>1.077</td>
</tr>
</tbody>
</table>