Effects of diet and age-sex class on fecal particle size of wild Japanese macaques (*Macaca fuscata yakui*)

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Abstract:

Fecal particle size provides important information on the feeding and digestion of herbivores. Understanding the effects of the potential proximate determinants on fecal particle size helps us interpret this widely used measurement. In folivores, previous studies found that diet composition, dietary toughness, and age-sex related factors, such as body size and tooth wear, influenced fecal particle size. However, the role of these factors remains unknown in frugivorous and omnivorous primates. This study aims to clarify how age-sex class and diet influence fecal particle size in omnivorous Japanese macaques in Yakushima. We expected that their variable diet and differences among age-sex classes would cause variations in fecal particle size. We simultaneously documented Japanese macaques' diet, dietary toughness, and fecal particle size in the lowland area of Yakushima in the period from March 2018 to April 2019. Unexpectedly, fecal particle size showed limited differences across months and no difference among age-sex classes. Dietary toughness showed no effects on fecal particle size, while the consumption of fruits showed only a marginally significant negative effect. Our data indicate that the results of chewing were not affected by dietary toughness in our study subjects, while age-sex classes showed no difference in food comminution. This lack of variation might derive from a diet with low dietary toughness. We also found that the physical structure of preferred foods played an important role in fecal particle size variations. These results suggest that food comminution is less variable in frugivorous and omnivorous primates compare to highly specialized species (e.g., geladas). Factors other than what we examined in this study, such as food physical structure and chewing behavior, should also be taken into consideration.

Keywords: Chewing; Fecal particle size; Intra-specific variation; Toughness; Japanese macaque

INTRODUCTION

Chewing is the most important step in reducing digesta particle size. It facilitates digestion by enlarging the surface area of food for chemical digestion (Bjorndal, Bolten, & Moore, 1990; Emanuele & Staples, 1988; Gerson, King, Kelly, & Kelly, 1988; Pond, Ellis, & Akin, 1984; Udén, 1992). As a result, chewing efficiency is a key factor that affects energy intake (Bjorndal et al., 1990; Bjorndal & Bolten, 1992; Schwarm, Ortmann, Wolf, Streich, & Clauss, 2009). Thus, a decrease in chewing efficiency may cause undernutrition and impact the fitness of animals (King et al., 2005; Venkataraman et al., 2014). Chewing efficiency is affected by a multitude of factors such as dental morphology, tooth wear, bite force, and chewing behavior (Pérez-Barbería & Gordon, 1998). However, it is difficult to directly measure chewing efficiency in free-ranging animals because digesta particle size cannot be measured with non-invasive methods. Since the digestion processes other than mastication have little influence on digesta particle size in terrestrial mammalian herbivores (McLeod & Minson, 1988a, 1988b; Poppi, Norton, Minson, & Hendricksen, 1980; Spalinger & Robbins, 1992), fecal particle size has been repeatedly used as a proxy for chewing efficiency (Dunbar & Bose, 1991; Fritz et al., 2009; Matsuda et al., 2014; Venkataraman et al., 2014; Weary, Wrangham, & Clauss, 2017). Previous studies have found that several factors affect fecal particle sizes in different primate species, such as diet composition (Thiry et al., 2018; Weary et al., 2017), dietary toughness and age (Venkataraman et al., 2014), and re-mastication (Matsuda et al., 2014). However, some of these factors only pertained to species with a highly specialized lifestyle (Matsuda et al., 2014; Venkataraman et al., 2014), while the determinants of fecal particle size remain unclear in other species (He, Honda, Kurihara, & Thiery, 2020). These results indicate that the role of ecological factors in the determination of fecal particle size warrants further investigation. Such knowledge could offer an insight into the ecology of primates from the perspective of chewing.

Previous studies have found fecal particle size variations among different species. With any given food, fecal particle size has been modeled to depends on tooth surface area and the number of chewing times per quantity of food (Pérez-Barbería & Gordon, 1998). As these two factors both scale to body mass with an exponent, the model predicted that fecal particle size would scale against body mass with an exponent. A comparison among mammalian taxa found that fecal particle size scaled to body mass with an exponent of 0.22 as the model predicted even without controlling diet, while rumination, complicated molar design, and specialized chewing behavior also played important roles in fecal particle size variations (Fritz et al., 2009). In a comparison among nine simple-stomached primates, fecal particle size scaled to body mass with an exponent of 0.33 (Matsuda et al., 2014).

Diet can induce intra-specific variation in fecal particle size. Fecal particle size differs between captive and free-ranging ruminants that rely on different diets (Hummel et al., 2008). A study of chimpanzees (*Pan troglodytes*) found that they had larger fecal particle sizes during drupe seasons compared to the fig season (Weary et al., 2017). Proboscis monkeys (*Nasalis larvatus*) had smaller fecal particle sizes during the wet season, when a higher percentage of fecal samples containing intact seeds, than in the dry season (Thiry et al., 2018). Food mechanical properties, specifically, fracture toughness (i.e., the energy required for crack propagation), and the combination of toughness and stiffness (i.e., the ability to resist deformation), are critical factors influencing food comminution (Agrawal, Lucas, Prinz, & Bruce, 1997; Lucas, Prinz, Agrawal, & Bruce, 2002; Pérez-Barbería & Gordon, 1998). These factors are expected to link diet to fecal particle size. Toughness is also expected to play an important role in the feeding ecology of primates as a potential sensory cue for food selection (Dominy, Lucas, Osorio, & Yamashita, 2001; Lucas et al., 2012), but few studies have measured toughness and fecal particle size simultaneously. A study on a specialized graminoid-eater, the gelada (*Theropithecus gelada*), found that the consumption of tougher food during the dry season led to larger fecal particle sizes (Venkataraman et al., 2014). It remains unknown whether similar correlations exist in other less dietary specialized species. Japanese macaques (*Macaca fuscata yakui*) that relied on a potentially tough bamboo-dominant diet indeed had larger fecal particle sizes compared to their conspecifics that consumed a different diet (He et al., 2020). However, dietary toughness was not measured in that study.

Different age-sex classes may have different needs and abilities in reducing particle size. A few studies have provided equivocal evidence of age or sex differences in fecal particle size. Digestive physiology studies of Nubian ibex (*Capra ibex nubiana*) suggest that the females may modify their chewing behavior to compensate for the disadvantage of smaller body size, even though fecal particle size was not reported (Gross, Demment, Alkon, & Kotzman, 1995; Gross, Alkon, & Demment, 1996). In contrast, another study found no significant fecal particle size difference between sexes in dimorphic Soay sheep (*Ovis aries*) (Pérez-Barbería, Pérez-Fernández, Robertson, & Alvarez-Enríquez, 2008). A study of geladas found that older individuals suffered a more obvious increase in fecal particle size when consuming tough food than did younger individuals (Venkataraman et al., 2014), while two studies of chimpanzees found no difference among individuals of different ages and sexes (Schulz‐Kornas, Stuhlträger, Clauss, Wittig, & Kupczik, 2019; Weary et al., 2017).

Japanese macaques in Yakushima, Japan, are considered dietary generalists that have temporal and age-sex variations in their diet (Agetsuma, 1995, 2001; Hanya, 2003; Hanya, Noma, & Agetsuma, 2003; Hill, 1997; Maruhashi, 1980). Body size variations in this species (Hazama, 1964; Iwamoto, 1971) indicate potential age and sex differences in tooth size and digestive capacity. These variations make *M. fuscata yakui* a suitable model for investigating fecal particle size differences at the intra-specific level. The previous study of Japanese macaques in Yakushima found larger fecal particle sizes in the summit zone compared to the lowland in autumn and speculated that dietary composition and toughness were the causes (He et al., 2020). However, that speculation was based on fecal content analysis, which cannot assess diet composition in detail. Without direct observation of the macaques, that study could not examine the determinants of fecal particle size, including diet composition, toughness, and individual differences.

This study aims to reveal the determinants of fecal particle size in Japanese macaques in Yakushima, an omnivore. With fecal particle size, food toughness, diet, and demographic data that cover a full year, we examined the effects of dietary toughness, diet composition, age, and sex on fecal particle size. Base on the hypothesis that dietary toughness impedes chewing (Pérez-Barbería & Gordon, 1998; Venkataraman et al., 2014), we predicted that fecal particle size increases when dietary toughness increases. A previous study found smaller fecal particle sizes when proboscis monkeys consumed preferred food (Thiry et al., 2018). We predicted that higher fruit consumption leads to smaller fecal particle size, while higher mature leaf consumption results in larger fecal particle size. Based on the hypothesis that animals with smaller body mass have better chewing ability (Fritz et al., 2009; Gross et al., 1995, 1996; Matsuda et al., 2014), we predicted that fecal particle size would be larger in males than in females, while also being larger in mature individuals than in immature ones. Based on the hypothesis that tooth wear accumulation hinders chewing (King et al., 2005;

Millette, Sauther, Cuozzo, & Ness, 2012; Venkataraman et al., 2014), we predicted that fecal particle size increases along with senescence. These results contribute to the interpretation of fecal particle size in omnivores and thus a better understanding of the mechanisms that determine fecal particle size.

MATERIALS AND METHODS

Study site and subject

Yakushima island is located in the southwestern part of Japan (30°N, 131°E). According to the records from the Yakushima Meteorological Station in the eastern lowland area, from May 2018 to April 2019, the mean temperature was 21°C, and the temperature of the coldest month (January 2019) was 13.7°C, while the temperature of the hottest month (July 2018) was 27.4°C (Japan Meteorological Agency, 2019). The mean monthly precipitation was 288.6 mm (minimum in January 2019: 35.5 mm; maximum in July 2018: 816.5 mm) in this area during this period (Japan Meteorological Agency, 2019).

This study was conducted on a group of Japanese macaques (Umi-C) living in the lowland zone of western Yakushima from March 2018 to April 2019, except for April 2018. Demographic data came from the records of the annual census, information shared among researchers, and our observations during the study period. The individuals were divided into mature and immature based on the observed year of birth. Specifically, because individuals that were born in and after 2013 (i.e., ≤ six and half years old at the end of the study period) were younger than the reported average age of the complete eruption of permanent teeth (Iwamoto, Watanabe, & Hamada, 1987), we classified them as immature. The group consisted of 18 individuals when we started the study (six mature females, five mature males, three immature females, and four immature males, Table 1). Among them, two females were very old (23 and 26 years old, Table 1). Two immature males and a mature male emigrated, while two immature males and two mature males immigrated in November 2018.

Behavioral observation and fecal sample collection

We conducted 1-hour focal sampling on individuals older than 12 months by continuous observation (*N* = 1260, Table 2). The times of the start and end of each behavior were recorded to the nearest second. For feeding behavior, we recorded the species and the part eaten by the subjects. When direct counting was possible, we counted the number of food units consumed (Kurihara & Hanya, 2017; Nakagawa, 2009). A unit was operationally defined according to the particular food item (e.g., a leaf, a seed, a fruit) (Kurihara & Hanya, 2017; Nakagawa, 2009). We recorded feeding rates (units per second) when it is possible to continue direct counting for at least 60 consecutive seconds. When changing the focal subject, to equalize the observation time of different age-sex classes, we selected individuals from the age-sex class with the fewest observation hours.

Fecal samples were collected whenever possible right after defecation and preserved in sealed tubes with 75% ethanol. We noted the individual ID, collection date and time during collection. At least three samples were collected for each age-sex class in each month except for May and June 2018 (N = 381, Table 2). There was no biased pattern in the timing of collection within each month, except for May 2018. We must note that the sample sizes of behavioral observation and fecal sampling were small in May 2018, while we conducted the data collection at the end of that month.

Dietary toughness and dry weight of food items

To ensure that the plant samples came from the consumed plant and the location where the macaques foraged, we collected most samples from the intact food items dropped by the macaques during feeding. We then preserved them in plastic bags and tested their fracture toughness within 10 hours after collection. To ensure the toughness measurements reflected the toughness that macaques faced during chewing, we measured the parts that macaques would actually chew and swallow during foraging based on behavioral observation. For example, we observed that macaques always removed the midrib of the *Ficus superba* leaves. Thus, we also removed the midrib and tested the remaining part (lamina along with secondary ribs). We recorded 46 major food items that occupied more than 5% of monthly dry weight intake. At least 10 samples were measured for the 44 of these items. A rheometer (COMPAC100II, SUN Scientific Co., Ltd., Tokyo) was used for the measurement (Taniguchi, 2015). It drove a single blade cutting through samples and recorded the force every millisecond. The speed of cutting was set as 60 mm/min or 90 mm/min depending on the size of the sample. When the cross-section was a regular shape, we measured the length and thickness of the cut with a digital caliper (N-500, NAFCO Co., Japan) to calculate the intersectional area. When the cross-section was an irregular shape, we took a picture of it along with a caliper. We then calculated the area by comparing the pixel counts of the shape with the pixel counts of a 1 cm^2 area (determined by the scale on the caliper) in the photo. It was difficult to measure the toughness of insects due to their small size and complicated structure. Thus, we used a published value of 390 J/m^2 (Strait & Vincent, 1998), which did not change the overall results as insects occupied only 0.89% of the annual diet.

We measured the fresh weight of plant samples and then desiccated them in an oven until their weight became constant. Then we measured the dry weight of food intake units. We later used these values to estimate dry weight intake.

Fecal particle size

We employed wet sieving analysis to measure fecal particle size (Fritz, Streich, Schwarm, & Clauss, 2012). To assess the proportion of dry mass in a sample, approximately half of the sample was weighed and then dried to a constant weight. The remaining part was weighed and suspended in a beaker with water until it was fully dispersed. The suspension was then poured into a cascade consisting of nine sieves with square holes (from bottom to top, the hole sizes of sieves 1-9 are 0.063 mm, 0.125 mm, 0.250 mm, 0.500 mm, 1.000 mm, 2.000 mm, 4.000 mm, 8.000 mm and 16.000 mm, respectively, Retsch Test sieve, Germany). We rinsed the beaker with 1 L of water. We used a vibratory sieve shaker to shake the sieve cascade (Retsch AS200 digit, Germany) for 10 minutes with a vibration amplitude of 40% (approximately 2 mm). The water throughput was set to approximately 2 L/min. Unchewed large seeds and pulp pieces on sieves 7 and 8 were weighed separately before and after drying. We did not find any particles on sieve 9. Other particles on each sieve were transferred onto a pre-weighed tinfoil dish and desiccated at 103℃ in a vacuum oven for 24 h. After cooling to room temperature in the desiccator, we weighed samples using an analytical balance with a measuring accuracy of 0.0001 g (Shimadzu Libror AEG-220, Shimadzu Scientific Instruments, Japan). A previous study of Japanese macaques in Yakushima reported fecal particle size data in the lowland zone in all seasons, as well as data in the highland zone and the summit zone in summer and autumn (He et al., 2020). We used the fecal particle size data from that study for comparison.

Data analysis

Quantification of dietary composition, dietary toughness, and fecal particle size

We expressed the dietary composition based on the proportion of dry weight intake. First, we calculated the mean feeding rate of each food item for each age-sex class. Second, for feeding records that did not include the number of intake units, this was calculated by multiplying the time and the mean feeding rate. Then, we multiplied that number with the dry weight of the corresponding food intake unit to obtain the dry weight intake of each record. To simplify the analysis, we sorted food items into 7 categories: mature leaves, young leaves, seeds, unripe seeds, fruits, fungus, and others. The “others” category includes rare food items that occupied less than 5% of monthly dry weight intake, such as animal matter, bark, and soil. Finally, for each category, we summed all records for a month and then calculated its proportion of total intake. For the food items without data, we used the average value of the relevant food category.

To quantify dietary toughness, we calculated the cumulative fracture toughness (Venkataraman et al., 2014; Vogel et al., 2008), which is a weighted mean of fracture toughness during a certain period. This method integrates the food intake and the toughness of food items to ensured the dietary toughness became large only when macaques ate enough of the tough foods. First, the fracture toughness R (J/m^2) of food items was calculated as follows:

$$\mathrm{R}=\frac{\sum_{i=1}^{n}\mathrm{V}_c\times 0.001\times \mathrm{F}_i}{S_c} \qquad (1)$$

where V (m/s) is the speed of cutting, F_i (N) is the i-th force recorded, and S_c (m^2) is the intersectional area. Then, we calculated the weighted fracture toughness of each record by multiplying the fracture toughness of the item with the proportion of the dry weight intake during the record in the total intake of that session. Finally, to analyze the temporal variation of toughness, we summed these values to generate the sessional cumulative fracture toughness. To analyze the effects of toughness on fecal particle size, we used daily cumulative fracture toughness. This was calculated by the same method of sessional values but based on all of the diet data from one age-sex class in a day instead of a focal session. We used these daily cumulative values as the representative toughness for fecal samples because we could not accurately designate a fecal sample to a specific session without data on gut passage and the diet does not change drastically over several days.

To analyze fecal particle size, we chose the discrete mass particle size (dMEAN) as the measurement of mean particle size following a previous study (Fritz et al., 2012):

$$\mathrm{dMEAN} = \sum_{i=1}^{n} \mathrm{p}(i) \times \frac{S(i+1)+S(i)}{2} \qquad (2)$$

where *p(i)* is the proportion of particle mass retained on sieve *i* over the total mass of particles retained on all sieves and *S(i)* is the hole size of sieve *i* (mm). Thus, *S(1)* refers to the minimum hole size while *S(9)* refers to the maximum hole size. During calculation, we excluded the large seeds and pulp pieces that did not pass the pores of 8 mm size (i.e., on sieve 7 and sieve 8). These materials were only briefly chewed and were reported to cause larger values that do not appropriately reflect the chewing ability of a species (Weary et al., 2017).

Statistical analysis

To determine the variation in dietary toughness, diet composition, and fecal particle size, we performed the Kruskal-Wallis test with Dunn’s test (Bonferroni correction) for post-hoc comparison with different determinants as the factors.

Specifically, we tested the variations in the sessional cumulative fracture toughness across months, among age-sex classes, and among absolute ages. We examined whether the proportion of dry weight intake of each food category differed among age-sex classes, respectively. We also examined fracture toughness differences among the food categories. Then, we determined fecal particle size variations across months, among age-sex classes, and among absolute ages. For the Kruskal-Wallis test showed marginally significant results, we calculated epsilon-squared as effect size.

We compared the fecal particle size in our study group with the previous results from Japanese macaques in Yakushima. For each season, we used the Kruskal-Wallis test with Dunn’s test (Bonferroni correction) to examine the differences between our data and the data from other altitude zones.

To determine the effects of measured factors on fecal particle size, we ran two generalized linear mixed-effect model (GLMM) analyses with fecal particle size as the dependent variable and with either the daily cumulative fracture toughness, or the intake of each food category as the independent variables. To control the effects of age, sex, and sampling time, we used individual ID and sampling month as the random factors in both analyses. The values of measurements have very different scales, which impeded the proper fitting of GLMM. Thus, we rescaled data by dividing them by the root square mean of each data category. All of the statistical analyses were performed in R 3.6.1 (R Core Team, 2019) with $\alpha = 0.05$.

Ethical note

This research was noninvasive and adhered to the legal requirements of Japan. This study complies with the American Society of Primatologists Principles for the ethical treatment of nonhuman primates and conformed to the Guidelines for Field Research established by the Ethics Committee of the Primate Research Institute, Kyoto University.

RESULTS

Diet and dietary toughness variation

We found significant differences across months in the sessional cumulative fracture toughness (Kruskal-Wallis test, $X^2 = 151.48$, df = 12, $P < 0.001$, for the results of Dunn's tests, Fig. 1). The lowest monthly mean cumulative fracture toughness was in May 2018 ($N = 24$, 385.31 ± SE 28.26 J/m^2), when fruits were dominant in the diet (*Myrica rubra* fruits and *Machilus thunbergii* fruits, 85% and 13% of the food intake, respectively, Fig. 2). We must note that this result only covered the end of that month. Excluding May 2018, the lowest monthly mean was in August 2018 ($N = 156$, 587.23 ± SE 27.96 J/m^2), when unripe seeds and fruits were important foods (41% and 34% of the food intake, respectively, Fig. 2). The highest monthly mean cumulative fracture toughness was in February 2019 ($N = 64$, 1479.34 ± SE 146.26 J/m^2), when the proportion of mature leaf intake reached the highest value (40%) in the study. The dietary toughness increased in June and July but remained lower than in November, January, and February (Dunn's tests, Fig. 1). During these summer months, the fruits of *Machilus thunbergii* (>60% in June and July) were the most important foods.

We detected no significant difference in cumulative fracture toughness among age-sex classes (Kruskal-Wallis test, $X^2 = 2.66$, df = 3, $P = 0.45$, Fig. 3). We found a marginally significant difference among absolute ages, while the effect size was small (Kruskal-Wallis test, $X^2 = 17.47$, df = 10, $P = 0.06$, epsilon-squared = 0.01). The dietary composition was in general similar among age-sex classes, except that immature males consumed more seeds than mature males (Kruskal-Wallis test, $X^2 = 11.25$, $P < 0.05$; Dunn's test, two-tailed, Z = 3.19, $P < 0.005$).

We found differences in toughness among the food categories. Fruit had lower toughness than mature leaf, fungi and seed, while unripe seed had a lower toughness than mature leaves (Kruskal-Wallis test, $X^2 = 30.43$, df = 5, $P < 0.001$; Dunn's test, two-tailed, fruit vs mature leaf, Z= -4.78, $P < 0.001$; fruit vs fungi, Z= -3.78, $P < 0.0005$; fruit vs seed, Z= -3.38, $P < 0.025$; unripe seed vs mature leaf, Z= -3.07, $P< 0.025$, Fig. 4).

Fecal particle size variations

We found a significant difference in fecal particle size across months (Kruskal-Wallis test, $X^2 = 80.32$, df = 12, $P < 0.001$, for the results of Dunn's tests, Fig. 5A). The largest monthly mean fecal particle size was in July 2018 ($N = 40$, 1.47 ± SE 0.06 mm), while the smallest value was in March 2018 ($N = 36$, 0.93 ± SE 0.08 mm). In general, the values were lower in spring than in any other period of the year, while it was higher in June and July than it was in most of the months. We did not find significant fecal particle size difference across age-sex classes (Kruskal-Wallis test, $X^2 = 3.82$, df = 3, *P* = 0.28,Fig. 6). The analysis on absolute ages found marginally significant differences, while the effect size was small (Kruskal-Wallis test, $X^2 = 17.47$, df = 10, *P* = 0.06, epsilon-squared = 0.05).

In comparison with the previous study (He et al., 2020), we found that, in autumn, the fecal particle size in the summit zone was significantly larger than the result in our study group (Umi-C) (Kruskal-Wallis test, $X^2 = 25.60$, df = 3, *P* <0.001; Dunn's test, two-tailed, summit vs this study, Z= 4.54, *P* < 0.0005, Fig. 5B). The results of this study were similar to the previous data collected in the lowland zone in all seasons (Kruskal-Wallis test, spring, $X^2 = 1.64$, df = 1, *P* = 0.20; summer, $X^2 = 2.43$, df = 3, *P* = 0.49; autumn, $X^2 = 25.60$, df = 3, *P* <0.001, Dunn's test, lowland vs this study, Z=0.45, *P* = 1.00; winter, $X^2 = 0.12$, df = 1, *P* = 0.73,Fig. 5B).

Factors affecting fecal particle size

The results of GLMM analysis showed toughness did not influence fecal particle size (Table 3A), and only the intake of fruits showed a marginally significant negative effect on fecal particle size (Table 3B).

DISCUSSION

Contrary to our prediction, although dietary toughness had significant temporal variations, it had no effect on fecal particle size. This result indicates that the food toughness is not a limiting factor for the chewing of Japanese macaques in the lowland area of Yakushima. This may be due to the relatively low dietary toughness (monthly cumulative weighted toughness: $N = 13$, mean = 867.43 ± SE 81.55 $\mathrm{J/m^2}$; minimum = 385.00 $\mathrm{J/m^2}$; maximum = 2191.00 $\mathrm{J/m^2}$). In comparison, geladas had a constantly high dietary toughness (mean = 1707.00 $\mathrm{J/m^2}$; minimum = 1133.00 $\mathrm{J/m^2}$; maximum = 2128.00 $\mathrm{J/m^2}$) and a fecal particle size that increased when the dietary toughness was higher (Venkataraman et al., 2014). The less mechanically challenging diet of our study subjects may not obviously influence chewing. Although the gelada study supported the idea that tough food impedes chewing, our results highlight the possibility that such an impediment does not appear in other primates, especially in frugivores and omnivores that consume a diet with lower toughness (Fig. 2). However, the effects of toughness may also appear in these species under certain conditions. For example, we found that the fecal particle size in the summit area of Yakushima was larger than in our study group (Fig. 5B). This suggests Japanese macaques that rely on a potentially tougher diet may show a different variation pattern in fecal particle size than our study subjects. Unfortunately, existing studies of those groups have reported neither diet information nor data on dietary toughness. Further studies in the summit area of Yakushima or northern Japan could provide a better understanding of the role of toughness in the determination of fecal particle size.

Chewing behavior is another factor that may limit the effects of toughness (Pérez-Barbería & Gordon, 1998). Spending a longer time chewing tougher food may counterbalance the influence of dietary toughness. The fact that our study subjects foraged more slowly (less dry weight intake per second) when dietary toughness became higher supports this conjecture. A similar correlation has also been reported in other primates (Coiner-Collier et al., 2016). However, the foraging rate was influenced by many undocumented factors such as food distribution, food processing, and the size of cheek pouch. Without data on these variables and chewing time, we cannot draw a solid conclusion on the effects of chewing behavior.

We predicted that higher fruit consumption would reduce fecal particle size, while mature leaf consumption would increase it. However, among all of the food categories, only fruit consumption had a marginally significant negative effect on fecal particle size, which means only the increase in fruit consumption had potential to decrease fecal particle size. Unexpectedly, the largest fecal particle size appeared in June and July when fruits were the dominant food (80% and 64% *Machilus thunbergii* pulp, respectively). In the fecal samples, we noticed many broken pieces of the exocarp of *Machilus thunbergii*, which could explain this unexpectedly large fecal particle size. While consuming *Machilus thunbergii* pulps, monkeys can extract the paste-like mesocarp without too much chewing. We found that p(1~2)/p(0.5~1) (i.e., the ratio of the mass with particle size between 1 mm and 2 mm to the mass between 0.5 mm and 1 mm) and p(2~4)/p(0.5~1) in June and July were about twice as high as the mean value of the entire study period. This indicates that large particles of exocarp were swallowed. Although the large unchewed fragments were excluded during the experiments, the smaller insufficiently chewed exocarp still influenced the result. Such an effect of fruits could also explain why the previous study detected a larger fecal particle size in summer in this habitat (He et al., 2020). In contrast to folivores (Clauss, Nunn, Fritz, & Hummel, 2009; Clauss et al., 2015), it seems that the increased fecal particle size does not necessarily indicate difficulty in the digestion of Japanese macaques. Similar to chimpanzees (Weary et al., 2017), they consumed their preferred food regardless of how much it impedes the reduction of digesta particle size. These results suggest that the characteristics of specific food items and chewing behavior play an important role in determining fecal particle size. However, the diversity of food structures has complicated effects on digestion, where particle size reduction is not always important. Therefore, frugivory may have different needs in food processing compared to typical folivory. In particular, particle size reduction of indigestible components (such as exocarp) may not be as easy, nor as important, as the particle size reduction of digestible components (such as endocarp), though it will influence the results of overall particle size.

Age and sex variation do not support the hypothesis that individuals with smaller body mass have better chewing ability. We did not find fecal particle size differences among age-sex classes. These results indicate that juveniles with deciduous teeth had neither an advantage nor a disadvantage in food comminution. Considering that juveniles were not much different from adults in terms of the balance of food intake and energy requirement, their similar fecal particle size also supports the idea that juveniles are not more vulnerable to starvation (Hanya, 2003). Together with previous studies of Soay sheep (Pérez-Barbería et al., 2008), equids, rhinos, and elephants (Clauss et al., 2015), our results suggest that no correlation with body mass appears at the intra-specific level. Effects at the inter-specific level are derived from the tooth surface area, as well as chewing numbers per quantity of food, dental morphology, and other aspects of chewing physiology, in particular rumination (Fritz et al., 2009; Matsuda et al., 2014; Pérez-Barbería & Gordon, 1998). Such a lack of correlation is understandable, since the correlation between tooth size and body size is weaker at the intra-specific level (Lauer, 1975; Wood, 1979), and dental morphology and chewing physiology are likely to be similar between conspecifics. Again, it is also possible that the low dietary toughness was insufficient to produce detectable differences among age-sex classes, as mentioned in the previous section.

The six-year-old border of age classes may not be a precise reflection of tooth differences. The two classes have different total tooth surface areas since deciduous teeth are smaller than permanent teeth. Specifically, in this study, mature individuals had all of their permanent teeth, while immature individuals at least lacked their permanent third molars. However, the main occluding areas (i.e., the areas on the dental crowns that make contact during mastication) (Kato, Furuki, & Hasegawa, 1996) in Japanese macaques remain unknown, not to mention changes in the main occluding areas used during the eruptions of permanent teeth (1.5 to 6.5 years old, Iwamoto et al., 1987). The exact timing of the increase in the actual functional occlusal area remains unknown. In this study, we also did not find any difference among individuals of different absolute ages. Thus, the conclusion that individuals of different ages are similar in terms of food comminution remains reasonable. But for future studies, caution must be taken in defining age classes.

Old individuals in our study subjects did not suffer from an increase in fecal particle size, either. According to the reported association between tooth wear and age in captive Japanese macaques (Nass, 1981), they are likely to have worn teeth. This result does not support the hypothesis that tooth wear accumulation hinders chewing. Similar to the results in chimpanzees, they support the hypothesis that for frugivorous or omnivorous species, the factor of tooth wear in maintaining their longevity is less critical (Weary et al., 2017). By consuming a less mechanically challenging diet, worn teeth can still produce adequate chewing ability. Meanwhile, without specialized tooth structures, senescence may not significantly diminish tooth function. The lack of age-related fecal particle size variation in frugivorous chimpanzees agrees with this conjecture (Schulz-Kornas et al., 2019; Weary et al., 2017); the fact that wild ring-tailed lemurs can survive with tooth loss also support this notion (Cuozzo & Sauther, 2004; Sauther, Sussman, & Cuozzo, 2002). In comparison, the geladas' highly specialized dentition plays an important role in adapting to their graminoid-based diet (Jablonski, 1994, 2005; Swindler, 2002). They produced smaller fecal particle sizes compared to other primates of similar body mass, while old individuals with severely worn teeth produced larger fecal particle sizes in dry seasons (Venkataraman et al., 2014). However, the comparisons of tooth wear and fecal particle size are rare, and further investigation is necessary to examine a relevant hypothesis directly.

Although this study provided a much more comprehensive dataset compared to the previous work on fecal particle size in Japanese macaques in Yakushima (He et al., 2020), we recognize the limitations imposed by the small sample size and the biased timing of data collection in May 2018. Considering the results of April 2019, compared to our results, the diet in May should include more young leaves and mature leaves, which may lead to higher dietary toughness. Because fruits are the dominant food for at least one-third of the month, such differences are not likely to be large enough to influence the statistical results of other months and the subsequent conclusions. However, the data of diet composition, dietary toughness, and fecal particle size in that month should be considered tentative.

In conclusion, we found only limited fecal particle size variations in Japanese macaques in the lowland area of Yakushima, despite the existence of temporal differences in dietary toughness. We did not detect the effects of age or sex. These results indicate that the chewing ability of Japanese macaques is adequate to process most of their food into similar particle sizes in the Yakushima lowland area, despite some variations. Our analysis also showed that diet and dietary toughness were not important determinants for fecal particle size in such a condition. In general, primates are not outstanding in terms of food comminution compared to taxa specialized for folivory (e.g., ruminants and equids) (Dunbar & Bose, 1991; Fritz et al., 2009; Matsuda et al., 2014). They are very likely to compensate for such disadvantages with other strategies, such as avoiding mechanically challenging food (Glowacka et al., 2017; Hill & Lucas, 1996; Teaford, Lucas, Ungar, & Glander, 2006). Therefore, although fecal particle size has proved to be a useful tool for ecophysiology studies of many mammalian herbivores, detailed data from more primate species and populations are necessary for interpreting the relevance of this measure in this taxon.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Tables

Table 1 Demographic data of the study group (Umi-C)

Age-sex class	Individual ID	Age	Year of birth	Note
Mature Male	Yubinashi	15	2004	
	Betamale	14	2005	
	Sora23	11	2008	Immigrated in Nov. 2018
	Beautyspot	9	2010	
	Brokennose	9	2010	Emigrated in Nov. 2018
	Tearspot	9	2010	
	Tara	7	2012	Immigrated in Nov. 2018
Mature Female	Mizu	26	1993	
	Stem	23	1996	
	Squid	11	2008	
	Dot	17	2002	
	Dolphin	9	2010	
	Dola	7	2012	
Immature male	Scarear	5	2014	Emigrated in Nov. 2018
	Alex	5	2014	Immigrated in Nov. 2018
	Ted	5	2014	Immigrated in Nov. 2018
	Blackface	4	2015	Emigrated in Nov. 2018
	Squid2016	3	2016	
	Dot2016	3	2016	
Immature female	Stem2014	5	2014	
	Stem2016	3	2016	
	Dolphin2016	3	2016	

Note: Age refers to the age at the end of the study period (April 2019).
Four infants were born in 2018, they are not included because they mainly consumed milk, and therefore were not the subjects of this study.

Table 2 Number of the focal sessions and fecal samples examined in each month and age-sex classes (the numbers of fecal samples are enclosed by parentheses)

Year	Month	Mature Female	Mature Male	Immature Female	Immature Male	Total
2018	**Mar**	26 (9)	26 (8)	26 (6)	26 (6)	104 (29)
	Apr	-	-	-	-	-
	May	6 (6)	6 (4)	6 (0)	6 (1)	24 (11)
	Jun	33 (11)	33 (5)	33 (4)	33 (1)	132 (21)
	Jul	23 (14)	23 (10)	23 (9)	23 (7)	92 (40)
	Aug	39 (9)	39 (11)	39 (9)	39 (13)	156 (42)
	Sep	29 (12)	29 (8)	29 (9)	29 (9)	116 (38)
	Oct	25 (6)	25 (6)	25 (5)	25 (6)	100 (23)
	Nov	29 (8)	29 (8)	29 (8)	29 (8)	116 (32)
	Dec	21 (4)	21 (5)	21 (5)	21 (5)	84 (19)
2019	**Jan**	24 (8)	24 (8)	24 (8)	24 (8)	96 (32)
	Feb	16 (7)	16 (7)	16 (7)	16 (7)	64 (28)
	Mar	23 (9)	23 (9)	23 (9)	23 (9)	92 (36)
	Apr	21 (9)	21 (7)	21 (6)	21 (8)	84 (30)
Total		315 (112)	315 (96)	315 (85)	315 (88)	1260 (381)

Table 3 Summary of the generalized linear models for the effect of daily cumulative fracture toughness, food intake on fecal particle size (dMEAN)

Independent Variables	Coefficient Estimate	Standard Error	t-values	*P*-values	
A) Effects of cumulative fracture toughness on fecal particle size					
(Intercept)	0.99	0.05	19.80	<0.001	***
Cumulative Toughness	-0.01	0.02	-0.27	0.79	
B) Effects of food intake on fecal particle size					
(Intercept)	0.98	0.04	21.84	<0.001	***
Seed	0.01	0.02	0.64	0.52	
Unripe Seed	0.02	0.02	1.52	0.13	
Fruit	-0.04	0.02	-1.92	0.05	•
Other	-0.00	0.01	-0.23	0.82	
Mature Leaf	-0.02	0.02	-1.16	0.25	
Young Leaf	0.01	0.01	0.87	0.38	
Fungi	-0.00	0.01	-0.14	0.89	

Note: GLMM with Gamma distribution family, and identity link function.

*** $P < 0.001$; **$0.001 < P < 0.01$; * $0.01 < P < 0.05$; • $0.05 < P < 0.10$

FIGURES

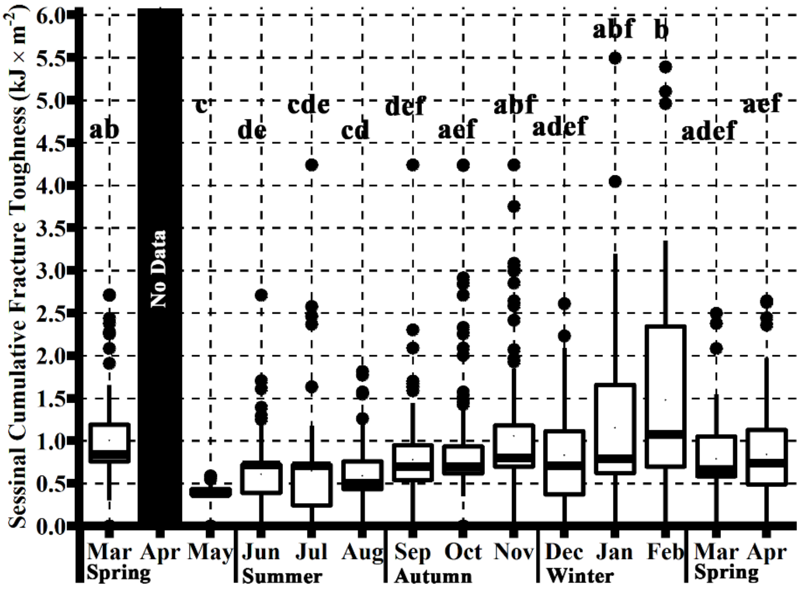

Figure 1. Monthly difference in dietary fracture toughness (sessional cumulative fracture toughness). Each observation represents the dietary fracture toughness of a focal session. Central horizontal lines in the plot represent medians, and the box indicates the first and third quartiles. The lines represent the range of the data and the outliers are indicated with dots. The results of the Dunn's test (Bonferroni correction) are shown by a compact letter display: shared letters represent statistically indistinguishable months (*P* > 0.025).

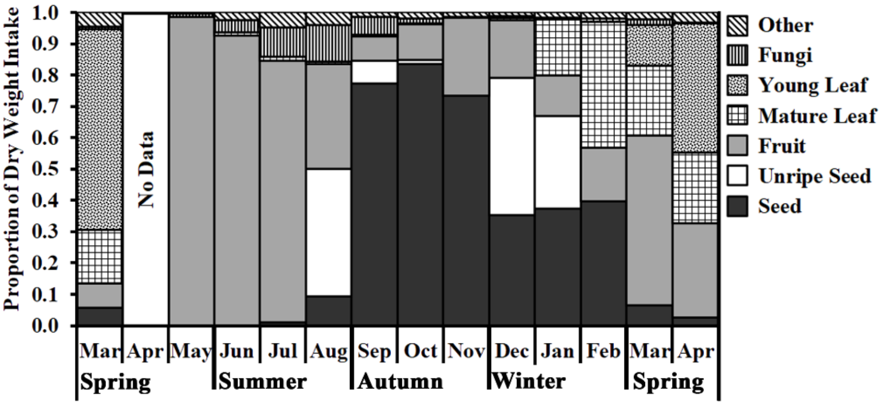

Figure 2. Monthly change in diet composition based on the dry weight of each food category.

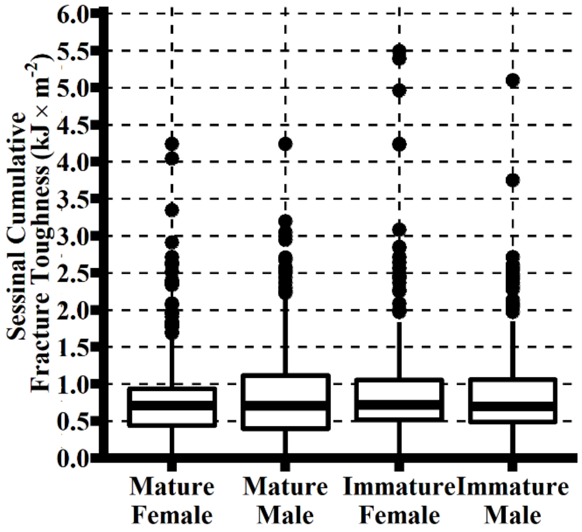

Figure 3. Dietary toughness (sessional cumulative fracture toughness) in different age-sex classes. Each observation represents the dietary fracture toughness of a focal session. Central horizontal lines in the plot represent medians, and the box indicates the first and third quartiles. The lines represent the range of the data and the outliers are indicated with dots.

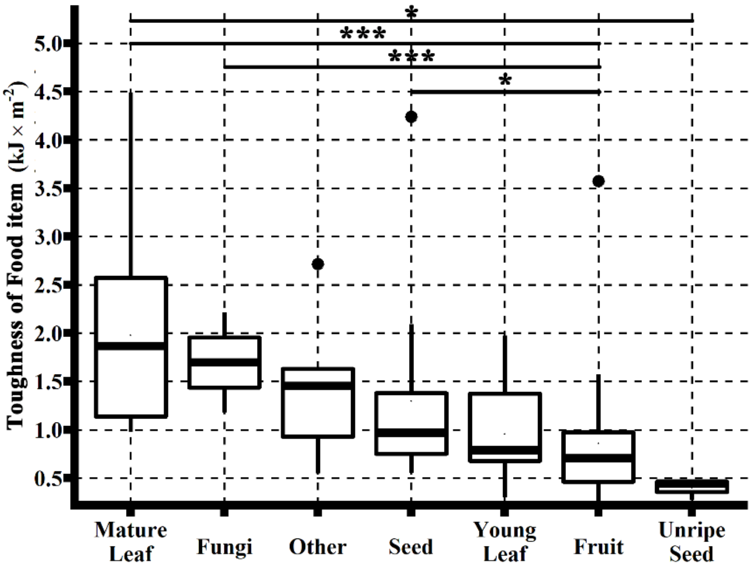

Figure 4. Toughness difference among food categories. Sorted from the highest to the lowest. Each observation represents the fracture toughness of a food item. Central horizontal lines in the plot represent medians, and the box indicates the first and third quartiles. The lines represent the range of the data and the outliers are indicated with dots. * $0.005 \leq P < 0.025$, ** $0.0005 \leq P < 0.005$, *** $P < 0.0005$.

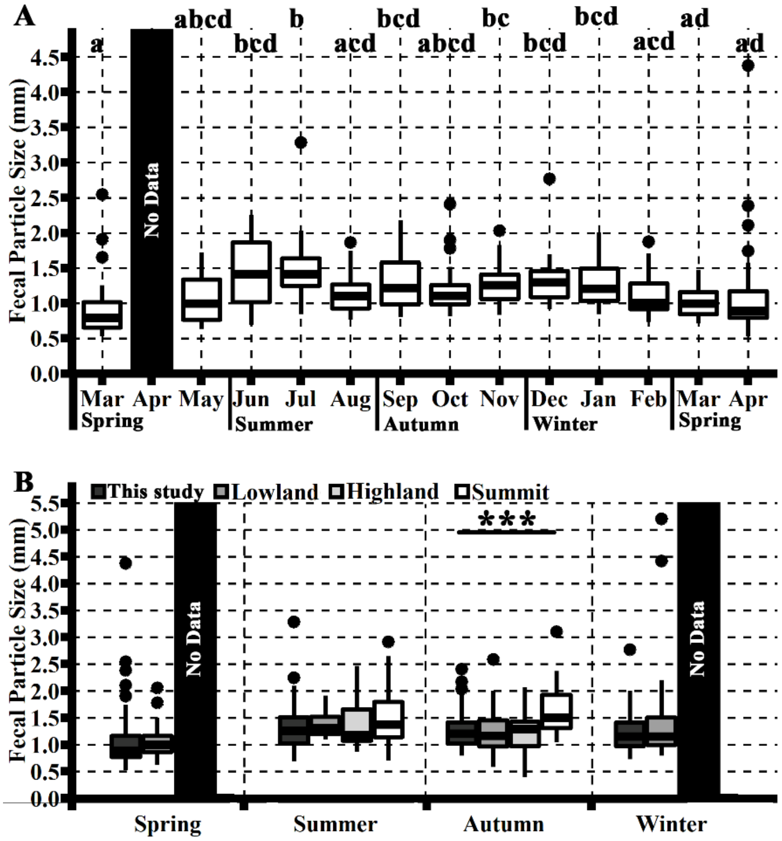

Figure 5. The monthly difference in fecal particle size (dMEAN). Each observation represents the dMEAN of a fecal sample. Central horizontal lines in the plot represent medians, and the box indicates the first and third quartiles. The lines represent the range of the data and the outliers are indicated with dots. A) Monthly differences in our study subjects. The results of the Dunn’s test (Bonferroni correction) are shown by a compact letter display: shared letters represent statistically indistinguishable months (*P* > 0.025). B) Comparison with a previous study on Japanese macaques in Yakushima. The data of the lowland, the highland, and the summit habitat came from a previous study (He et al., 2020). * 0.005 ≤ *P* < 0.025, ** 0.0005 ≤ *P* < 0.005, *** *P* < 0.0005.

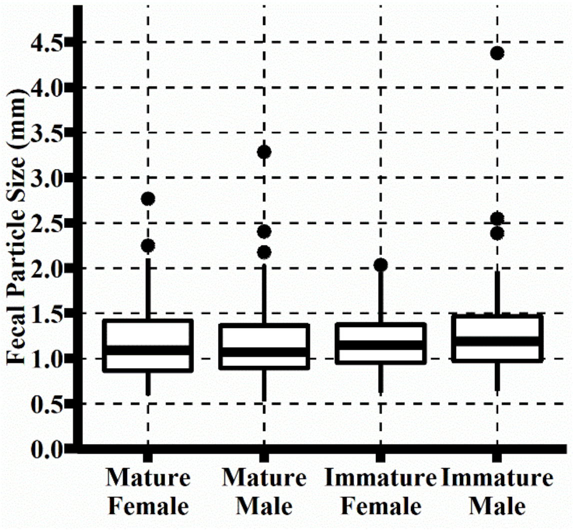

Figure 6. Fecal particle size (dMEAN) in different age-sex classes. Each observation represents the dMEAN of a fecal sample. Central horizontal lines in the plot represent medians, and the box indicates the first and third quartiles. The lines represent the range of the data and the outliers are indicated with dots.