Effects of the physical characteristics of seeds on gastrointestinal passage time in captive Japanese macaques

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Short title: Seed characteristics and passage time of Japanese macaques

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Abstract

The time it takes seeds to pass through the gut of vertebrates is an important aspect of endozoochorous seed dispersal because it influences seed dispersal distance. The physical characteristics of seeds (e.g., dry seed weight, volume, and specific gravity) vary among plant species, which might cause a difference in seed movement through the gastrointestinal system. We conducted feeding experiments with captive female Japanese macaques (*Macaca fuscata*) (n = 5) using eight different types of seeds to evaluate the effects of the physical characteristics of seeds on their passage time. Median seed recovery percentage for the real seeds was 35.5 % (range, 24%–78 %). Among three passage time variables examined, mean retention time (*MRT*) (37–54 hr) and time of last appearance of a seed (*TLA*) (53–109 hr) differed significantly among seed types, and the latter differed significantly among individuals. Transit time (*TT*) (22–35 hr) did not. The generalized linear models (GLM) selected dry seed weight as the most important factor affecting *MRT*, while specific gravity of seeds as the most important factor affecting *TLA*. This implies that 1) heavier seeds and (or) seeds with greater specific gravity remain in the gut longer and are likely to be dispersed farther from the parent plant, and 2) the lighter seeds and (or) seeds with lower specific gravity are dispersed nearer the parent. Our study demonstrated the importance of considering the effects of the physical characteristics of seeds on the manner which primates disperse plant species, though we should consider the effect of the individual variation in the passage time, too.

Key words: Japanese macaque, passage time, seed dispersal, seed size, specific gravity

Introduction

Endozoochory is the dispersal of seeds that pass unharmed through digestive tracts of animals. According to Pollux *et al*. (2007), endozoochory depends on the following four stages: 1) the probability that seeds are ingested by animals; 2) the time of seed retention in the digestive system (i.e., passage time); 3) the resistance of seeds to digestion, and 4) the viability and germination rate of seeds after passage through the gut.

Among these factors, passage time influences the dispersal distance of seeds (Link & Di Fiore, 2006). In several plant species, seeds moved further away from the parent plants have a greater chance of survival (Garber, 1986), though this may not be the case for all plant species and (or) every season (Augspurger, 1984; Chapman & Chapman, 1996). To date, the retention time of seeds in the primate gut has been studied particularly through feeding experiments using particle markers that imitate real seeds (e.g., Dierenfeld, Koontz & Goldstein, 1992; Maisels, 1993) and on a few occasions by direct observation of wild primates (Garber, 1986; Julliot, 1996).

There are numerous variables which should affect the passage time: time of day, health / age / sex of the animal, stress, quantity / quality of foods, etc. Among these factors, our focus in this study is on the physical characteristics of seeds (e.g., seed size, shape, coat hardness, and external structure). The physical characteristics of seeds differ among plant species. These variations in seed characteristics may have an important influence on the passage time in the gut of animal dispersers (Traveset, 1998). For example, Leavy & Grajal (1991) and Schwarm *et al*. (2008) showed negative correlations between seed size (mm) and passage time in cedar waxwings *Bombycilla cedrorum* and the pygmy hippopotamus *Hexaprotodon liberiensis*, respectively. In such cases, smaller seeds would be dispersed farther from parent plants. In contrast, Julliot (1996), Wotton, Clout & Kelly (2008), and Schwarm *et al*. (2008) provided evidence of a positive correlation between seed size and passage time in red howler monkeys *Alouatta seniculus*, New Zealand pigeons *Hemiphaga novaezeelandiae*, and bantengs *Bos javanicus*. Finally, Garber (1986) and Gardener, McIvor & Jansen (1993) showed a negative correlation between the specific gravity of seeds and the passage time for two species of tamarin monkeys, *Saguinus mystax* and *S. fuscicollis* and cattle *Bos taurus*. Other studies have found no clear relationships between the physical characteristics of seeds and passage time (emu *Dromaius novaehollandiae*: Wilson (1989); arctic fox *Alopex lagopus*: Graae, Pagh & Bruun (2004); two species of fox *Pseudolopex gymnocerus* and *Cerdocyon thous*: Varela & Bucher (2006); carp *Cyprinus carpio*: Pollux *et al*. (2007)). Thus, it appears that the relationships between the physical characteristics of seeds and passage time vary among animal species and may depend on difference in the size / morphology of the digestive system and the digestive processes of the subject animals. Thus, this relationship must be studied in individual species.

Primates commonly consume large amounts of fleshy fruit, and often void the intact seeds while moving or during rest periods (*Alouatta seniculus* and *Lagothrix lagotricha*: Yumoto, Kitamura & Nishimura, 1999; *A. guariba* and *Brachyteles arachnoides*: Martins, 2006; *Macaca fascicularis*: Lucas & Corlett, 1998; *Papio anubis*: Kunz & Linsenmair, 2008a; *Cercopithecus* spp. and *Pan troglodytes*: Lambert, 2002; *Gorilla gorilla*: Remis, 2000). Differences in the ranging patterns and dietary preference of individual primate species, along with physical characteristics of seeds swallowed influence their passage time through the digestive system. Since primates move from several hundred meters to several kilometers daily (e.g., Raemakers, 1980), a difference in passage time can result in marked difference of several hundred meters in seed dispersal distance. For example, Link & Di Fiore (2006) reported that seed dispersal distances of 38 plant species averaged 443 m, with about 2 % of seeds retained in the gut for over 6 hours and dispersed more than 1250 m away from the parent plant. However, few studies thus far have considered the effects of the physical characteristics of seeds on primate gut passage time. A better understanding of the effects of the physical characteristics of seeds on passage time is necessary for evaluating the dispersal distance of seeds by primates.

In the present study we conducted feeding experiments with captive Japanese macaques (*Macaca fuscata*), an important seed disperser in temperate forests of Japan (Yumoto, Noma & Maruhashi, 1998; Otani & Shibata, 2000; Otani, 2003). We tested one prediction: passage time through the macaque gut differed based on the physical characteristics of the seeds.

Materials and methods

Our methodology complied with protocols approved by the guidelines (Guide for the Care and Use of Laboratory Primates, Second Edition) of the Primate Research Institute, Kyoto University, Japan, and adhered to Japan's legal requirements.

Study animals and their housing conditions

Study animals were 5 adult (>10 years) female Japanese macaques housed at the Primate Research Institute, Kyoto University, Japan (body weight: 6.7 – 10.5 kg). Each animal was reared in an individual cage (W 760 mm × L 900 mm × H 850 mm) in an air-conditioned (20°C) experimental room. All 5 animals were active and in good condition. None were lactating or pregnant. Difference in the body weight of the monkeys before and after the experiments were not significant (paired *t*-test, $df = 4$, $t = 1.18$, $P >$

0.05).

The monkeys were fed 100 g of monkey chow twice a day (10:00 and 14:00). They were also fed 35 g of sweet potatoes for morning meals three times per week. These are high quality foods relative to many of the foods consumed by Japanese macaques in the wild (Mori, 1979; Nakagawa *et al*. 1996). We alone entered the experimental room as a health precaution and to minimize any stress to the animals that might affect gut retention times.

Seed administration

Eight experimental trials were conducted from June to September 2008 (Table 1). We did not change the macaques' housing conditions or diet composition during the experiments, except that the sweet potatoes were replaced by chunks of banana (ca. 100g) in which we inserted experimental seeds (see below). During each experiment, we continued to feed the chunks of banana without seeds three times per week.

A trial consisted of providing the monkeys with nontoxic plastic seeds (two sizes of white plastic beads) or real seeds (six types of commercial seeds) with varied dimensions (Table 1). We used commercial seeds to assure similar-sized seeds. Before the experiment we measured the length, width, and height of each seed (n = 10) with a vernier caliper

126 (THS-30, Niigata Seiki Co., Japan) to the nearest 0.05 mm. We also weighed dry seeds on
127 an electric balance (UX4200H, Shimadzu Co., Japan) to the nearest 0.01 mg. We
128 estimated seed volume based on the following formula suggested by Garber (1986):

129 $$V = \pi R^2\left(L - \frac{2}{3}R\right),$$

130 where R = (seed width + height)/4, and L = seed length. The specific gravity of the seed
131 (mg·mm^{-3}) was calculated as (dry seed weight/seed volume).

132 We inserted both plastic and real seeds into chunks of banana and fed them to the
133 monkeys. We adjusted the number of seeds inserted into the banana to total
134 approximately 400–500 mm^3 to eliminate the effect of total seed volume on the passage
135 time (Table 1). Each animal was fed seed-loaded bananas once during the morning meal
136 (10:00). Single type of seeds was fed to a given animal for each experiment, and same
137 type of seeds was fed to all macaques on a given experiment. We visited the experimental
138 room at least every two hours from 6:00 to 18:00 to determine whether the monkeys had
139 defecated. Since we did not observe defecating behavior, the passage time was estimated
140 at two-hour intervals. Though we did not observe the monkeys during the night
141 (18:00–6:00), this would not affect the results because the monkeys rarely (only twice)
142 defecated during this time. Each fecal sample collected was washed and screened by a
143 sieve (mesh size, 0.5 mm) to determine whether seeds were present. If we found seeds,

the time of the sample collection and the number of seeds were recorded. We ended an experimental session when no new seed was found in the feces within 24 hours from the last seed appearance. We started the next experimental session after at least a one-day interval from the previous experiment.

From the obtained data we calculated time of first appearance of a seed: transit time (*TT*), time of last appearance of a seed (*TLA*), and mean retention time (*MRT*). The *MRT* was calculated according to the following formula (Lambert, 2002):

$$MRT = \sum_{i=1} m_i t_i \Big/ \sum_{i=1} m,$$

where m_i = the number of seeds excreted at the ith defecation at time t_i (hr) after ingestion.

Statistical analyses

We tested the effects of the seed type (plastic and real) on the percentage of seed recovery and on the three variables associated with passage time (*TT*, *MRT*, and *TLA*), and the effects of individual macaque on the passage times. For these analyses, we calculated the median instead of the mean and standard deviation, and employed Friedman's two-way ANOVAs since our data were not normally distributed (tested by Shapiro-Wilk normality test, $P < 0.05$). We tested correlations between the percentage of seed recovery and physical dimensions of seeds, and correlations among the three passage time variables using Spearman's correlation analyses. Significance levels were set at 5% for these analyses. We examined the effects of the physical characteristics of seeds (dry weight, volume, and specific gravity) on the passage time using the generalized linear models (GLM). We assumed gamma distributions for the three variables associated with passage time. Then the best model was determined by removing independent variables that did not improve Akaike's information criterion (*AIC*) compared to that for the full model. All data analyses were carried out using the statistical software R version 2.4.1 (R Development Core Team, 2006).

Results

Administration and recovery of seeds

The monkeys readily and immediately consumed the real and plastic seeds concealed in chunks of banana. Spitting out was rarely observed for the real seeds (n = 5, mean ± SD = 2.8 ± 6.4 % of seeds in a banana (six types mean)), while for the plastic seeds relatively many of the seeds were spat out (29.0 ± 17.6 % of larger and 10.5 ± 12.1 % of smaller seeds) (Table 1).

Among eight feeding trials, the median of seed recovery percentage for the real seeds per individual monkey was 35.5 % (range, 24%–78 %). The median percentage of plastic seeds recovered was 81.5 % (range, 65%–86 %) (Table 1). The percentage of seed recovery varied significantly among the real seed types (Friedman's two-way ANOVA, $\chi^2 = 18.94$, $df = 5$, $P < 0.01$), although none of the physical dimensions of the real seeds correlated with the percentage of recovery (Spearman's correlation analyses, $df = 5$, dry seed weight: $r_s = 0.26$, $P = 0.658$; seed volume: $r_s = 0.03$, $P = 1.000$; specific gravity of seed: $r_s = 0.60$, $P = 0.242$).

Gut passage time and physical characteristics of seeds

The passage rates measured by *TT*, *MRT*, and *TLA* were 22–35 hr (Fig. 1a), 37–54 hr (Fig. 1b), and 53–109 hr (Fig. 1c), respectively. We treated the three passage time variables as independent of each other because there were no correlations among them (Spearman's correlation analyses, $df = 7$, *TT* vs *MRT*: $r_s = 0.68$, $P = 0.062$; *TT* vs *TLA*: $r_s = 0.24$, $P = 0.568$; *MRT* vs *TLA*: $r_s = 0.70$, $P = 0.069$). Among these variables, *MRT* (median: 44.7 hr) and *TLA* (median: 70.0 hr) differed significantly among the seed types (Friedman's two-way ANOVA, $df = 7$, *MRT*: $\chi^2 = 14.58$, $P = 0.042$; *TLA*: $\chi^2 = 21.32$, $P = 0.003$). *TT* (median: 24.0 hr), however, did not differ significantly among the seed types ($\chi^2 = 11.63$, $P = 0.114$). On the other hand, *MRT* differed significantly among individual macaques (Friedman's two-way ANOVA, $df = 4$, $\chi^2 = 11.66$, $P = 0.020$), while *TT* and *TLA* did not

(TT: $\chi^2 = 1.60$, $P = 0.809$; TLA: $\chi^2 = 8.94$, $P = 0.062$). When we omitted the plastic beads from the analysis, only *TLA* differed significantly among the real seed types (Friedman's two-way ANOVA, $df = 5$, TT: $\chi^2 = 8.43$, $P = 0.634$; MRT: $\chi^2 = 6.91$, $P = 0.243$; TLA: $\chi^2 = 20.24$, $P = 0.001$). In this case, *TLA* also differed significantly among individual macaques (Friedman's two-way ANOVA, $df = 4$, $\chi^2 = 10.40$, $P = 0.034$), while *TT* and *MRT* did not (TT: $\chi^2 = 2.48$, $P = 0.648$; MRT: $\chi^2 = 8.48$, $P = 0.076$).

The GLM selected dry seed weight as a factor affecting *MRT* (positive effect), and specific gravity as a factor affecting *TLA* (positive effect) (Table 2). When we omitted the plastic beads from the analysis of the *TLA*, the GLM again selected specific gravity as the sole factor (positive) (Table 2).

Discussion

The median percentage of real seeds recovered per individual monkey was 36 %. This result is probably due to the fact that the Japanese macaques act both as seed dispersers and as seed predators (Yumoto *et al*., 1998; Otani and Shibata, 2000; Otani, 2003). Otani (2003), for example, found some cracked seeds of relatively large-seeded species (e.g., *Akebia trifoliata* (mean cubic diameter: 3.6 mm) and *Berchemia racemosa* (3.2 mm)) with their intact seeds in macaque feces. Percent seed recovery was not significantly correlated with dry seed weight, volume, or specific gravity. Kunz & Linsenmair (2008a) also reported the absence of a linear correlation between seed size and that of seed damage in their study of olive baboons *Papio anubis*. It is possible that characteristics of seeds, such as hardness or shape, may help to explain the low seed recovery rate of several seed types reported in our study. Corlett & Lucas (1990) reported that captive studies exaggerate seed predation, and this might also be the case in our study. Seed spitting is another handling behavior in cercopithecine monkeys, including macaques (Corlett & Lucas, 1990; Lucas & Corlett, 1998). For Japanese macaques in our study, however, spitting out of the seeds was rarely observed (3%), though the plastic beads were spat out more (29%) (Table 1). Small seed size might contribute to the lower percentage of seed spitting (Lucas & Corlett, 1998).

Measurements of the transit time (*TT*) and the mean retention time (*MRT*) in the female Japanese macaques in our study (*TT*, 22–35 hr, *MRT*, 37–54 hr) (Fig. 1a, 1b) were similar to those found in previous studies of cercopithecine monkeys (*TT*, 20 hr for *Cercopithecus ascanius*, 17 hr for *C. mitis*, 21 hr for *C. neglectus* (Lambert, 2002), and 23 hr for *Lophocebus albigena* (Maisels, 1993); *MRT*, 27 hr for *C. ascanius*, 25 hr for *C. mitis*, 34 hr for *C. neglectus* (Lambert, 2002), 38 hr for *L. albigena* (Maisels, 1993), and 39 hr for *Macaca fuscata* (Otani, 2004)). In general, seed passage time of Old World

234 monkeys is considerably longer than that reported for most species of New World
235 monkeys (Lambert, 1998; Chapman & Russo, 2007). Further, when the effects of body
236 size were removed, the passage time of Old World monkeys also is significantly greater
237 than that of apes (Lambert, 2002). Relatively long retention time is one of physiological
238 traits of cercopithecine monkeys, including Japanese macaques, and this might facilitate
239 more efficient consumption of a high fiber non-fruit diet and parts while maintaining a
240 greater capacity to detoxify secondary metabolites (Lambert, 2002).

241 Among the three variables of the passage time of seeds treated in this study, *TT* did
242 not differ among seed types used in the feeding experiments and among individual
243 macaques ($P > 0.05$). In contrast, *MRT* and *TLA* of a seed differed significantly among
244 seed types, and, for the latter, among individuals ($P < 0.05$): dry seed weight showed a
245 positive effect on *MRT*, while specific gravity of seeds showed a positive effect on *TLA*
246 (Table 2). Furthermore, it is noteworthy that the positive effect of the specific gravity on
247 *TLA* was irrelevant to the plastic beads, which had a quite high specific gravity. Our study
248 is the first to demonstrate the effect of the physical characteristics of seeds on the passage
249 time in cercopithecine monkeys, though we should consider the effect of the individual
250 variation in the passage times, too. Our results imply that 1) (at least) seeds with higher
251 specific gravity and (or) heavier seeds will be dispersed farther from the parent plants,

and 2) (at least) seeds with lower specific gravity and (or) the lighter seeds will be dispersed nearer the parent. Interestingly, the effect of specific gravity of seeds on the passage time was opposite to Garbers' (1986) result which suggested that the specific gravity of seeds was negatively correlated with passage time through the gut of two callitrichid species. The difference in the effect of specific gravity of seeds between the macaques and tamarins would be attributed to the difference in relative gut volume and digestive systems (Strier, 2000; Lambert, 1998), or due to the difference in metabolism between them.

On the basis of present results, testing the relationship between dispersal distribution of seeds and their performance (e.g., germination and growth of seedling) in the field is needed to draw conclusions about the adaptive significance of the effects of physical characteristics of the seeds on gut passage time.

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FIGURE LEGENDS

Figure 1. Relationships between the seed type (eight types) and the passage time variables: a) transit time (*TT*), b) mean retention time (*MRT*), and c) time of last appearance of a seed (*TLA*). Filled circles show mean values and bars show standard deviations (SD).

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Table 1. The physical characteristics of seeds used in feeding experiments and their fate.

	Physical characteristics of seeds						Dose		Fate of seeds (# of seeds, mean ± SD)		
	Size (mm, mean ± SD)			Dry weight	Volume[a]	Specific gravity[b]	No. of seeds in a banana	Total volume	Defecated	Spitted out	Destructed
Seed type	Length	Width	Height	(mg, mean ± SD)	(mm^3)	($mg \cdot mm^{-3}$)		(mm^3)			
Small beads	2.10 ± 0.09	2.12 ± 0.09	1.45 ± 0.12	9.60 ± 0.46	3.75	3.33	110	412.5	94.6 ± 18.2	11.6 ± 13.3	4.0 ± 5.2
Large beads	2.84 ± 0.11	2.76 ± 0.14	1.88 ± 0.13	25.70 ± 6.58	8.72	2.95	40	348.8	25.8 ± 11.1	11.6 ± 7.1	2.6 ± 4.7
Radish	4.14 ± 0.21	3.26 ± 0.21	2.53 ± 0.28	18.20 ± 2.78	20.84	0.87	20	416.8	7.2 ± 3.0	0.0 ± 0.0	12.8 ± 3.0
Eggplant	3.45 ± 0.17	2.89 ± 0.27	0.93 ± 0.12	4.04 ± 0.47	8.03	0.50	50	401.5	39.0 ± 6.9	0.2 ± 0.5	10.8 ± 6.8
Spinach	3.84 ± 0.40	3.23 ± 0.29	2.31 ± 0.30	11.35 ± 1.97	17.58	0.65	25	439.5	6.0 ± 2.9	0.4 ± 0.6	18.6 ± 2.5
Melon	6.24 ± 0.59	3.22 ± 0.16	0.99 ± 0.15	8.67[c]	19.21	0.45	20	384.2	9.4 ± 3.9	1.0 ± 2.2	9.6 ± 3.8
Potherb Mustard	1.62 ± 0.18	1.43 ± 0.12	1.43 ± 0.12	1.74 ± 0.35	1.83	0.95	230	420.9	56.0 ± 13.4	12.2 ± 27.3	161.8 ± 40.3
Bermuda grass	1.38 ± 0.09	0.71 ± 0.11	0.68 ± 0.11	0.19[d] ± 0.02	0.44	0.44	1300	572.0	538.0 ± 247.9	0.0 ± 0.0	762.0 ± 247.9

The sample number of seed measurements was ten for each species.

[a] Seed volume was calculated by using the following formula: $V = \pi R^2 (L - 2/3R)$; V = volume, R = (width + height) / 4, L = length.

[b] Calculated as dry weight / volume.

[c] Dry seed weight was measured only once.

[d] Dry seed weight was measured five times.

Table 2. Selected physical characteristics of seeds affecting passage time variables by GLM: mean retention time (*MRT*) and time of last appearance (*TLA*).

	Dependent variable		
	MRT	*TLA*	*TLA* (without beads)
Independent variable	Estimate ± SE	Estimate ± SE	Estimate ± SE
Intercept	3.736 ± 0.062***	4.071 ± 0.072***	3.915 ± 0.199***
Dry weight	0.011 ± 0.005	—	—
Volume	—	—	—
Specific gravity	—	0.167 ± 0.043**	0.416 ± 0.294

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$

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