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

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7 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

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7 32 characteristics of seeds on the manner which primates disperse plant species, though we
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10 33 should consider the effect of the individual variation in the passage time, too.
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16 35 Key words: Japanese macaque, passage time, seed dispersal, seed size, specific gravity
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Review Copy

36 Introduction

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

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

50 There are numerous variables which should affect the passage time: time of day,
51 health / age / sex of the animal, stress, quantity / quality of foods, etc. Among these
52 factors, our focus in this study is on the physical characteristics of seeds (e.g., seed size,
53 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.

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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.

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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* >

108 0.05).

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

115

116 Seed administration

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

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

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

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

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

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

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

where m_i = the number of seeds excreted at the i th 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 $\pm$ 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

180 plastic seeds recovered was 81.5 % (range, 65%–86 %) (Table 1). The percentage of seed
181 recovery varied significantly among the real seed types (Friedman’s two-way ANOVA,
182 $\chi^2 = 18.94$, $df = 5$, $P < 0.01$), although none of the physical dimensions of the real seeds
183 correlated with the percentage of recovery (Spearman’s correlation analyses, $df = 5$, dry
184 seed weight: $r_s = 0.26$, $P = 0.658$; seed volume: $r_s = 0.03$, $P = 1.000$; specific gravity of
185 seed: $r_s = 0.60$, $P = 0.242$).

186

187 Gut passage time and physical characteristics of seeds

188 The passage rates measured by *TT*, *MRT*, and *TLA* were 22–35 hr (Fig. 1a), 37–54 hr (Fig.
189 1b), and 53–109 hr (Fig. 1c), respectively. We treated the three passage time variables as
190 independent of each other because there were no correlations among them (Spearman’s
191 correlation analyses, $df = 7$, *TT* vs *MRT*: $r_s = 0.68$, $P = 0.062$; *TT* vs *TLA*: $r_s = 0.24$, $P =$
192 0.568 ; *MRT* vs *TLA*: $r_s = 0.70$, $P = 0.069$). Among these variables, *MRT* (median: 44.7 hr)
193 and *TLA* (median: 70.0 hr) differed significantly among the seed types (Friedman’s
194 two-way ANOVA, $df = 7$, *MRT*: $\chi^2 = 14.58$, $P = 0.042$; *TLA*: $\chi^2 = 21.32$, $P = 0.003$). *TT*
195 (median: 24.0 hr), however, did not differ significantly among the seed types ($\chi^2 = 11.63$,
196 $P = 0.114$). On the other hand, *MRT* differed significantly among individual macaques
197 (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).

208

209 Discussion

210 The median percentage of real seeds recovered per individual monkey was 36 %. This
211 result is probably due to the fact that the Japanese macaques act both as seed dispersers
212 and as seed predators (Yumoto *et al.*, 1998; Otani and Shibata, 2000; Otani, 2003). Otani
213 (2003), for example, found some cracked seeds of relatively large-seeded species (e.g.,
214 *Akebia trifoliata* (mean cubic diameter: 3.6 mm) and *Berchemia racemosa* (3.2 mm))
215 with their intact seeds in macaque feces. Percent seed recovery was not significantly

216 correlated with dry seed weight, volume, or specific gravity. Kunz & Linsenmair (2008a)
217 also reported the absence of a linear correlation between seed size and that of seed
218 damage in their study of olive baboons *Papio anubis*. It is possible that characteristics of
219 seeds, such as hardness or shape, may help to explain the low seed recovery rate of
220 several seed types reported in our study. Corlett & Lucas (1990) reported that captive
221 studies exaggerate seed predation, and this might also be the case in our study. Seed
222 spitting is another handling behavior in cercopithecine monkeys, including macaques
223 (Corlett & Lucas, 1990; Lucas & Corlett, 1998). For Japanese macaques in our study,
224 however, spitting out of the seeds was rarely observed (3%), though the plastic beads
225 were spat out more (29%) (Table 1). Small seed size might contribute to the lower
226 percentage of seed spitting (Lucas & Corlett, 1998).

227 Measurements of the transit time (*TT*) and the mean retention time (*MRT*) in the
228 female Japanese macaques in our study (*TT*, 22–35 hr, *MRT*, 37–54 hr) (Fig. 1a, 1b) were
229 similar to those found in previous studies of cercopithecine monkeys (*TT*, 20 hr for
230 *Cercopithecus ascanius*, 17 hr for *C. mitis*, 21 hr for *C. neglectus* (Lambert, 2002), and 23
231 hr for *Lophocebus albigena* (Maisels, 1993); *MRT*, 27 hr for *C. ascanius*, 25 hr for *C.*
232 *mitis*, 34 hr for *C. neglectus* (Lambert, 2002), 38 hr for *L. albigena* (Maisels, 1993), and
233 39 hr for *Macaca fuscata* (Otani, 2004)). In general, seed passage time of Old World

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

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

- Augspurger, C.K. (1984). Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. *Ecology* **65**, 1705-1712.
- Chapman, C.A. & Chapman, L.J. (1996). Frugivory and the fate of dispersed and non-dispersed seeds of six African tree species. *J. Trop. Ecol.* **12**, 491-504.
- Chapman, C.A. & Russo, S.E. (2007). Primate seed dispersal. In *Primate in Perspective*: 510-525. Campbell, C.J., Fuentes, A., MacKinnon, K.C., Pager, M. & Boarder, S.K. (Eds.). Oxford: Oxford University Press.
- Corlett, R.T. & Lucas, P.W. (1990). Alternative seed-handling strategies in primates: seed-spitting by long-tailed macaques (*Macaca fascicularis*). *Oecologia* **82**, 166-171.
- Direnfeld, E.S., Koontz, F.W. & Goldstein, R.S. (1992). Feed intake, digestion and passage of the proboscis monkey (*Nasalis larvatus*) in captivity. *Primates* **33**, 399-405.
- Garber, P.A. (1986). The ecology of seed dispersal in two species of Callitrichidae primates (*Saguinus mystax* and *Saguinus fuscicollis*). *Am. J. Primatol.* **10**, 155-170.
- Gardener, C.J., McIvor, J.G. & Jansen, A. (1993). Passage of legume and grass seeds

- 288 through the digestive tract of cattle and their survival in feces. *J. Appl. Ecol.* **30**,
- 289 63-74.
- 290 Graae, B.J., Pagh, S. & Bruun, H.H. (2004). An experimental evaluation of the arctic fox
- 291 (*Alopex lagopus*) as a seed disperser. *Arc. Antarc. Alpine. Res.* **36**, 468-473.
- 292 Julliot, C. (1996). Seed dispersal by red howling monkeys (*Alouatta seniculus*) in the
- 293 tropical rain forest of French Guiana. *Int. J. Primatol.* **17**, 239-258.
- 294 Kaplin, B.A. & Moermond, T.C. (1998). Variation in seed handling by two species of
- 295 forest monkeys in Rwanda. *Am. J. Primatol.* **45**, 83-101.
- 296 Kunz, B.K. & Linsenmair, K.E. (2008a). The role of the olive baboon (*Papio anubis*,
- 297 Cercopithecidae) as seed disperser in a savanna-forest mosaic of West Africa. *J.*
- 298 *Trop. Ecol.* **24**, 235-246.
- 299 Kunz, B.K. & Linsenmair, K.E. (2008b). Seed size selection by olive baboons. *Primates*
- 300 **49**, 239-245.
- 301 Lambert, J.E. (1998). Primate digestion: interactions among anatomy, physiology, and
- 302 feeding ecology. *Evol. Anthropol.* **7**, 8-20.
- 303 Lambert, J.E. (2001). Red-tailed guenons (*Cercopithecus ascanius*) and *Strychnos mitis*:
- 304 evidence for plant benefits beyond seed dispersal. *Int. J. Primatol.* **22**, 189-201.
- 305 Lambert, J.E. (2002). Digestive retention times in forest guenons (*Cercopithecus* spp.)

- 306 with reference to chimpanzees (*Pan troglodytes*). *Int. J. Primatol.* **23**, 1169-1185.
- 307 Leavy, D.J. & Grajal, A. (1991). Evolutionary implications of fruit-processing limitation
- 308 in cedar wax wings. *Am. Nat.* **138**, 171-189.
- 309 Link, A. & Di Fiore, A. (2006). Seed dispersal by spider monkeys and its importance in
- 310 the maintenance of Neotropical rain-forest diversity. *J. Trop. Ecol.* **22**, 235-246.
- 311 Lucas, P.W. & Corlett, R.T. (1998). Seed dispersal by long-tailed macaques. *Am. J.*
- 312 *Primatol.* **45**, 29-44.
- 313 Maisels, F. (1993). Gut passage rate in guenons and mangabeys: another indicator of a
- 314 flexible feeding niche? *Folia Primatol.* **61**, 35-37.
- 315 Martins, M.M. (2006). Comparative seed dispersal effectiveness of sympatric *Alouatta*
- 316 *guariba* and *Brachyteles arachnoids* in Southeastern Brazil. *Biotropica* **38**, 57-63.
- 317 Mori, A. (1979). An experiment on the relation between the feeding speed and the caloric
- 318 intake through leaf eating in Japanese monkeys. *Primates* **20**, 185-195.
- 319 Nakagawa, N., Iwamoto, T., Yokota, N. & Soumah, A.G. (1996). Inter-regional and
- 320 inter-seasonal variation of food quality in Japanese macaques: with reference to the
- 321 constraints of digestive volume and feeding time. In *Evolution and Ecology of*
- 322 *Macaque species*: 207-234. Fa, J.E. & Lindberg, D.G. (Eds.). Cambridge:
- 323 Cambridge University Press.

- 324 Otani, T. & Shibata, E. (2000). Seed dispersal and predation by Yakushima macaques,
325 *Macaca fuscata yakui*, in a warm temperate forest of Yakushima Island, southern
326 Japan. *Ecol. Res.* **15**, 133-144.
- 327 Otani, T. (2003). Seed dispersal and predation of fleshy-fruited plants by Japanese
328 macaques in the cool temperate zone of northern Japan. *Mamm Study* **28**, 153-156.
- 329 Otani, T. (2004). Effects of macaque ingestion on seed destruction and germination of a
330 fleshy-fruited tree, *Eurya emarginata*. *Ecol. Res.* **19**, 495-501.
- 331 Pollux, B.J.A., Ouborg, N.J., van Groenendael, J.M. & Klaassen, M. (2007).
332 Consequences of intraspecific seed-size variation in *Sparganium emersum* for
333 dispersal by fish. *Func. Ecol.* **21**, 1084-1091.
- 334 Raemakers, J. (1980). Causes of variation in the distance traveled daily by gibbons. *Folia*
335 *Primatol.* **23**, 46-60.
- 336 R Development Core Team. (2006). R: A language and environment for statistical
337 computing. Version 2.4.1. Vienna, Austria, R Foundation for Statistical Computing.
- 338 Remis, M.J. (2000). Initial studies on the contributions of body size and gastrointestinal
339 passage rates to dietary flexibility among gorillas. *Am. J. Phys. Anthropol.* **112**,
340 171-180.
- 341 Schwarm, A., Ortmann, S., Wolf, C., Streich, W.J. & Clauss, M. (2008). Excretion

- patterns of fluid and different sized particle passage markers in banteng (*Bos javanicus*) and pygmy hippopotamus (*Hexaprotodon liberiensis*): two functionally different foregut fermenters. *Comp. Biochem. Physiol. A.* **150**, 32-39.
- Strier, K.B. (2000). *Primate Behavioral Ecology*. 1st edn. Boston: Allyn & Bacon.
- Traveset, A. (1998). Effect of seed passage through vertebrate frugivores' guts on germination: a review. *Persp. Plant Ecol. Evol. Syst.* **1/2**, 151-190.
- Varela, O. & Bucher, E.H. (2006). Passage time, viability, and germination of seeds ingested by foxes. *J. Arid. Environ.* **67**, 566-578.
- Wilson, M.F. (1989). Gut retention times of experimental pseudoseeds by emus. *Biotropica* **21**, 210-213.
- Wotton, D.M., Clout, M.N. & Kelly, D. (2008). Seed retention times in the New Zealand pigeon (*Hemiphaga novaeseelandiae novaeseelandiae*). *NZ. J. Ecol.* **32**, 1-6.
- Yumoto, T., Noma, N. & Maruhashi, T. (1998). Cheek-pouch dispersal of seeds by Japanese monkeys (*Macaca fuscata yakui*) on Yakushima Island, Japan. *Primates* **39**, 325-338.
- Yumoto, T., Kimura, K. & Nishimura, A. (1999). Estimation of the retention times and distances of seed dispersed by two monkey species, *Alouatta seniculus* and *Lagothrix lagotricha*, in a Colombian forest. *Ecol. Res.* **14**, 179-191.

360 FIGURE LEGENDS

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

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

Seed type	Physical characteristics of seeds							Dose		Fate of seeds (# of seeds, mean ± SD)					
	Size (mm, mean ± SD)			Dry weight (mg, mean ± SD)	Volume ^{a)} (mm ³)	Specific gravity ^{b)} (mg-mm ⁻³)	No. of seeds in a banana	Total volume (mm ³)		Defecated		Spitted out		Destructed	
	Length	Width	Height												
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*).

Independent variable	Dependent variable		
	<i>MRT</i>	<i>TLA</i>	<i>TLA</i> (without beads)
	Estimate $\pm$ SE	Estimate $\pm$ SE	Estimate $\pm$ SE
Intercept	3.736 $\pm$ 0.062***	4.071 $\pm$ 0.072***	3.915 $\pm$ 0.199***
Dry weight	0.011 $\pm$ 0.005	—	—
Volume	—	—	—
Specific gravity	—	0.167 $\pm$ 0.043**	0.416 $\pm$ 0.294

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

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