Gastrointestinal passage time of seeds ingested by captive Japanese martens *Martes melampus*

Yamato TSUJI, Toshiaki SHIRAISHI, and Sayako MIURA

The time it takes for ingested seeds to pass through the gut of animals is an important aspect of endozoochorous seed dispersal because it influences seed dispersal distance. Variations in the physical characteristics of seeds, such as their weight, volume, and specific gravity, can affect their movement through the gastrointestinal system of a given animal. We conducted feeding experiments with captive Japanese martens, *Martes melampus* (n = 4), at Toyama Municipal Family Park Zoo, central Japan, to examine the effects of the physical characteristics of seeds on their passage times. The mean (± SD) transit time, mean retention time, and time of last appearance of four different types of commercial seeds were 2.6 ± 0.3 hr (range: 0.6 – 5.4), 9.7 ± 1.1 hr (3.8 – 17.3), and 23.8 ± 3.1 hr (12.2 – 51.8), respectively. All of these values are greater than those found during previous experiments conducted with mustelids. Similar to previous studies, however, none of these passage time variables was correlated with the physical characteristics of

seeds. Our results thus indicate that martens disperse seeds of different plant species, whose size, volume, and specific gravity all fall within the range of those used in the present study, from parent plants at similar distances.

Primate Research Institute, Kyoto University, Aichi 484-8506, Japan, e-mail: ytsuji@pri.kyoto-u.ac.jp (YT); Toyama Municipal Family Park Zoo, Toyama 930-0151, Japan (TS, SM)

Key words: feeding experiments, *Martes melampus*, physical characteristics, seed dispersal

Running page headline: Seed passage time of Japanese martens

Introduction

The physical characteristics of seeds, such as size, weight and specific gravity, vary among plant species. These variations can have a significant impact on passage time through the gut of a given animal (Traveset 1998). Leavy and Grajal (1991), for example, showed a negative correlation between the size of seeds ingested by cedar waxwings *Bombycilla cedrorum* and their subsequent passage times. Smaller seeds would thus be dispersed farther from parent plants. In contrast, Julliot (1996) provided evidence of a positive correlation between seed size and passage time in red howler monkeys *Alouatta seniculus*. To further confuse the issue, the relationship between passage time and specific gravity, another physical characteristic relevant to seed dispersal, was positive for seeds ingested by Japanese macaques *Macaca fuscata* (Tsuji *et al*. 2010) but negative for those ingested by two species of tamarin monkeys *Saguinus mystax* and *S. fuscicollis* (Garber 1986). Other studies have found no clear relationships between the physical characteristics of seeds and their passage times (emus *Dromaius novahollandiae*: Wilson 1989; arctic foxes *Alopex lagopus*: Graae *et al*. 2004; Pampa foxes *Lycalopex* *gymnocercus* and crab-eating foxes *Cerdocyon thous*: Varela and Bucher 2006). Thus, it appears that the effects of physical characteristics on seed passage time vary among animal species, and this may reflect variation in the size and/or morphology of the digestive system and the digestive strategies of the animals under investigation.

Mustelids are generally regarded as carnivorous, but they also commonly consume large amounts of fruit (Rosalino and Santos-Reis 2009), often voiding intact seeds with their feces (pine martens *Martes martes*: Schaumann and Heinken 2002; stone martens *M. foina*: Schaumann and Heinken 2002; Japanese martens *M. melampus*: Arai *et al*. 2002; Koike *et al*. 2008; yellow–throated martens *M. flavigula*: Zhou *et al*. 2008a). The passage of seeds through the guts of martens can have a positive effect on germination and/or seedling growth (Hickey *et al*. 1999, Schaumann and Heinken 2002, but see Rosalino *et al*. 2010). Therefore, the marten appears to be an important seed disperser within its habitat (Hickey *et al*. 1999). Since mustelids travel between several hundred meters and several kilometers daily (Hickey *et al*. 1999, Zalewski *et al*. 2004), and have relatively large home ranges amounting to between 1 and 4 km^2 (Zalewski and Jedrzejewski 2006), variation in passage time can markedly affect seed dispersal distance, which in turn affects the seed shadow of a given plant species. Thus, evaluating the effects of the physical characteristics of seeds on passage time through marten digestive tracts is essential to understanding their role in endozoochorous seed dispersal.

In this study we conducted feeding experiments with captive Japanese martens, which are considered an important mammalian seed disperser throughout Japanese forests (Kusui and Kusui 1998, Otani 2002, Koike *et al*. 2008, Tsuji *et al*. in press). We tested one simple prediction: that seed passage time through the gut of Japanese martens differs among seed types because of variation in their weights, volumes, and specific gravities.

Materials and methods

Four adult martens (three males and one female) with which we conducted our feeding experiments were housed at the Toyama Municipal Family Park Zoo, central Japan. All animals were reared in individual wire mesh pens (length 1.80 m × width 1.8 m

× height 2.8 m), and were active and in good condition. The estimated ages and body weights of the three animals for which data were available (two males and one female, respectively) were 16, 3, and 3 yr and 2.0, 1.8, and 1.0 kg. The female marten was neither lactating nor pregnant during the study period. The martens were normally fed dead chicks, chicken heads, boiled sweet potatoes and fresh fruit (fresh weight: 230 – 300 g· individual^{-1}) once a day (at 10:00). We only entered the pen during the experiments, to minimize stressing the animals and thereby affecting gut passage times. About one month before conducting the experiments, we set security cameras on the roofs of each pen to continuously monitor the movements of each animal. Videos were continuously recorded onto hard disks, so we could accurately record their defecation times and locations (see below).

In light of a previous review of fruit feeding by martens (Koike and Masaki 2008), and the results of our own recent study (Tsuji *et al*. in press), we selected the seeds of four commercial plant species (spinach, water spinach, kiwi fruits, and okra), with seed lengths ranging from 2.2 – 5.9 mm (Table 1) and shapes similar to those of many of the plant species on which martens are known to ingest in the wild. Because of restrictions relating to experimentation with captive animals, we could not provide seeds collected in wild in this study. We measured the length, width, and height of 30 randomly-picked seeds with a vernier caliper (THS–30, Niigata Seiki Co., Japan) to the nearest 0.05 mm. We also weighed 30 randomly-picked dry seeds with an electric balance (UX4200H, Shimadzu Co., Japan) to the nearest 1 mg. We then calculated seed volume according to the formula:

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

where R = (seed width + height) / 4, and L = seed length (Garber 1986). Finally, we calculated the specific gravity of seeds (mg·mm^{-3}) by dividing the seed volume by the seed weight (Table 1). After taking these measurements, we put seeds into the stomachs of dead chicks, since preliminary experiments showed that the martens would not ingest the seeds alone. We must thus accept the possibility that this treatment might have affected the results of our experiments. Information concerning the size, weight, and amount of seeds used in each experiment is shown in Table 1.

We conducted four experimental sessions between late September and early October, 2010. We did not change the martens' housing conditions or diet composition during the experiments. Our methodology adhered to Japan's legal requirements for animal welfare. On the first day of a given experiment, each animal was fed as usual at 10:00, and we regularly entered the pen twice a day (10:00 and 16:00) to collect both non-ingested seeds and all feces found within the enclosure. We recorded the locations of defecation and cleaned the floor of the pen in order to facilitate subsequent monitoring of defecation events. We rinsed fecal samples with water immediately after collection and screened them using a 0.5 mm sieve, recording the number of intact seeds, defined as those with undamaged embryos, whenever present. We considered seeds that were ingested but not observed in faecal samples to be destroyed through mastication or digestion. We initiated subsequent experimental sessions after 24 hr had passed since the onset of the previous experimental session (i.e., 10:00). We recorded the time of defecation for each fecal sample from the videos taken for each animal.

According to previous studies of seed dispersal, the quantitative evaluation of seed passage time is based on three parameters: transit time, mean retention time, and time of last appearance. We obtained the mean retention time via the following formula:

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

where MRT = mean retention time, m_i = the number of seeds excreted in the ith defecation at time t_i (hr) after ingestion (Lambert 2002).

We tested the effects of seed type on the percentage of seeds recovered and on the three passage time variables (transit time, mean retention time, and time of last appearance), and the effects of individual marten on the passage times. We employed Friedman's two-way ANOVAs to account for the non-normality of our data. We used Spearman's correlation analyses to test for relationships between the physical characteristics of seeds and both the percentage of seeds recovered and the three passage time variables. We could not test for differences in seed recovery and passage time variables between sexes because of our small sample size of four animals. We set the level of significance for these analyses (α) at 0.05. All data analyses were conducted using the statistical software R version 2.9.1 (R Development Core Team 2009).

Results

The martens in our study readily and immediately consumed the dead chicks when presented with them, but not all of the seeds concealed therein were ingested because several dropped out of the chick during feeding (Table 1). The martens defecated more than half of their ingested seeds, The mean percentage of seeds recovered for each seed type ranged from 70 – 86 %, and did not vary significantly among seed types (Friedman's two-way ANOVA, $\chi^2 = 4.3$, $p = 0.233$, df = 3) or among animals ($\chi^2 = 4.9$, $p = 0.181$, df = 3). Furthermore, none of the physical dimensions of the seeds correlated with the percentage of seeds recovered (Spearman's correlation analyses, dry weight: $r_s = -0.20$, $p = 0.917$; volume: $r_s = 0.40$, $p = 0.750$; specific gravity: $r_s = -0.40$, $p = 0.750$, df = 2 for all analyses).

The mean transit time, mean retention time, and time of last appearance were 2.6 ± 0.3 hr (range: 0.6 – 5.4 hr, Fig. 1a), 9.7 ± 1.1 hr (3.8 – 17.3 hr, Fig. 1b) and 23.8 ± 3.1 hr (12.2 – 51.8 hr, Fig. 1c), respectively. We treated the three passage time variables as independent of each other because there were no significant correlations among them (Spearman's correlation analyses, transit time vs mean retention time: r_s = -0.20, p = 0.917; transit time vs time of last appearance: r_s = -0.20, p = 0.917; mean retention time vs time of last appearance: r_s = -0.20, p = 0.917, df = 2 for all analyses). None of these passage time variables differed significantly among seed types (Friedman's two-way ANOVA, transit time: χ^2 = 1.5, p = 0.682; mean retention time: χ^2 = 1.2, p = 0.753; time of last appearance: χ^2 =2.1, p = 0.552, df = 3 for all analyses). Among these variables, mean retention time differed significantly between individual animals (Friedman's two-way ANOVA, χ^2 = 9.9, p = 0.019, df = 3), being longest in the heavier males and shortest in the lighter female. Finally, transit time and time of last appearance did not differ between individual animals (transit time: χ^2 = 4.4, p = 0.219; time of last appearance: χ^2 = 5.7, p = 0.127).

Discussion

In this study, none of the three passage time variables differed significantly among seed types in our experiments. Our results differ from those concerning other animal species such as birds (Leavy and Grajal 1991) and primates (Garber 1986, Tsuji *et al*. 2010), in which the physical characteristics of seeds were shown to affect passage times. Our results imply that plant seeds with physical characteristics falling within the ranges of those used in the present study have the same probability of being dispersed by Japanese martens a similar distance away from parent plants. Previous studies of carnivorous mammals have also showed that the passage times of seeds ingested by their subject animals did not vary across seed types (Graae *et al*. 2004, Varela and Bucher 2006, Zhou *et al*. 2008b). Thus, the lack of a relationship between seed type and passage time appears to be a common trait of carnivorous mammals. However, we cannot deny the possibility that our results might have been affected by our small number of experiments. Another possibility is that factors that we did not consider in this study, such as fruit pulp properties (e.g. texture and chemistry), the amount of other fiber-rich compounds of the wild marten diet (e.g., meat and fur), and the amount and/or shape of seeds, may have masked the effects of seed size, volume, and specific gravity. Thus, further and more detailed experimentation considering these effects should be conducted in the future (Traveset 1998). We also encourage cooperation between researchers and zoological gardens, as this will be invaluable in furthering our understanding of endozoochorous seed dispersal among carnivorous mammals.

By combining data on passage time and animal movement obtained through radio telemetry, Hickey *et al*. (1999) estimated a range of seed dispersal distances (i.e., seed shadow) created by American martens. However, there have been few quantitative studies to date evaluating ranging patterns among Japanese martens (Kawauchi *et al*. 2003, Okumura and Kitahara 2006). Furthermore, few studies have tested the effects of ingestion and passage through the digestive systems of Japanese martens on the germination and/or growth of those plant species. Given this lack of empirical data, our study can contribute to an estimation of the seed shadows created by Japanese martens. To be an effective seed disperser, fruit consumers cannot destroy the seeds they ingest, but they are also expected to enhance (or at least have a neutral effect on) seed germination during transit through the gut (Pollux *et al*. 2007, Rosalino *et al*. 2010). In the future, testing the relationship between the seed dispersal distribution and seed performance (e.g. germination and seedling growth) in the field is a prerequisite to any evaluation of the efficacy of Japanese martens as seed dispersers.

Acknowledgements

We thank all staff at the Toyama Municipal Family Park Zoo, especially H. Murai, M. Horiuchi, and Y. Ishihara, for their cooperation in our feeding experiments. We are grateful to M.A. Huffman and A.J.J. McIntosh, and three anonymous reviewers for proofreading and generally improving this manuscript.

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Figure legends

Figure 1: Relationships between seed type (n=4) and passage time variables: a) transit time, b) mean retention time, and c) time of last appearance of a seed. Filled circles indicate mean values and bars indicate standard deviations.

Figure

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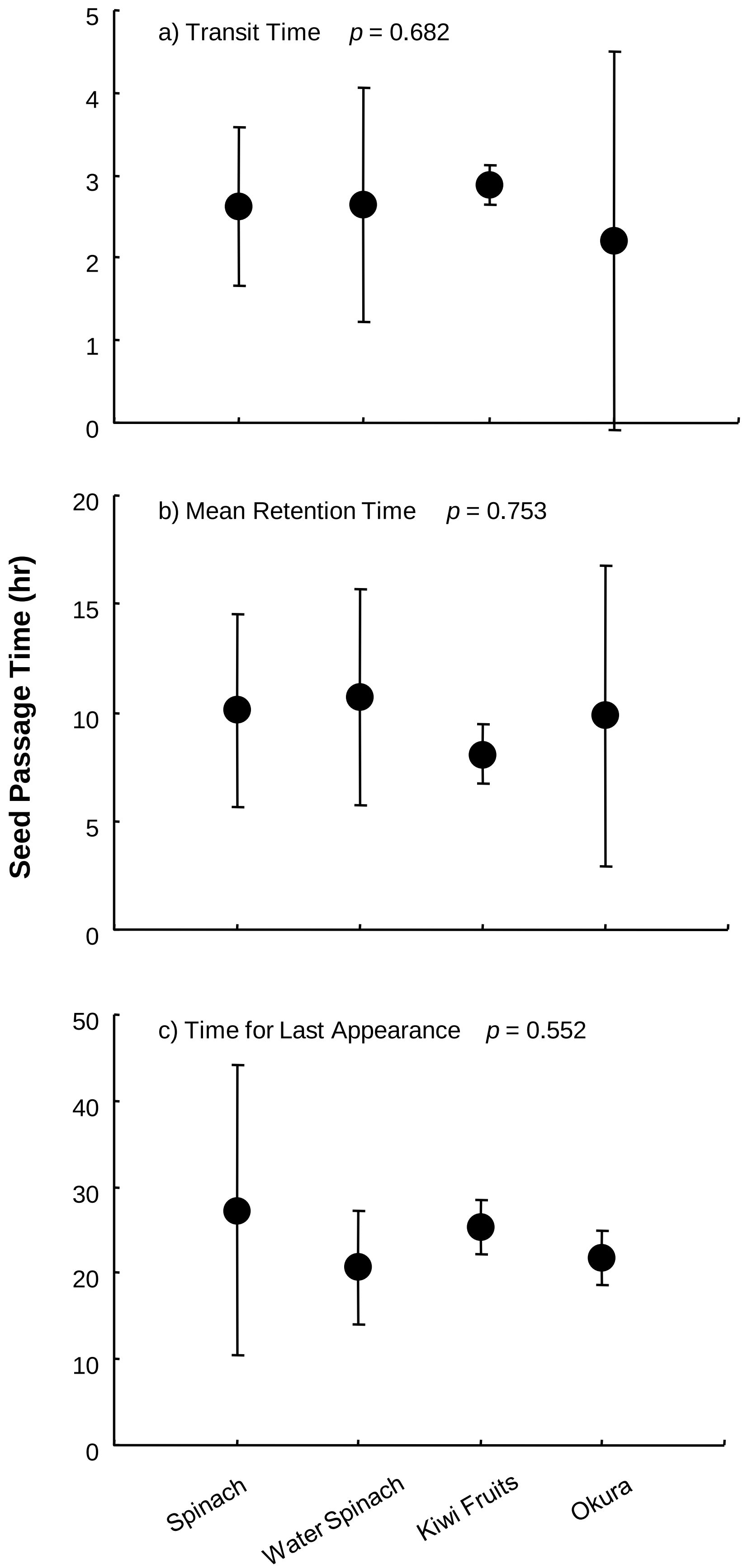

Table

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

Seed type	Physical characteristics of seeds						Dose	Seeds fate					
	Size (mm, mean ± SD)			Dry weight	Volume[a)]	Specific gravity[b)]	# seeds in a chick	Ingested		Defecated		% Recovary	
	Length	Width	Height	(mg, mean ± SD)	(mm^3)	($mg \cdot mm^{-3}$)		mean ± SD	range	mean ± SD	range	mean ± SD	range
Spinach	3.58 ± 0.41	3.12 ± 0.33	2.26 ± 0.22	11.47 ± 3.17	15.25	0.75	40	39.8 ± 0.5	39 - 40	31.5 ± 3.5	28 - 35	79.2 ± 8.4	70.0 - 87.5
Water spinach	5.92 ± 0.32	4.09 ± 0.34	3.55 ± 0.24	51.67 ± 8.53	53.29	0.97	40	39.5 ± 0.0	39 - 40	27.8 ± 4.0	23 - 32	70.4 ± 11.2	57.5 - 82.1
Kiwi fruits	2.24 ± 0.21	1.23 ± 0.15	0.71 ± 0.08	1.00 ± 0.31	1.43	0.70	400	400.0 ± 0.0	-	278.8 ± 61.2	210 - 350	78.4 ± 10.0	52.5 - 87.8
Okura	4.84 ± 0.31	4.23 ± 0.26	4.29 ± 0.31	43.69 ± 9.64	48.64	0.90	20	20.0 ± 0.0	-	16.3 ± 1.2	15 - 20	86.3 ± 10.3	75.0 - 100.0

The sample number of seed measurements was 30 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.