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

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

2 *melampus*

3 Yamato TSUJI, Toshiaki SHIRAISHI, and Sayako MIURA

4 The time it takes for ingested seeds to pass through the gut of animals is an important

5 aspect of endozoochorous seed dispersal because it influences seed dispersal distance.

6 Variations in the physical characteristics of seeds, such as their weight, volume, and

7 specific gravity, can affect their movement through the gastrointestinal system of a given

8 animal. We conducted feeding experiments with captive Japanese martens, *Martes*

9 *melampus* ($n = 4$), at Toyama Municipal Family Park Zoo, central Japan, to examine the

10 effects of the physical characteristics of seeds on their passage times. The mean ($\pm$ SD)

11 transit time, mean retention time, and time of last appearance of four different types of

12 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

13 ± 3.1 hr (12.2 – 51.8), respectively. All of these values are greater than those found during

14 previous experiments conducted with mustelids. Similar to previous studies, however,

15 none of these passage time variables was correlated with the physical characteristics of

16 seeds. Our results thus indicate that martens disperse seeds of different plant species,
17 whose size, volume, and specific gravity all fall within the range of those used in the
18 present study, from parent plants at similar distances.
19
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23
24 Key words: feeding experiments, *Martes melampus*, physical characteristics, seed
25 dispersal
26 Running page headline: Seed passage time of Japanese martens

27 Introduction

28 The physical characteristics of seeds, such as size, weight and specific gravity, vary
29 among plant species. These variations can have a significant impact on passage time
30 through the gut of a given animal (Traveset 1998). Leavy and Grajal (1991), for example,
31 showed a negative correlation between the size of seeds ingested by cedar waxwings
32 *Bombycilla cedrorum* and their subsequent passage times. Smaller seeds would thus be
33 dispersed farther from parent plants. In contrast, Julliot (1996) provided evidence of a
34 positive correlation between seed size and passage time in red howler monkeys *Alouatta*
35 *seniculus*. To further confuse the issue, the relationship between passage time and
36 specific gravity, another physical characteristic relevant to seed dispersal, was positive
37 for seeds ingested by Japanese macaques *Macaca fuscata* (Tsuji *et al.* 2010) but negative
38 for those ingested by two species of tamarin monkeys *Saguinus mystax* and *S. fuscicollis*
39 (Garber 1986). Other studies have found no clear relationships between the physical
40 characteristics of seeds and their passage times (emus *Dromaius novahollandiae*: Wilson
41 1989; arctic foxes *Alopex lagopus*: Graae *et al.* 2004; Pampa foxes *Lycalopex*

42 *gymnocercus* and crab-eating foxes *Cerdocyon thous*: Varela and Bucher 2006). Thus, it
43 appears that the effects of physical characteristics on seed passage time vary among
44 animal species, and this may reflect variation in the size and/or morphology of the
45 digestive system and the digestive strategies of the animals under investigation.

46 Mustelids are generally regarded as carnivorous, but they also commonly consume
47 large amounts of fruit (Rosalino and Santos-Reis 2009), often voiding intact seeds with
48 their feces (pine martens *Martes martes*: Schaumann and Heinken 2002; stone martens *M.*
49 *foina*: Schaumann and Heinken 2002; Japanese martens *M. melampus*: Arai *et al.* 2002;
50 Koike *et al.* 2008; yellow-throated martens *M. flavigula*: Zhou *et al.* 2008a). The passage
51 of seeds through the guts of martens can have a positive effect on germination and/or
52 seedling growth (Hickey *et al.* 1999, Schaumann and Heinken 2002, but see Rosalino *et*
53 *al.* 2010). Therefore, the marten appears to be an important seed disperser within its
54 habitat (Hickey *et al.* 1999). Since mustelids travel between several hundred meters and
55 several kilometers daily (Hickey *et al.* 1999, Zalewski *et al.* 2004), and have relatively
56 large home ranges amounting to between 1 and 4 km² (Zalewski and Jedrzejewski 2006),

57 variation in passage time can markedly affect seed dispersal distance, which in turn
58 affects the seed shadow of a given plant species. Thus, evaluating the effects of the
59 physical characteristics of seeds on passage time through marten digestive tracts is
60 essential to understanding their role in endozoochorous seed dispersal.

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

67

68 Materials and methods

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

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

83 In light of a previous review of fruit feeding by martens (Koike and Masaki 2008),
84 and the results of our own recent study (Tsuji *et al.* in press), we selected the seeds of four
85 commercial plant species (spinach, water spinach, kiwi fruits, and okra), with seed
86 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 $R = (\text{seed width} + \text{height}) / 4$, and $L = \text{seed length}$ (Garber 1986). Finally, we calculated the specific gravity of seeds ($\text{mg} \cdot \text{mm}^{-3}$) 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.

102 We conducted four experimental sessions between late September and early October,
103 2010. We did not change the martens' housing conditions or diet composition during the
104 experiments. Our methodology adhered to Japan's legal requirements for animal welfare.
105 On the first day of a given experiment, each animal was fed as usual at 10:00, and we
106 regularly entered the pen twice a day (10:00 and 16:00) to collect both non-ingested seeds
107 and all feces found within the enclosure. We recorded the locations of defecation and
108 cleaned the floor of the pen in order to facilitate subsequent monitoring of defecation
109 events. We rinsed fecal samples with water immediately after collection and screened
110 them using a 0.5 mm sieve, recording the number of intact seeds, defined as those with
111 undamaged embryos, whenever present. We considered seeds that were ingested but not
112 observed in faecal samples to be destroyed through mastication or digestion. We initiated
113 subsequent experimental sessions after 24 hr had passed since the onset of the previous
114 experimental session (i.e., 10:00). We recorded the time of defecation for each fecal
115 sample from the videos taken for each animal.

116 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:

$$MRT = \frac{\sum_{i=1} m_i t_i}{\sum_{i=1} m_i}$$

where MRT = mean retention time, m_i = the number of seeds excreted in the i th 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: $\chi^2 = 1.5$, $p = 0.682$; mean retention time: $\chi^2 = 1.2$, $p = 0.753$; time of last appearance: $\chi^2 = 2.1$, $p = 0.552$, $df = 3$ for all analyses). Among these variables, mean retention time differed significantly between individual animals (Friedman's two-way ANOVA, $\chi^2 = 9.9$, $p = 0.019$, $df = 3$), 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: $\chi^2 = 4.4$, $p = 0.219$; time of last appearance: $\chi^2 = 5.7$, $p = 0.127$).

Discussion

In this study, none of the three passage time variables differed significantly among

162 seed types in our experiments. Our results differ from those concerning other animal
163 species such as birds (Leavy and Grajal 1991) and primates (Garber 1986, Tsuji *et al.*
164 2010), in which the physical characteristics of seeds were shown to affect passage times.
165 Our results imply that plant seeds with physical characteristics falling within the ranges
166 of those used in the present study have the same probability of being dispersed by
167 Japanese martens a similar distance away from parent plants. Previous studies of
168 carnivorous mammals have also showed that the passage times of seeds ingested by their
169 subject animals did not vary across seed types (Graae *et al.* 2004, Varela and Bucher 2006,
170 Zhou *et al.* 2008b). Thus, the lack of a relationship between seed type and passage time
171 appears to be a common trait of carnivorous mammals. However, we cannot deny the
172 possibility that our results might have been affected by our small number of experiments.
173 Another possibility is that factors that we did not consider in this study, such as fruit pulp
174 properties (e.g. texture and chemistry), the amount of other fiber-rich compounds of the
175 wild marten diet (e.g., meat and fur), and the amount and/or shape of seeds, may have
176 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.

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References

- Arai S., Adachi T., Kuwahara Y. and Yoshida K. 2003. Food habit of the Japanese marten (*Martes melampus*) at Kuju Highland in Kyushu, Japan. *Honyurui Kagaku* 43: 19-28.
- [In Japanese with English summary]
- Garber P. A. 1986. The ecology of seed dispersal in two species of Callitrichidae primates (*Saguinus mystax* and *Saguinus fuscicollis*). *American Journal of Primatology* 10:

- 208 155-170.
- 209 Graae B. J., Pagh S. and Bruun H. H. 2004. An experimental evaluation of the arctic fox
- 210 (*Alopex lagopus*) as a seed disperser. Arctic, Antarctic, and Alpine Research 36:
- 211 468-473.
- 212 Hickey J. R., Flynn R. W., Buskirk S. W., Gerow K. G. and Willson M. F. 1999. An
- 213 evaluation of a mammalian predator, *Martes americana*, as a disperser of seeds.
- 214 Oikos 87: 499-508.
- 215 Julliot C. 1996. Seed dispersal by red howling monkeys (*Alouatta seniculus*) in the
- 216 tropical rain forest of French Guiana. International Journal of Primatology 17:
- 217 239-258.
- 218 Kawauchi N., Yamamoto Y. and Imai K. 2003. Annual changes in testicular size, plasma
- 219 testosterone concentration, home range and active time in wild male marten, *Martes*
- 220 *melampus melampus*. Honyurui Kagaku 43: 93-98. [In Japanese with English
- 221 summary]
- 222 Koike S. and Masaki T. 2008. Frugivory of carnivora in central and southern part of Japan

- 223 analyzed by literature search. *Nihon Shinrin Gakkaishi* 90: 26-35. [In Japanese with
224 English summary]
- 225 Koike S., Morimoto H., Goto Y., Kozakai C. and Yamazaki K. 2008. Frugivory of
226 carnivores and seed dispersal of fleshy fruits in cool-temperate deciduous forests.
227 *Journal of Forest Research* 13: 215-222.
- 228 Kusui H. and Kusui Y. 1998. Food habits of the Japanese marten, *Martes melampus*
229 (Wagner, 1840) in Mt. Yamato-Katsuragi (2) studies of year to year variation. *Kii*
230 *Hantou No Yasei Doubutsu* 4: 13-19. [In Japanese]
- 231 Lambert J. E. 2002. Digestive retention times in forest guenons (*Cercopithecus* spp.) with
232 reference to chimpanzees (*Pan troglodytes*). *International Journal of Primatology* 23:
233 1169-1185.
- 234 Leavy D. J. and Grajal A. 1991. Evolutionary implications of fruit-processing limitation
235 in cedar wax wings. *American Naturalist* 138: 171-189.
- 236 Okumura T. and Kitahara M. 2006. Food habits and home range of sympatric red fox and
237 martens in north foot of Mt. Fuji: a preliminary report. *Wildlife Forum* 10: 99-100.

- 238 [In Japanese]
- 239 Otani T. 2002. Seed dispersal by Japanese marten *Martes melampus* in the subalpine
- 240 shrubland of northern Japan. *Ecological Research* 17: 29-38.
- 241 Pollux B.J.A., Ouborg N.J., van Groenendael J.M. and Klaassen M. 2007. Consequences
- 242 of intraspecific seed-size variation in *Sparganium emersum* for dispersal by fish.
- 243 *Functional Ecology* 21: 1084-1091.
- 244 R Development Core Team. 2009. R: A language and environment for statistical
- 245 computing. Version 2.9.1. R Foundation for Statistical Computing, Vienna, Austria.
- 246 Rosalino L.M. and Santos-Reis M. 2009. Fruit consumption by carnivores in
- 247 Mediterranean Europe. *Mammal Review* 39: 67-78.
- 248 Rosalino L.M., Rosa S. and Santos-Reis M. 2010. The role of carnivores as
- 249 Mediterranean seed dispersers. *Annales Zoologici Fennici* 47: 195-205.
- 250 Schaumann F. and Heinken T. 2002. Endozoochorous seed dispersal by martens (*Martes*
- 251 *foina*, *M. martes*) in two woodland habitats. *Flora* 197: 370-378.
- 252 Traveset A. 1998. Effect of seed passage through vertebrate frugivores' guts on

- 253 germination: a review. *Perspectives in Plant Ecology, Evolution and Systematics* 1/2:
254 151-190.
- 255 Tsuji Y., Morimoto M. and Matsubayashi K. 2010. Effects of the physical characteristics
256 of seeds on gastrointestinal passage time in captive Japanese macaques. *Journal of*
257 *Zoology* 280: 171-176.
- 258 Tsuji Y., Tatewaki T. and Kanda E. in press. Endozoochorous seed dispersal by sympatric
259 mustelids, *Martes melampus* and *Mustela itatsi*, in western Tokyo, central Japan.
260 *Mammalian Biology*.
- 261 Varela O. and Bucher E. H. 2006. Passage time, viability, and germination of seeds
262 ingested by foxes. *Journal of Arid Environment* 67: 566-578.
- 263 Wilson M. F. 1989. Gut retention times of experimental pseudoseeds by emus. *Biotropica*
264 21: 210-213.
- 265 Zalewski A., Jedrzejewski W. and Jedrzejewska B. 2004. Mobility and home range use by
266 pine martens (*Martes martes*) in a Polish primeval forest. *Ecoscience* 11: 113-122.
- 267 Zalewski A. and Jedrzejewski W. 2006. Spatial organization and dynamics of the pine

- 268 marten *Martes martes* population in Białowieża Forest (E Polen) compared with
- 269 other European woodlands. *Ecography* 29: 31-43.
- 270 Zhou Y. B., Slade E., Newman C., Wang X. M. and Zhang S. Y. 2008a. Frugivory and
- 271 seed dispersal by the yellow-throated marten, *Martes flavigula*, in a subtropical
- 272 forest of China. *Journal of Tropical Ecology* 24: 219-223.
- 273 Zhou Y. B., Zhang L., Kaneko Y., Newman C. and Wang X. 2008b. Frugivory and seed
- 274 dispersal by a small carnivore, the Chinese ferret-badger, *Melogale moschata*, in a
- 275 fragmented subtropical forest of central China. *Forest Ecology and Management*
- 276 255: 1595-1603.

277 Figure legends

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

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		range	range
	Length	Width	Height	(mg, mean ± SD)		(mm ³)	(mg·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.