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Effects of food intake on digesta passage time in captive Japanese martens (*Martes melampus*) and implications for endozoochorous seed dispersal

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Abstract. To examine the effects of food intake on the gastrointestinal passage time of seeds in the Japanese marten (*Martes melampus*), we conducted four feeding experiments using captive animals ($n = 4$). We estimated passage time variables (transit time and mean retention time) of plant seeds (two types) using two different numbers of chicks (single and three) representing two seasons when the animal prey is abundant/scarce. There was no significant relationship between food intake and passage time, and seed type did not affect the passage time variables. Our results were different to those for herbaceous/omnivorous mammals, in which a shorter passage time was observed when food intake increased. The stability in the passage time of the martens could be attributed to the higher level of digestibility of the animal prey. Our data also suggests that martens possess an elastic gut that can expand in volume, which leads to a consistent passage time despite the increase in food intake and enables them to efficiently assimilate nutrients from the consumed food. The results of the present study suggest that the dispersal distance of seeds and defecation site density of martens is influenced by their ranging/activity pattern and not by their digestive physiology.

Key words: amount of prey, endozoochory, feeding experiment, food intake, *Martes melampus*.

In the wild, the availability of food for mammals varies according to the season, particularly in temperate and boreal regions (Currie and Fritz 1993; Ting et al. 2008). To cope with temporal variations in food availability, mammals often alter their searching/feeding behaviour, including their foraging time and food preference to satisfy their nutritional requirements (Jedrzejewski and Jedrzejewska 1993; Agetsuma and Nakagawa 1998; Zhou et al. 2010).

Increasing food intake does not always lead to higher nutrient intake. Previous studies have reported a negative correlation between food intake and gastrointestinal passage time (Clauss et al. 2007, 2008). The degree of this relationship varies among animal taxa: in some foregut fermenting herbivores (such as hippos and cattle), an increase in food intake leads to a marked shortening of ingesta passage, whereas in some hindgut fermenters (such as elephants and horses), a much more moderate shortening of ingesta passage with increasing food intake was observed (Clauss et al. 2007). This suggest that the feeding mechanism of animals in relation to seasonal changes might be influenced by their digestive system. If food is digestible, it is absorbed in the gut rather than pushing along (as indigestible material), and therefore the passage-shortening effect should be more pronounced when indigestible or less-digestible food is eaten than when highly digestible food is eaten (Sawada et al. 2011). There may also be animal factors that prevent such an effect, e.g., gut distension.

This intra-specific variation in passage time can also affect endozoochorous seed dispersal, in terms of defecation site density and dispersal distance. Seasonal changes in the distribution of defecation sites could also affect the survival and/or growth of specific plant species (Tsujino and Yumoto 2009; Nakashima et al. 2010) and might ultimately affect the floral diversity of the habitat (Schupp et al. 2010).

The family Mustelidae is one of the major groups of

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mesopredators in temperate and boreal regions (Zhou et al. 2010). They are generally regarded as carnivorous, but they also consume large amounts of fruit (Willson 1993; Remonti et al. 2007; Zhou et al. 2010); they void intact seeds with their faeces. Consequently, they play a role as endozoochorous seed dispersers in their habitats (Willson 1993; Hickey et al. 1999; Schaumann and Heinken 2002; Tsuji et al. 2011b). Variations in the mustelids diet are mainly attributable to seasonal changes in food availability, particularly relating to animal prey such as small rodents (Helldin 1999; Lanszki and Heltai 2011; Caryl et al. 2012). Animal prey contains a low amount of fiber (0.2–2.2% dry matter, Davison et al. 1978) and is generally easy to digest. Fishers (*Martes pennanti*), for example, digest about 80% of dry matter ingesta (Davison et al. 1978). Hickey et al. (1999) once predicted that high food volume should reduce the passage time of mustelids, but few studies have tried to confirm their prediction. Evaluating the effects of the amount of food intake on the passage time of mustelids is thus essential in understanding their feeding mechanisms and in determining their role as endozoochorous seed dispersers (Hickey et al. 1999). The objective of this study was to examine the effects of the different amounts of animal material in the diet on gastrointestinal passage time in captive Japanese martens (*Martes melampus*), an endemic mustelid species in mainland Japan, by conducting a series of feeding experiments.

Methods and materials

Study animals

Four adult Japanese martens (three males and one female, age $\geq$ 3 years old) housed at the Toyama Municipal Family Park Zoo in Central Japan were included in a series of feeding experiments conducted in 2011. They were reared in individual wire-meshed pens (length 1.8 m × width 1.8 m × height 2.0 m) and were active and in good condition during the study period. The female marten was neither lactating nor pregnant during our experiments. The body weights of the four animals in 2010 were 2.0, 1.8, and 1.6 (males) and 1.0 kg (female) (Tsuji et al. 2011a), and their body weights were stable during the study periods. In the zoo, the martens were fed with dead chicks, chicken heads, boiled sweet potatoes, and fresh fruit (total fresh weight: 100–150 g·individual⁻¹) once a day. We only entered the individual pens during the experiments when necessary to avoid inducing stress in the animals, which could also affect their digestion.

Two weeks before the experiments, we placed security cameras on the roofs of the individual pens to continuously monitor the movements of each animal. The video was recorded onto a hard disk and was reviewed to accurately record each marten's defecation time and location (see Tsuji et al. 2011a for details on the methodology).

Feeding experiments

To test the effects of food intake on the passage time of the martens, we conducted four different experiments using varying numbers of chicks/different types of seeds. We obtained a permission for the experiments from Toyama Municipal Family Park Zoo, and our methodology adhered to Japan's legal requirements for animal welfare.

The regular diet during the feeding experiments was a cut sweet potato. In addition we either gave one or three chicks. Except diet we did not change the martens' housing conditions during the feeding experiments. To test the effect of seed size on digestive passage time, we repeated the two experiments using two different-sized seeds, spinach (large seeds hereafter) and kiwi seeds (small seeds). Large seeds and small seeds simulated *Vitis* sp. (Vitaceae) and *Actinidia* sp. (Actinidaceae), which wild Japanese martens readily defecate (Yamagishi 1990; Nakamura et al. 2001; Arai et al. 2003; Tsuji et al. 2014). Mean seed size, weight, and volume (calculated by $V = \pi r^2 (L - 2/3R)$, where R = (seed width + seed height)/4; and L = seed length [Garber 1986]), were 3.6 mm, 15.3 mg, and 5.3 mm³ for the large seeds ($n = 10$), and 2.2 mm, 1.0 mg, and 1.4 mm³ for the small seeds ($n = 10$), respectively.

The seeds ($n = 40$ for the large seeds and 400 for the small seeds) were placed in the stomachs of the dead chicks because our previous study had shown that the martens did not ingest exposed seeds alone (Tsuji et al. 2011a). When we fed three chicks during an experimental session, we evenly distributed the seeds among the chicks. Each food item weighed by 0.5 g in all the experiments. On the first day of the experimental session, chick (43–49 g in fresh weight for "single chick", and 130–143 g in fresh weight for "three chicks" experiments) and a piece of boiled sweet potato (26–60 g in fresh weight, Table 1) were given at 10:00, after which we entered the pen twice (16:00 on the first day and 10:00 on the second day) to collect both non-ingested material and all faeces found within the pen. The moisture of the chick and sweet potato was 81.9% and 67.4%, respectively. By deleting the weight of non-ingested materials from the weight of the

Table 1. Diet composition and results of two passage time variables (shown as the mean ± *SD*) for four experiments (two seed types × two amount of chick) for captive Japanese martens (*n* = 4)

	Single chick		Three chicks	
Diet composition (fresh weight, g)				
Chick	45.5 ± 2.3	(43.0–49.0)	136.1 ± 3.6	(130.0–142.5)
Sweet potato	45.1 ± 11.3	(27.5–60.0)	39.6 ± 11.2	(25.5–55.0)
Large Seeds (Spinach, dosage: 40)				
DM (g/kg$^{0.75}$/day)	19.0 ± 5.6	(6.8–11.9)	29.7 ± 9.0	(23.5–43.0)
DMI (g/kg$^{0.75}$/day)	19.0 ± 5.6	(6.8–11.9)	24.2 ± 7.9	(15.0–34.1)
TT (hr)	2.72 ± 1.05	(1.50–3.62)	1.32 ± 1.64	(0.05–3.17)
MRT (hr)	4.39 ± 0.45	(3.82–4.85)	7.47 ± 2.92	(6.40–11.75)
Ingested seeds	40.0 ± 0.0	–	30.0 ± 13.5	(1–40)
Seed recovery rate (%)	51.9 ± 11.4	(40.0–65.0)	52.5 ± 30.3	(8.3–72.5)
# Defecation	11.0 ± 4.1	(7–15)	10.5 ± 3.7	(6–14)
Small Seeds (Kiwi Fruit, dosage: 400)				
DM (g/kg$^{0.75}$/day)	15.0 ± 4.8	(11.1–21.6)	26.0 ± 7.9	(18.4–37.4)
DMI (g/kg$^{0.75}$/day)	15.0 ± 4.8	(11.1–21.6)	22.3 ± 10.1	(14.5–37.1)
TT (hr)	2.45 ± 0.49	(2.03–3.00)	1.94 ± 1.27	(0.80–3.27)
MRT (hr)	6.03 ± 2.85	(3.02–9.18)	4.18 ± 1.57	(1.96–5.59)
Ingested seeds	327.3 ± 67.1	(237–398)	376.0 ± 38.9	(318–400)
Seed recovery rate (%)	44.9 ± 28.2	(17.3–73.6)	42.7 ± 18.8	(17.5–57.5)
# Defecation	9.0 ± 4.7	(6–16)	12.5 ± 6.5	(8–22)

Values in parentheses represent ranges. *DM*: daily dry matter as fed, *DMI*: daily dry matter intake, *MRT*: mean retention time, *TT*: transit time. Moisture of chick and sweet potato are 81.9% and 67.4%, respectively (unpublished data).

total amount of food fed, we calculated the total daily dry matter intake (*DMI*). We recorded the locations of the faeces and cleaned the floor of the pen to facilitate subsequent monitoring of defecation events using a video camera. We recorded the time of defecation for each faecal sample using video recordings of each animal. On the basis of a previous study (Tsuji et al. 2011a), we initiated subsequent experimental sessions after 24 hr had passed since the onset of the previous experimental session (10:00 on the second day).

We transported the faeces to our laboratory and carefully picked out the seeds directly from stirred faeces without rinsing because we had to keep the faecal samples in fresh condition for another study. According to previous studies on other mammals (Lambert 2002), the quantitative evaluation of digestive passage time was based on two parameters, transit time (time of first marker appearance, *TT*) and mean retention time (*MRT*). We calculated *MRT* using the following formula:

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

where m_i = the number of seeds excreted in the *i*th defecation time at time t_i (hr) after ingestion (Lambert 2002).

Statistical analyses

We examined the relationship between *DMI* and the passage time variables, *TT* and *MRT*, using Pearson's correlation analysis using all data points obtained from the four individuals in the four experiments. On the other hand, we tested the effects of seed size, number of chicks, and their interaction on the passage time variables using generalized linear mixed models (GLMMs). In this analysis, we treated individual martens as a random effect because we conducted four experiments using the same individuals, and each experiment should thus not be considered as independent. Because the digestive passage time variables in our study follow a normal distribution (K-S Lilliefors test in the 'nortest' package, *P* > 0.05), we assumed the Gaussian distribution as the error structure (once called the repeated measures ANOVAs). In all analyses, we set the level of significance (α) to 0.05. All data analyses were conducted using the R version 2.15.0 statistical software (R Developmental Core Team 2012).

Results

The four martens immediately consumed chicks upon presentation, but not all of the seed markers concealed
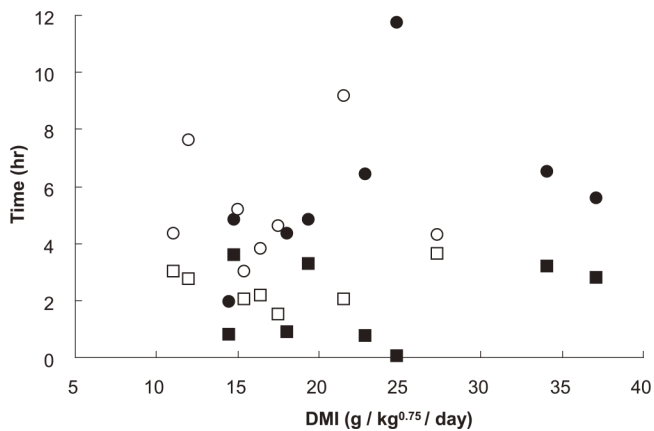
Fig. 1. Relationship of daily dry matter intake (*DMI*, g/kg$^{0.75}$/day) and transit time (*TT*, squares) and mean retention time (*MRT*, circles) obtained from captive Japanese martens (*n* = 4). Open circles/squares represent the single-chick condition, and filled circles/squares represent the three-chick condition.

therein were ingested because several dropped out of the chicks during feeding (Table 1). During the experiments, we recorded 172 defecation events in total (6–22 defecation per experiment, Table 1). The recovery rates of the seeds were within the range of 40–50%.

The mean values of *TT* and *MRT* in the two conditions with large seeds were 2.02 and 5.93 h, respectively, whereas corresponding values for small seeds were 2.19 and 5.11 h, respectively (Table 1). Correlation analysis using two digestive passage variables and average values for the four experiments did not detect a significant relationship between *TT* and *MRT* (Pearson's rank correlation test: *r* = −0.300, *df* = 13, *P* = 0.331). Thus, we used both passage time variables in the next analyses.

We found no significant negative correlation between *DMI* and *TT* (Pearson's rank correlation test: *r* = 0.300, *df* = 14, *P* = 0.229) and between *DMI* and *MRT* (*r* = 0.100, *df* = 13, *P* = 0.710) (Fig. 1). Next, we examined the effects of seed size and the number of chicks on the passage time variables (*TT* and *MRT*) using GLMMs. The two passage time variables did not differ significantly among seed types (GLMM, *TT*: *t* = 0.559, *P* = 0.592; *MRT*: *t* = −1.015, *P* = 0.337, *df* = 8 for each), or with the number of chicks (*TT*: *t* = 0.999, *P* = 0.347; *MRT*: *t* = −1.015, *P* = 0.337, *df* = 8 for each). The interaction between seed type and the number of chicks also showed no significant effect on the passage time variables (*TT*: *t* = −0.730, *P* = 0.487; *MRT*: *t* = 0.019, *P* = 0.985, *df* = 8 for each).

Discussion

The digestive passage time variables *TT* and *MRT* in captive Japanese marten showed no significant relationships with the *DMI*. Furthermore, these passage time variables did not change in response to the number of chicks or seed type, and the ingesta passed through the gastrointestinal systems at a constant time (ca. 2.1 h for *TT* and 5.5 h for *MRT*). In our previous study, we found a lack of significant relationship between seed type and passage time (Tsuji et al. 2011a), and the present study reconfirmed our previous finding. The short, straight digestive tract of carnivores facilitates a rapid rate of food transit but a relatively short period of time for digestion and absorption of protein and fat (Davis et al. 1992).

Unlike Hickey et al. (1999)'s prediction, the passage time of seeds were stable regardless of the amount of diet. Our result was different to those obtained for foregut herbivores (hippopotami and cattle) (Clauss et al. 2007), beavers (Fryxell et al. 1994) and primates (Clauss et al. 2007, 2008; Sawada et al. 2011), in which the passage time was shorter when the animals were fed larger amounts of fibrous foods, and similar to those of elephants and horses (Clauss et al. 2007), in which the passage time moderately increased when the animals consumed a larger amount of food. There are two possibilities that could explain why the passage time of our martens was stable. One possibility is the higher digestibility of animal matter. The fiber content of animal prey (0.2–2.2% dry matter, Davison et al. 1978) is much lower than that of plant materials (tens of percent, Begon et al. 2005), and the martens might have digested the animal prey more efficiently regardless of its amount. In this study, we controlled only the amount of animal prey and we have no data on the effects of consuming fibrous food such as fruits on the passage time and digestion (see also Clauss et al. 2010). To ensure the effect of higher digestibility, we should test whether the passage time remained stable even if we changed the amount of both animal and plant food. Another possibility involves the gut morphology of martens. Mammals possess elastic guts that can expand in volume, which ensures that higher food intake does not significantly impede passage time and enables them to efficiently assimilate nutrients from the consumed food. Unfortunately, we have no anatomical knowledge on the martens' gastrointestinal tract, and future clinical studies addressing this issue are thus warranted. By accumulating such information, we can establish the mechanism underlying the relationship between food intake and passage

time in martens and other mustelids. Number of subject animals in this study was four, and a possibility that lack of significant effects in the GLMM analysis was attributed to the small sample size cannot be denied. Reanalysis using large number of animals is required to confirm our interpretation.

It is known that home range of the Japanese martens expanded when they fed on mammals (Nakamura 2001). Thus, despite the stability in passage time, the dispersal distance of seeds from feeding sites can vary seasonally. The microhabitats that martens prefer also change seasonally (Rondinini and Boitani 2002; Miyoshi and Higashi 2005), primarily because of seasonal changes in the availability and behaviour of their prey (Miyoshi and Higashi, 2005; Caryl et al. 2012). Thus, the characteristics of microhabitats in which martens defecate in a given season can affect seed germination and subsequent growth (Nakashima et al. 2010).

References

Agetsuma, N. and Nakagawa, N. 1998. Effects of habitat differences on feeding behaviors of Japanese monkeys: comparison between Yakushima and Kinkazan. Primates 39: 275–289.

Arai, S., Adachi, T., Kuwahata, Y. and Yoshida, K. 2003. Food habit of the Japanese marten (*Martes melampus*) at Kuju Highland in Kyushu, Japan. Honyurui Kagaku [Mammalian Science] 43: 19–28 (in Japanese with English abstract).

Begon, M., Townsend, C. R. and Harper, J. L. 2005. Ecology: From Individuals to Ecosystems, 4th ed. Blackwell Publishing, Malden, 752 pp.

Caryl, F. M., Raynor, R., Quine, C. P. and Park, K. J. 2012. The seasonal diet of British pine marten determined from genetically identified scats. Journal of Zoology 288: 252–259.

Clauss, M., Kleffner, H. and Kienzle, E. 2010. Carnivorous mammals: nutrient digestibility and energy evaluation. Zoo Biology 29: 687–704.

Clauss, M., Streich, W. J., Nunn, C. L., Ortmann, S., Hohmann, G., Schwarm, A. and Hummel, J. 2008. The influence of natural diet composition, food intake level, and body size on ingesta passage in primates. Comparative Biochemical Physiology A 150: 274–281.

Clauss, M., Streich, W. J., Schwarm, A., Ortmann, S. and Hummel, J. 2007. The relationship of food intake and ingesta passage predicts

feeding ecology in two different megaherbivore groups. Oikos 116: 209–216.

Currie, D. J. and Fritz, J. T. 1993. Global patterns of animal abundance and species energy use. Oikos 67: 56–68.

Davis, H. G., Aulerich, R. J., Bursian, S. J., Sikarskie, J. G. and Stuht, J. N. 1992. Feed consumption and food transit time in northern river otters (*Lutra canadensis*). Journal of Zoo and Wildlife Medicine 23: 241–244.

Davison, R. P., Mautz, W. W., Hayes, H. H. and Holter, J. B. 1978. The efficiency of food utilization and energy requirements of captive female fishers. Journal of Wildlife Management 42: 811–821.

Fryxell, J. M., Vamosi, S. M., Walton, R. A. and Doucet, C. M. 1994. Retention time and the functional response of beavers. Oikos 71: 207–214.

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: 155–170.

Helldin, J. O. 1999. Diet, body condition, and reproduction of Eurasian pine martens *Martes martes* during cycles in microtine density. Ecography 22: 324–336.

Hickey, J. R., Flynn, R. W., Buskirk, S. W., Gerow, K. G. and Wilson, M. F. 1999. An evaluation of a mammalian predator, *Martes americana*, as a disperser of seeds. Oikos 87: 499–508.

Jedrzejewski, W. and Jedrzejewska, B. 1993. Predation on rodents in Bialowieza primeval forest, Poland. Ecography 16: 75–78.

Lambert, J. E. 2002. Digestive retention times in forest guenons (*Cercopithecus* spp.) with reference to chimpanzees (*Pan troglodytes*). International Journal of Primatology 23: 1169–1185.

Lanszki, J. and Heltai, M. 2011. Feeding habits of sympatric mustelids in an agricultural area of Hungary. Acta Zoologica Academiae Scientiarum Hungaricae 57: 291–304.

Miyoshi, K. and Higashi, S. 2005. Home range and habitat use by the sable *Martes zibellina brachyura* in a Japanese cool-temperate mixed forest. Ecological Research 20: 95–101.

Nakamura, T. 2001. Food Habits and Home Ranges of Japanese Marten (*Martes melampus*) on the North Foot of Mt. Fuji. Master thesis. Tokyo University of Agriculture and Technology (in Japanese).

Nakamura, T., Kanzaki, N. and Maruyama, N. 2001. Seasonal changes in food habits of Japanese martens in Hinode-cho and Akiruno-shi, Tokyo. Yasei Seibutsu Hogo [Wildlife Conservation Japan] 6: 15–24 (in Japanese with English abstract).

Nakashima, Y., Inoue, E., Inoue-Murayama, M. and Abd Sukor, J. R. 2010. Functional uniqueness of a small carnivore as seed dispersal agents: a case study of the common palm civets in the Tabin Wildlife Reserve, Sabah, Malaysia. Oecologia 164: 721–730.

R Developmental Core Team 2012. R: A Language and Environment for Statistical Computing. Version 2.15.0. R Foundation for Statistical Computing, Vienna.

Remonti, L., Balestrieri, A. and Prigioni, C. 2007. Role of fruits in the diet of small mustelids (*Mustela* sp.) from the western Italian Alps. Eurasian Journal of Wildlife Research 53: 35–39.

Rondinini, C. and Boitani, L. 2002. Habitat use by beech martens in a fragmented landscape. Ecography 25: 257–264.

Sawada, A., Sakaguchi, E. and Hanya, G. 2011. Digesta passage time, digestibility, and total gut fill in captive Japanese macaques (*Macaca fuscata*): effects of food type and food intake level. International Journal of Primatology 32: 390–405.

Schaumann, F. and Heinken, T. 2002. Endozoochorous seed dispersal by martens (*Martes foina*, *M. martes*) in two woodland habitats. Flora 190: 370–378.

Schupp, E. W., Jordano, P. and Gómez, J. M. 2010. Seed dispersal

effectiveness revisited: a conceptual review. New Phytologist 188: 333–353.

Ting, S., Hartley, S. and Burns, K. C. 2008. Global patterns in fruiting seasons. Global Ecology and Biogeography 17: 648–657.

Tsuji, Y., Shiraishi, T. and Miura, S. 2011a. Gastrointestinal passage time of seeds ingested by captive Japanese martens *Martes melampus*. Acta Theriologica 56: 353–357.

Tsuji, Y., Tatewaki, T. and Kanda, E. 2011b. Endozoochorous seed dispersal by sympatric mustelids, *Martes melampus* and *Mustela itatsi*, in western Tokyo, central Japan. Mammalian Biology 76: 628–633.

Tsuji, Y., Yasumoto, Y. and Takatsuki, S. 2014. Multi-annual variation in the diet composition and frugivory of the Japanese marten (*Martes melampus*) in western Tokyo, central Japan. Acta Theriologica 59: 479–483.

Tsujino, R. and Yumoto, T. 2009. Topography-specific seed dispersal by Japanese macaques in a lowland forest on Yakushima Island, Japan. Journal of Animal Ecology 78: 119–125.

Willson, M. F. 1993. Mammals as seed-dispersal mutualists in North America. Oikos 67: 159–176.

Yamagishi, M. 1990. Seasonal food habits of the Japanese marten. Bulletin of Experimental Forest of University of Tokyo 83: 9–18 (in Japanese with English abstract).

Zhou, Y. B., Newman, C., Xu, W. T., Buesching, C. D., Zalewski, A., Kaneko, Y. and Macdonald, D. W. 2010. Biogeographical variation in the diet of Holarctic martens (genus *Martes*, Mammalia: Carnivora: Mustelidae): adaptive foraging in generalists. Journal of Biogeography 38: 137–147.