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Original article

Hares promote seed dispersal and seedling establishment after volcanic eruptions

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ABSTRACT

Although seed dispersal through animal guts (endozoochory) is a process that determines plant establishment, the behaviour of carriers mean that the seeds are not always dispersed to suitable habitats for germination. The germinable seeds of *Gaultheria miqueliana* were stored in the pellets of a hare (*Lepus timidus ainu*) on Mount Koma in northern Japan. To clarify the roles of hares in seed dispersal and germination, field censuses and laboratory experiments were conducted. The field observations were conducted on pellets and seeds in four habitats (bare ground, *G. miqueliana* shrub patch, *Salix reinii* patch, and *Larix kaempferi* understory), and the laboratory experiments were conducted on seed germination with different light, water potential and cold stratification treatments. The laboratory experiments confirmed that seed germination began a few weeks after the sowing of seeds, independent of cold stratification, when light was sufficient and the water potential was low. The seeds did not germinate at high water potential. The pellets were gradually degraded *in situ*. More seeds germinated from crushed than from intact pellets. Therefore, over the long term, seeds germinated when exposed to light due to the degradation of pellets. The pellets were proportionally dispersed among the four studied habitats. More seeds sown in the field germinated more in shaded habitats, such as in the *Gaultheria* patch and the *Larix* understory, and seeds did not germinate on bare ground, where drought often occurred. Thus, the hares had two roles in the dispersal and germination of seeds: (1) the expansion of *G. miqueliana* populations through seed dispersal to various habitats and (2) the facilitation of delayed seed germination to avoid risks of hazards such as drought. The relationships between small mammals represented by the hare and the shrubs that produce berries are likely to be more mutually evolved than was previously thought.

1. Introduction

Seeds dispersed by animals often determine the spatial structures and dynamics of plant populations (Godinez-Alvarez et al., 2002; Levin et al., 2003). The fates of seeds after dispersal are determined by the suitability of environments for seedling emergence (Schupp et al., 2010). In endozoochory, diaspores transit through the digestive system of animals, and a seed is dispersed to a given habitat, depending on the behaviour of the animal (Wenny, 2001). For example, seeds of *Rhus trichocarpa* (Anacardiaceae) pass through the guts of crow to immigrate to bare grounds on the summit of Mount Koma in northern Japan, a suitable habitat for seed germination (Nishi and Tsuyuzaki, 2004). Therefore, understanding the dynamics of plant population requires the identification of the habitats for seed dispersal.

Gaultheria miqueliana Takeda (Ericaceae) forms evergreen shrub patches. The shrubs produce enormous endozoochorous berries during summer and fall on Mount Koma, and the seed banks are found mostly beneath the shrub patches (Uesaka and Tsuyuzaki, 2004). Additionally, mammal pellets are distributed abundantly on the southwestern slope. Preliminary investigations of the pellets confirmed that more than one hundred *G. miqueliana* seeds germinated from the mammalian pellets collected in the early summer of 2001 on Mount Koma (Tsuyuzaki, unpublished data). *G. miqueliana* seeds require light for germination (Tsuyuzaki and Miyoshi, 2009). Based on the morphological traits of pellets and the fauna of Mount Koma, the pellets were eliminated from the hare, *Lepus timidus ainu* (Leporidae). The common European rabbit, *Oryctolagus cuniculus* (Leporidae), disperses seeds of *Corema album*

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(Empetraceae) to bare ground (Calvino-Cancela, 2002). However, these mammals are herbivores or frugivores that feed on plants and often reduce seed germinability (Izhaki and Ne'eman, 1997; Chang et al., 2005). Therefore, evaluating the effectiveness of seed dispersal by hares requires assessing the germinability in dispersed habitats.

These results suggested that the feeding on seeds by hares had two effects on the population maintenance and enlargement of *G. miqueliana*: the plant population was enlarged by seed dispersal in hare pellets and was maintained by the development of a seed bank in the pellets in the absence of light. To understand the relationship between seed dispersal by the hare and the germination of *G. miqueliana* seeds, the following three hypotheses were examined: (1) pellets were distributed evenly across the habitats because hares eliminated at random, (2) seeds germinated in any habitat soon after dispersal when light and moisture were suitable, and (3) the absence of light delayed the germination of seeds embedded in pellets.

2. Materials and methods

2.1. Study sites and plant materials

The study site is located on the southwestern slope of Mount Koma (42°04′N, 140°42′E, 1131 m asl) in southwestern Hokkaido Island, Japan. The climate is warm-cool temperate, and between 1981 and 2010, average annual precipitation was 1097 mm, and the mean annual temperature was 8.1 °C (Mori Climatological Observatory, 8 km north from the summit of Mount Koma). Snow generally accumulates from late November to late May. During the survey period, the annual precipitation was 1029 mm and 1278 mm in 2012 and 2013, respectively, and was equivalent to the typical year. However, the rainfall pattern during June and August 2013 was unusual because the total rainfall was only 80 mm, and the daily maximum of 30 mm was recorded during June and July, with a daily average of 1.3 mm. Therefore, the ground surface was dry in non-vegetated areas during this period except after rainfalls. In contrast, total rainfall during August and September was 682 mm, with a maximum of 174 mm and an average of 11.2 mm/day. The total rainfall during October and November was 262 mm.

The mountain is an active, andesitic volcano. The most recent major eruption in 1929 produced 0.38 km^3 of ash and 0.14 km^3 of volcanic ejecta and induced blast and debris avalanches, which destroyed the former vegetation (Kondo and Tsuyuzaki, 1999). Small-scale eruptions were recorded in 1996, 1998 and 2000, but the effects on vegetation were minimal in the study area (Nishi and Tsuyuzaki, 2004). The recovery of the vegetation was slow, and the amount of bare ground increases with increasing elevation. Four major habitats were recognized for plant colonization (Uesaka and Tsuyuzaki, 2004; Akasaka and Tsuyuzaki, 2009): *G. miqueliana* patches (hereafter *Gaultheria* patches), the *Larix kaempferi* understory (*Larix* understory), the *Salix reinii* patches (*Salix* patches) and bare ground. The former three habitats were vegetated with the respective vascular plants, and the bare ground was covered with mosses and lichens, or with nothing. *G. miqueliana* is an evergreen shrub, *L. kaempferi* a tall tree, and *S. reinii* a deciduous shrub.

G. miqueliana produces fruits (berries) during early summer and late fall on Mount Koma, and most seeds are dispersed before snowfall. The mature fruits each contain more than 125 seeds, and the seeds are small, approximately 0.065 mg (Tsuyuzaki and Miyoshi, 2009).

2.2. Fate of pellets

To investigate the dynamics of hare pellets, 30 2 m × 1 m plots were established on the southwestern slope at approximately 680 m in early July 2012. The silhouettes of habitats in each plot were sketched on cross-section paper with 10-cm accuracy. The area of each habitat was measured with scan of the silhouettes. All the pellets were removed from the plots before the pellet counts began.

The census was conducted monthly during snow-free periods from August 2012 to November 2013. The census was divided in two phases: the first phase was before June 2013 and the second phase was after July 2013, because the sampling design changed. The locations of newly immigrated pellets were recorded at every census. In the first phase, 42 immigrated pellets were randomly marked and left in the plots to evaluate the natural degradation of the pellets, and 65 pellets were taken to the laboratory. The degradation of pellets in the first phase was evaluated by visually checking each pellet left in the plots until the final census was conducted on November 6, 2013. In the second phase, all immigrated pellets were removed to the laboratory. The seed germination tests of the pellets in the laboratory were performed immediately. The tests were conducted on three-layered filter paper (Whatman #1) in petri dishes at 25 °C/5 °C with an 18:6 h light:dark cycle.

2.3. Seed germination from pellets in laboratory experiments and on habitats in the field

The temperature and light intensity were measured at 1-h intervals with three automatic data loggers (HOBO, UA-002-XX, Onset, Bourne) randomly placed on the ground surface in each habitat during the snow-free period from May 23 to November 8, 2013. The photosynthetic photon flux density (PPFD) was calculated from the comparisons between the data loggers and a PAR sensor (S-LIA-M003, Onset). For each habitat, the daily mean temperature and the daily maximum PPFD were calculated.

Seed germination tests were conducted with pellets collected from the southwestern slope between 480 and 900 m asl in early August 2013. The tests were conducted for two months, from late August to late October. Because pellets degraded with time *in situ*, two treatments of pellets were prepared intact and degraded. The degraded pellets were prepared by crushing gently by hands. The germination tests of pellets were conducted in an incubator at 25 °C/5 °C with an 18:6 h light:dark cycle. In the experiment, one pellet was placed on three layers of filter paper in a petri dish. Each dish was wrapped with Parafilm after 3 ml of distilled water was poured on the filter papers. The seedlings were counted every two weeks until no new seedlings were observed for 10 days. Each treatment included 45 replications.

The fruits of *G. miqueliana* were randomly collected from more than 20 patches in November 2012, and were stored in an incubator at 4 °C in the dark. The seeds were extracted from the fruits before sowing. Fifty seeds each were placed in twenty plastic cups (6.5 cm in diameter and 3.5 cm in depth), which were placed in each habitat in June 2013. Each cup was filled with volcanic ash collected from bare ground near the study site. The germinated seeds were counted every two weeks until early November 2013. The viability of the seeds used in the cup experiment was determined using a seed germination test that included eight replicates of 50 seeds in the laboratory at 25 °C/5 °C with an 18:6 h light:dark cycle.

2.4. Seed germination characteristics

Fruits were collected from more than 20 individuals in early August or early October 2012. The fruits were air-dried in paper bags for a week and were stored in an incubator at 4 °C in the dark until use. The seeds were separated from pulps just before the