The Seed Germination of Berry-Producing Ericaceous Shrubs in Relation to Dispersal by Hare

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ABSTRACT

Hare pellets contribute greatly to the seed dispersal of a berry-producing shrub, *Gaultheria miqueliana* Takeda (Ericaceae), on Mount Koma, northern Japan. Numerous seeds of *G. miqueliana* germinated from the hare pellets (122.2 ± 7.3/pellet, mean ± SE), confirming that the pellets potentially had a role in the seed dispersal. Lab-experiments confirmed that the seeds of *G. miqueliana* needed light for full germination without cold stratification. Besides, hare pellets were collected from a post-mined peatland in Sarobetsu Mire, northern Japan, where *G. miqueliana* was not distributed but two berry-producing ericaceous species were distributed. No seeds germinated on the pellets collected from the Mire. These results suggested that the tight relationships between hare and berry-producing shrubs were species-specific.

Keywords

Berry fruit; Hare pellet; Light; Seed dispersal and germination; Volcano; Wetland

Introduction

The potential of seeds dispersed through mammalian guts, i.e., endozoochory, often determines plant population dynamics, even though the seed survival is reduced by the digestion (Cosyns et al. 2005). Of the mammals, hares (*Lepus* spp.) act as seed dispersers of *Retama raetum* (Fabaceae) in the east Mediterranean deserts (Izhaki and Ne'eman 1997). A dwarf shrub, *Gaultheria miqueliana* Takeda, produces berries containing numerous seeds that are dispersed by mountain hare (*Lepus timidus ainu*) on Mount Koma, northern Japan, and the hare pellets facilitate delayed seed germination, i.e., seed germination occurs with the degradation of pellets for long-term to avoid risks such as drought (Nomura and Tsuyuzaki 2015). Therefore, the hare and shrub, *G. miqueliana*, are more mutually evolved than previously thought.

Since light and cold stratification are two major determinants on seedbank development and delayed seed germination (Baskin and Baskin 2014), further lab-experiments were performed to confirm the effects of light and cold stratification on the seed germination of *G. miqueliana*. *G. miqueliana* is distributed in northern Japan, Sakhalin, Kuril Islands and Aleutian Islands (Satake et al. 1981), while hare (*Lepus timidus*) is distributed in the arctic, subarctic and boreal regions of the Old Continent (Thulin 2003). *L. t. ainu* is endemic subspecies to Hokkaido (Yamada et al. 2002). Therefore, a distributional gap between the shrub and hare exists. Due to the behaviors of animals, the dispersal distances of endozoochorous seeds are determined (Jordano 2017). The seeds of *G. miqueliana* dispersed by a migrant bird, *Turdus naumanni*, which moves between Siberia and southern China via Japan, has been reported (Nishi and Tsuyuzaki 2004). The home ranges of *L. t. ainu* are a few tens of hectares (Chapman and Flux 1990). Therefore, the short-distance seed dispersal by hare that maintains the population of *G. miqueliana* is expected, as compared with the migrant bird.

Ericaceous shrubs often establish in nutrient-poor habitats, such as volcanoes and bogs, using symbiotic mycorrhizal fungi (Keddy 2007). A post-mined peatland in

Sarobetsu Mire, where nutrients were poor (Nishimura and Tsuyuzaki 2015), allows the establishment of two ericaceous shrub species producing berries, *Empetrum nigrum* L. and *Vaccinium oxycoccos* L., but lacks *G. miqueliana* (Egawa et al. 2017). Therefore, pellets collected from the post-mined peatland were also investigated to examine if germinable seeds were available in the pellets. The seed dormancy types of these three species are categorized into physiological dormancy with firm seed coats (Vander Kloet and Hill 2000; Tsuyuzaki and Miyoshi 2009; Baskin and Baskin 2014). Therefore, the seed characteristics of these three species were expected to be comparable.

Using the seed germination tests of hare pellets and fruits collected from the volcano and pellets collected from the peatland, I reported: 1) the responses of *G. miqueliana* seeds to light and cold stratification; and 2) species-specific interactions between hare pellets and berry-producing shrubs. Although this study did not examine the seasonal and annual variations of seed production, it detected that species -specific relationships between hare and berry-producing shrubs were present.

Methods

Sampling sites in relation to berry-producing ericaceous shrubs

The samples were collected from the two locations, Mount Koma and Sarobetsu Mire, northern Japan. Mount Koma is an andesite stratovolcano of which last catastrophic eruption occurred in 1929 (Table 1). The revegetation progresses slowly by *Larix kaempferi* (Lam.) Carr. but the nutrients are still poor (Kwon and Tsuyuzaki 2016). Although seven ericaceous species are recorded from Mount Koma (Tsuyuzaki et al. 2001), all species except for *G. miqueliana* produce capsules.

Sarobetsu Mire, occupied mostly by bog, is distributed in the northernmost part of Hokkaido, Japan (Table 1). In the mire where *Sphagnum* mosses were predominant, peat mining was conducted from 1970 until 2003. The succession after peat mining starts with pioneer sedges, such as *Rhynchospora alba* (L.) Vahl. (Nishimura and

Tsuyuzaki 2014). Four ericaceous species establish in this post-mined peatland (Egawa et al. 2017). Of the four species, *E. nigrum* and *V. oxycoccos* produce berries.

Sample preparations and seed germination experiments

The pellets were collected from the southwestern slope of Mount Koma in May 5 2001 and from the post-mined peatlands of Sarobetsu Mire in early April 2019. The pellets were collected from different locations that were apart more than 5 m to each other. The timings of pellet collections were just after snowmelt in both sites. The hare infrequently utilizes grassy habitats, including Sarobetsu Mire, during snow season to avoid the predators (Abe and Ota 1987). The three berry-producing shrubs, *G. miqueliana*, V. *oxycoccos* and *E. nigrum*, produce their fruits during middle summer to late autumn. Since the hare pellets were collected in early spring before the fruit production began in the current year, seeds in the pellets should be produced in the last year or earlier if available. In addition, most seeds were exposed naturally to cold stratification for more than a few months.

The inter-annual variations of fruit production are often observed for berry-producing shrubs, including these three examined species (Nestby et al. 2019). However, annual monitoring on Mount Koma and Sarobetsu Mire to date confirms that the fruit production of the examined species is not poor every year (Tsuyuzaki and Hase 2005, Nishimura and Tsuyuzaki 2014). The food of hare, *L. t. ainu*, is diverse among seasons and among regions (Chapman and Flux 1990). When numerous seeds germinated from the pellets, the results also suggested that the hare depend the food on the berries.

The seed germination test of pellets collected from Mount Koma started on May 12 2001. A pellet crushed gently by hands was placed on a moistened three-layered filter paper (Whatman #1) in a petri dish. Thirty replications of pellets were used. The pellets in dishes were kept at a 5°C/25°C cycle with continuous light in an incubator.

Distilled water was poured into the petri dishes when the pellet surface was slightly dried. Seed germination was counted every day until the peak of germination and thereafter at ever few days until no seeds germinated for one week for all the seed germination experiments. Pellets collected from Sarobetsu Mire were treated as the same way with these collected from Mount Koma except for the temperatures. Two treatments were conducted, i.e., 10ºC/25ºC (12h/12h) with continuous light and 5ºC/20ºC (12h/12h) with discontinuous light (12h/12h). Ten replications were used in each treatment. When each experiment was completed, the pellets on all petri dishes were observed under a binocular microscope to check if viable seeds remained.

The mature fruits of *G. miqueliana* on Mount Koma were collected from more than 20 shoots, which were apart more than 5 m to each other, on the southwestern slope in the end of October 2001. About five fruits were collected from each shoot. Seeds were removed gently from the fruits soon after the collection and kept in a paper bag at room temperature for one month until use. In each petri dish, 50 seeds were sown on a moistened three-layered filter paper without any pre-treatments on November 26 2001. The seeds were randomly selected from the paper bag. The remainders of seeds were sown after cold stratification at 2ºC for one month under the darkness. Then, the seeds were placed into an incubator with light and with darkness on December 26 2001. On each combination of treatments (light × cold stratification), 50 seeds were placed into each dish and 10 replications were used. While the germination was recorded under dim green light on the dark treatment, it was done under fluorescent light on the light treatment. Soon after completing the experiment, the viability of ungerminated seeds was estimated from the extent to which they were firm and intact (Ishikawa-Goto and Tsuyuzaki 2004).

Final germination (FG; %) and the time to onset of germination (t_0; d) were calculated for each petri dish. Final germination was calculated as germination percentage in relation to total viable seeds and t_0 was the day for the first germination. Differences in the final seed germination between the treatments were examined by a generalized linear model (GLM) with a binomial distribution and logit-link function (R version 3.6.1) (R Core Team 2019). The equation is:

$$P(g) = 1/(1 + \exp(-A)), \text{ and}$$

$$A = \alpha_c \cdot \log t + \alpha_l \cdot \log l + \alpha_{c \times l} \cdot (\log c \times \log l) + \beta,$$

where $P(g)$ is the probability of seed germination rate; c and l indicate cold stratification and light, respectively. Cross symbols (×) indicate interactions. α_i is the slope of each explanatory variable; and β is the intercept. In the same way, differences in t_0 was investigated by GLM with and without light and cold stratification, respectively, with the assumption of binomial distribution and logit-link function.

Results

In total, 3853 seeds germinated from the 30 pellets for five months. The number of germinated seeds per pellet averaged 122.2 ± 7.3 (mean with SE; range: 64-210 seeds). The first seeds germinated 13 days after sowing but was *Anaphalis margaritacea* Benth. et Hook. fil. var. *angustior* Nakai. Except this seeding, all the seeds were *G. miqueliana* . The t_0 of *G. miqueliana* averaged 18.9 ± 0.3 days after sowing the seeds (Figure 1). Thereafter, the seed germination occurred enormously for two weeks and ceased 119 days after sowing the seeds.

The pellets collected from Sarobetsu Mire did not show any seeds germinated for five months under the two temperature fluctuation patterns with continuous and discontinuous light. The microscopic observations of pellets after completing the seed germination experiments conducted by the pellets collected from Mount Koma and Sarobetsu Mire confirmed that germinable or viable seeds were least in the pellets, showing that most seeds germinated by the experiments.

On the seeds of *G. miqueliana* removed from fruits, t_0 of all the treatments averaged 13.9 ± 0.5 days. The t_0 indicated that the cold-stratified seeds germinated earlier (GLM, slope = -1.143, $P < 0.01$), while t_0 did not changed by light exposure ($p = 0.365$).

The interaction was not significant (p = 0.447). These results showed that the cold stratification promoted early germination and the light did not. The final seed germination percentage was 86.2% ± 1.8 under the light and 29.2% ± 2.7 under the darkness when the seeds were not exposed to cold stratification (Figure 2). When the seeds experienced cold stratification, the percentages were 85.0% ± 1.2 and 46.4% ± 3.5 under the light and darkness, respectively. The seed germination percentages were affected by light but not by cold stratification (intercept = +1.742, significant at $P < 0.001$), viz; the seed germination was decreased by the darkness (slope = -1.887, $P < 0.001$) and was not affected by cold stratification ($P = 0.618$). The interaction between light and cold stratification was also significant (slope = -0.783, $P < 0.001$), showing that the cold stratification enhanced the seed germination under the darkness. The microscopic observations after the experiments confirmed that 1.9 and 0.6 seeds per dish had been rotten in average with and without light, respectively. Therefore, nearly all seeds germinated with light and 1/2 or less seeds were still dormant without light.

Discussion

The pellets collected from Mount Koma in early spring emerged 64 seedings or more per pellet, indicating that the hare has a high potential role in the seed dispersal of *G. miqueliana*. Even though the seed survival is decreased after mammalian digestion (Cosyns et al. 2005), the hare should be considered to contribute to the seed dispersal of *G. miqueliana* on Mount Koma. Concurrently, these meant that the hare depended its food greatly on the berries of *G. miqueliana* in late spring. In contrast, no seeds germinated from the pellets collected in early spring on Sarobetsu Mire. Since the pellets collected from the mire, where the two berry-producing ericaceous species established, did not contain viable seeds, the diet of hare should be examined (Green et al. 2013). The seeds of *G. miqueliana* required light for full germination and did not require cold stratification, although the seed germination became earlier by the cold stratification. This meant that the cold stratification promoted the seeds germinate quickly so that seedlings are large before dry summer.

By contrast, the hare pellets prolong the seed germination period when the pellets degraded slowly, because of no light in the inside. The decay rates of *Lepus americanus* pellets, of which decay is defined as visually disappeared, are 1-14% per year, increase with soil moisture in a tundra shrubland (Prugh and Krebs 2004). Since moisture in the volcanic deposits of Mount Koma is dry in summer (Uesaka and Tsuyuzaki 2004), the decay rates should be lower. This seed germination strategy is not observed on other species and/or in other regions, including Sarobetsu Mire, although berry-producing ericaceous species establish well in the mire. The responses of sedge seeds to cold stratification and daily temperature fluctuation differ greatly between wetland and dryland species (Schutz and Rave 1999). As the soil moisture is different between the volcano and peatland, the different seedbank development in hare pellets is possible. Therefore, the optimal seed germination timings and patterns seemed to be determined by a balance between seed germination shortened by cold stratification and prolonged by burial in the hare pellets.

In Sarobetsu Mire, the two ericaceous species usually produce numerous berries. However, these seeds were not present in the hare pellets collected in early spring. The two species in Sarobetsu Mire do not develop seedbanks in the pellets, as shown in this study, and in the peat on the post-mined peatland (Egawa et al. 2009). The hares in Sarobetsu Mire do not rely on ericaceous berries as food resources. In contrast, the seedlings of *E. nigrum* emerge from scats provided by passerine, gull, mink and fox in a wide range of Nova Scotia (Hill et al. 2012). These results suggest that the behaviors of hare, related to the development of seedbank in pellets, are different between the habitats or regions.

Numerous *G. miqueliana* seeds were viable in the pellets collected from Mount

Koma. The t_0 was a few days earlier for the seeds removed from fruits than for the seeds germinated from the pellets. These results suggested that the digestion by hare damaged less seeds. Hare pellets contribute to the dispersal and accumulation of nutrients through mineral cycling in nutrient-poor ecosystems such as tundra and then increase shrub growth (Krebs et al. 2001). Nutrients are poor in the volcanic ejecta and *Sphagnum* peat. In fact, nitrogen and phosphorus are poor on the volcanic bareground (Uesaka and Tsuyuzaki 2004) and the post-mined peatland (Nishimura and Tsuyuzaki 2014). The nutrient supply from pellets is likely to contribute to the establishment and growth of *G. miqueliana*. In conclusion, the firm interactions between the shrub and hare on the volcano and the least interactions between them on the mire show the regional and species differences in interspecific interactions between the plants and hares.

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Table 1. The characteristics of study sites, Mount Koma and Sarobetsu Mire in northern Japan .

Study site	Mount Koma	Sarobetsu Mire
Latitude	42°03’N	45°06’N
Longitude	140°40’E	141°42’E
Elevation (m)	650-450[a]	7
Mean annual temperature (°C)	10.2[b]	12.3[b]
Mean annual precipitation (mm)	1072.5[b]	1097.3[b]
Disturbance	Eruption in 1929	Peat mining during 1970 to 2003
Number of ericaceous species	7	4
Ericaceous species producing berry	*Galutheria miqueliana*	*Empetrum nigrum* *Vaccinium oxycoccos*

a) the top of mountain is 1133 m a.s.l. The study area is located on the southwestern slope of mountain.

b) the mean annual temperature and mean annual precipitation calculated for the period from 1981 and 2010 on weather stations located in Mori Town (7 km from Mount Koma, 10 m a.s.l.) and in Toyotomi Town (5 km far from Sarobetsu Mire, 16 m a.s.l.) (Japan Meteorological Agency 2019).

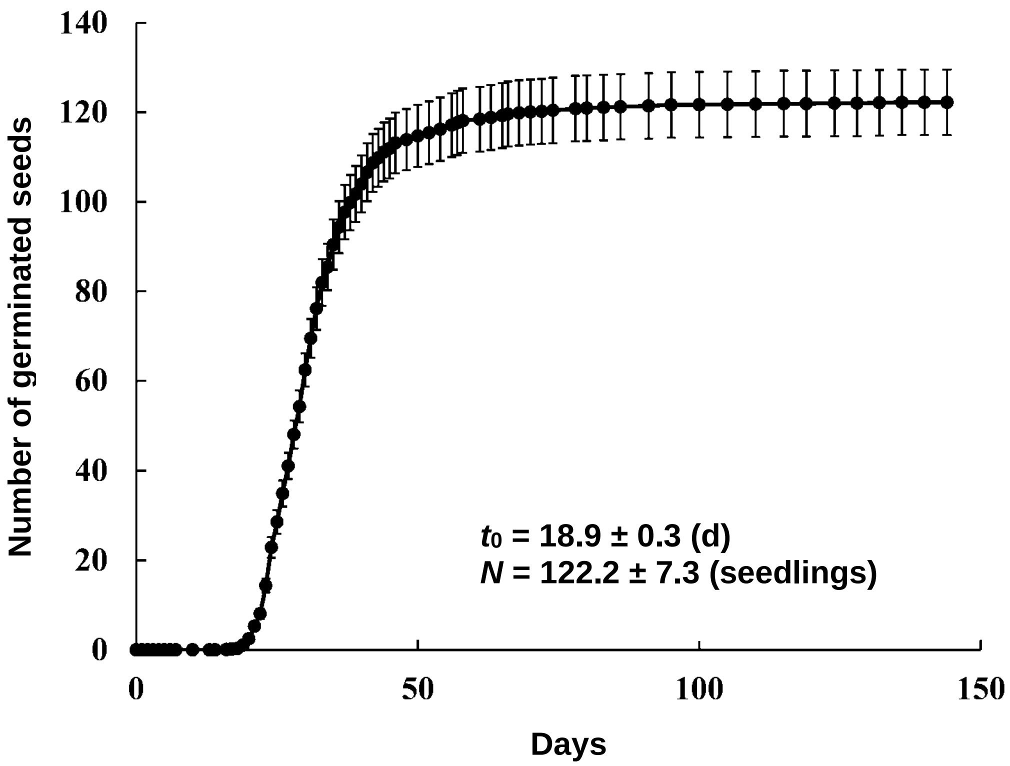

Figure 1. Cumulative number of seedling emergence of *Gaultheria miqueliana* by 30 hare pellets collected on May 5 2001from Mount Koma. The lab-experiment started on May 12 2001 and ceased on September 20 2001. The mean of cumulative number of seedling emergence is shown with standard error (vertical bars). *N* = number of seeds germinated at the final investigation (mean ± SE).

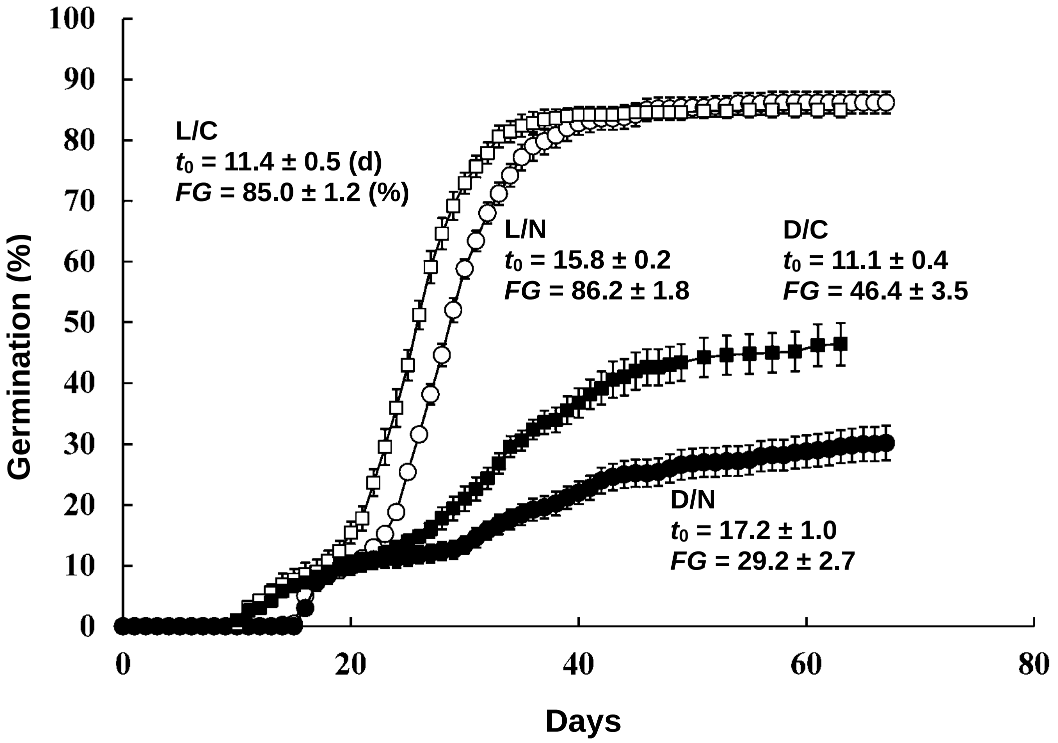

Figure 2. The cumulative germination percentage of *G. miqueliana* seeds treated by cold stratification and light, confirmed by lab-experiments. The mean number of ten replicates at each treatment is shown with standard error (vertical bars). Open and closed symbols indicate seeds under light (L) and darkness (D), respectively. Circles and squares indicate non-cold (N) and cold (C) stratification, respectively. On the statistical results, see in the text.