Germinability of Seeds in a Glacial Relict *Dryas octopetala* var. *asiatica*: Comparison with a Snowbed Alpine Plant *Sieversia pentapetala* in a Middle-Latitude Mountain Area of Central Japan

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Abstract

Dryas octopetala var. *asiatica*, a glacial relict plant species, is now fragmentally distributed in wind-blown fellfields in middle-latitude mountains in northeastern Asia. Because of the specific habitat requirements of this species, its isolated distribution, and therefore restricted gene flow between populations, there is concern that the population will decline with global warming. To assess the reproductive viability of *D. octopetala* var. *asiatica* growing in a southernmost population in its geographical distribution in the Tateyama Range of central Japan, we examined the maturity and germinability of seeds under various thermal conditions in laboratory experiments. We compared the viability and germination behavior of the seeds of this relict species with those of a snowbed alpine species, *Sieversia pentapetala*, commonly distributed in alpine regions in Japan. The seeds of *D. octopetala* var. *asiatica* showed lower maturity and greater susceptibility of seed germination, i.e., fewer seeds germinated in the warmest thermal condition (35) in *D. octopetala* var. *asiatica* than in *S. pentapetala*.

Keywords: *Alpine Plants, Conservation, Dryas, Glacial Relict, Seed Germination*

Introduction

In the middle-latitude mountains of northeastern Asia, some species of plants or animals have isolated distributions with long distances between populations within a single species. A glacial relict, *Dryas octopetala* L. var. *asiatica* (Nakai) Nakai (Rosaceae), is one such plant species. As suggested by Hultén (1959) in Alaskan *Dryas*, it is speculated that Asian *Dryas* species emigrated from high arctic regions to middle-latitude ones during the last glaciation; they were restricted to nonglaciated refugia in northeastern Asia but achieved widespread distribution again following glacial retreat.

Dryas octopetala L. var. *octopetala* is now widely distributed in circumpolar regions of the

Northern Hemisphere (Hultén, 1968), while the Asian endemic variety *D. octopetala* var. *asiatica* is now fragmentally distributed in alpine regions of northeastern Asia (Shimizu, 1982). Because *D. octopetala* var. *asiatica* appears to adapt to cool thermal environments (e.g., Wada and Hohnoki, 2001), and genetic diversity within a population is likely to be low (Max et al., 1999; Wada and Watanabe, personal observation in 2003), there is concern that populations could decline or become extinct with global warming. To assess the healthiness of a population for conservation purposes, it is first important to clarify its reproductive activity and the viability of its offspring. The productivity of viable seeds and their manner of germination are considered to have an important impact on population dynamics and recruitment success (Harper, 1977; Washitani and Yahara, 1996). *Dryas octopetala* var. *octopetala* has little capability of vegetative reproduction (Hagen, 2002), so the regeneration of this species depends mostly on sexual reproduction to produce viable seeds. In the high arctic tundra, extremely low germinability of seeds, i.e., less than 10% germination, has been reported in *D. octopetala* var. *octopetala* (Hagen, 2002). However, to date, there are no reports on *D. octopetala* var. *asiatica* growing in a middle-latitude alpine region.

The aim of this study was to clarify the ability of seeds of a glacial relict, *D. octopetala* var. *asiatica,* growing in the Tateyama Range of central Japan, to germinate under various thermal conditions. We compared the viability of the offspring of this species with that of a snowbed alpine species, *Sieversia pentapetala* (L.) Greene, which belongs to the same Rosaceae family and is commonly distributed in alpine regions of central to northern Japan.

The alpine ecosystem consists of two contrasting habitats, fellfields and snowbeds (Körner, 1999). Fellfields are located on ridges or around mountaintops where little snow accumulates, resulting in freezing soil during winter, and cool air temperatures and frost in spring (Shimono and Kudo, 2003). *Dryas octopetala* var. *asiatica* is found mainly in this harsh environment. In contrast, snowbeds are located on leeward slopes or at the bottom of valleys where a large amount of snow accumulates. This protects plants and soils from freezing during winter, and results in moist soil in summer and warm air temperatures when seeds are exposed after the snow melts, but a shortened growing season. *Sieversia pentapetala* favors this environment (Kudo and Ito, 1992). Because their habitat conditions are so different, we expected to find considerable differences in the germination behavior of the seeds of these two species; i.e., higher response of seeds to lower temperatures and/or lower tolerance to higher temperatures in *D. octopetala* var. *asiatica* compared to *S. pentapetala*. The second aim of this study was to examine this hypothesis in laboratory experiments.

Study area

The study area was located in the alpine zone of the Tateyama Range, Toyama Prefecture, central Japan (latitude 36°33′42″N; longitude 137°36′29″E). There is a population of *D. octopetala* var. *asiatica* on the north-facing slope of the ridge (i.e., fellfield habitat) of Mt. Johdo at 2700 m a.s.l. It is a small population with less than 150 patches observed in a ca. 20 × 50-m area (Matsuzato and Wada, personal observation in 2003); there is no other population of *D. octopetala* var. *asiatica* in the study

area. The population is isolated from the nearest populations by at least 10 km of unsuitable habitat. In contrast, *Sieversia pentapetala* is commonly continuously distributed in snowbed habitats in this study area; we selected one population of this species growing on the east-facing slope of Mt. Johdo at 2820 m a.s.l. (Wada, 2000; Taguchi and Wada, 2001).

In the fellfield habitat dominated by *Dryas octopetala* var. *asiatica*, the vegetation consists of evergreen shrubs such as *Arcteria nana* (Maxim.) Makino, *Diapensia lapponica* L. var. *obovata* Fr. Schm., and *Loiseleuria procumbens* (L.) Desv.; deciduous shrubs such as *Arctous alpinus* (L.) Niedenzu and *D. octopetala* var. *asiatica*; forbs such as *Gentiana algida* Pall., *Geum calthifolium* Smith var. *nipponicum* (F. Bolle) Ohwi, *Polygonum viviparum* L., and *Potentilla matsumurae* Th. Wolf; graminoids; and mosses.

In the snowbed habitat dominated by *S. pentapetala*, the vegetation consists of evergreen shrubs such as *Empetrum nigrum* L. var. *japonicum* K. Koch, *Loiseleuria procumbens,* and *Phyllodoce aleutica* (Spreng.) A. Heller; the deciduous shrub, *S. pentapetala*; forbs such as *Aletris foliata* (Maxim.) Bureau et Franch., *Anaphalis lactea* Maxim., *Anemone narcissiflora* L. var. *nipponica* Tamura, *Fauria crista-galli* (Menzies) Makino subsp. *japonica* (Franch.) Gillet, *Potentilla matsumurae* Th. Wolf, and *Schizocodon soldanelloides* Sieb. et Zucc. f. *alpinus* Maxim.; a sedge *Carex blepharicarpa* Franch.; and grasses.

Materials and methods

1. Soil surface temperature

To clarify seasonal variations in soil-surface temperature in each habitat (fellfield at 2700 m a.s.l. and snowbed at 2820 m a.s.l.), we placed a temperature logger (StowAway TidbiT, Onset Computer Co., MA, USA) at a depth of 0.5 cm below the soil surface, and measured the temperature at one-hour intervals from 6 October, 2001, to 10 October, 2002. We then compared seasonal variations in the soil temperature of the two habitats.

2. Plant materials

Dryas octopetala L. var. *octopetala* (Rosaceae), an evergreen (Rønning, 1996) or semi-evergreen (Jonasson, 1989) dwarf shrub, is a circumpolar species widely distributed in arctic and subarctic tundra (Hultén, 1968). The Asian endemic variety, *D. octopetala* L. var. *asiatica* (Nakai) Nakai, is a deciduous dwarf shrub (Shimizu, 1982; Wada et al., 2003) fragmentally distributed in middle-latitude alpine regions of northeastern Asia: Honshu and Hokkaido Islands of Japan, northern Korea (northeastern China), Kuril Islands, Sakhalin, Kamchatka, Ussuri, and east Siberia (Shimizu, 1982). This species favors gravel habitats such as fellfields.

Sieversia pentapetala (L.) Greene (Rosaceae), a deciduous alpine shrub, is a representative species in alpine zones of Japan and is distributed in the northern part of Far East Asia: Honshu and Hokkaido Islands of Japan, Kuril Islands, Sakhalin, Kamchatka, and Aleutian Islands (Hultén, 1968; Shimizu, 1982). This species favors wet habitats such as snowbeds (Kudo and Ito, 1992).

The optimum temperature for pollen germination is lower in *D. octopetala* var. *asiatica* (= 15°C) than in *S. pentapetala* (20°C) growing in the study area (Wada and Hohnoki, 2001), suggesting an adaptation of temperature-dependent physiological activity to a cooler thermal regime in *D. octopetala* var. *asiatica*.

The morphology of the fruit of *D. octopetala* var. *asiatica* is similar to that of *S. pentapetala*. The fruit is a pubescent achene including a nutlet (seed) with a feather-like style (plume: a wind-dispersal device at maturity) that is several centimeters long. The achene is longer in *D. octopetala* var. *asiatica* (ca. 4 mm) than in *S. pentapetala* (ca. 2.5 mm; Shimizu, 1982). Thirty to fifty achenes were produced by single fruiting shoots in both species in this study area.

3. Maturity and germinability of intact seeds

We collected 15 fruiting shoots of *D. octopetala* var. *asiatica* in the fellfield habitat and 20 fruiting shoots of *S. pentapetala* in the snowbed habitat on 19 September, 2002. These samples were immediately taken to a laboratory at Toyama University. For each species, we picked the achenes from all the fruiting shoots sampled, mixed them together, and then randomly chose 225 achenes. These samples were used to determine (1) the number of germinated seeds per achene, and (2) the number of mature seeds per achene (seeds that appeared to be of mature size (embryos) when non-germinated achenes were dissected), without treatment or consideration of any physiological dormancy in either species. The remaining achenes were used in the experiment described below. We arbitrarily divided the 225 achenes into 15 achenes × 15 sets. Fifteen achenes, with plumes removed, were numbered and individually placed on a 0.3% agar medium (Shimono and Kudo, 2003) in Petri dishes (8.6 cm in diameter and 1.0-cm deep). The Petri dishes were tightly lidded to maintain moist conditions. In this manner we replicated 15 sets in each species: 15 achenes per Petri dish × 15 dishes. We then immediately inserted these dishes into an incubator with five separate chambers (Temperature Gradient Chamber TG-200-ADFL, Nippon Medical & Chemical Instruments Co. Ltd., Tokyo) under five thermal conditions, 5, 10, 15, 20, and 25°C, which were designed to mimic temperatures for seeds and achenes exposed in autumn after dispersal in each habitat. For each species, three arbitrarily chosen dishes were inserted in each chamber; i.e., three replicates per thermal condition. The achenes (seeds) were incubated using a regime of 12-h light and 12-h dark for three weeks. In this experiment, "germination" was defined as an elongated root visible outside the achene. We checked each numbered achene in each dish daily from the beginning of the experiment, and recorded germination dates until the end of the experiment. After three weeks, we dissected non-germinated achenes and checked for the presence of a mature embryo inside. If we found an achene with a very small (undeveloped) embryo, the achene was considered non-viable, and the achene was removed from the sample (dish) when the germination rates were calculated. In each thermal condition for each species, we calculated (1) the mean number of mature seeds per achene, and (2) the mean number of germinated seeds per achene with mature seeds. The former value roughly reflects the seed-ripening ratio, i.e., efficiency in pollination and fertilization, and the latter indicates the ability of seeds to germinate, i.e., the germinability of apparently viable seeds. We also calculated the speed of germination for each germinated seed; $1/D$ (day^{-1}), where D is days taken for germination. We

compared the rate and speed of germination between thermal conditions and between species, and seed maturity between species.

4. Germinability of seeds after cold-moist stratification

In the field, achenes (seeds) naturally experience cold stratification during winter. We therefore subjected achenes to a moist-chilling treatment in this germination experiment (Shimono and Kudo, 2003). From the remaining achenes of each species (as described in section 3), we selected large achenes with apparently mature seeds. These achenes were placed in Petri dishes on filter paper moistened with 7-ml de-ionized water for two months at 0°C before being transferred to the incubator (Shimono and Kudo, 2003). The Petri dishes were wrapped in aluminium foil to eliminate any light (Shimono and Kudo, 2003). After the moist-chilling treatment, we arbitrarily selected 250 achenes from each species and divided them into 10 achenes × 25 sets. Ten achenes, with plumes removed, were numbered and individually placed on a 0.3% agar medium in Petri dishes in the same manner as for the germination test described above. There were thus 25 replicates for each species: ten achenes per Petri dish × 25 dishes. We inserted these dishes into the incubator chambers under five thermal conditions: 15, 20, 25, 30, and 35°C, which were designed to mimic temperatures for seeds and achenes exposed in summer in each habitat. Five arbitrarily chosen dishes were inserted in each chamber, i.e., five replicates per thermal condition for each species, with a 12-h light and 12-h dark regime. Daily observations were then recorded for each achene for two weeks. After two weeks, we dissected each non-germinated achene and checked the maturity of the seeds or embryos inside. Then we calculated the mean number of mature seeds per achene, the mean number of germinated seeds per achene with mature seeds, and the mean germination speed in each thermal condition for each species. We then compared the rate and speed of germination between thermal conditions and between species.

5. Statistical analysis

All statistical analyses were performed using StatView version 5.0 (SAS Institute Inc., Cary, NC, USA). For any percent data, the values were arcsine-transformed, and then the transformed values were used for statistical analyses, such as analysis of variance (ANOVA) (Sokal and Rohlf, 1995; Baskin and Baskin, 2001).

Results

1. Seasonal variation in soil-surface temperature

Seasonal variation in soil-surface temperature was considerably different between the fellfield habitat dominated by *D. octopetala* var. *asiatica* and the snowbed habitat dominated by *S. pentapetala* (Fig. 1). In the fellfield, the average daily soil-surface temperature was less than 0°C during winter, from late October to mid-April. The minimum temperature of 15.7°C was recorded in mid-February. In contrast, in the snowbed area, the soil-surface temperature stayed at around 0°C from late October to early June. These results clearly indicate that the snow cover serves to protect

plants and soils from freezing in the snowbed habitat, while the smaller accumulation of snow in the wind-blown fellfield habitat tends to result in plants and soils freezing in winter.
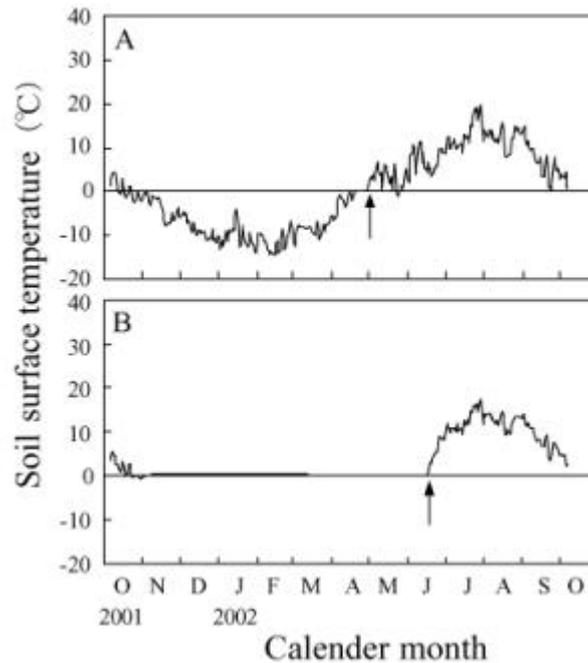

Figure 1 Seasonal variations in soil-surface temperature (daily average temperature based on 24 hourly data) from 7 October, 2001, to 9 October, 2002, in the fellfield (A) dominated by *Dryas octopetala* var. *asiatica* (at 2700 m a.s.l.) and in the snowbed (B) dominated by *Sieversia pentapetala* (at 2820 m a.s.l.), in the Tateyama Range, central Japan. Arrows show snowmelt in each habitat, defined as when the average daily soil-surface temperature changes from minus to plus degrees.

Snowmelt, defined as when the average daily soil-surface temperature changes from minus to plus degrees ($> 0°C$), occurred on 2 May, 2002, in the fellfield habitat, while it occurred ca. 1.5 months later in the snowbed habitat on 19 June, 2002 (Fig. 1). This indicates a longer growing season for plants in the fellfield than in the snowbed. However, the physiologically critical lowest temperature for seed germination in tundra and alpine plants is considered to be $5°C$ (Larcher, 1995). Therefore, the length of the growing season for seedlings, calculated by the number of days with a daily mean temperature above $5°C$, was 102 days in the fellfield and 95 days in the snowbed. The difference was only 1 week, mostly because of cool temperatures from early May to early June after snowmelt in the fellfield habitat. The accumulated temperature above $5°C$ in daily mean temperature during the snow-free period was 585.8 day degrees in the fellfield habitat while it was 570.3 day degrees in the snowbed habitat.

The daily mean, daily maximum, and daily minimum temperatures during the growing season (from June to September) are summarized in Table 1. Larger daily fluctuations in soil-surface temperatures during the snow-free period were found in the fellfield habitat, especially in June; the daily maximum temperature reached 23°C, but the daily minimum temperature dropped to around

0°C within a day. The maximum temperatures were 32.4°C in the fellfield habitat and 26.6℃ in the snowbed habitat, both recorded on 28 July, 2002. At the end of the growing season in September, soil-surface temperatures tended to be warmer in the snowbed habitat than in the fellfield habitat (Table 1).

Table 1. Daily mean (DM), daily maximum (MX), and daily minimum (MN) soil-surface temperatures during the 2002 growing season. Means with standard deviations (°C) are shown for each month for the two habitats.

	June	July	August	September
Fellfield				
DM	6.3 ± 2.7	12.4 ± 3.4	12.0 ± 2.2	7.4 ± 3.9
MX	13.0 ± 5.9	17.4 ± 7.4	18.4 ± 4.7	12.4 ± 5.9
MN	1.5 ± 3.3	8.7 ± 3.2	7.7 ± 3.3	4.1 ± 4.1
Snowbed				
DM	5.3 ± 2.5*	11.8 ± 2.6	12.4 ± 1.6	8.8 ± 2.9
MX	9.5 ± 4.9*	16.7 ± 5.6	18.0 ± 3.4	14.0 ± 4.1
MN	1.8 ± 2.7*	8.9 ± 2.5	9.1 ± 2.4	5.7 ± 3.3

*after snowmelt (n = 12 days)

2. Maturity and germinability of intact seeds

In *D. octopetala* var. *asiatica*, the maturity ratio of seeds was 0.48 ± 0.15 (mean ± SD, n = 15 samples with 225 achenes in total), which varied between samples (i.e., Petri dishes) incubated in five thermal conditions, ranging from 0.13 in a sample incubated at 10°C to 0.67 at 5℃. However, there was no statistically significant difference in the maturity of seeds between thermal conditions (F = 3.10, P = 0.069, df = 4, 10; one-way ANOVA). Moreover, there was no significant relationship between the number of mature seeds and the temperature regime (Kendall's = 0.211, P = 0.273, n = 15). Thus, our findings showed that the maturity of seeds did not depend significantly on the thermal conditions in which the achenes were incubated. This suggests that half the achenes, 0.52 on average, had naturally failed to produce seeds capable of germinating before the germination experiment began.

In *S. pentapetala*, the maturity ratio of seeds was 0.82 ± 0.09 (mean ± SD, n = 15 samples with 225 achenes in total), and was 1.7 times higher than the maturity in *D. octopetala* var. *asiatica* (t = 7.68, df = 28, P < 0.001; Student-t test). Although the variation was small, maturity varied between samples incubated in the five thermal conditions, ranging from 0.67 in samples incubated at 20°C and 25°C to 0.93 at 5, 10, and 15°C. There was a marginally significant difference in the maturity of seeds between thermal conditions (F = 3.46, P = 0.051, df = 4, 10; one-way ANOVA). A significant relationship between the number of mature seeds and temperature regime was detected (Kendall's = 0.546, P = 0.005, n = 15). The results did not show that seed maturity did not depend on the

thermal conditions used for incubating the achenes; seeds incubated in colder thermal conditions showed higher maturity than seeds incubated in warmer conditions. However, all *S. pentapetala* samples showed equal or higher seed maturity for every thermal condition compared to samples from *D. octopetala* var. *asiatica*.

The germination rate for mature seeds differed remarkably between the two species and between thermal conditions (Table 2 and Fig. 2A). Although the germination rate was higher with higher incubation temperature in both species, *D. octopetala* var. *asiatica* showed ca. 90% germination (mean $\pm$ SD = 87.8 $\pm$ 11.2%, *n* = 3 replications) at 25°C, while *S. pentapetala* showed only 6% germination (6.1 $\pm$ 5.4%, *n* = 3 replications) under the same temperature regime (Fig. 2A). No mature seeds germinated at either 5 or 10°C in both species, and only one seed germinated at 15°C in *S. pentapetala*. Overall, 25 out of 108 mature seeds (23%) germinated in *D. octopetala* var. *asiatica,* while only four out of 184 mature seeds (2%) germinated in *S. pentapetala* in the five thermal conditions from 5 to 25°C.
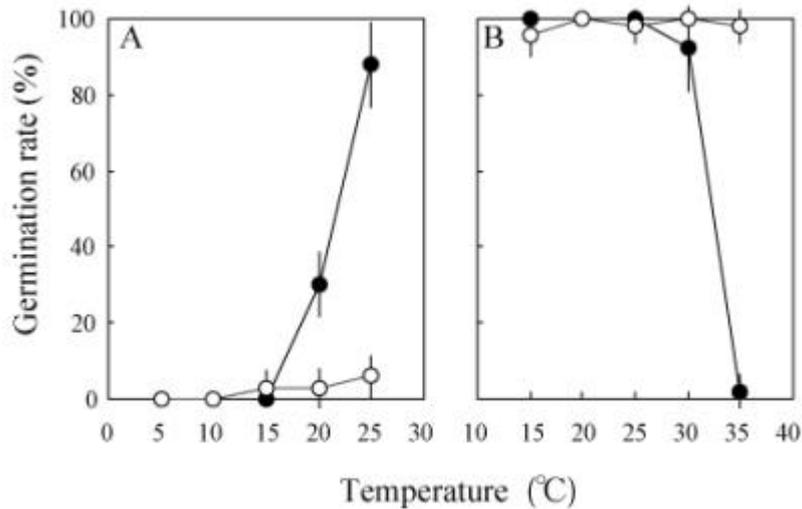

Figure 2. Effects of temperature and moist chilling treatment on germination of mature seeds in *Dryas octopetala* var. *asiatica* (   ) and in *Sieversia pentapetala* (   ). The average values are shown with standard deviations (vertical lines). (A) intact fresh seeds (achenes) without any treatment (*n* = 3 replicates); (B) seeds after cold-moist stratification for two months at 0°C (*n* = 5 replicates).

Table 2. Two-way ANOVA of the effect of temperature (Tm.) and species (Sp.) on the germination rate of mature seeds with no treatment. Seeds were incubated under five thermal conditions: 5, 10, 15, 20, and 25°C.

	df	*MS*	*F*	*P*
Temperature	4	0.416	28.624	< 0.0001
Species	1	0.547	37.621	< 0.0001
Tm. × Sp.	4	0.348	23.937	< 0.0001
Error	20	0.015		

The period required for germination was 17.6 ± 4.5 days (mean $\pm$ SD, $n = 5$ seeds) at 20°C, and 12.0 ± 3.8 days ($n = 20$ seeds) at 25°C in *D. octopetala* var. *asiatica.* Seeds incubated at 25°C (mean $\pm$ SD $= 0.09 \pm 0.03$ day^{-1}, $n = 20$ seeds) germinated faster than at 20°C (0.06 ± 0.02 day^{-1}, $n = 20$ seeds). In *S. pentapetala*, the period required for germination was 10.5 ± 3.1 days (mean $\pm$ SD, $n = 4$ seeds), and the germination speed was 0.10 ± 0.02 day^{-1} ($n = 4$ seeds) in the 15-25°C thermal conditions. Because of the small number of seeds that germinated in *S. pentapetala*, we were unable to assess differences in the above values between thermal conditions, but there appeared to be no large differences between them.

3. Germinability of seeds after cold-moist stratification

Because of a shortage of large (mature) achenes in *D. octopetala* var. *asiatica*, we were unable to prepare enough mature seeds for the 15°C thermal condition before this experiment began. As a result, only eight seeds out of 50 achenes, including apparently small-sized fruit, germinated (maturity ratio: mean $\pm$ SD $= 0.16 \pm 0.05$, $n = 5$ replications). We therefore omitted these samples and analyzed differences in the number of mature seeds between achenes incubated in four thermal conditions. The maturity ratio of the seeds was 0.77 ± 0.23 (mean $\pm$ SD, $n = 20$ samples with 200 achenes in total), ranging from 0.30 in a sample incubated at 30°C to 1.0 at 20, 30, and 35°C. There was no statistically significant difference in the maturity of seeds between the four thermal conditions ($F = 1.56$, $P = 0.237$, $df = 3, 16$; one-way ANOVA). Moreover, there was no significant relationship between the number of mature seeds and the temperature regime (Kendall's $= 0.237$, $P = 0.143$, $n = 20$). Because we deliberately chose large achenes for this experiment, the higher rate of mature seeds, 0.77 on average, was as a matter of course compared to the mean maturity ratio of 0.48 in the previous germinability experiment for *D. octopetala* var. *asiatica*.

Similarly, a higher maturity ratio of seeds was also observed in *S. pentapetala*. The maturity ratio of the seeds was 0.96 ± 0.07 (mean $\pm$ SD, $n = 25$ samples with 250 achenes in total). This ratio showed little variation between samples, and was 1.2 times higher than the maturity in *D. octopetala* var. *asiatica* (with the exception of samples in the 15°C thermal condition) ($z = 3.38$, $P = 0.0007$; Mann-Whitney U test because of the significant difference in variance between the species ($F = 2.75$, $P = 0.024$; F test)). There was no statistically significant difference in the maturity of seeds between the five thermal conditions ($F = 0.517$, $P = 0.724$, $df = 4, 20$; one-way ANOVA). Moreover, there was no significant relationship between the number of mature seeds and the temperature regime (Kendall's $= 0.129$, $P = 0.367$, $n = 25$).

After the cold-moist stratification, the seed germination rate increased considerably in both species, except for seeds of *D. octopetala* var. *asiatica* at 35°C (Fig. 2B). This species showed high germinability, more than 90% on average, from the 15 to 30°C thermal regimes. In the 35°C thermal condition, however, the germination rate was 2.2%; only one seed germinated instead of most of the mature seeds (46 out of 50 achenes had apparently viable seeds). Thus, the germination rate for *D. octopetala* var. *asiatica* was significantly different between thermal conditions ($F = 90.23$, $P < 0.0001$, $df = 4, 20$; one-way ANOVA); most seeds did not germinate and failed to develop roots in the warmest thermal condition.

In contrast, there was no significant difference in the germination rate for mature seeds between thermal conditions in *S. pentapetala* (*F* = 1.02, *P* = 0.419, *df* = 4, 20; one-way ANOVA), which showed more than 95% germinability for the thermal regimes from 15 to 35°C. Thus, we found significant differences in germinability between species and temperature regime (temperature× species interaction; see Table 3).

Table 3. Two-way ANOVA of the effect of temperature (Tm.) and species (Sp.) on the germination rate of mature seeds with cold-moist stratification. Seeds were incubated under five thermal conditions: 15, 20, 25, 30, and 35°C.

	df	*MS*	*F*	*P*
Temperature	4	1.161	42.721	< 0.0001
Species	1	1.034	38.024	< 0.0001
Tm. × Sp.	4	1.141	41.990	< 0.0001
Error	40	0.027		

The number of days needed for germination at 15, 20, 25, 30, and 35°C were 5.5 ± 1.2 days (mean ± SD, *n* = 8 seeds), 3.3 ± 0.5 days (*n* = 33), 2.8 ± 0.4 days (*n* = 42), 4.0 ± 2.1 days (*n* = 31), and 11 days (*n* = 1), respectively, in *D. octopetala* var. *asiatica*. Most of the mature seeds germinated rapidly after the moist chilling treatment. For instance, at 25°C, the period required for germination went from 12.0 days to 2.8 days on average, i.e., it was shortened by as much as nine days after cold-moist stratification. This trend was also observed in *S. pentapetala*.

Figure 3 shows the temperature dependency of the germination speed in both species. The highest germination speed was found at 30°C in *S. pentapetala,* while at 25°C it was 5°C lower in *D. octopetala* var. *asiatica*. In the range from 15 to 25°C, the germination speed was slightly higher in *D. octopetala* var. *asiatica* than in *S. pentapetala*. However, the opposite occurred in the range from 30 to 35°C with larger differences between species. The relationship between incubating temperature (T°C) and germination speed (GS day^{-1}) could be expressed significantly for *D. octopetala* var. *asiatica* using the following formula (R^2 = 0.967, *F* = 60.45, *P* = 0.016, *n* = 5);

$$GS = \ 0.0022 \, T^2 + 0.1062 \, T \ 0.9837.$$

According to this formula, the optimum temperature for germination speed was calculated as 24.1°C. For *S. pentapetala*, the regression formula was:

$$GS = \ 0.0012 \, T^2 + 0.0719 \, T \ 0.6854.$$

The optimum temperature in relation to germination speed was estimated to be 30°C. However, this regression formula was not statistically significant (R^2 = 0.816, *F* = 9.86, *P* = 0.092, *n* = 5). Based

on the calculated value (24.1°C) in *D. octopetala* var. *asiatica* and the actually observed value (30°C) in *S. pentapetala* (Fig. 3), we estimated that the difference in optimum temperature in relation to germination speed was 5.9°C between the two species.
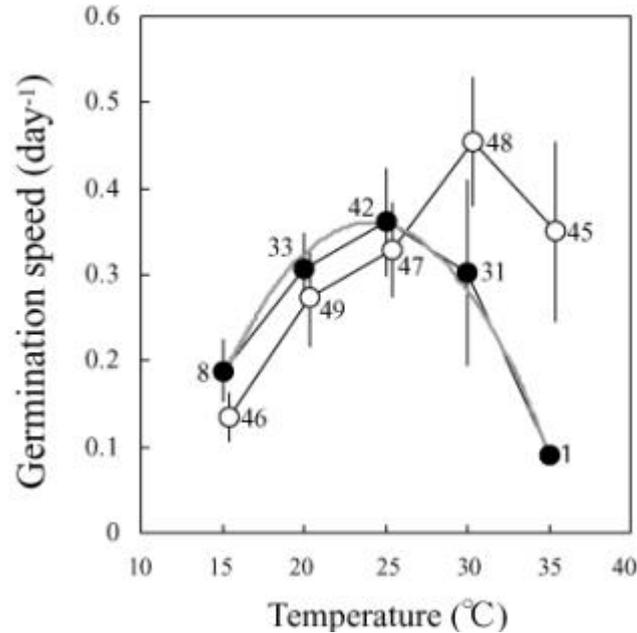

Figure 3. Germination speed (day^{-1}) of seeds with moist chilling treatment. The average values are shown with standard deviations (vertical lines). , *Dryas octopetala* var. *asiatica*; , *Sieversia pentapetala*. The regression line (gray bold; $P <$ 0.02, $n = 5$) is shown only for *D. octopetala* var. *asiatica*. The numerals positioned near each circle in this figure show the sample size ($n =$ number of seeds sampled in each thermal condition).

Discussion

The maturity ratio of seeds in *D. octopetala* var. *asiatica*, as shown in the germination experiment using intact seeds and randomly sampled achenes, was 0.58 times lower than that in *Sieversia pentapetala*. This tendency was also found when we deliberately chose large achenes (with no samples for the 15°C thermal condition in *D. octopetala* var. *asiatica*) for the germination experiment after cold-moist stratification. These results suggest that reproductive efficiency in producing viable offspring is lower in the glacial relict *D. octopetala* var. *asiatica* than in *S. pentapetala*, which is commonly distributed continuously in snowbed habitats in this study area. However, on the basis of only one year's observation, we cannot determine whether these results are attributable to species-specific characteristics in *D. octopetala* var. *asiatica*, such as low efficiency of pollination and fertilization, resource limitation and abortion, inbreeding depression owing to the isolated population, and habitat-specific differences relating to other factors between the fellfield and snowbed. A preliminary experiment showed that *S. pentapetala* growing in a snowbed habitat could produce seeds even though insect visitors were excluded from the flowers (Motoki and Wada, 2000),

suggesting that this species is capable of autogamy. This breeding system may result in high seed-setting success whenever effective pollinators are scarce (Motoki and Wada, 2000; Wada and Watanabe, 2003). In the subarctic tundra of northern Sweden, autogamous reproduction has been reported in *D. octopetala* var. *octopetala* (Molau, 1993). For *D. octopetala* var. *asiatica*, however, it is not known what breeding system the flowers have and how many seeds they can produce in a normal year, or what insect visitors are important in producing viable seeds. Thus, for the conservation of this small population, we need further basic information about the factors that affect seed-setting success before we can assess the species' ability to produce viable offspring.

In the high arctic tundra of Svalbard, Hagen (2002) reported extremely low germinability of seeds (less than 10% germination) when, after cold stratification, seeds were grown under optimum environmental conditions for seedling emergence in *D. octopetala* var. *octopetala*; the maturity of the seeds was not examined. These results suggest that many of the achenes used in this germination experiment may not have had mature seeds. The seed-setting success of *D. octopetala* var. *octopetala* is greatly affected by ambient temperatures during the flowering period rather than by out-crossing pollination (Wada, 1999), and the seed viability is markedly improved by elevated ambient air temperature (Wookey et al., 1995) in the high arctic tundra of Svalbard. Thus, the low germinability of *D. octopetala* var. *octopetala* reported in the Arctic (Eurola, 1972; Khodachek, 1997; Hagen, 2002) is likely due to immature seeds produced under cool temperatures that limit physiological activities such as pollen germination, tube elongation, fertilization, and developmental growth of embryos.

Only a few of the intact seeds of *S. pentapetala* germinated, while those of *Dryas octopetala* var. *asiatica* germinated under warmer thermal conditions (30% at 20°C and 88% at 25°C) without being cold stratified (Fig. 2A). Another trend has also been reported in *D. octopetala* var. *octopetala*: 48% of freshly matured seeds germinated in light at 25°C (Elkington, 1971). This suggests a weak adaptation to snow cover in *D. octopetala* var. *asiatica* growing in wind-blown fellfields where little snow accumulates. In nature, however, achenes and seeds dispersed on the soil surface in autumn will never be exposed to such warm thermal conditions as 20°C and above (Fig. 1 and Table 1). Therefore, germination soon after seed dispersal may not occur in *D. octopetala* var. *asiatica*.

It is known that most alpine species require high temperatures to germinate, and only a few germinate at low temperatures (Bliss, 1985; Nishitani and Masuzawa, 1996). In both species studied, no germination was found at temperatures as low as 10°C and below. This may be a selective mechanism to prevent germination in fall or spring when the high frequency of frost will increase seedling mortality (Bliss, 1985; Shimono and Kudo, 2003). In contrast to intact seeds, after cold-moist stratification, seeds show higher germination rates in both species under the same thermal conditions, ranging from 15 to 25°C (Table 4), indicating physiological dormancy in both species according to the definition of Baskin and Baskin (2001). However, very few seeds of *D. octopetala* var. *asiatica* germinated at 35°C, indicating a lower tolerance to high temperature in this species. In other words, *S. pentapetala* may have a higher tolerance to high temperatures. Most of the non-germinated seeds of *Dryas* appeared to be of appropriate size and color, making it difficult to determine whether these seeds had died or were still viable. We were unable to account for the differences in the physiological mechanism of tolerance to high temperature between the two species studied.

Table 4. Multiple ANOVA of the effect of treatment (cold-moist stratification: Tr.), temperature (Tm.), and species (Sp.) on the germination rate of mature seeds. Seeds incubated under three thermal conditions (15, 20, and 25°C) were used for this analysis.

	df	*MS*	*F*	*P*
Treatment	1	17.862	903.765	< 0.0001
Temperature	2	0.399	20.177	< 0.0001
Species	1	0.826	41.489	< 0.0001
Tr. × Tm.	2	0.342	17.303	< 0.0001
Tr. × Sp.	1	0.361	18.257	< 0.0001
Tm. × Sp.	2	0.301	15.240	< 0.0001
Tr.× Tm.× Sp.	2	0.357	18.059	< 0.0001
Error	36	0.020		

From ecological and evolutionary points of view, however, this difference in tolerance might reflect the thermal conditions naturally experienced by these seeds in their individual habitats. Seeds of *D. octopetala* var. *asiatica* experience a gradually cooler thermal regime over a relatively long period after snowmelt in the fellfield, while those of *S. pentapetala* are rapidly exposed to a warmer thermal regime over a short period after the disappearance of snow from the snowbed (Fig. 1). These variations in thermal regimes may exert selective pressure on the seeds of both species, resulting in differences in tolerance to temperature and in germination performance, as described below.

Based on the germination speed of seeds after cold stratification, the optimum temperature was estimated to be 24.1°C in *Dryas octopetala* var. *asiatica,* and 30°C in *Sieversia pentapetala*. Moreover, the germination speed of *D. octopetala* var. *asiatica* was slightly higher in thermal conditions below 25°C, while that of *S. pentapetala* was much higher in thermal conditions above 25°C. These results support our prediction: seed germination in *D. octopetala* var. *asiatica* is higher in lower thermal conditions, but is less tolerant of higher thermal conditions compared to seed germination in *S. pentapetala*. These findings indicate adaptive traits to the thermal regimes in each species' habitat. As a next step, we will examine the linkage between these germination patterns and the growth and survival of seedlings in the field and in laboratory experiments.

The temperature regimes measured in the field differed considerably from the thermal conditions in the germination experiments. In *D. octopetala* var. *asiatica*, naturally dispersed seeds in the following spring may be exposed on the soil surface where temperatures range from 1.5°C (daily minimum mean) to 13.0°C (daily maximum mean), with a daily average of 6.3°C in June (first month with an average daily mean temperature above 5°C) in the fellfield (Table 1). In *S. pentapetala*, the field temperature for seed after snowmelt may range from 8.9°C (daily minimum mean) to 16.7°C (daily maximum mean), with a daily average of 11.8°C in July (first month above 5°C) in the snowbed (Table 1). Moreover, soil-surface temperatures fluctuate widely within a day and within a month. We therefore need to carry out germination experiments under fluctuating temperature regimes, as suggested in a review by Baskin and Baskin (2001). However, the difference in soil-surface temperatures between the habitats was 5.5°C when we compared daily mean temperatures above 5°C

for one month in each habitat; i.e., 6.3°C in June at the fellfield and 11.8°C in July at the snowbed (Table 1). This difference of 5.5°C in habitat temperature seems to be related to the difference in optimum temperature, estimated as 5.9°C, between the species, which was derived from our data on germination speed. Thus, our results suggest that the optimum temperature for germination in each species may reflect the thermal regimes in each habitat.

We conclude that the glacial relict, *Dryas octopetala* var. *asiatica,* produced offspring that showed high germinability under cooler thermal conditions, while the opposite applied under warmer conditions in the population studied. However, the productivity of viable seeds showed lower efficiency compared to that in *Sieversia pentapetala*. Therefore, further studies are required to examine reproductive activity from flowering to seeding and from germination to seedling growth and survival if we are to protect this small and isolated population of *D. octopetala* var. *asiatica* from decline or extinction.

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