RESEARCH PAPER

Seed dormancy and germination of *Asarum sieboldii*, a disjunct relict species in East Asia

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ABSTRACT

- Disjunct species in the same genus are a common feature of the flora in the temperate forests of East Asia and eastern North America. This study aimed to evaluate whether the kind of seed dormancy was maintained after species in the genus *Asarum* (*Aristolochiaceae*) were separated from their common ancestor.
- We classified the seed dormancy of *Asarum sieboldii*, an East Asian species, based on a phenology study and experiments in controlled temperature conditions, and then compared it to that of the previously studied *A. canadense*, an eastern North American species.
- The underdeveloped embryo of *A. sieboldii* grew and germinated (radicle emergence) in autumn but shoot emergence did not occur until the following spring. The seeds of *A. sieboldii* had deep simple epicotyl morphophysiological dormancy because the seeds with emerged radicle required a relatively long period of cold stratification to break epicotyl dormancy and produce a shoot.
- Although the seed of *A. sieboldii* had weaker radicle dormancy and stronger epicotyl dormancy compared to *A. canadense*, the kind of seed dormancy was the same for the two species. The trait of seed dormancy was inherited from a shared common ancestor and maintained in populations well after the two species (or their ancestors) separated. However, quantitative differences in temperature requirements for radicle and shoot emergence suggest the possibility of adaptation to the environment.

INTRODUCTION

Seeds of many herbaceous plants that are native to temperate regions of the Northern Hemisphere have an underdeveloped embryo, which has to grow before germination can occur (Baskin & Baskin, 2014). If these underdeveloped embryos grow and germination (radicle emergence) is completed within about 30 days under appropriate conditions, the seeds have only morphological dormancy (MD). However, if germination takes more than 30 days the seeds probably require some type of treatment to break physiological dormancy (PD) before, during or after embryo growth. In this case, seeds with underdeveloped embryos have morphophysiological dormancy (MPD) (Nikolaeva, 1969; Baskin & Baskin, 2014). There are nine levels of MPD, classified according to the temperature requirements for embryo growth and germination; the timing of embryo growth, radicle emergence and shoot emergence; and dormancy release when treated with gibberellic acid (GA₃) (Baskin & Baskin, 2014).

Two of these nine levels of MPD are characterized by temporal differences in radicle emergence (germination) and shoot emergence (Baskin & Baskin, 2014). While the radicle emerges in autumn, shoot emergence is delayed for a month or more until spring. These two levels of MPD differ in whether cold stratification is required to break shoot dormancy (deep simple epicotyl MPD) or is not required (nondeep simple epicotyl

MPD). Seeds having epicotyl MPD have been reported in 62 species in 13 diverse families, such as *Aristolochiaceae*, *Caprifoliaceae*, *Liliaceae* and *Ranunculaceae* (Baskin & Baskin, 2014). These families also contain genera that exhibit the classical disjunction between Asia and eastern North America (Wen, 1999; Donoghue & Smith, 2004). Phylogenetic relationships among several of these Asian/North American disjuncts have been conducted on morphological and molecular data. While many of these disjunct species have experienced stasis in their morphological traits, stasis/divergence in other characters has not been as well studied (Wen, 1999; Walck *et al.*, 2002). In recent years, several studies have examined the stasis/divergence of ecological traits related to seed dormancy, and some of these have been done within a phylogenetic framework (Adams *et al.*, 2005; Hidayati *et al.*, 2005; Vandelook *et al.*, 2007; Vandelook & Van Assche, 2008; Walck *et al.*, 2002; Walck *et al.*, 2012).

In *Aristolochiaceae*, the genus *Asarum* consists of approximately 100 species of low-growing and rhizomatous herbs distributed in northern temperate regions (Cheng & Yang, 1983; Kelly, 2001; Hwang *et al.*, 2003). The majority of the members of this genus are distributed in Asia (~65 species) and others are distributed in North America (15 species) and Europe (one species), thus exhibiting a disjunct distribution (Sinn *et al.*, 2015). The seed germination ecology of two North American species of *Asarum*, *A. canadense*, and *A. minus* (*Hexastylis*

heterophylla; The Plant List, 2013), have been studied and reported to have deep simple epicotyl MPD (Baskin & Baskin, 1986; Adams *et al.*, 2003). More than 60 species of *Asarum* are distributed across China, Taiwan, Japan, Vietnam and the Korean Peninsula (Sinn *et al.*, 2015). However, species of Asian *Asarum* have not been investigated for seed dormancy and germination. Although Liu *et al.* (1993) mentioned the germination of the Asian *A. heterotropoides*, there was only a brief statement that shoots emerged the following year after sowing in autumn. This implies that seeds of *A. heterotropoides* have deep simple epicotyl MPD (Baskin & Baskin, 2014).

The focus of the present study was *A. sieboldii* Miq. This species is one of seven distributed on the Korean Peninsula (Oh, 2008). Its roots were traditionally used to treat headaches and indigestion and were also used as raw material for peppermint flavouring. There have been many reports on various applications, such as antifungal and insecticidal activity, and inhibition of melanin biosynthesis by *A. sieboldii* extract (Choi *et al.*, 2007; Kang *et al.*, 2012). The shape of the fruit resembles a traditional hat, so it also has ornamental value.

The main purpose of our study was to classify the kind of dormancy found in *A. sieboldii*. To do so, we examined: (i) the phenology of embryo growth and of radicle and shoot emergence under natural conditions in Korea; (ii) the temperature requirements for embryo growth and for radicle and shoot emergence through move-along temperature sequences under laboratory conditions; (iii) the effects of different periods of warm and cold stratification on radicle and shoot emergence, respectively, in the laboratory; and (iv) the effects of gibberellic acid (GA_3) on radicle emergence. Finally, we compared our results to those previously obtained for *A. canadense* and *A. minus* (*Hexastylis heterophylla*).

MATERIAL AND METHODS

Seed collection

Mature seeds of *A. sieboldii* were harvested on Mt. Sogaebang, Hongcheon-gun, Gangwon-do, Korea (37°75′ N, 128°44′ E) on 23 June 2015. After collection, seeds were dried at room temperature (20–25 °C) for 1 week and stored in sealed plastic bags in a 5 °C refrigerator until used for each experiment.

Phenology

This experiment was performed to monitor embryo growth, radicle emergence and shoot emergence from seeds exposed to seasonal changes in natural conditions. On 13 July 2015, 12 fine-mesh polyester bags containing ten seeds each were buried under the soil to about 3-cm depth in the experimental garden of the Korea National Arboretum in Gyeonggi-do, Korea (37°47′ N, 127°59′ E); one bag was exhumed to monitor embryo growth every month. Seeds were cut in half with a razor blade, and the length of each seed and embryo measured under a digital microscope (AM4515 series; AnMo Electronics, Hsinchu, Taiwan) equipped with a calibrated micrometre, which allowed the ratio of embryo lenght to seed length (E:S ratio) to be calculated. To monitor radicle emergence under natural conditions, three sets of 30 seeds each were placed in fine-mesh polyester bags and buried to a depth of 3 cm in soil. The bags were exhumed every month from 13 July 2015 and

then checked each week. After radicle emergence began, the germinated seeds were counted and removed from the bags. Radicle emergence was scored when at least 1 mm of radicle protruded from the seed. Shoot emergence was observed by sowing 20 seeds at 1-cm depth in plastic pots filled with horticultural medium. Four replicates (pots) were buried at soil level in a shady site in the experimental garden. Shoot emergence was taken as the time when at least one cotyledon was observed above the ground. The emerged shoots were counted each week from July 2015 to May 2016. The soil temperature at a depth of 3 cm was monitored every hour with a temperature data logger (Watch Dog Model 450; Spectrum Technologies Plainfield, IL, USA), and daily maximum and minimum temperatures were recorded.

General laboratory procedures

Laboratory experiments were conducted in four light- and temperature-controlled incubators (Multi-Room Incubator; WiseCube, Wonju, Korea) using a 12-h daily photoperiod (PPFD 30–40 mol·m^{-2}·s^{-1}, with light provided by cool white fluorescent lamps). The incubators were set at 25 °C to simulate summer temperature for warm stratification, 20 °C to simulate early autumn and late spring, 15 °C to simulate late autumn and early spring, and 5 °C to simulate winter temperatures for cold stratification. Unless otherwise stated, three replicates of 30 seeds each were placed in 9.0-cm diameter plastic Petri dishes containing sand. The sand used in experiments was washed with distilled water and sterilized in a hot air oven at 80 °C for more than 48 h. This sand was moistened with distilled water and Petri dishes sealed with Parafilm (Pechiney Plastic Packaging, Menasha, WI, USA) to retard water loss during incubation; the sand was watered as needed to retain moisture. Radicle and shoot emergence were scored when at least 1 mm of radicle or at least one cotyledon protruded from the seed, respectively.

Move-along test

This experiment simulated seasonal temperature changes with temperature sequences in incubators, which enabled us to verify whether warm and/or cold temperatures were required for embryo growth, radicle emergence and shoot emergence from seeds. On 13 July 2015, three treatments were started with seeds being moved (→) through the following sequences: (i) Move 1: 25 °C for 12 weeks 20 °C for 4 weeks 15 °C for 4 weeks 5 °C for 12 weeks 15 °C for 14 weeks; (ii) Move 2: 5 °C for 12 weeks 15 °C for 4 weeks 20 °C for 4 weeks 25 °C for 12 weeks 20 °C for 14 weeks; and (iii) constant 15 °C for 46 weeks. We did not have controls at 25, 20 or 5 °C, but only at a constant temperature of 15 °C. The alternating temperature regime of 20/10 °C (12/12 h) was optimum for radicle emergence in *A. canadense* (Baskin & Baskin, 1986). To compare the experiment on *A. canadense* to our experiment, we selected 15 °C to correspond to the average maximum and minimum temperature of 20/10 °C. To determine E:S ratios, five seeds were selected and measured at 2-week intervals during the constant condition and during the first three temperature regimes of Move 1 and Move 2. At weekly intervals, the emergence of radicles and shoots was scored.

Effects of warm stratification on radicle emergence

On 15 July 2015, seeds were incubated at 25 °C for 0, 4, 8 and 12 weeks. After each temperature treatment period, seeds were moved to 15 °C and scored for radicle emergence weekly until the percentage emergence no longer increased.

Effects of GA₃ on radicle emergence

On 14 July 2015, seeds were soaked in distilled water (control) or a solution of 10, 100 and 1000 mg·l⁻¹ GA₃ for 24 h at room temperature. After imbibition, all seeds were washed with distilled water and placed on sand moistened with distilled water. Then seeds were incubated at 20 °C because this temperature is generally used in experiments to test if GA₃ overcomes dormancy in seeds (Baskin & Baskin, 2014). In addition, seeds of *A. sieboldii* had relatively rapid germination at 15 °C. At 20 °C, radicle emergence was relatively slow, which allowed us to more adequately measure the effectiveness of GA₃. After 16 weeks of incubation, the final radicle emergence was assessed.

Effects of cold stratification on shoot emergence

On 14 July 2015, seeds were incubated at 25 °C for 12 weeks and then moved to 15 °C for 4 weeks until radicles emerged from the seeds. On 3 November 2015, seeds with a protruding radicle of >5 mm were incubated at 5 °C for 0, 4, 8 and 12 weeks. After each cold stratification period, seeds were moved to 15 °C and the emergence of the shoot was scored each week for 12 weeks.

Statistical analyses

At the end of each experiment, seeds without an emerged radicle and/or shoot were examined for viability using a pinch test: viable seeds were firm and non-viable seeds were soft. Percentages of radicle and shoot emergence were calculated based on the number of viable seeds. The radicle emergence percentage across the GA₃ concentrations and the shoot emergence percentage across the cold stratification periods were analysed using Fisher exact test (significance level = 0.05) followed by pairwise comparisons between the conditions. We did *post-hoc* tests using Fisher exact test with the *P*-value adjustment using Holm's method. Statistical analyses were performed using R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Phenology

When seeds were sown, the initial E:S ratio was 0.06; by 13 August 2015, the E:S ratio was 0.09 with daily maximum and minimum temperatures between sowing and 13 August being 36.4 °C and 22.1 °C, respectively (Figs. 1, 2). By 7 September 2015, the E:S ratio had increased to 0.37 with daily maximum and minimum temperatures between 13 August 2015 and 7 September 2015 being 22.3 °C and 19.4 °C, respectively. Radicle emergence began on 14 September 2015 and percentages of emergence increased to 79.5% and 98.4% by 21 September and

12 October 2015, respectively, during which time daily maximum and minimum temperatures were 24.2 °C and 9.4 °C, respectively. Following winter, shoot emergence began on 14 March 2016 with daily maximum and minimum temperatures between 29 February 2016 and 14 March 2016 of 6.5 °C and −1.9 °C, respectively. Shoot emergence rose to 88% by 4 April 2016, with temperatures between 14 March and 4 April being 14.3 °C and −0.9 °C, respectively.

Move-along test

During constant incubation at 15 °C, embryos grew little during the first 4 weeks, but they grew rapidly between 4 and 8 weeks, with the E:S ratio increasing to near 0.4 (Fig. 3). In the Move 1 sequence, which was subjected to 25 °C first, the E:S ratio was <0.2 for 12 weeks. After moving to 20 °C, the E:S ratio increased to 0.4. In the Move 2 sequence subjected to 5 °C first, embryos hardly grew during the cold temperature, and the E:S ratio slightly increased to 0.14 when seeds were moved to 15 °C. When seeds were moved to 20 °C, the E:S ratio increased rapidly to 0.5.

In the Move 1 sequence, radicle emergence started in week 14, when seeds were transferred from 25 °C to 20 °C, and radicle emergence was 90% after moving to 15 °C (Fig. 4). Shoot emergence began in this sequence when seeds with an emerged radicle were transferred to 15 °C after 12 weeks of 5 °C, with shoot emergence reaching 60%. In the Move 2 sequence,
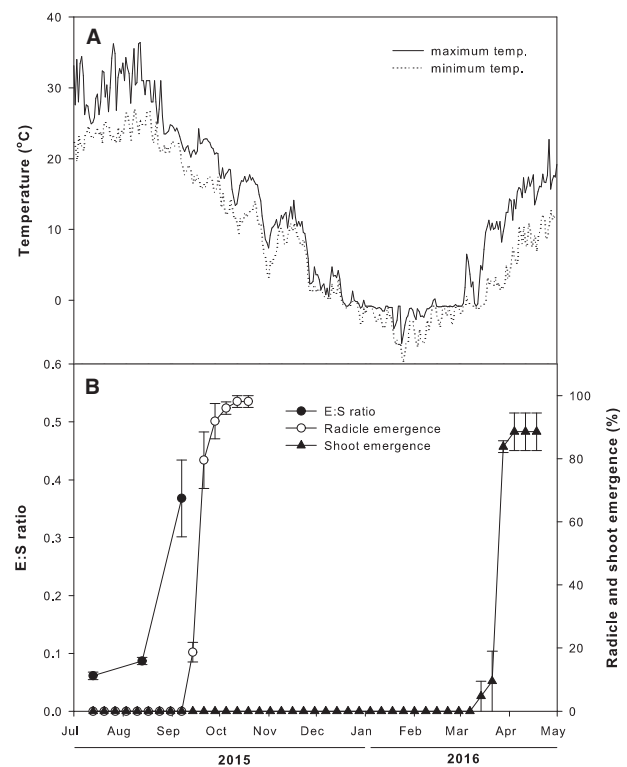

Fig. 1. The solid and dotted line indicates mean daily maximum and minimum soil temperatures at a depth of 3 cm, respectively (A). E:S ratio (embryo length:seed length) and cumulative percentages of radicle emergence and shoot emergence of *Asarum sieboldii* seeds under natural conditions (B). Vertical bars represent ± SE.
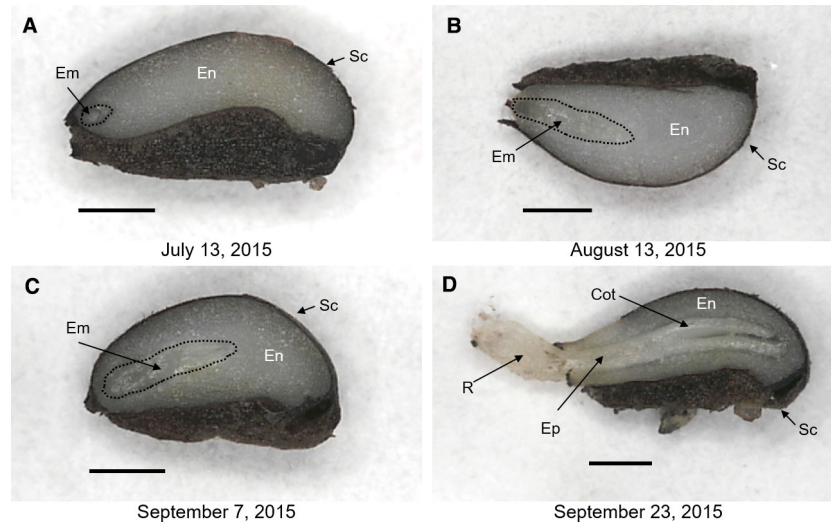
Fig. 2. Longitudinal sections of *Asarum sieboldii* seeds showing the cotyledon (Cot), embryo (Em), endosperm (En), epicotyl (Ep), radicle (R) and seed coat (Sc). Embryo growth in the seeds under natural conditions in Gyeonggi-do, Korea, on 13 July 2015 (A), 13 August 2015 (B) and 7 September 2015 (C); radicle emergence on 23 September 2015 (D). Bars represent 1 mm.
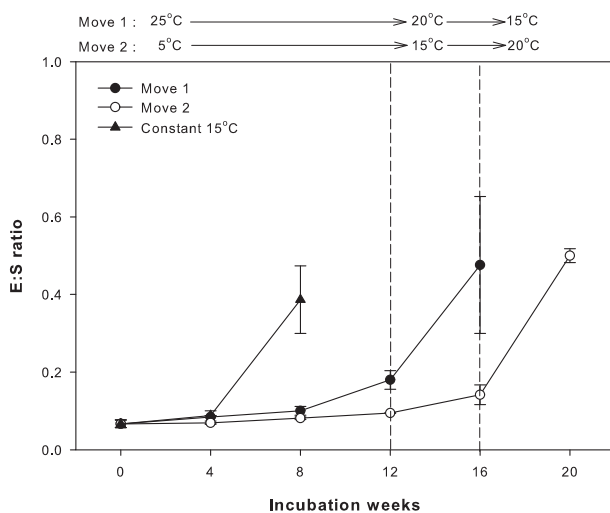

Fig. 3. E:S ratio (embryo length:seed length) of *Asarum sieboldii* seeds incubated under two temperature sequences and one constant temperature. Vertical error bars represent ± SE.

Fig. 4. The cumulative radicle and shoot emergence from *Asarum sieboldii* seeds incubated under two temperature sequences. Vertical error bars represent ± SE.

radicle emergence did not occur during 5 °C for 12 weeks or 15 °C for 4 weeks. Radicle emergence began when seeds were transferred to 20 °C, reaching 80%; however, no shoots emerged in the Move 2 sequence.

Effects of warm stratification on radicle emergence

In the treatment receiving 0 weeks of warm stratification, radicle emergence started at week 6 when seeds were placed at 15 °C and emergence increased to 82.5% by week 12 (Fig. 5). On the other hand, following 4, 8 and 12 weeks of warm stratification at 25 °C, radicles emerged quickly when seeds were moved to 15 °C, reaching approximately 90% at week 6.
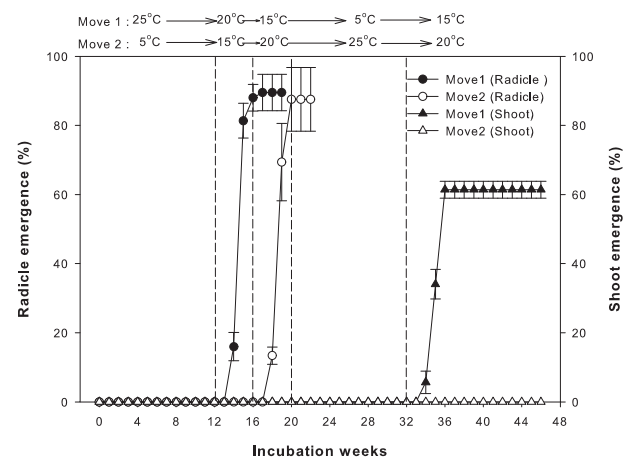
Effects of GA$_3$ on radicle emergence

There was a significant difference in the percentage of radicle emergence among GA$_3$ concentrations ($P < 0.001$). The radicle emergence percentage of seeds soaked in 0, 10, 100 and 1000 mg·l^{-1} GA$_3$ was 0, 12.7, 87.8 and 51.7%, respectively (Fig. 6). The highest radicle emergence occurred in the 100 mg·l^{-1} GA$_3$ treatment.

Effects of cold stratification on shoot emergence

The percentage of shoot emergence differed among the periods of cold stratification ($P < 0.001$). Shoot emergence was not observed from seeds with an emerged radicle subjected to 0, 4 and 8 weeks of cold stratification at 5 °C (Figs. 7, 8). The percentage of shoot emergence at 15 °C following 12 weeks of cold stratification was 45.1% for seeds with an emerged radicle.
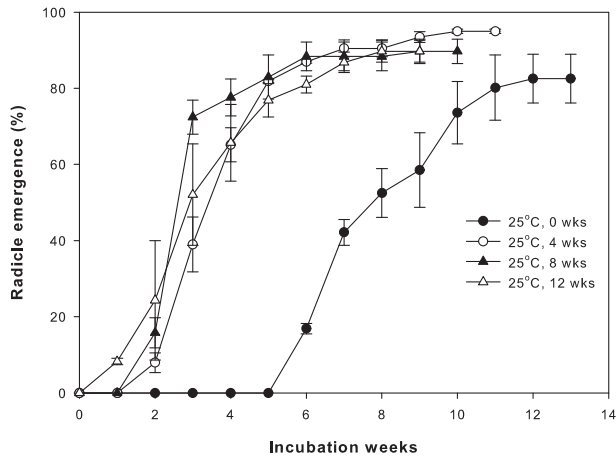
Fig. 5. Cumulative radicle emergence from *Asarum sieboldii* seeds incubated at 15 °C after warm stratification treatments at 25 °C for 0, 4, 8 and 12 weeks. Vertical error bars represent ± SE.
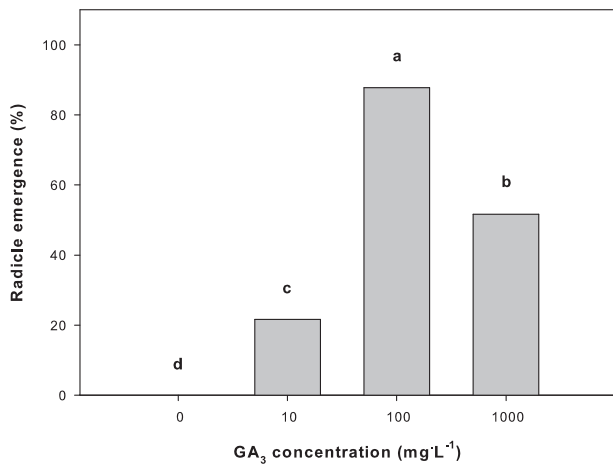

Fig. 6. Radicle emergence from *Asarum sieboldii* seeds treated with 0, 10, 100 and 1000 mg·l⁻¹ GA₃. Vertical error bars represent ± SE. Bars with different letters are significantly different ($P < 0.001$) using Fisher exact test with P-value adjustment using Holm's method.

Fig. 7. Shoot emergence from *Asarum sieboldii* seeds incubated at 15 °C after treatment at 5 °C for 0, 4, 8 and 12 weeks. Before starting the experiment, seeds were incubated at 25 °C for 12 weeks and 15 °C for radicle emergence. Bars with different letters are significantly different ($P < 0.001$) using Fisher exact test with P-value adjustment using Holm's method.

Fig. 8. Epicotyl dormancy was maintained in radicle-emerged seeds of *Asarum sieboldii* without cold stratification (A) and shoot emergence only occurred after treatment at 5 °C for 12 weeks (B). R: radicle, L: leaves.

DISCUSSION

At the time of dispersal, seeds of *A. sieboldii* had underdeveloped embryos with an E:S ratio of 0.06 (Figs. 1B, 2A). The embryo had to grow at least 600% (E:S ratio = 0.37) for the radicle to emerge, so seeds had MD. If germination does not occur within 30 days under appropriate environmental conditions, then it is determined that the seeds have PD (Baskin & Baskin, 2014). Since the seeds did not germinate within 30 days in any of the conditions tested, because even at 5 °C, 15 °C or 25 °C the seeds did not germinate immediately (Figs. 4, 5), they can be classified as having MPD, *i.e.* with both PD and MD. Types of MPD can be divided into simple and complex, depending on the temperature regimes for embryo growth. Embryos of *A. sieboldii* grew in natural conditions at relatively warm temperatures from July to September in the range of 19.4 °C to 36.5°C (Fig. 1). In a move-along test, embryos grew at a constant 15 °C, or at 25 °C followed by 15 °C in the Move
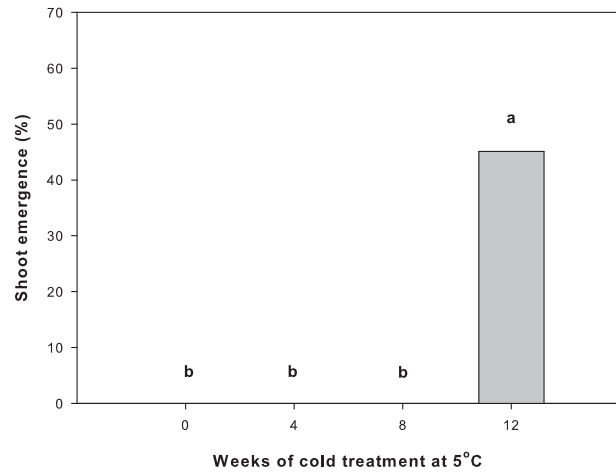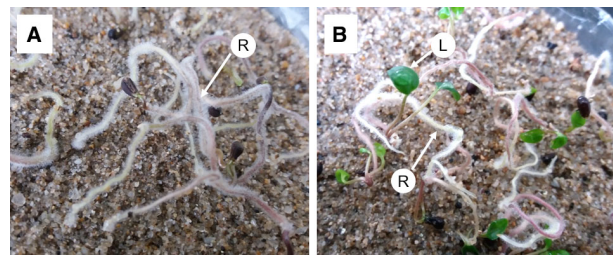
1 sequence (Fig. 3). In the Move 2 sequence, embryos hardly grew at 5 °C, and they only began to grow when moved to 15 °C and grew rapidly at 20 °C. Therefore, seeds of *A. sieboldii* have simple MPD, with embryos growing only at warm temperatures (≥15 °C).

In the phenology experiment, radicles of *A. sieboldii* emerged in the autumn, but shoots were not found until spring (Fig. 1B), indicating that seeds had epicotyl dormancy. Epicotyl MPD can be divided into two levels, non-deep simple and deep simple, depending on whether cold stratification is needed to break shoot dormancy (Baskin & Baskin, 2014). For *A. sieboldii*, cold stratification was required to break shoot dormancy in seeds with an emerged radicle (Fig. 7). Thus, seeds of *A. sieboldii* can be classified as deep simple epicotyl MPD. The dormancy formula for *A. sieboldii* is $C_{1b}B(root) - C_3(epicotyl)$, which means that the root (radicle) of the underdeveloped embryo (B) has PD (C) of non-deep type (subscript 1) that is broken during a relatively short period of warm stratification (subscript b). Further, GA₃, regardless of concentration, promoted radicle emergence from seeds (Fig. 6), indicating that the dormancy of the radicle was non-deep PD. The category $C_3(epicotyl)$ means that epicotyl dormancy in the seed with a fully developed embryo and with an emerged radicle was deep

PD (C$_3$) and is broken by a relatively long period of cold stratification (Nikolaeva, 2001; Baskin & Baskin, 2008).

Radicle emergence in seeds of *A. canadense*, which is a North American congener, required warm temperatures, such as alternate temperatures of 20 °C/10 °C (12 h/12 h) (Baskin & Baskin, 1986) or constant temperatures of 15 °C or 20 °C (Barton, 1944). After the radicle emerged, cold stratification at 5 °C was required to break epicotyl dormancy of the seeds (Baskin & Baskin, 1986). Therefore, the seeds of *A. canadense* also have deep simple epicotyl MPD like the Asian species (*A. sieboldii*).

Among the plant species belonging to *Aristolochiaceae*, it was reported that seeds of *Hexastylis heterophylla* also have deep simple epicotyl MPD (Adams *et al.*, 2003). The genus *Hexastylis* is not monophyletic and is considered as one of the segregates of *Asarum sensu lato* (*s.l.*) through nuclear and plastid genomes (Kelly, 1998; Sinn *et al.*, 2018; Takahashi & Setoguchi, 2018) as well as floral morphology (Kelly, 1997). Therefore, the nomenclature of *Hexastylis heterophylla* is currently *A. minus* Ashe (The Plant List, 2013). *Asarum s.l.* was organized into two main clades: *Asarum sensu stricto* (*s.s.*) clade and *Asiasarum + Hexastylis + Heterotropa* clade (Kelly, 1997; Kelly, 1998). *Asarum s.s.* segregate has 17 species distributed over a wide range of regions, including North America, Europe and Asia (mainly China). The *Hexastylis* segregate (eight to nine species) is found only in North America. *Heterotropa* (~50 species), *Asiasarum* (three to four species) and *Geotaenium* (three to four species) segregates are exclusive to Asia (Kelly, 1997). *Asarum canadense*, *A. minus* (accepted name of *Hexastylis heterophylla*) and *A. sieboldii*, whose seeds have deep simple epicotyl MPD, belong to different segregates of *Asarum s.s.*, *Hexastylis* and *Asiasarum*, respectively.

Based on ITS data and morphological traits, *Hexastylis* segregate is more closely related to Asian species than to *Asarum s.s.* Thus, *Hexastylis* (*A. minus*) is regionally located in North America but has a close relationship with the *Asiasarum* (*A. sieboldii*) and is evolutionarily less related to *Asarum s.s.* (*A. canadense*). The genus *Asarum* is postulated to have originated from Asia and expanded its range into North America and Europe (Kelly, 1998). The species were separated, and various distinctive traits appeared in chromosome number (Maekawa, 1963) and in flower and stem morphology, such as internode elongation, stigma position and ovary position (Cheng & Yang, 1983), but they showed stasis of seed dormancy derived from the common ancestor. *Asarum europaeum* is the only species of *Asarum* native to Europe. Research from Nikolaeva and colleagues indicated that seeds of *A. europaeum* required cold stratification (Nikolaeva *et al.*, 1985; Rosbakh *et al.*, 2020), and Adams *et al.* (2003) considered that this European species may have intermediate simple MPD. However, no definitive data could be found for *A. europaeum* and further research will be needed to ascertain the kind of dormancy found in this species. From a phylogenetic perspective, *Asarum* originated in Asia and was later separated into European and American lineages. Thus, *A. europaeum* may have the ancestral condition of deep simple epicotyl MPD, which is the same type as found in Asian and American species. However, we cannot refute the alternative hypothesis that this kind of dormancy might be a derived trait that evolved multiple times in species growing in similar environments.

Among the disjunct relict species, studies have compared seed dormancy for species that had been separated for long periods. Species of *Jeffersonia* from East Asia (*J. dubia*) and North America (*J. diphylla*) have the same seed dormancy type (Grushvitzky, 1967; Baskin & Baskin, 1989; Rhie *et al.*, 2015). However, seeds of *Osmorhiza aristata* (Asian species), *O. occidentalis* and *O. chilensis* (western North American species) differed in dormancy from those of *O. longistylis* and *O. claytonia* from eastern North America (Baskin & Baskin, 1984; Baskin *et al.*, 1995; Walck *et al.*, 2002). The genus *Aristolochia*, a derived member of the sister group of *Asarum*, existed in the early Tertiary according to the fossil record of the middle Eocene (MacGinitie, 1969), and is distributed in Asia, North America and Europe, as is the case of *Asarum*. However, unlike *Asarum*, this genus showed divergence in seed dormancy among species. Two eastern North American species (*Aristolochia tomentosa* and *Ar. macrophylla*) and one eastern Asian species (*Ar. manshuriensis*) had MD or non-deep simple MPD, but the western North American species (*Ar. californica*) had intermediate or deep complex MPD (Adams *et al.*, 2005).

Although Asian and North American species of *Asarum* have the same seed dormancy type, their specific requirements for warm or cold stratification differ. (i) In *A. canadense*, warm stratification for 6 weeks at 30 °C/15 °C was insufficient to break radicle dormancy, so that an additional period of 8 weeks at 20 °C/10 °C was required for radicle emergence of 84% (Baskin & Baskin, 1986). However, the seeds of *A. sieboldii* lost radicle dormancy after only 4 weeks of warm stratification at 25 °C, with rapid radicle emergence in over 80% of seeds when incubated at 15 °C (Fig. 5). These results suggest that the radicles of *A. sieboldii* have weaker PD than those of *A. canadense*. (ii) Shoot emergence from *A. canadense* seeds was close to 80% following 8 weeks of cold stratification at 5 °C (Baskin & Baskin, 1986). However, in *A. sieboldii* no shoots emerged following 4 and 8 weeks of cold stratification, and only 50% emerged following 12 weeks of stratification (Fig. 7). Thus, *A. sieboldii* had deeper epicotyl dormancy compared with *A. canadense*. This quantitative difference (divergence) might be due to adaptation to the environment. In Hongcheon-gun, Korea, the native home of *A. sieboldii*, it requires about 5 months at below 5 °C, based on average monthly temperature data for 20 years from 2000 to 2019 (KMA, 2020). On the other hand, in eastern Kentucky in the USA, the native home of *A. canadense*, this was about 3 months from December to February of the following year (UK Ag Weather Center, 2020). *Asarum sieboldii*, which grows in a long winter period, required longer cold stratification to break epicotyl dormancy than *A. canadense*. Temperature requirements for breaking dormancy often differ depending on geographic region, even within the same species. Among populations of *Viburnum opulus* spread across Asia, North America and Europe, there was variation in the germination responses according to geographic region (Walck *et al.*, 2012). In seeds from the northern-most populations of *V. opulus*, relatively longer cold stratification was required for shoot emergence compared to other populations.

CONCLUSIONS

The underdeveloped embryo of *A. sieboldii* grew during late summer and radicle emergence occurred in autumn following seed dispersal in early summer. However, due to epicotyl dormancy shoot emergence was delayed until spring of the following year when the temperature rose after winter. To break

epicotyl dormancy, 12 weeks of cold stratification were required, indicating that seeds of this species had deep simple epicotyl MPD. This Asian *Asarum* has the same kind of dormancy as the North American species *A. canadense* and *A. minus*, showing that strong stasis of seed dormancy has occurred even after the long time since the species became separated.

ACKNOWLEDGEMENTS

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