Morphophysiological dormancy in seeds of three eastern North American *Sanicula* species (Apiaceae subf. Saniculoideae): evolutionary implications for dormancy break

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Abstract

Dormancy breaking and germination requirements were determined for seeds of the eastern North American (eNA) species *Sanicula canadensis*, *Sanicula gregaria* and *Sanicula trifoliata*, and the data compared to those available for the European–Asian (EurA) congener *Sanicula europaea*. Seeds of the three eNA species had underdeveloped embryos that were physiologically dormant, i.e., morphophysiological dormancy (MPD). Warm (25/15°C) followed by cold (5/1°C) stratification was effective in breaking dormancy in 100% of the *S. canadensis* seeds, but in only 29.3% of *S. gregaria* seeds and 43.3% of *S. trifoliata* seeds. Cold stratification alone broke dormancy in 38.7, 12.0 and 0% of *S. canadensis*, *S. gregaria* and *S. trifoliata* seeds, respectively. Thus, some seeds of *S. canadensis* and of *S. gregaria* that germinated have non-deep complex MPD, and others have deep complex MPD. All seeds of *S. trifoliata* that germinated have non-deep complex MPD. Within a phylogenetic context, the kind (level) of MPD may or may not differ between eNA *Sanicula* sister species because conspecific variation in the kind of MPD exists in seeds of *S. canadensis* and *S. gregaria*. Similarly, the kind of MPD in seeds of eNA *S. canadensis* and *S. gregaria* may or may not differ with the deep complex MPD in seeds of the EurA *S. europaea*. However, the non-deep complex MPD in all seeds of eNA *S. trifoliata* and deep complex MPD in seeds of *S. europaea* represent a distinct difference in this trait between two of the five clades comprising the genus *Sanicula*.

Keywords: Apiaceae, morphophysiological dormancy, *Sanicula*, Saniculoideae, seed germination.

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Introduction

Seeds of many temperate plant species are dormant at the time of seed dispersal, and specific temperature requirements must be met before they will germinate (Baskin & Baskin 1998). Furthermore, within the temperate forest biome, seeds of many herbaceous species contain underdeveloped embryos at the time of seed dispersal. In addition to being underdeveloped, embryos may be physiologically dormant, thus requiring a period of warm and/or cold stratification before the seed can germinate (Nikolaeva 1977). Embryo growth and breaking of

physiological dormancy may be synchronous (Baskin *et al*. 1992), embryo growth may be delayed until after physiological dormancy break is completed (Baskin & Baskin 1990; Walck *et al*. 1999), or embryo growth may be completed during the first phase of physiological dormancy break, but radicle emergence does not occur until the second phase of physiological dormancy is broken (Phartyal *et al*. 2009; Vandelook *et al*. 2009). Seeds with underdeveloped embryos that require these dormancy breaking and germination treatments have morphophysiological dormancy (MPD), one of the five classes of seed dormancy (Baskin & Baskin 2004).

Nine kinds (levels) of MPD have been identified, and they are distinguished based on the temperatures required for embryo growth and dormancy break and their responses to gibberellic acid (Baskin & Baskin 2004; Baskin *et al*. 2008). Of the nine known levels of MPD, three

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have been found to occur in eastern North American (eNA) temperate herbaceous Apiaceae species. For example, embryo growth and dormancy break in seeds of *Chaerophyllum tainturieri* (Baskin & Baskin 1990) and *Chaerophyllum procumbens* (Baskin *et al.* 2004) occur at warm temperatures, and these seeds have non-deep simple MPD. In contrast, embryo growth and dormancy break in seeds of *Cryptotaenia canadensis* (Baskin & Baskin 1988b) and *Perideridia americana* (Baskin & Baskin 1993) occur at low temperatures, and these seeds have deep complex MPD. A period of warm stratification followed by a period of cold stratification promotes germination in seeds of *Osmorhiza claytonii* (Baskin & Baskin 1991) and *Osmorhiza longistylis* (Baskin & Baskin 1984), and embryos grow during the low temperature period. Therefore, these seeds have non-deep complex MPD. Nikolaeva (1977) found that gibberellic acid (GA$_3$) promoted germination in some seeds with a low temperature requirement for embryo growth and classified them as having intermediate complex MPD. Nikolaeva (1977) also found that GA$_3$ promoted germination in some seeds with a high temperature requirement for embryo growth and classified these as having intermediate simple MPD. However, these dormancy kinds (intermediate complex MPD and intermediate simple MPD) have not yet been reported in members of the Apiaceae (Nikolaeva *et al.* 1985; Walck *et al.* 2008; Phartyal *et al.* 2009).

When comparing seeds with MPD in genera with an Arcto-Tertiary distribution, seed dormancy breaking and germination requirements may or may not differ between geographically disjunct congeners. Level of MPD does not differ between the closely related east-Asian (eA) *Aristolochia manshuriensis* and eNA *Aristolochia macrophylla* (Adams *et al.* 2005) or between eA–eNA disjunct *Jeffersonia* or *Panax* species (Baskin & Baskin 1998). Within an evolutionary context, this suggests stasis of the level of MPD, and seemingly supports hypotheses of little to no change in ecophysiological traits throughout a taxon's history (e.g. Axelrod 1983; Ricklefs & Latham 1992). In contrast, deep complex MPD reported for the eA *Osmorhiza aristata* (Walck *et al.* 2002) differs from the non-deep complex MPD observed in the eNA species *O. claytonii* and *O. longistylis* (Baskin & Baskin 1984, 1991), suggesting changes in the kind of seed dormancy in response to changes in selective pressures (adaptations) through geological time (e.g. Wolfe 1975; Wen *et al.* 2002).

Although the kind of seed dormancy has been determined for numerous species in the subfamily Apioideae (Apiaceae), members of the subfamily Saniculoideae remain largely unstudied. Baskin and Baskin (1988a) suggested that cold stratification was required for *Sanicula canadensis* seeds to germinate. Recently, Vandelook and Van Assche (2008) reported that seeds of the Eurasian (EurA) polycarpic perennial, *Sanicula europaea*, have deep

complex MPD, and Hawkins *et al.* (2007) suggested that three eNA *Sanicula* species have non-deep complex MPD. The latter was inferred from the results of a seed germination phenology study; therefore, dormancy breaking and the germination requirements for seeds of eNA *Sanicula* species have yet to be empirically determined. Furthermore, if the geographical pattern of the kind of dormancy as reported by Vandelook and Van Assche (2008) and Hawkins *et al.* (2007) is confirmed, stasis of seed dormancy kind would not be indicated among the EurA–eNA disjuncts. Therefore, the primary objective of our research was to determine the dormancy breaking and germination requirements for the eNA temperate deciduous forest species *S. canadensis*, *Sanicula gregaria* and *Sanicula trifoliata*. A secondary objective was to compare levels of MPD in these species with that of the EurA congener *S. europaea*. Such information in combination with recently published phylogenetic research by Calviño *et al.* (2008) and Kadereit *et al.* (2008) may provide insight into the evolution of seed dormancy in Saniculoideae.

The study species

Saniculoideae consists of two tribes, Saniculeae and Steganotaenieae, and *Sanicula* is one of seven genera in the former tribe (Calviño & Downie 2007). *Sanicula* consists of approximately 40 species that grow in the north temperate zone of both the Old and New World (Pryor & Phillippe 1989) and four species endemic to Hawaii (Wagner *et al.* 1999). The fruit of the three study species, *S. canadensis* L., *S. gregaria* Bickn. and *S. trifoliata* Bickn., is a schizocarp densely covered with hooked bristles, and consisting of two mericarps that separate at maturity. Fruit lengths are 2–5 mm for *S. canadensis*, 3–5 mm for *S. gregaria* and 6–8 mm for *S. trifoliata* (Radford *et al.* 1968; Gleason & Cronquist 1991).

Sanicula canadensis is a strict biennial (Hawkins 2003) that grows in dry-mesic to wet-mesic deciduous forests (Petranka & Holland 1980; Phillippe *et al.* 1999; Edgin & Ebinger 2001). Its range extends from Florida to Texas, north to Massachusetts, New Hampshire, Vermont, Pennsylvania, Ohio, Kentucky, Missouri and Oklahoma (Fig. 1a). The seeds are dispersed in late summer to early autumn, although some seeds (mature mericarps) may be retained on upright stems of the dead mother plant for 1 year or longer (Hawkins 2003).

Sanicula gregaria is a polycarpic perennial that grows in rich, mesic to wet-mesic deciduous forests (Phillippe 1978; Magee & Ahles 1999; Phillippe *et al.* 1999). It ranges from Quebec to Minnesota and South Dakota, south to Florida, Alabama, Missouri, Kansas and Texas (Fig. 1b). Dispersal of *S. gregaria* seeds begins in late summer and is
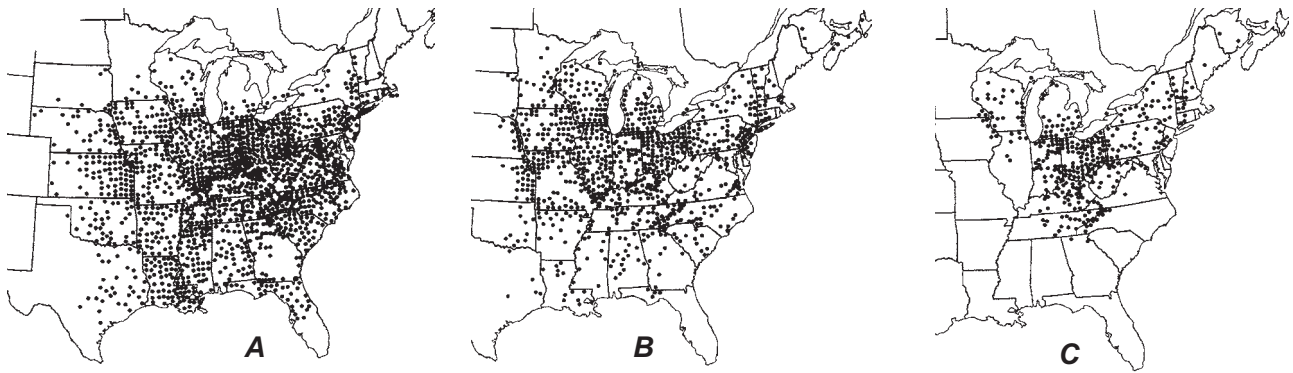
Fig. 1 Geographical distribution of (a) *Sanicula canadensis*, (b) *Sanicula gregaria* and (c) *Sanicula trifoliata* based on specimen information from AUA, BE, DOV, EK, GA, IA, KA, KY, LAF, LL, LO, LSU, LTU, MISS, MO, MU, NBM, NK, NLU, NMNH, NO, QK, TAMU-BWG, TEX, TROY, UNA, UNB, WIS and WEEK (*sensu* Holmgren *et al.* 1990) and published reports from Deam (1940), Steyermark (1963), Radford *et al.* (1968), Great Plains Flora Association (1977), Mohlenbrock and Ladd (1978), Strausbaugh and Core (1978), Scoggan (1979), Harvill *et al.* (1981), Hough (1983), Roland and Smith (1983), Voss (1985), Smith (1988), Pryor and Phillippe (1989), Ownbey and Morley (1991), Rhoads and Klein (1993), Cooperrider (1995), Hawkins and Richards (1995), Chester *et al.* (1997), Magee and Ahles (1999), Hinds (2000), Weldy *et al.* (2002) and Wunderlin and Hansen (2002). Each symbol (●) represents one or more county records.

generally complete by early autumn. Seeds are not retained on the mother plant for an extended period of time (Hawkins 2003).

Sanicula trifoliata grows in mesic deciduous forests (Magee & Ahles 1999; Edgin & Ebinger 2001) and it is typically an indicator of rich, mature forests (Phillippe 1978). It ranges from Vermont and adjacent Quebec to southern Wisconsin and northeast Iowa, south to North Carolina and Tennessee (Fig. 1c). Although often described in taxonomic manuals as a biennial, *S. trifoliata* may act as a facultative biennial, whereby it remains in the rosette growth stage for more than one winter before bolting and producing seeds (Hawkins 2003). Seeds are dispersed in late summer to early autumn, and dispersal may last for 1 year or longer, with mature mericarps retained on dead, upright stems (Hawkins 2003).

Materials and methods

General

Mature fruits (hereafter referred to as seeds) of *S. canadensis*, *S. gregaria* and *S. trifoliata* were collected in September 2002 and stored dry at room temperature (22–24°C) for approximately 12 days before they were used in the experiments. The seeds were harvested from plants growing on a north slope in a second growth broad-leaved deciduous forest in the Breathitt County (Kentucky) tract of the University of Kentucky's Robinson Forest. This forest is located in the Eastern Rugged Area of the Cumberland Plateau in Braun's (1950) Mixed Mesophytic Forest Region. *Sanicula canadensis* most often grows at the edge of a *Quercus–Liriodendron–Acer* forest. Codominant

canopy components in the areas where *S. gregaria* and *S. trifoliata* grow are *Fagus grandifolia* and *Tsuga canadensis*.

For germination studies, seeds were placed in 9-cm-diameter plastic Petri dishes on sand moistened with distilled water. Emergence of the radicle was the criterion for germination. Embryo growth studies were carried out by placing seeds in 9-cm-diameter plastic Petri dishes on Whatman number 1 filter paper moistened with distilled water. Three replicates of 25 seeds per dish were used in each treatment and control for the germination studies. For the embryo growth study, one dish of 25 seeds was used each time measurements were made. All dishes were wrapped in plastic film to retard water loss during incubation and stratification.

Both studies were conducted in three temperature-controlled and light-controlled incubators and in a refrigerator. The incubators were set at 12 h/12 h daily alternating temperature regimes of 15/6°, 20/10° and 25/15°C, and the refrigerator was set at an alternating 5/1°C. At 15/6°, 20/10° and 25/15°C, seeds were exposed to 14 h of light (~ 40 µmol/m² /s) diurnally, extending from 1 h before the beginning of the high temperature period to 1 h after the beginning of the low temperature period. At 5/1°C, seeds were exposed to 12 h of light (~ 40 µmol/m² /s) each day during the high-temperature period.

Temperature requirements for dormancy break, embryo growth and germination

Seeds of each species were moved through a sequence of temperature treatments to simulate the seasonal

temperature fluctuations experienced by field populations starting during summer: 25/15°C for 12 weeks → 20/10°C for 8 weeks → 15/6°C for 4 weeks → 5/1°C for 12 weeks → 15/6°C for 4 weeks → 20/10°C for 8 weeks. For each species, 12 Petri dishes each containing 25 seeds were placed in the initial 25/15°C temperature. As Petri dishes were moved through the sequence of temperature treatments, one Petri dish of seeds for each species was removed every 4 weeks. Embryos were excised from these seeds, and their lengths were measured using a dissecting microscope equipped with an ocular micrometer. Seeds used as control groups remained continuously in each of the four temperature treatments for the duration of the study (48 weeks). At the end of the study embryos in seeds in the control groups were excised and measured. An additional three Petri dishes of 25 seeds each in the 5/1°C temperature regime were removed at 4, 8 and 12 weeks, and embryos were excised and measured.

The effect of cold stratification (5/1°C) and of warm (25/15°C) followed by cold stratification on seed germination was evaluated. In the cold stratification procedure, seeds of each species received 12 weeks of cold, after which they were incubated at each of the test temperature regimes (15/6°C, 20/10°C, 25/15°C). Seeds that received the warm + cold treatment were warm stratified for 12 weeks, then moved to cold stratification for 12 weeks, after which they were placed at the three test temperatures. Seeds used as controls were placed directly at test temperatures, where they remained for the duration of the study. All seeds were monitored for germination at 2-week intervals throughout the study, at which time any germinated seeds were counted and discarded. Distilled water was added to the dishes as needed to keep the seeds moist. At the end of the experiments, non-germinated seeds were checked to determine whether the embryos were white and firm (viable) or grayish brown and soft (non-viable).

Statistical analyses

Means and standard errors were calculated for embryo lengths and germination percentages. In the dormancy break and embryo growth study, a one-way ANOVA ($P = 0.05$) was used to compare final embryo lengths in seeds of the control groups. In the germination study, the square root of the germination percentages was arcsine transformed before analysis and actual values were used for presentation. A one-way ANOVA was used to compare germination percentages (based on the number of viable seeds) among stratified and non-stratified seeds. In both studies, the ANOVAs were followed by a protected least significant difference test (PLSD, $P = 0.05$). The SAS

procedure Generalized Linear Model (GLM) was used to carry out the statistical analyses (SAS Institute 2001).

Results

Temperature requirements for dormancy break, embryo growth and germination

At the beginning of the study seeds contained underdeveloped embryos with mean lengths of 0.36 ± 0.01 mm, 0.38 ± 0.02 mm and 0.50 ± 0.0 mm for *S. canadensis*, *S. gregaria* and *S. trifoliata*, respectively. In week 32 (5/1°C) of the sequence of temperature regimes, 22% of the embryos in the *S. canadensis* seeds ($n = 25$) had grown to critical length (1.58 ± 0.11 mm; embryo length just prior to radicle emergence), 16% had not grown, but were viable, 45% of the seeds had germinated and 17% contained no embryo or were rotten (Fig. 2). Thirty-five percent and 18% of the seeds had germinated in the week 36 (5/1°C) and week 40 (15/6°C) Petri dishes, respectively (Fig. 2). In addition, in week 36, 18% of the embryos had reached critical length. All ungerminated seeds in weeks 36 and 40 remained viable. In seeds used as control groups, embryos of *S. canadensis* showed a significant amount of growth after 8 weeks at 5/1°C ($P = 0.0024$; Fig. 3).

Embryos in all seeds of *S. gregaria* grew during the $20/10° \rightarrow 15/6° \rightarrow 5/1° \rightarrow 15/6°$C portion of the temperature sequence (weeks 16–40), but most growth occurred at 5/1°C (weeks 24–40). Six percent of the seeds had germinated in week 40 (15/6°C), and 15% and 6% had germinated in weeks 44 (20/10°C) and 48 (20/10°C), respectively (Fig. 4). Embryos grew to critical length (2.54 ± 0.09 mm) and remained viable in all ungerminated seeds. In the control groups, embryo lengths in *S. gregaria* seeds were significantly longer in the 15/6°C (48 weeks) temperature regime ($P < 0.0001$) than in the other control temperatures (Fig. 4). Furthermore, the *S. gregaria* seeds at 15/6°C (48 weeks) contained embryos that had grown to approximately half of critical length.

After seeds of *S. trifoliata* were moved from 15/6°C to 5/1°C, approximately 27% of the embryos grew to critical length (2.99 ± 0.19 mm) after 8–12 weeks at 5/1°C. In week 40 (15/6°C), 16% of the seeds in the Petri dish had germinated (Fig. 5), and embryos in the remaining seeds had not grown, but remained viable. In the controls, embryos in seeds of *S. trifoliata* showed little to no growth at any temperature; although mean embryo length for seeds in the 5/1°C (48 weeks) and alternating temperatures was significant ($P = 0.0052$) relative to mean initial length (Fig. 3).

Following 12 weeks of cold stratification, mean germination percentages for seeds of *S. canadensis* were significantly higher ($P \leq 0.0412$) at 15/6°C and 20/10°C than at 25/15°C (Table 1). For *S. gregaria*, cold-stratified seeds
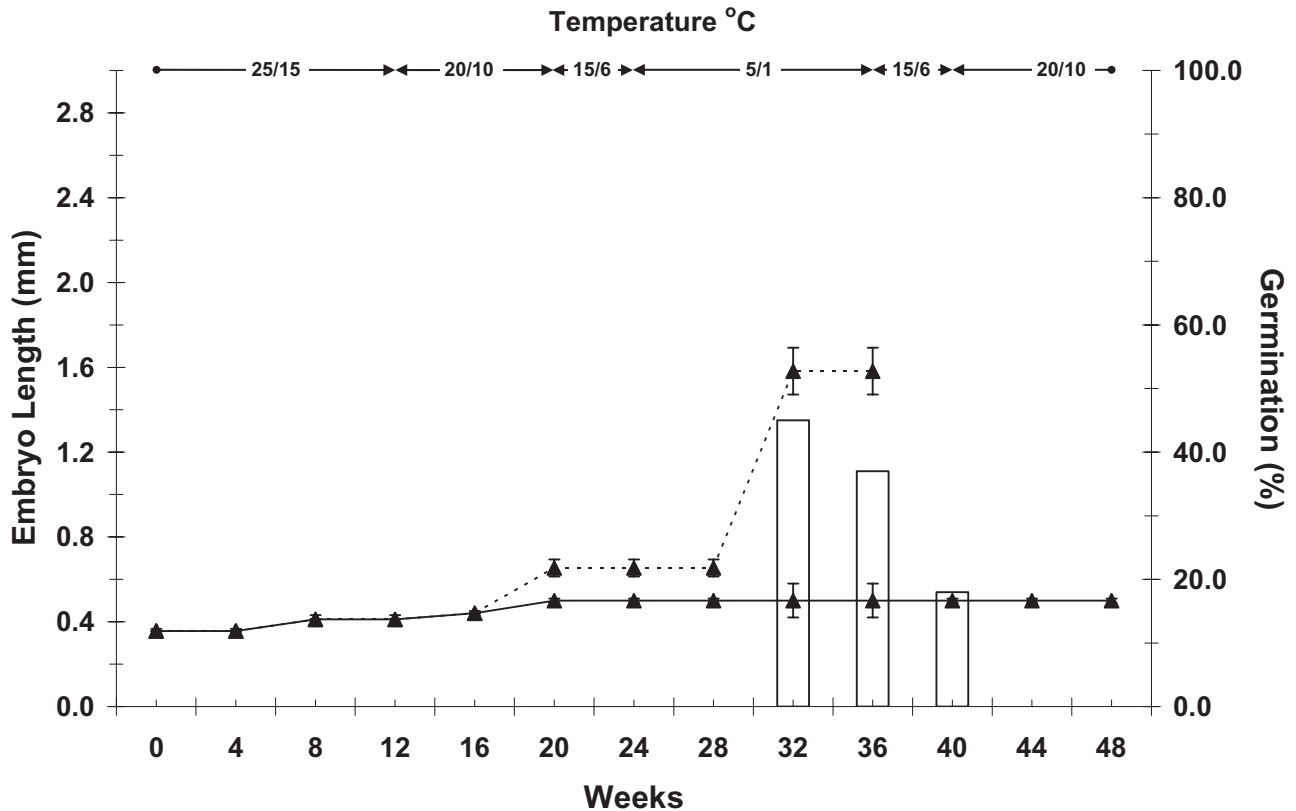
Temperature °C

Fig. 2 Embryo length (▲; mean ± standard error [SE]) in seeds of *Sanicula canadensis* during a sequence of temperature regimes. Dotted line, mean length ± SE of embryos that grew to critical length; solid line, mean length ± SE of embryos that did not grow; bars, non-cumulative percentage of seeds (*n* = 25) that germinated.

incubated at 15/6°C germinated to a higher percentage (12.0 ± 0.0%) than those incubated at 20/10°C and 25/15°C (Table 1). After 12 weeks of warm stratification followed by 12 weeks of cold stratification, germination percentages for seeds of *S. canadensis* and *S. gregaria* incubated at 15/6°C and 20/10°C were significantly higher ($P \leq 0.0226$) than they were for seeds that received only cold stratification (Table 1). Furthermore, seeds of these two species that received only cold stratification did not germinate at 25/15°C, whereas those receiving warm + cold stratification germinated to low percentages (≤16.0%) at 25/15°C. *Sanicula trifoliata* seeds that received no treatment or 12 weeks of cold stratification did not germinate, whereas those warm stratified for 12 weeks and then cold stratified for 12 weeks germinated equally well (20.7–43.3%; $P = 0.1470$) at the three alternating temperature regimes (Table 1).

Discussion

Baskin and Baskin (1988a) reported that cold stratification was required for dormancy break in seeds of *S. canadensis*, suggesting that these seeds have deep complex MPD.

However, results of a germination phenology study prompted Hawkins *et al.* (2007) to infer that seeds of this species, as well as those of *S. gregaria* and *S. trifoliata*, have non-deep complex MPD. In the present study, seeds of *S. canadensis*, *S. gregaria* and *S. trifoliata* contained underdeveloped embryos that were dormant at the time of seed dispersal. In all three species, embryo growth occurred during cold stratification, indicating that these seeds have a kind of complex MPD. However, in a portion of the *S. canadensis* and *S. gregaria* seed populations, only cold stratification was required for dormancy break, whereas warm stratification followed by cold stratification was required for dormancy break in the remaining portion of these cohorts. Therefore, both deep complex MPD (or intermediate complex MPD) and non-deep complex MPD may be found within a seed population of these two *Sanicula* species, which resolves the conflicting reports of Baskin and Baskin (1988a) and Hawkins *et al.* (2007) with regard to the kind of dormancy in seeds of *S. canadensis*. In seeds that require cold stratification to break the physiological dormancy part of MPD, GA_3 is used to help distinguish whether seeds have deep complex MPD or intermediate complex MPD (i.e. GA_3 promotes
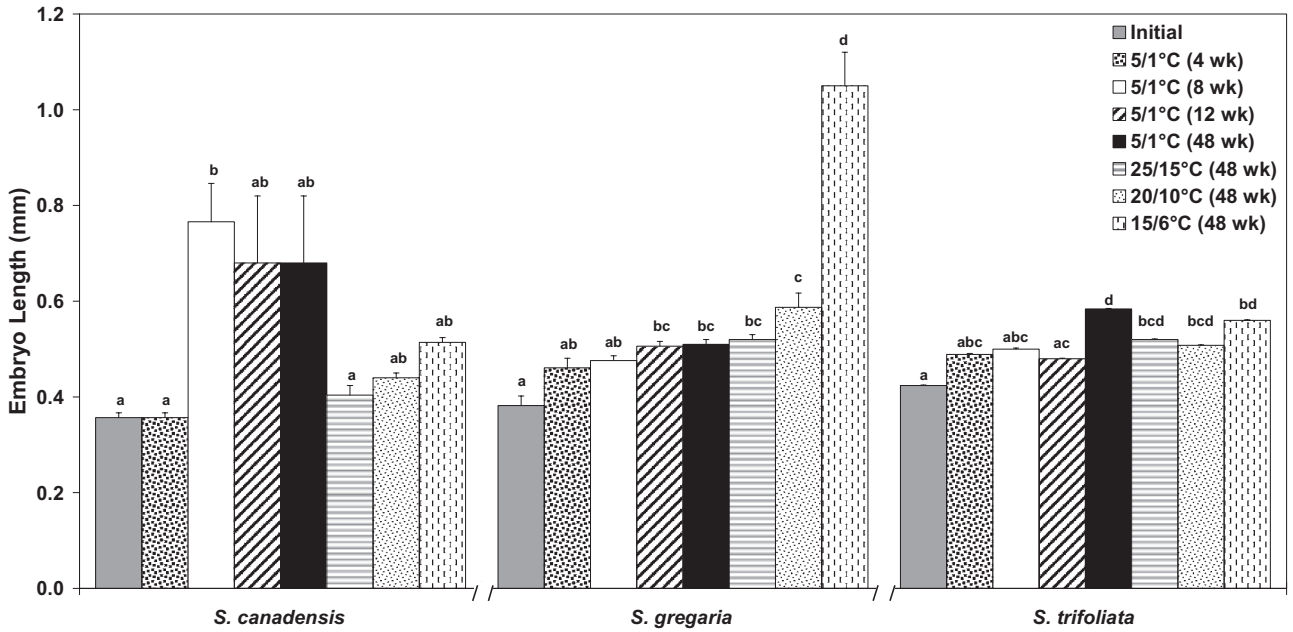
Fig. 3 Initial and final embryo lengths (mean ± standard error) for seeds of *Sanicula canadensis*, *Sanicula gregaria* and *Sanicula trifoliata* maintained in control temperatures. For each species, embryo length values with different lowercase letters are significantly different (protected least significant difference test, *P* < 0.05).
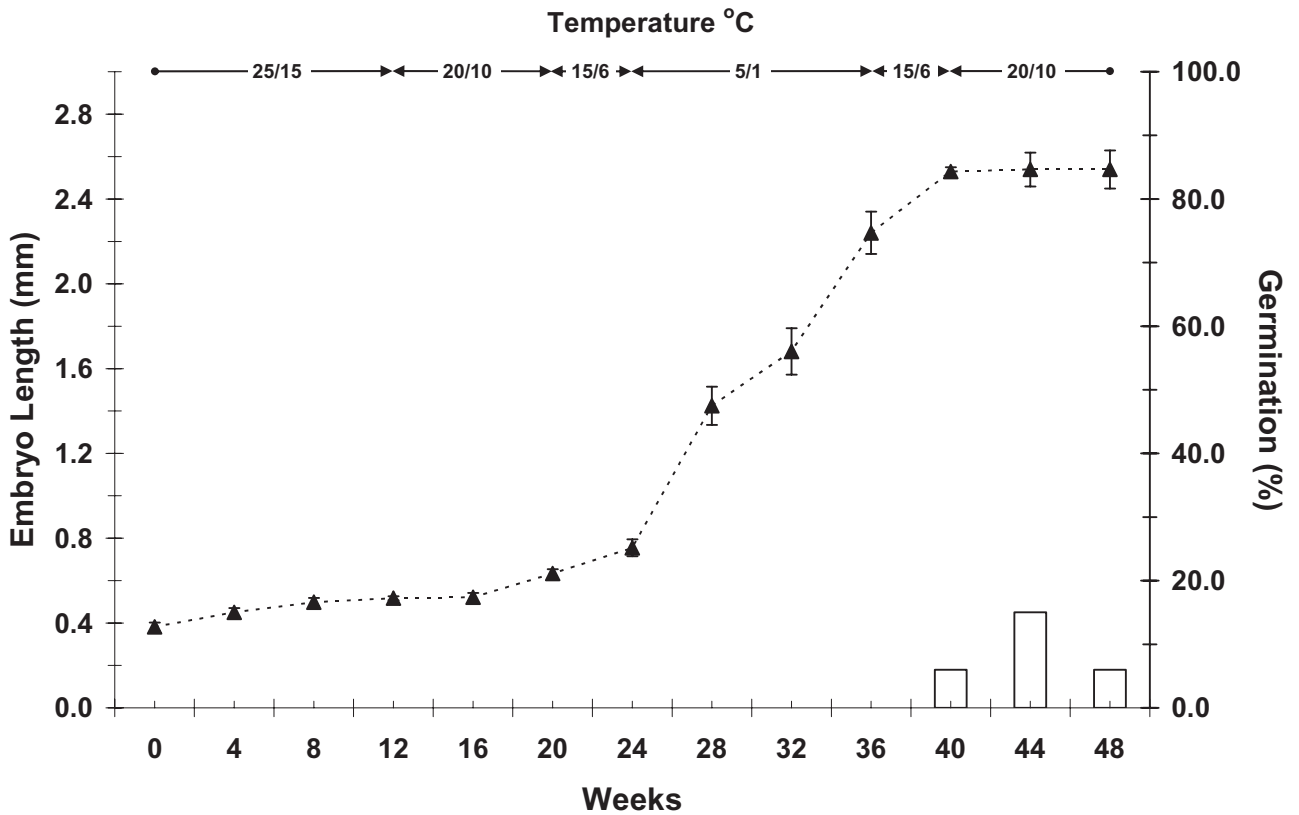

Fig. 4 Embryo length (▲; mean ± standard error) in seeds of *Sanicula gregaria* during a sequence of temperature regimes. Bars represent the non-cumulative percentage of seeds (*n* = 25) that germinated. Embryos in all seeds that did not germinate grew to critical length.
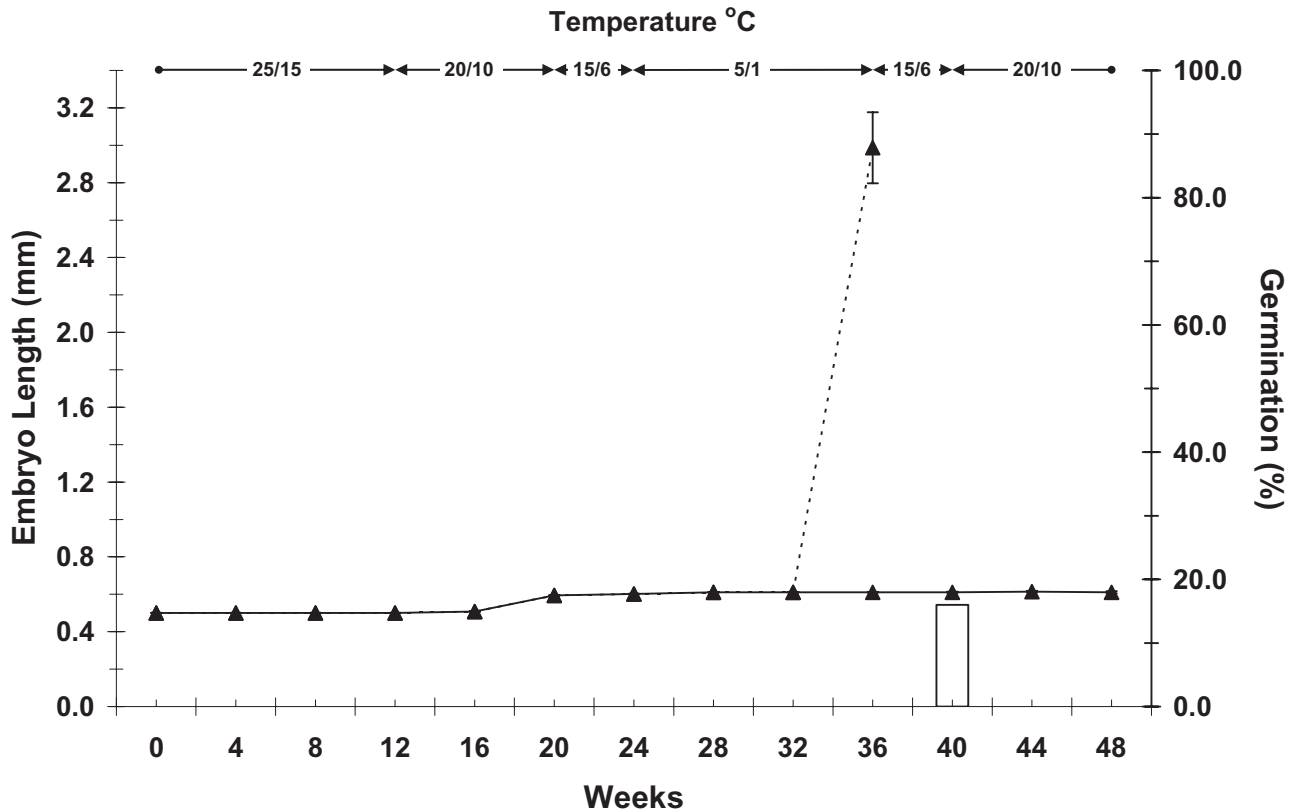
Temperature °C

Fig. 5 Embryo length (▲; mean ± standard error [SE]) in seeds of *Sanicula trifoliata* during a sequence of temperature regimes. Dotted line, mean length ± SE of embryos that grew to critical length; solid line, mean length ± SE of embryos that did not grow; bars, non-cumulative percentage of seeds (*n* = 25) that germinated.

Table 1 Cumulative germination percentages (mean ± standard error) for seeds of *Sanicula canadensis*, *Sanicula gregaria* and *Sanicula trifoliata* after 4 weeks at the test temperature regimes

		Temperature regime (°C)		
Species	Treatment†	15/6	20/10	25/15
S. canadensis				
	Control	0.0[Aa]	0.0[Aa]	0.0[Aa]
	C	38.7 ± 11.9[Ab]	22.3 ± 7.5[Ab]	0.0[Ba]
	W + C	100.0 ± 0.0[Ac]	61.1 ± 1.0[Ac]	2.9 ± 1.5[Ba]
S. gregaria				
	Control	0.0[Aa]	0.0[Aa]	0.0[Aa]
	C	12.0 ± 0.0[Ab]	1.3 ± 1.0[Ba]	0.0[Ba]
	W + C	29.3 ± 4.8[Ac]	17.3 ± 3.5[Ab]	16.0 ± 6.1[Ab]
S. trifoliata				
	Control	0.0[Aa]	0.0[Aa]	0.0[Aa]
	C	0.0[Aa]	0.0[Aa]	0.0[Aa]
	W + C	43.3 ± 1.7[Ab]	20.7 ± 5.5[Ab]	35.3 ± 10.7[Ab]

† C, 12 weeks 5/1°C; W + C, 12 weeks 25/15°C + 12 weeks 5/1°C; Control, 24 weeks at each test temperature. For each species, values in a row with different uppercase letters are significantly different, and values in a column with different lowercase letters are significantly different (protected least significant difference test, *P* < 0.05).

germination in seeds with intermediate complex MPD, but not those with deep complex MPD). However, given that few species have been found to have intermediate complex MPD, none of which are in Apiaceae, we will refer only to deep complex MPD in future discussion, with the caveat that this description is in the absence of a GA$_3$ treatment.

Warm stratification followed by cold stratification (~12 weeks at 5/1°C) was required for dormancy break and to promote embryo growth in *S. trifoliata* seeds. In this species, embryo growth was delayed until dormancy loss was complete. This aspect of dormancy break differed from that in seeds of *S. canadensis* and *S. gregaria*, where embryo growth and dormancy loss was synchronous, beginning shortly after seeds were moved to cold temperatures. Furthermore, warm stratification prior to cold stratification was required to break dormancy in seeds of *S. trifoliata*; therefore, seeds of this species have only non-deep complex MPD and do not display the intraspecific variation in the kinds of dormancy observed in *S. canadensis* and *S. gregaria*. Conspecific variation in levels of MPD has also been found in *Aristolochia macrophylla* (Adams *et al.* 2005), a polycarpic perennial that is ecologically sympatric with the eNA *Sanicula* species. In *A. macrophylla*, a portion of the seed cohort is morphologically dormant (MD), and a portion has non-deep simple MPD. Although *A. macrophylla* embryos had grown to critical length in all seeds, only 65% of them germinated (Adams *et al.* 2005), a phenomenon also observed in *S. gregaria*.

Some seeds of *S. canadensis* and of *S. gregaria* germinated following 12 weeks of cold stratification; however, the germination percentages were significantly lower than those for seeds that received 12 weeks of warm stratification followed by 12 weeks of cold stratification. Seeds of *S. trifoliata* germinated only in the warm followed by cold stratification treatment. Although cumulative germination percentages were relatively low for *S. canadensis* following only cold stratification, and for *S. gregaria* and *S. trifoliata* in both cold and warm + cold treatments, the ungerminated seeds were viable (contained firm, white embryos). This is in accordance with the results of our embryo growth and dormancy break experiment, as well as with the results reported by Hawkins *et al.* (2007) for a study of the germination phenology of these species. In the latter study, germination of some seeds of *S. gregaria* was delayed until the fourth spring following dispersal, and until the sixth spring for seeds of *S. canadensis* and *S. trifoliata*. Based on the results of our current study, the seed physiology underlying the capacity to form a short-lived, persistent soil seed bank differs between the monocarpic perennials and the polycarpic perennial. In *S. canadensis* and *S. trifoliata*, a percentage of the embryos grew to critical length and seeds germinated following cold and/or warm + cold treatment, whereas embryos in

the remaining ungerminated seeds remained viable, but underdeveloped. In contrast, ungerminated seeds of *S. gregaria* remained viable with embryos that had reached critical length.

The low germination percentages observed for *S. gregaria* and *S. trifoliata* seeds in our germination study, as well as those reported for a germination phenology study (Hawkins *et al.* 2007) cannot be explained relative to the known level(s) of MPD. However, because our study showed that ungerminated seeds were viable, it is possible that they had some kind (or combination of kinds) of MPD yet to be described.

Timing of seed dormancy break and germination is particularly significant in that it ensures that seedling emergence occurs when conditions are optimum for seedling survival. In the case of the three *Sanicula* species, seeds are dispersed in late summer to early autumn. For seeds with non-deep complex MPD, if the warm stratification requirement is satisfied prior to the onset of winter, germination will occur the following spring. However, in seeds that are dispersed in autumn, it is possible that germination will be delayed until the second spring following dispersal because the warm stratification requirement may not be satisfied prior to cold stratification during winter. On the other hand, in the portion of the *S. canadensis* and *S. gregaria* seed population that has deep complex MPD, the cold stratification requirement will be satisfied during winter, and seeds will germinate the following spring. This ecological adaptation is well illustrated in seeds of the eNA *Sanicula* spp., when seeds were sown on soil in a non-temperature controlled greenhouse. Seeds sown in early autumn and thus exposed to warm temperatures prior to winter germinated the following spring. However, seed germination was delayed until the second spring in seeds that were sown on soil in early winter (i.e. did not receive a warm stratification period); although some germination (<5%) was observed in seeds of *S. gregaria* (Hawkins *et al.* 2007).

Calviño *et al.* (2007) have shown that *Sanicula* is a monophyletic genus comprised of four clades: eA *S. chinensis*, eNA *Sanicula* spp.., EurA *Sanicula/Hacquetia* spp. (including *S. europaea*) and wNA *Sanicula* spp. The monotypic genus *Petagnaea* (Calviño *et al.* 2008; Kadereit *et al.* 2008), a relict of Tertiary flora endemic to northeastern Sicily, is ancestral to *Sanicula* (De Castro *et al.* 2009). Utilizing this framework, we find that within a seed population, some seeds of *S. canadensis* and *S. gregaria* have the same kind of MPD as that found in the EurA *S. europaea*, and other seeds differ from *S. europaea* in kind of MPD (Vandelook & Van Assche 2008). The kind of seed dormancy in *S. trifoliata* differs completely from that of *S. europaea*, and from some of the seed population of its sympatric sister taxa, *S. canadensis* and *S. gregaria*. Collectively, the pattern of seed dormancy found among the eNA *Sanicula* and between

the eNA–EurA clades does not appear to support stasis of this trait *sensu stricto*.

Recent biogeographic reconstructions suggest a South African origin of Saniculoideae (and Apioideae), but subsequent migration routes remain unclear (Calviño *et al.* 2008; Kadereit *et al.* 2008). Migration of Saniculeae to North America may have occurred either via South Africa → Asia → Europe, or South Africa → Europe → Asia (Calviño *et al.* 2008). Given the current unresolved dispersal routes in combination with knowledge of the kinds of seed dormancy in only four *Sanicula* species, an attempt to reconstruct the evolution of MPD in this subfamily would be purely speculative. However, by comparing the kinds of seed dormancy in *S. canadensis*, *S. gregaria*, *S. trifoliata* and *S. europaea* within the phylogeny provided by Calviño *et al.* (2008), we can formulate hypotheses regarding selective pressures that have influenced the evolution and occurrence of the levels of MPD among these congeners.

First, migration and diversification of *Sanicula* progressed during the mid to late Miocene (Vargas *et al.* 1998; Kadereit *et al.* 2008), an epoch characterized by periods of warming and cooling; therefore, selection may have favored seeds with a warm, cold or warm + cold dormancy breaking requirement, dependent on time and the location of the species. Second, the appearance of deep complex MPD in seeds of *S. europaea* (Vandelook & Van Assche 2008) suggests similar selective pressures in the eNA and EurA *Sanicula* clades. Interestingly, Vandelook and Van Assche (2008) also mention that embryo growth is 'more synchronized' in *S. europaea* seeds placed at high temperatures prior to incubation at a cold temperature (5°C). Therefore, this may be an indication of a relict warm + cold stratification requirement no longer selected for during the dispersal event of *S. europaea*, but selected for in eNA *Sanicula*.

A final point is that the lack of intraspecific variation in the kind of seed dormancy in *S. trifoliata* may indirectly represent other species-specific physiological requirements that are not found in the eNA sister taxa. *Sanicula trifoliata* is considered to be an indicator of rich, mature forests (Phillippe 1978); therefore, an affinity for this type of habitat may have directed historical dispersal events, or impeded the rate of migration relative to that of *S. canadensis* and *S. gregaria*. In turn, selective pressures may have differed among the three congeners, resulting in the variations in present-day kinds of seed dormancy.

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