

Germination ecophysiology of the western North American species *Osmorhiza depauperata* (*Apiaceae*): implications of preadaptation and phylogenetic niche conservatism in seed dormancy evolution

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Abstract

Requirements for dormancy break and embryo growth were determined for seeds of the western North American species, *Osmorhiza depauperata*. Seeds were collected in August 2001 from Sandia Crest (3200 m elevation) and Las Huertas (2300 m), New Mexico (USA). Embryos in fresh seeds were c. 0.6 mm long, and they had to grow to c. 9–10 mm before the radicle emerged from the mericarp. Embryo growth occurred at low temperatures (1 and 5°C), and seeds germinated to high percentages at 1°C during 32 weeks of incubation in the light. No seeds germinated at 5, 15/6, 20/10, 25/15 or 30/15°C during 32 weeks of incubation. Although a 4–18 week warm-temperature (25/15°C) pretreatment increased germination rates at 1°C, it was unnecessary for a high percentage of seeds to germinate. Gibberellic acid (GA₃, 10–1000 mg l⁻¹) did not substitute for cold stratification. Seeds from the low-elevation population contained larger embryos and required less time to germinate than those from the high-elevation population. *O. depauperata* seeds have deep complex morphophysiological dormancy (MPD), which is similar to two other western North American congeners and an Asian congener, but different from two eastern North American congeners. Results from this study suggest that: (1) phylogenetic niche conservatism has played a role in the persistence of deep complex MPD in the three western North American species of *Osmorhiza*; and (2) the stimulatory effect from a warm pretreatment in species needing only cold stratification for dormancy break is a preadaptation that initiated the development of an absolute warm requirement in species needing both warm and cold stratification.

Keywords: *Apiaceae*, morphophysiological seed dormancy, *Osmorhiza depauperata*, phylogenetic niche conservatism, preadaptation

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Introduction

Diverse evolutionary processes have produced a wide array of ecophysiological traits, such as the different types of seed dormancy, observed among species at local, regional and global scales (Baskin and Baskin, 1998; Ackerly *et al.*, 2000). The evolution of these traits is influenced by adaptive processes and by the evolutionary history of a lineage. Divergence of traits between closely related taxa is interpreted as adaptive, and similarity of traits as a phylogenetic constraint. Similarity also could arise by the persistence of ancestral traits that are adaptive in particular environments, namely phylogenetic niche conservatism. However, niche conservatism permits change when taxa inhabit different environments, especially if they are preadapted to them (Lord *et al.*, 1995; Westoby *et al.*, 1995). Unravelling the mechanism of evolution that influences seed dormancy and germination is facilitated by mapping traits on independently derived phylogenies (Feder *et al.*, 2000).

The genus *Osmorhiza* (*Apiaceae*) consists of ten species of perennial woodland herbs: one in Asia, eight in North America (three of which also occur in South America), and one restricted to the central Andes (Constance and Shan, 1948; Lowry and Jones, 1984; Wen *et al.*, 2002). Phylogenetic analyses based on nuclear ribosomal DNA (nrDNA) and chloroplast DNA (cpDNA) found that the Asian species, *O. aristata*, occupied a basal position within the genus and was sister to the New World species, which formed a monophyletic group (Downie *et al.*, 2000; Wen *et al.*, 2002; Yoo *et al.*, 2002). The nrDNA data set showed that the New World species grouped into three clades, forming a trichotomy: eastern North American clade (*O. claytonii*, *O. longistylis*), western North American clade (*O. berteroi*, *O. brachypoda*, *O. depauperata*, *O. mexicana*, *O. occidentalis*, *O. purpurea*), and central Andean clade (*O. glabrata*) (Wen *et al.*, 2002). Utilizing cpDNA, relationships among the

New World species were mostly unresolved, and discordance between the two molecular phylogenies was unexplained (Yoo *et al.*, 2002).

Morphophysiological dormancy (MPD), which is the most complex kind of dormancy in nature, has been found in seeds of all *Osmorhiza* species studied so far (Baskin and Baskin, 1984, 1991; Baskin *et al.*, 1995; Walck *et al.*, 2002). Seeds with this type of dormancy have tiny embryos and much endosperm. The embryos have distinguishable cotyledons and a radicle, and are described as underdeveloped, since they must grow to a critical length before radicle emergence occurs. Warm ($\geq 15^{\circ}\text{C}$) and/or cold ($0\text{--}10^{\circ}\text{C}$) stratification overcomes dormancy that is present when the seed is freshly matured. Embryo dormancy is broken and then growth occurs in some species (e.g. Baskin and Baskin, 1984), whereas embryo dormancy and growth take place at the same time in other species (e.g. Baskin *et al.*, 1995). In general, eight types of MPD have been distinguished on the basis of: (1) temperatures required to break dormancy and stimulate embryo growth; and (2) whether gibberellic acid (GA_3) overcomes dormancy (Nikolaeva, 1977; Baskin and Baskin, 1998). Of the eight types of MPD, two have been reported in *Osmorhiza*.

Seeds of the eastern North American species, *O. claytonii* (Baskin and Baskin, 1991) and *O. longistylis* (Baskin and Baskin, 1984), have non-deep complex MPD: they require warm stratification followed by cold stratification for dormancy break, their embryos grow at cold temperatures, and GA_3 substitutes for warm stratification. In contrast, seeds collected from western North American populations of *O. berteroi* and *O. occidentalis* (Baskin *et al.*, 1995) and the Asian species, *O. aristata* (Walck *et al.*, 2002), have deep complex MPD: they need cold stratification for dormancy break and embryo growth, and GA_3 does not overcome dormancy.

The focus of the present study was to investigate the seed germination ecophysiology of *O. depauperata* Phil. This species is found primarily from the south-western portion of the Mackenzie District (Canada), south to north-eastern California and southern Arizona and New Mexico (USA). Disjunct ranges occur in southern Alaska, north central North America, the Great Lakes region, north-eastern North America and southern South America (Lowry and Jones, 1984). The goal of our research was to determine whether *O. depauperata* seeds have MPD and, if so, which one of the eight types. Specifically, we investigated: (1) the temperature requirements for dormancy break, germination and embryo growth; and (2) the effects of GA_3 on dormancy break and germination. We then compared the germination characteristics of *O. depauperata* with its previously studied congeners in a phylogenetic context.

Materials and methods

Seed collection

Freshly matured seeds (mericarps) of *O. depauperata* were collected on 12 August 2001 from two sites, differing in elevation, in the Sandia Mountains of New Mexico (USA): Sandia Crest in Bernalillo County (c. 3200 m above mean sea level) and Las Huertas in Sandoval County (c. 2300 m). The vegetation of the Sandia Crest site was subalpine (spruce-fir) forest, whereas that of the Las Huertas site was riparian woodland. Seeds were kept dry between collection and the initiation of laboratory studies. About 32% of the seeds collected at Sandia Crest were devoid of embryos even though the endosperm was intact, whereas nearly all of those collected at Las Huertas contained embryos.

Temperature and light schemes during experiments

Laboratory experiments were conducted in four temperature- and light-controlled incubators and in two refrigerators. The incubators were set at 12 h/12 h daily alternating thermoperiods of 15/6, 20/10, 25/15 and 30/15°C. The daily photoperiod in the incubators was 14 h, lasting from 1 h before the beginning of the high-temperature period to 1 h after the beginning of the low-temperature period. One refrigerator was set at a constant temperature of 1°C, and the other one at 5°C; each refrigerator was equipped with lights and a time clock, with seeds being exposed to light for 14 h each day. Cool white, 20 W fluorescent tubes, which produced a photosynthetic photon flux density (400–700 nm) at seed level of c. 50–70 $\mu\text{mol m}^{-2} \text{s}^{-1}$, were used as the light source in the incubators; 15 W tubes producing c. 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were used in the refrigerators.

Temperatures simulated mean maximum and minimum monthly air temperatures near Cline's Corner, New Mexico (2160 m) and Santa Fe, New Mexico (2130 m): January, February and December, 1°C; March and November, 5°C; April and October, 15/6°C; May and September, 20/10°C; June and August, 25/15°C; and July, 30/15°C (Weather Channel, 2003). Both Cline's Corner and Santa Fe are located approximately 70 km south-east and north-east, respectively, of the collection sites. The 25/15°C thermoperiod was used for warm stratification, and 1 or 5°C for cold stratification, because they are effective in seeds of species that require either warm and/or cold temperatures, respectively, for dormancy release and embryo growth (Stokes, 1965; Baskin and Baskin, 1998).

Requirements for dormancy break and germination

Seeds were placed in 6.0-cm-diameter plastic Petri dishes on white quartz sand that had been moistened

previously with distilled water. The dishes were wrapped with plastic film to retard water loss during incubation and stratification. Three replications of 25 seeds/dish were used in each treatment. The criterion for germination was emergence of the radicle. Viability of ungerminated seeds was determined by pinching them with forceps under a dissecting microscope to see if they contained firm, white embryos (viable) or soft, light-brown ones (non-viable). Tetrazolium tests confirmed that white embryos were viable and brown ones were not.

Two experiments were performed to examine germination responses to temperature regimes. The first experiment was started on 24 August 2001 and the second experiment on 13 May 2002, after the results from the first one were evaluated. In the first experiment, seeds collected from Sandia Crest were kept in light at 1, 5, 15/6, 20/10, 25/15 and 30/15°C for 32 weeks, and examined for germination at 2-week intervals. In the second experiment, seeds from Sandia Crest and Las Huertas were placed for 4, 8, 12 and 16 weeks at 25/15°C in light, followed by 36, 32, 28 and 24 weeks, respectively, at 1°C in light. Seeds were monitored for germination every 2 weeks for the entire 40-week period. In addition, seeds that acted as controls were also maintained at 1 and 25/15°C in light for 40 weeks and examined for germination at 2-week intervals. Seedlings in both experiments were counted and removed from the Petri dishes, and water was added to the dishes as needed to keep the seeds moist.

Requirements for embryo growth

Studies on embryo growth were started on 24 August 2001 for seeds collected at Sandia Crest. Seeds were placed in 10.0-cm-diameter plastic Petri dishes on two sheets of Whatman number 1 filter paper, moistened with distilled water, and incubated in light at 5 or 25/15°C. The dishes were wrapped with plastic film to reduce water loss during the experiment, and additional water was added if needed. One dish of 25 seeds was used each time measurements were made. Embryos were excised from seeds with a razor blade, and their lengths measured under a dissecting microscope equipped with a micrometer. Measurements of embryos were made following 2, 4, 6, 8 and 12 weeks of incubation at each temperature regime.

In addition, embryos were removed from seeds and measured at the end of the first germination experiment (see above), i.e. following 32 weeks of incubation at 1, 5 and 25/15°C. Ten ungerminated (viable) seeds were randomly selected from one Petri dish incubated at 5°C and from one dish at 25/15°C. Embryos in all ungerminated seeds in the three dishes at 1°C were examined.

Embryos were excised (and subsequently measured) on 24 August 2001 from fresh seeds

collected at Sandia Crest, and on 22 April 2004 from seeds stored dry in glass jars under laboratory conditions, collected at Sandia Crest and Las Huertas. The fresh and dry-stored seeds were allowed to imbibe water at room temperature for 24 h before embryo measurements were obtained. The length of seeds (mericarps without the caudate appendages; cf. Lowry and Jones, 1984) was recorded for those collected at Sandia Crest and Las Huertas ($N = 25$).

Effects of GA₃ on dormancy break and germination

Seeds used in the GA₃ experiment were collected from Sandia Crest, and this experiment started on 10 May 2002. Twenty-five seeds were placed on two sheets of Whatman number 1 filter paper in 10.0-cm-diameter glass Petri dishes. Three replications (Petri dishes) were used per treatment. The paper was moistened with either distilled water (control) or a solution of 10, 100 or 1000 mg l⁻¹ of GA₃ (GA₃, potassium salt) dissolved in distilled water. Dishes were wrapped with plastic film to reduce water loss. Seeds were incubated in light on distilled water or the GA₃ solutions at 1 or 25/15°C for 40 weeks. Germination was monitored at 4-week intervals. To test whether GA₃ could substitute for warm or cold stratification, a temperature of 1°C was used, since it is too low to be effective for warm stratification, and 25/15°C was used because it is too high for cold stratification (Stokes, 1965). The criterion for germination was emergence of the radicle, and viability was determined as described previously (see above).

Statistical analyses

Germination data were transformed to percentages on the basis of the number of viable seeds. Means and standard errors were calculated for germination percentages, embryo lengths and number of days until the start of germination and until maximum germination. Means were compared by analyses of variance (ANOVAs) followed by *t*-tests or protected least significant difference tests (PLSDs, $P = 0.05$) (SPSS, 2000). Two-way ANOVAs were used to examine the effects and interaction of temperature regime and length of incubation on germination percentages (first experiment) and on embryo growth, and the effects and interaction of length of warm-temperature pretreatment and elevation on timing and percentages of germination (second experiment). A three-way ANOVA tested the effects and interactions of thermoperiod, incubation length and concentrations on germination of seeds in the GA₃ experiment. An one-way ANOVA examined differences of embryo lengths among fresh seeds measured in 2001 (from Sandia Crest) and dry-stored

seeds measured in 2004 (from Sandia Crest and Las Huertas).

Germination percentages were arcsine square-root transformed for statistical analyses. Transformations were unsuccessful in correcting for heteroscedasticity for the following parameters: embryo growth and timing for start of germination and for maximum germination. However, ANOVA results are robust, especially when sample sizes are equal (Neter *et al.*, 1990). Variances among embryo lengths for fresh and dry-stored seeds were homogeneous.

Results

Requirements for dormancy break and germination

In the first experiment, temperature regime, length of incubation and their interaction had significant effects on germination of seeds collected from Sandia Crest ($P < 0.001$). Seeds germinated to 82% at 1°C during 32 weeks of incubation, whereas none germinated at 5, 15/6, 20/10, 25/15 or 30/15°C (Fig. 1).

In the second experiment, the length of warm-temperature pretreatment and elevation had significant effects on the number of days until the start of germination, number of days for maximum germination and maximum germination percentages ($P \leq 0.013$). The germination response with regard to the number of days until start of germination and until maximum germination was similar between the two elevations (interaction, $P \geq 0.074$), but it was dissimilar for maximum germination percentages ($P < 0.001$). Start of germination for seeds from the high-

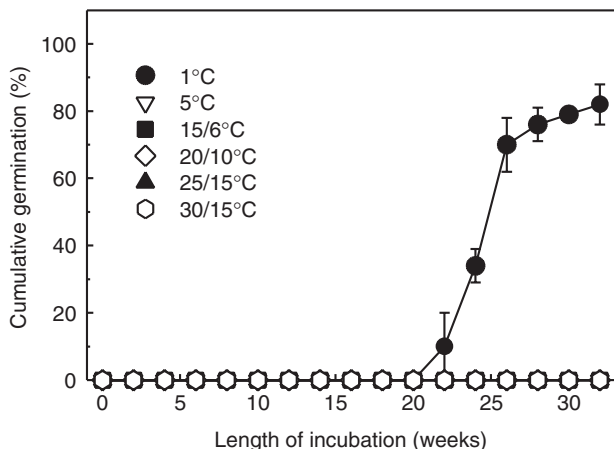


Figure 1. Cumulative germination percentages (mean \pm SE; SE shown if $\geq 5\%$) of *Osmorhiza depauperata* seeds incubated in light at 1, 5, 15/6, 20/10, 25/15 and 30/15°C for 32 weeks. Seeds were collected at Sandia Crest, New Mexico in August 2001. Germination of seeds at 1°C differed significantly from that of seeds at the other temperature regimes at week 32 of incubation (one-way ANOVA, $P < 0.001$; PLSD, $P = 0.05$).

elevation population was significantly delayed by 6–9 d compared to the low-elevation population, regardless of length of warm pretreatment, but length of time until maximum germination was about the same (Fig. 2, Table 1). Maximum germination percentages did not differ between the two elevations, except for seeds given a 16-week pretreatment, in which the high-elevation population germinated significantly less than the low-elevation population. Times for start of germination and for maximum germination were significantly less, by 3–6 and 5–12 d, respectively, for seeds given warm-temperature pretreatment than for those given none, in both high- and low-elevation populations. Germination percentages did not differ significantly among any of the warm pretreatments (i.e. 0–16 weeks) for the high- and low-elevation populations, except for 16 weeks' pretreatment in the high-elevation population.

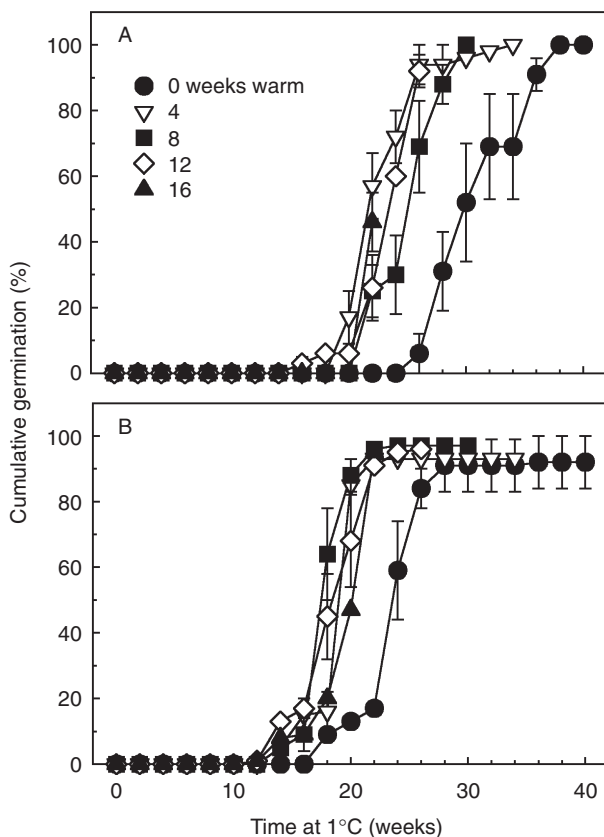


Figure 2. Cumulative germination percentages (mean \pm SE; SE shown if $\geq 5\%$) of *Osmorhiza depauperata* seeds given 0, 4, 8, 12 and 16 weeks of warm stratification in light at 25/15°C and then placed in light at 1°C for 40, 36, 32, 28 and 24 weeks, respectively. Control seeds maintained at 25/15°C did not germinate during the 40-week incubation period. Seeds were collected in August 2001 from New Mexico either at (A) Sandia Crest (3200 m above mean sea level) or (B) Las Huertas (2300 m).

Table 1. Number of days until start of germination and until maximum germination and percentage of germination for seeds of *Osmorhiza depauperata* given 0–16 weeks of warm stratification at 25/15°C in light before being transferred to 1°C in light for the remainder of the 40-week test period

Parameter	Warm stratification pretreatment (weeks)				
	0	4	8	12	16
Start of germination (days)					
High elevation	25 ± 1 ^{Aa}	21 ± 1 ^{Abc}	22 ± 0 ^{Ab}	19 ± 2 ^{Ac}	22 ± 0 ^{Ab}
Low elevation	18 ± 0 ^{Ba}	15 ± 1 ^{Bb}	15 ± 1 ^{Bb}	13 ± 1 ^{Bb}	13 ± 1 ^{Bb}
Maximum germination (days)					
High elevation	36 ± 2 ^{Aa}	31 ± 3 ^{Ab}	31 ± 1 ^{Ab}	28 ± 0 ^{Abc}	24 ± 0 ^{Ac}
Low elevation	31 ± 2 ^{Aa}	23 ± 1 ^{Ab}	25 ± 1 ^{Bb}	26 ± 1 ^{Ab}	24 ± 0 ^{Ab}
Maximum germination (%)					
High elevation	100 ± 0 ^{Aa}	100 ± 0 ^{Aa}	100 ± 0 ^{Aa}	92 ± 4 ^{Aa}	46 ± 9 ^{Ab}
Low elevation	91 ± 8 ^{Aa}	93 ± 3 ^{Aa}	97 ± 1 ^{Aa}	96 ± 2 ^{Aa}	95 ± 1 ^{Ba}

Seeds were collected in August 2001 from two elevations in New Mexico: high (3200 m; Sandia Crest) or low (2300 m; Las Huertas). Values are means ± SE. For each parameter, means with different upper-case letters within columns or with different lower-case letters within rows are significantly different (*t*-test or PLSD, $P \leq 0.05$). Seeds kept at 25/15°C during the entire 40-week incubation period did not germinate, and were not included in the statistical analyses.

Requirements for embryo growth

The mean (± SE) lengths of freshly matured seeds collected from Sandia Crest and Las Huertas were 9.62 ± 0.24 and 9.58 ± 0.20 mm, respectively. Embryos from Sandia Crest seeds (0.58 ± 0.02 and 0.44 ± 0.01 mm long measured in 2001 and 2004, respectively) were significantly smaller than those from Las Huertas seeds (0.64 ± 0.01 mm) ($P < 0.001$). Thus, embryos had to grow to approximately 9–10 mm before radicles would emerge from the seeds. Thermoperiod, length of incubation and their interaction had significant effects on embryo growth ($P < 0.001$). Embryos of seeds collected at Sandia Crest grew by 637% at 5°C during 12 weeks of incubation, whereas they grew by only 51% at 25/15°C (Fig. 3).

Eighty-two percent of the embryos in seeds incubated at 1°C for 32 weeks elongated to their critical length and their radicles emerged (they were classified as germinated) (Fig. 1). Embryos in all ungerminated (viable) seeds ($N = 7$) at the end of the 32-week incubation period at 1°C were 0.85 ± 0.15 mm long. At 5 and 25/15°C, embryos were 5.48 ± 0.43 and 1.41 ± 0.10 mm long, respectively, following 32 weeks of incubation.

Effects of GA₃ on dormancy break and germination

Length of incubation, temperature regime, concentration and all two- and three-way interactions had significant effects on germination of seeds ($P < 0.001$). No seeds germinated at 25/15°C during 40 weeks of incubation over the range of GA₃ concentrations, whereas they germinated to 38–73% at 1°C ($P = 0.190$) (Fig. 4).

Discussion

Seeds of *O. depauperata* contained linear embryos (cf. Martin, 1946) that occupied only 6% of the total seed length and were dormant at the time of dispersal. Dormancy break and embryo growth took place at the same time during exposure to low temperatures, with 1°C being optimum, and germination occurred at 1°C over a relatively long period of incubation in light (Figs 1–3). Seeds never gained the ability to germinate at 5, 15/6, 20/10, 25/15 or 30/15°C during 32 weeks of incubation. A combination of dormancy classes is

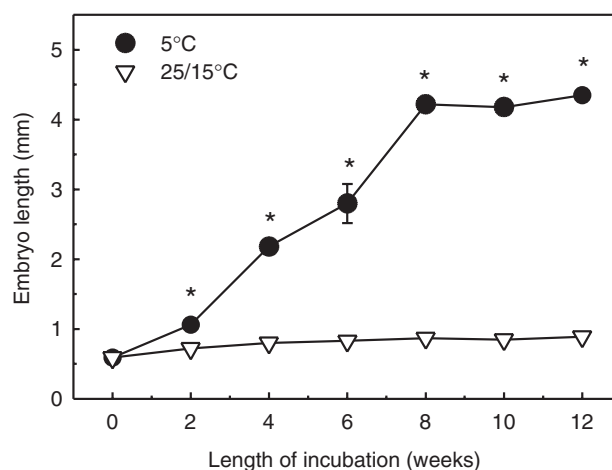


Figure 3. Embryo growth (mean length ± SE; SE shown if ≥ 0.2 mm) of *Osmorhiza depauperata* seeds collected at Sandia Crest, New Mexico in August 2001 and incubated in light at 5 or 25/15°C for 12 weeks. An asterisk indicates a significant difference between the two temperature regimes at a particular time period (*t*-test, $P < 0.001$); no asterisk specifies no significant difference.

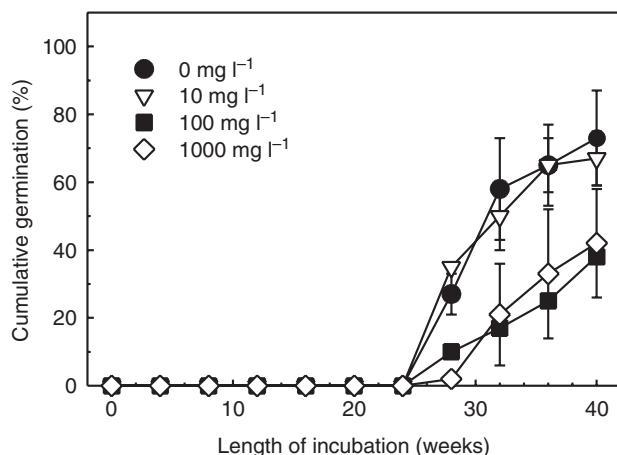


Figure 4. Cumulative germination percentages (mean \pm SE; SE shown if $\geq 5\%$) of *Osmorhiza depauperata* seeds collected at Sandia Crest, New Mexico in August 2001 and incubated in light at 1°C for 40 weeks on distilled water (0 mg l⁻¹ GA₃) or on three concentrations (10–1000 mg l⁻¹) of GA₃. The final germination percentages did not differ significantly among the control and three GA₃ solutions (one-way ANOVA, $P = 0.190$). No seeds germinated in light at 25/15°C during 40 weeks of incubation on distilled water or on any concentration of GA₃.

present in *O. depauperata* seeds. The embryos of this species are underdeveloped at maturity and must elongate before radicle emergence can occur (morphological dormancy). Moreover, cold temperatures are required to overcome the physiological block present in the embryos of seeds when freshly ripe. Thus, seeds of *O. depauperata* have morpho-physiological dormancy (MPD).

Classification of the type of MPD first requires an understanding of environmental conditions necessary for overcoming morphological dormancy: seeds with simple types of MPD require high temperatures ($\geq 15^\circ\text{C}$) for embryo growth, and those with complex types of MPD need low temperatures (0–10°C) (Nikolaeva, 1977; Baskin and Baskin, 1998). Because embryo growth in *O. depauperata* seeds occurred at low temperatures, they must have one of the three types of complex MPD. Division of complex MPD depends on the temperature regime and the effects of GA₃ for overcoming physiological dormancy. Non-deep complex MPD is broken by warm + cold temperatures, whereas intermediate and deep complex MPDs need only cold temperatures. GA₃ substitutes for warm- but not cold stratification in seeds with non-deep complex MPD, and it overcomes dormancy in seeds with intermediate complex MPD, but not in those with deep complex MPD (Nikolaeva, 1977; Baskin and Baskin, 1998). Seeds of *O. depauperata* required only cold stratification for dormancy break, and GA₃ did not substitute for it

(Fig. 4). The type of MPD in *O. depauperata* seeds would be classified as deep complex.

A warm-temperature pretreatment at 25/15°C given prior to placing seeds of *O. depauperata* at 1°C was not necessary for a high percentage of them to germinate (Fig. 2, Table 1). However, germination at 1°C was faster for seeds given 4–16 weeks of warm temperatures than for those exposed to none. A similar situation was found in seeds of the western North American species *O. berteroi* (*O. chilensis*), *O. occidentalis* and *Erythronium grandiflorum* (Baskin *et al.*, 1995). In contrast, seeds of the eastern North American *O. claytonii*, *O. longistylis* and *E. albidum* required at least 2–6 weeks of warm pretreatment for a high percentage of them to germinate at low temperatures. Warm temperatures that *O. claytonii* and *O. longistylis* seeds experienced while attached to dead upright shoots were ineffective in breaking dormancy (Baskin and Baskin, 1984, 1985, 1991). Apparently, seeds of *Osmorhiza* must be on the soil (and fully imbibed) during summer and early autumn for an absolute warm requirement to be fulfilled. Most of the plants in both low- and high-elevation populations of *O. depauperata* had not dispersed their seeds by mid-August (J. Walck, personal observation). Seeds of this species dispersed after August would receive a short period of warm temperatures, probably negating an absolute requirement for warm pretreatment in dormancy break.

The stimulatory effect of a warm-temperature pretreatment on germination of seeds from western North American *Osmorhiza* and *Erythronium* species was viewed by Baskin *et al.* (1995) as an ancestral condition that was partially retained. This idea was based on the premise that non-deep complex MPD (warm + cold stratification), found in seeds of eastern North American species of these two genera (Baskin and Baskin, 1984, 1985, 1991), was ancestral to deep complex MPD (cold stratification only) in the western North American congeners (Baskin *et al.*, 1995). However, Wen *et al.* (2002) showed that deep complex MPD was ancestral to non-deep complex MPD in *Osmorhiza*. Recently, Allen *et al.* (2003) found that the eastern North American and Eurasian *Erythronium* clades separated into two lineages following divergence from the western North American clade. Three eastern North American species of *Erythronium* (Baskin and Baskin, 1985, 1998) and an Eurasian species (Kondo *et al.*, 2002) require warm + cold temperatures to overcome dormancy, whereas a western North American taxon needs only cold temperatures. Like *Osmorhiza*, it appears that the requirement for warm + cold stratification is a derived condition in *Erythronium*. The stimulatory effect from a warm pretreatment in *Osmorhiza* and *Erythronium* species that only need cold stratification to overcome dormancy is perhaps best viewed as a

preadaptation (cf. Armbruster, 1997; Motychak *et al.*, 1999). That is, it initiated the development of an absolute warm requirement being essential prior to cold stratification in the other species of these two genera that require warm + cold conditions for dormancy break.

The type of MPD found in seeds of *O. depauperata* from low- versus high-elevation populations was identical, but germination rates were faster in the low-elevation population than in the high-elevation population (Table 1). Baskin *et al.* (1995) found that the type of MPD, deep complex, was the same for low-elevation (1740 m) and high-elevation (3250 m) populations of *E. grandiflorum*. However, seeds from low elevation required 16 weeks for >80% germination at 5°C, but those from high elevation needed 26 weeks. On the other hand, the germination characteristics observed between elevations of *O. depauperata* might be due to differences in initial embryo lengths and not dormancy level *per se*, or to a combination of both factors. A similar correlation between embryo length and germination has been reported in *O. aristata* (Walck *et al.*, 2002) and *Fraxinus excelsior* (Nikolaeva, 1999). In seeds from both of these species, embryo length decreased and dormancy (or germination rate) increased from southern to northern latitudes. Nevertheless, differences in embryo length and/or dormancy level between elevations of *O. depauperata* appear to be adaptive to local site conditions.

Seeds of *O. depauperata* are prevented from germinating after dispersal in late summer and autumn because they are dormant. Dormancy break and embryo growth occurred under simulated low temperatures of winter. Germination would begin approximately 3–5 months following dispersal, even if seeds received enough warm temperatures to stimulate germination slightly early. In field studies, seeds of *O. occidentalis* germinated to high percentages during the first spring following dispersal, while under snow at an elevation of 2400 m (Baskin *et al.*, 1995). Since the dormancy break and germination characteristics of *O. depauperata* are similar to those of *O. occidentalis*, germination in the former species should occur in nature when temperatures are very low, and perhaps under deep snow at 1°C. Meyer *et al.* (1995) suggested that the function of slow dormancy break at low temperature allows germination to occur in late winter, before snowmelt, to take advantage of the favourable moisture regime of early spring for seedling establishment.

The most favourable temperature for dormancy break in *O. depauperata* seeds was 1°C, and not 5°C, which is the temperature usually judged as optimum for most species (Stokes, 1965; Baskin and Baskin, 1998). Similarly, Baskin *et al.* (1995) reported that the optimum temperature for dormancy break and

germination of *O. occidentalis* seeds was 1°C. Moreover, they found that seeds of *O. berteroi* germinated to higher percentages at an alternating temperature of 5/1°C than at constant 5°C. In *Thalictrum mirabile*, a species with non-deep simple MPD, seeds germinated to 93–95% in light at 15/6, 20/10 and 25/15°C following 12 weeks of cold stratification at 1°C, but to only 3–25% following cold stratification at 5°C (Walck *et al.*, 1999).

The occurrence of deep complex MPD in populations of *O. depauperata*, *O. berteroi* and *O. occidentalis* is a symplesiomorphic condition. These western North American species and the Asian species (*O. aristata*) grow in habitats (cf. Eyre, 1968; Walter, 1985; Constance, 1993; Welsh *et al.*, 1993) for which there is a selective advantage to delay germination until spring, i.e. seedlings emerging in autumn would have insufficient time to form a perennating structure necessary for persistence over winter. Persistence of ancestral traits within lineages may be due either to a limit in design (phylogenetic constraint), such as a lack of genetic variation, or to continued success in a particular environment (phylogenetic niche conservatism) (McKittrick 1993; Ackerly and Donoghue, 1995; Patterson and Givnish, 2002). Apparently, an intrinsic barrier (constraint) to evolutionary change is not present in *Osmorhiza*: different types of MPD, as well as elevational and latitudinal variation in seed dormancy/germination, are present in the genus. We suggest that phylogenetic niche conservatism has played an important role in the persistence of deep complex MPD in the three western North American species of *Osmorhiza*.

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