

SEED GERMINATION ECOPHYSIOLOGY OF THE ASIAN SPECIES *OSMORHIZA ARISTATA* (APIACEAE): COMPARISON WITH ITS NORTH AMERICAN CONGENERS AND IMPLICATIONS FOR EVOLUTION OF TYPES OF DORMANCY¹

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Osmorhiza aristata is an herbaceous perennial that grows primarily in Japan, through southern China, to the Himalayas. It closely resembles the eastern North American species *O. claytonii* and *O. longistylis*, and, together, the three species are an example of the well-known North American–Asian pattern of disjunction. Requirements for dormancy break and embryo growth were determined for seeds of *O. aristata* collected in Japan during the summers of 1998–2000. Embryos in fresh seeds were ca. 0.5 mm long, and they had to grow to 9 mm before the radicle emerged from the mericarp. Embryo growth and germination occurred during cold stratification at 5°C, the optimum temperature for germination. Gibberellic acid did not substitute for cold stratification. Thus, *O. aristata* seeds have deep complex morphophysiological dormancy (MPD). The type of MPD in *O. aristata* is similar to that in two western North American congeners but different from that in eastern North American congeners (nondeep complex MPD). Mapping the types of MPD onto a phylogeny of the genus suggests that nondeep complex MPD is derived from deep complex MPD. Although eastern North American–Asian disjuncts often exhibit morphological stasis, the taxa may differ greatly in physiological traits, such as seed dormancy.

Key words: Apiaceae; Asia; disjunct taxa; evolution; morphological stasis; morphophysiological dormancy; North America; *Osmorhiza*; seed dormancy.

One of the classic patterns of biogeographic disjunction occurs in the temperate region of the Northern Hemisphere. Floristic similarities exist between two or more of the following areas: eastern North America, western North America, eastern Asia, western Asia, and southeastern Europe. The most notable disjunction pattern, and the one that has received much attention, is between eastern North America and eastern Asia (Graham, 1972; Thorne, 1972; Xiang, Soltis, and Soltis, 1998; Wen, 1999). Approximately 65 genera of seed plants exhibit the eastern North American–eastern Asian disjunction (Li, 1952; Hong, 1993; Wen, 1999). Phylogenetic, molecular, geologic, and fossil evidence support the belief that the disjunction is a relict of a mid-Tertiary temperate forest that became fragmented as a result of climatic and geologic changes. Estimates of divergence times indicate that the disjunction pattern was established primarily during the Miocene and Pliocene (Graham, 1993; Wen, 1999; Xiang et al., 2000).

The question of how plants that are similar morphologically come to occupy geographically distant areas has fascinated botanists and biogeographers since the Linnaean era (Boufford and Spongberg, 1983; Lee et al., 1996). Early workers treated many eastern North American–eastern Asian disjuncts as con-

specific, but most were later recognized as intercontinental sister pairs. Recent phylogenetic studies, however, indicate that rarely are disjunct pairs of species each other's closest relatives. Morphological stasis appears to be common among these congeners (Wen, 1999). Although the systematics and biogeography of eastern North American–eastern Asian disjuncts have been relatively well studied (see Wen, 1999), many other aspects of their biology remain unknown (Boufford, 1998). For example, how similar are the ecophysiological traits of congeners in eastern North America and eastern Asia? Unfortunately, very little comparative research has been conducted on such traits (Terui and Okagami, 1993; Wen, Jansen, and Kilgore, 1996; see also Baskin and Baskin, 1998), and only one within a phylogenetic framework (Wen, Jansen, and Kilgore, 1996). However, results of this limited set of studies suggest that, as with morphology, stasis has occurred in physiological traits, such as seed dormancy (Terui and Okagami, 1993; Baskin and Baskin, 1998) and thermogenesis (Wen, Jansen, and Kilgore, 1996).

Mechanisms that regulate seed germination through dormancy are important aspects of a species' life history and ecology. Germination occurs when plant growth, development, and reproduction are optimal. Dormancy prevents germination when seedlings would be unlikely to survive (Matthews, 1976; Allen and Meyer, 1998; Baskin and Baskin, 1998). Freshly matured seeds of some species have embryos that are very small relative to the size of the seed, and they have much endosperm. Although these tiny embryos have distinguishable cotyledons and radicle, they are underdeveloped since they must grow to a critical length before the radicle emerges from the seed. If the underdeveloped embryos are not dormant at

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maturity, the seeds have morphological dormancy (MD) and require no dormancy-breaking treatment for germination. If the underdeveloped embryos are dormant at maturity, the seeds have morphophysiological dormancy (MPD), which must be broken with warm ($\geq 15^{\circ}\text{C}$) and/or cold (0° – 10°C) stratification before the seeds can germinate (Nikolaeva, 1969, 1977; Baskin, Meyer, and Baskin, 1995; Baskin and Baskin, 1998). Embryo growth and the loss of dormancy may occur at the same time (e.g., Baskin, Chester, and Baskin, 1992), or embryo growth may be delayed until dormancy is broken (e.g., Baskin and Baskin, 1990). Nikolaeva (1977) initially described six types of MPD, and Baskin and Baskin (1990, 1991) subsequently described two others. The eight types of MPD are distinguished on the basis of (1) temperatures required to break dormancy and stimulate embryo growth and (2) whether gibberellic acid overcomes dormancy (Baskin and Baskin, 1998).

Seeds of numerous herbaceous species that grow in the deciduous forests of eastern North America have MPD, and seven of the eight types of MPD have been identified in them (Baskin and Baskin, 1988, 1998; Baskin, Meyer, and Baskin, 1995). In contrast, only one species from this habitat has been reported to have MD (Baskin and Baskin, 1986). Many of these eastern North American deciduous-forest herbs belong to genera with disjunct distributions in eastern Asia. If the same type of dormancy-breaking mechanism is present in the eastern North American–Asian congeners, then, apparently, it was present in the Tertiary before disjunction occurred. But if the disjunct congeners have different types of dormancy-breaking mechanisms, then the differences may have been present in the Tertiary or may have developed since then (Baskin, Meyer, and Baskin, 1995).

Osmorhiza (Apiaceae) is a genus of perennial woodland herbs whose distribution in the Northern Hemisphere is disjunct between eastern North America, western North America, and Asia (Constance and Shan, 1948; Lowry and Jones, 1984). Moreover, the types of MPD in seeds differ between eastern and western North American species. Seeds of *O. claytonii* (Michx.) C.B. Clarke and *O. longistylis* (Torr.) DC. in eastern North America have nondeep complex MPD: they require warm stratification followed by cold stratification for dormancy break, their embryos grow at cold temperatures, and gibberellic acid (GA_3) substitutes for warm stratification (Baskin and Baskin, 1984, 1991). In contrast, seeds collected from western North American populations of *O. berteroi* DC. and *O. occidentalis* (Nutt.) Torr. have deep complex MPD: they require cold stratification for dormancy break and embryo growth, and GA_3 does not overcome dormancy (Baskin, Meyer, and Baskin, 1995).

Three species of *Osmorhiza* have an eastern North American–Asian disjunction pattern. *Osmorhiza aristata* (Thunb.) Rydb., which is the focus of the present study, grows in moist deciduous woods from Sakhalin and the lower Amur basin (Russia), through Japan, Korea, Taiwan, and central and southern China, to the Himalayas of Bhutan, Nepal, India, and Pakistan. There are also disjunct populations in south-central and southwestern Russia (Constance and Shan, 1948; Lowry and Jones, 1984). Two monographs on the genus, by Constance and Shan (1948) and Lowry and Jones (1984), placed *O. aristata* in the same section as its eastern North American congeners, *O. claytonii* and *O. longistylis*. The three species exhibit remarkable morphological similarities and have been regarded as conspecific by several authors (Gray, 1859; Clarke, 1879; Kuntze, 1891; Boivin, 1968).

A phylogenetic analysis based on sequences of the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA found that *O. aristata* occupied a basal position within the genus and had a high level of sequence divergence from its congeners (Downie, Katz-Downie, and Spalik, 2000; Wen et al., in press). The antiquity of *O. aristata* and the high level of morphological similarity between it and *O. claytonii* and *O. longistylis* are consistent with morphological stasis. The New World species are monophyletic, with three clades forming a trichotomy: eastern North American clade, including *O. claytonii* and *O. longistylis*; western North American clade, including *O. berteroi*, *O. occidentalis*, and four other species; and central Andean clade, with one species (Wen et al., in press).

The goal of our research was to examine the germination ecophysiology of *O. aristata* and determine if seeds have MD or MPD and, if MPD, which one of the eight types. Specifically, we investigated (1) the light and temperature requirements for dormancy break and embryo growth, (2) the effects of GA_3 on dormancy break and embryo growth, and (3) the phenology of germination. With the results of those investigations, we then compared the germination of *O. aristata* with its North American congeners and examined two evolutionary questions: Have *O. aristata*, *O. claytonii*, and *O. longistylis* experienced stasis in an ecophysiological trait, such as seed dormancy, as they have in morphology? And how did the different types of dormancy found in the genus—and in plants in general—evolve? Wen et al. (in press) mapped the types of dormancy found in the genus on a phylogenetic tree. Knowledge of the seed biology of *O. aristata*, however, formed the basis for understanding the direction of evolutionary change (plesiomorphic vs. derived) for the dormancy trait in the genus.

MATERIALS AND METHODS

General—Freshly matured seeds (mericarps) of *O. aristata* were collected near the following locations in Japan: Matsuyama (Ehime Prefecture, Shikoku) on 24 July 1998; Mount Hakkoda (Aomori Prefecture, Honshu; plants sometimes recognized as *O. aristata* var. *montana* Makino) on 27 August 1998; Sendai (Miyagi Prefecture, Honshu) on 16 June 1999 and 26–29 June 1999; and Siroishi (Miyagi Prefecture, Honshu) on 20 July 1999. Collections of seeds were mailed to the University of Kentucky, Lexington, Kentucky, USA, where experiments were conducted. Seeds were kept dry during mailing and in the laboratory before studies were initiated. Experiments were started on 30 July 1998, 19 October 1998, and 4 September 1999 for seeds collected in Ehime, Aomori, and Miyagi Prefectures, respectively.

Laboratory experiments were conducted in four temperature- and light-controlled incubators and in a refrigerator. The incubators were set at 12 h/12 h daily alternating thermoperiods of $15^{\circ}/6^{\circ}$, $20^{\circ}/10^{\circ}$, $25^{\circ}/15^{\circ}$, and $30^{\circ}/15^{\circ}\text{C}$. 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. The refrigerator was set at a constant temperature of 5°C and was equipped with a light-and-time clock; seeds were exposed to light for 14 h each day. Cool white fluorescent tubes, which produced a photosynthetic photon flux density (400–700 nm) at seed level of ca. $40 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, were used as the light source: 20-W tubes in the incubators and 15-W in the refrigerator.

Germination tests on seeds collected in Ehime Prefecture were done at 5° , $15^{\circ}/6^{\circ}$, and $30^{\circ}/15^{\circ}\text{C}$. These temperatures simulate mean maximum and minimum seasonal air temperatures near Matsuyama: spring and autumn, $15^{\circ}/6^{\circ}$; summer, $30^{\circ}/15^{\circ}$; and winter, 5°C (Peterson and Vose, 1997). Germination tests on seeds collected in Miyagi Prefecture were done at 5° , $15^{\circ}/6^{\circ}$, $20^{\circ}/10^{\circ}$, $25^{\circ}/15^{\circ}$, and $30^{\circ}/15^{\circ}\text{C}$. These temperatures simulate mean maximum and minimum monthly air temperatures near Sendai: April and November, $15^{\circ}/6^{\circ}$;

May and October, 20°/10°; June and September, 25°/15°; July and August, 30°/15°; and December–March, 5°C (Peterson and Vose, 1997). The 30°/15°C thermoperiod was used for warm stratification and 5°C for cold stratification because both are near optimal for seeds of many species that require either warm or cold temperatures for dormancy release (Stokes, 1965; Baskin and Baskin, 1998).

Unless otherwise stated, seeds were placed in 5.5 cm diameter plastic petri dishes on soil (3 : 1, by volume, mixture of limestone-derived topsoil and river sand) for germination studies or in 9 cm diameter plastic petri dishes on two sheets of Whatman number 1 filter paper for embryo-growth studies. Both the soil and filter papers were moistened with distilled water before seeds were placed on them. Three replications of 20 seeds per dish were used in each treatment for germination studies, and one dish of 25 seeds was used each time measurements were made for embryo-growth studies. All dishes were wrapped with plastic film to retard water loss during incubation and stratification, and those incubated and stratified in darkness were also wrapped with two layers of aluminum foil.

Emergence of the radicle was the criterion for germination. 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 (nonviable). Tetrazolium tests confirmed that white embryos were viable and brown ones were not. Germination data were transformed to percentages on the basis of the number of viable seeds. For embryo-growth studies, embryos were excised from seeds with a razor blade and their lengths measured under a dissecting microscope equipped with a micrometer.

Requirements for dormancy break and embryo growth—Seeds collected from Ehime Prefecture were incubated in light at 5°, 15°/6°, and 30°/15°C for 30 wk and examined for germination at 2-wk intervals. Seedlings were counted and removed from the petri dishes, and water was added to the dishes as needed to keep the seeds moist. Because seeds of *O. aristata* ripen and are dispersed in summer-autumn, they can experience several weeks of warm stratification ($\geq 15^\circ\text{C}$; Baskin and Baskin, 1998) before receiving cold stratification. To mimic these conditions, we placed seeds for 8 wk at 30°/15°C in light followed by 22 wk at 5°C in light. Seeds were monitored for germination every 2 wk for the entire 30-wk (8 wk warm plus 22 wk cold) period.

Two types of experiments were conducted on seeds collected from Miyagi Prefecture. In one, seeds were placed in light at 5°C for 2, 4, 6, and 8 wk. At the end of each stratification period, the seeds were incubated in light for 2 wk at 15°/6°, 20°/10°, 25°/15°, and 30°/15°C and examined for germination afterward. In the second type of experiment, seeds were kept in light at 5°, 15°/6°, 20°/10°, 25°/15°, and 30°/15°C for 16 wk and examined for germination at 2-wk intervals. Seeds were also incubated in darkness at 5°, 15°/6°, 20°/10°, 25°/15°, and 30°/15°C for 2 wk and 12 wk and examined for germination only at the end of each incubation period.

Embryo growth was studied in seeds collected in Miyagi Prefecture. Seeds were placed in light at 5° or at 25°/15°C, and embryos were excised and measured following 2, 4, 6, and 8 wk of incubation at each temperature. Embryos were also excised (and subsequently measured) from fresh seeds collected in Ehime and Miyagi Prefectures after the seeds had been allowed to imbibe water at room temperature for 24 h. The length of seeds (mericarps without the caudate appendages; cf. Lowry and Jones, 1984) collected from Ehime and Miyagi Prefectures was also recorded ($N = 25$ for each collection).

Effects of GA₃ on dormancy break and embryo growth—Seeds used in the GA₃ experiment were collected in Aomori and Miyagi Prefectures. Twenty seeds each were placed on two sheets of Whatman number 1 filter paper in 9 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 of GA₃ (K-GA₃) dissolved in distilled water. Seeds collected in Aomori Prefecture were incubated on distilled water or on 1000 mg/L of GA₃ in light at 20°/10°C for 18 wk, and those collected in Miyagi Prefecture on distilled water or on 10, 100, or 1000 mg/L GA₃ for 10 wk. Germination was monitored at 2-wk intervals. Following 10 wk of incubation, embryo length in seeds collected in Miyagi Prefecture was determined for each treatment. The 20°/10°C thermoperiod was used because this

temperature regimen is too high to be effective for cold stratification (Stokes, 1965).

Germination phenology—In late August 1999, 12 caudices were collected near Siroishi. Plants from these caudices were grown to maturity during the 2000 growing season in a nursery in Siroishi and, after March 2000, in a nursery in Matsudo (Chiba Prefecture, Honshu). On 22 August 2000, 100 seeds were collected from the plants and were subsequently sown under approximately 2 cm of soil (vermiculite) in a plastic flat (15 × 35 × 15 cm deep) on 8 October 2000. The plastic flat was placed in the nursery at Chiba University, in Matsudo. The outer part of the flat was covered with grass to buffer against soil temperature elevation resulting from direct solar radiation on the side wall of the flat. Every week, until 2 July 2001, the flat was inspected for germinated seeds and any seedlings were counted. On 22 April 2001, an approximately 25% portion of the flat in which seeds were sown was dug up, and seeds were removed and examined for viability. Cumulative germination percentages were calculated on the basis of the estimated total number of viable seeds.

Throughout the study, the temperature of the soil was recorded at 15-min intervals by an SK-L200T datalogger (Sato Keiryoku, Tokyo, Japan). The temperature probe was placed 2 cm deep in the middle of the flat. Daily mean temperatures, calculated from 96 recordings each day, were used to determine the mean weekly temperature. The soil was watered on the first day of sowing; thereafter, it received only natural precipitation. During the winter, the flat was sometimes covered with snow.

Statistical analyses—Means and standard errors were calculated for germination percentages and embryo lengths. Means of germination percentages and embryo lengths were compared by analyses of variances (ANOVAs) and by protected least significant difference tests (PLSDs, $P = 0.05$) (SAS, 1996). Two-way ANOVAs were used to test the effects and interaction of temperature regimen and length of incubation on germination of seeds collected in the Ehime Prefecture and on embryo growth in seeds collected in the Miyagi Prefecture. A three-way ANOVA was used to test the effects and interactions of thermoperiod, incubation length, and light regimen on germination of seeds collected in the Miyagi Prefecture. Germination percentages were arcsine square-root transformed—and embryo lengths were log transformed—for statistical analyses.

RESULTS

Requirements for dormancy break and embryo growth—Temperature regimen, length of incubation, and their interaction had significant effects on germination of seeds collected in Ehime Prefecture (Table 1). Seeds germinated to 89–100% at 5°C during 6–8 wk of incubation in light; none germinated during 0–4 wk (Fig. 1). Seeds started to germinate at 15°/6°C after 14 wk of incubation in light and continued to germinate to 62% during an additional 16 wk of incubation. No seeds germinated at 30°/15°C during 30 wk of incubation in light. Seeds did not germinate at 30°/15°C during 8 wk of incubation in light, but 100% did so after they were moved and placed at 5°C for 8 wk.

Thermoperiod, incubation length, and their interaction had significant effects on germination of seeds collected in Miyagi Prefecture (Table 1). In addition, germination responses to thermoperiod and incubation length were similar in light and in darkness. Seeds incubated in light at 15°/6°, 20°/10°, 25°/15°, and 30°/15°C following 2, 4, and 6 wk of cold stratification in light germinated from 0 to $3 \pm 3\%$ (mean ± 1 SE) (data not shown). During 8 wk of cold stratification in light seeds germinated to $12 \pm 2\%$, but only an additional $2 \pm 1\%$ germinated after being transferred and incubated for 2 wk in light over the same range of thermoperiods. In contrast, seeds kept in light at 5°C for 16 wk germinated to 91%, but none

TABLE 1. Results of two- and three-way ANOVAs showing the effects of temperature, length of incubation, or light on germination and embryo growth of *Osmorhiza aristata* seeds.

Component	df	MS	F	P
GERMINATION (seeds collected in Ehime Prefecture)				
Temperature regimen	3	45266.888	3838.73	0.0001
Incubation length	14	6050.097	513.06	0.0001
Temperature × incubation length	42	1910.465	162.01	0.0001
Error	120	11.792		
GERMINATION (seeds collected in Miyagi Prefecture)				
Temperature regimen	4	0.485	249.41	
Incubation length	1	0.485	249.41	0.0001
Light regimen	1	0.003	1.36	0.0001
Temperature × incubation length	4	0.485	249.41	0.2499
Temperature × light	4	0.003	1.36	0.0001
Incubation length × light	1	0.003	1.36	0.2639
Temperature × incubation length × light	4	0.003	1.36	0.2499
Error	40	0.002		0.2639
EMBRYO GROWTH (seeds collected in Miyagi Prefecture)				
Temperature regimen	1	36.580	613.30	0.0001
Incubation length	4	17.194	288.27	0.0001
Temperature × incubation length	4	10.958	183.73	0.0001
Error	220	0.060		

kept at 15°/6°, 20°/10°, 25°/15°, and 30°/15°C germinated (Fig. 2). In darkness, no seeds germinated at 5°, 15°/6°, 20°/10°, 25°/15°, and 30°/15°C during 2 wk of incubation (data not shown). However, 55 ± 5% germinated in darkness at 5°C during 12 wk of incubation, compared with 67 ± 10% in light, and none germinated at 15°/6°, 20°/10°, 25°/15°, and 30°/15°C.

Mean (±1 SE) length of freshly matured seeds collected in Ehime and Miyagi Prefectures was 12.0 ± 0.2 and 11.1 ± 0.2 mm, respectively, and that of embryos was 0.64 ± 0.01 and 0.48 ± 0.01 mm, respectively. Temperature regimen, length of incubation, and their interaction had significant effects on embryo growth in seeds collected in Miyagi Prefecture (Table 1). Embryos grew 1142% at 5°C during 8 wk of incubation, whereas they grew only 31% at 25°/15°C (Fig. 3). When embryos had grown enough to start splitting the seed coats, they were approximately 9 mm long.

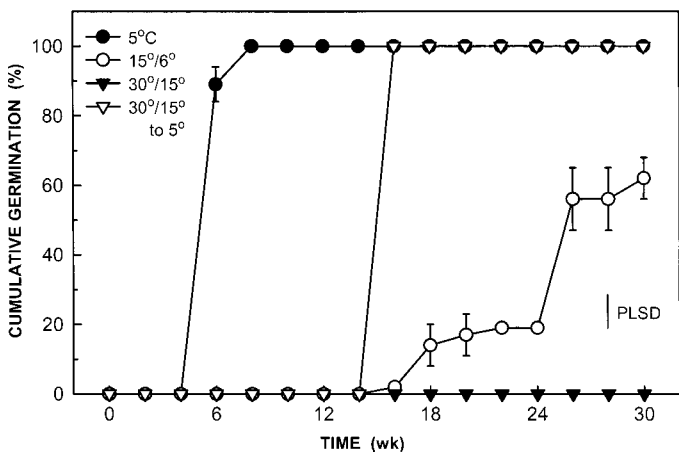


Fig. 1. Cumulative germination percentages (mean ± 1 SE; SE shown if ≥5%) of *Osmorhiza aristata* seeds incubated in light at 5°, 15°/6°, and 30°/15°C for 30 wk or incubated in light at 30°/15°C for 8 wk and then transferred to 5°C for an additional 22 wk. Seeds were collected in Ehime Prefecture, Japan, during summer 1998. Protected least significant difference (PLSD, $P = 0.05$) is indicated by bar.

Effects of GA₃ on dormancy break and embryo growth—None of the seeds collected from Aomori Prefecture germinated during 18 wk of incubation on distilled water or on 1000 mg/L of GA₃, and none from Miyagi Prefecture during 10 wk of incubation on distilled water or on 10, 100, or 1000 mg/L of GA₃. All embryos excised from Miyagi seeds following 10 wk of incubation on 0–1000 mg/L GA₃ were ≤1 mm long.

Germination phenology—Mean weekly temperature of the soil during the 2-wk period following sowing was 19.8°C (Fig. 4). Seeds began to germinate, and primarily did so, between 13 and 19 March 2001, when the mean weekly soil temperature was 8.2°C. No additional seeds germinated after 16 April 2001. Approximately 53 of the 100 seeds sown were viable. Thirteen ungerminated seeds were exhumed from a 25% portion of the flat: eight were nonviable and five were viable. In addition, four seeds germinated and emerged from the soil in

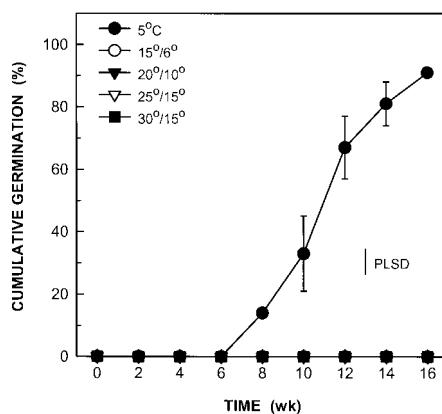


Fig. 2. Cumulative germination percentages (mean ± 1 SE; SE shown if ≥5%) of *Osmorhiza aristata* seeds incubated in light at 5°, 15°/6°, 20°/10°, 25°/15°, and 30°/15°C for 16 wk. Seeds were collected in Miyagi Prefecture, Japan, during summer 1999. Protected least significant difference (PLSD, $P = 0.05$) is indicated by bar.

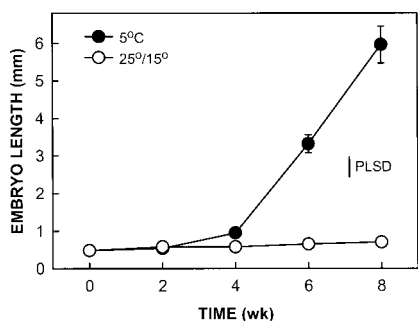


Fig. 3. Embryo growth (mean length \pm 1 SE; SE shown if ≥ 0.2 mm) of *Osmorhiza aristata* seeds collected in Miyagi Prefecture, Japan, during summer 1999 and incubated in light at 5° or 25°/15°C for 8 wk. Protected least significant difference (PLSD, $P = 0.05$) is indicated by bar.

the quarter area before the seeds were exhumed on 22 April 2001.

DISCUSSION

Seeds of *O. aristata* contained embryos that occupied only 4–5% of seed length and were dormant at the time of dispersal. Dormancy break and embryo growth occurred during a relatively short period at 5°C, which was also the optimum temperature for germination. Although seeds from Miyagi Prefecture needed to incubate longer at 5°C for high germination to occur than did those from Ehime Prefecture, seeds from both areas required only cold stratification and a warm period before the cold treatment was unnecessary. Embryos did not grow immediately when the seeds were placed in simulated suitable environmental conditions; instead, they required at least 4 wk at 5°C for appreciable growth to begin. In addition to being underdeveloped at maturity, embryos required cold stratification to overcome physiological dormancy and elongate. Thus, seeds of this species have morphophysiological dormancy (MPD).

Classification of the type of MPD requires an understanding of environmental conditions necessary for embryo growth. The eight types of MPD are initially divided into two categories, simple and complex, on the basis of temperature at the time of embryo growth. Seeds with simple MPD need relatively high temperatures ($\geq 15^\circ\text{C}$) for embryo growth, whereas those with complex MPD need low temperatures (0°–10°C) (Nikolaeva, 1977; Baskin, Meyer, and Baskin, 1995; Baskin and Baskin, 1998). Because embryo growth in *O. aristata* seeds occurred at a low temperature, they have complex MPD.

Further classification of the complex type of MPD into non-deep, intermediate, or deep depends on the temperature regimen required for dormancy break and whether GA₃ substitutes for warm or cold stratification. Nondeep complex MPD is broken by warm stratification followed by cold stratification, whereas intermediate complex MPD and deep complex MPD are broken only by cold stratification. GA₃ substitutes for warm but not cold stratification in seeds with nondeep complex MPD, and it overcomes dormancy in seeds with intermediate complex MPD but not in seeds with deep complex MPD (Nikolaeva, 1977; Baskin and Baskin, 1998). Seeds of *O. aristata* have deep complex MPD, because cold stratification broke physiological dormancy and GA₃ did not.

From an ecological perspective, seeds of *O. aristata* are prevented from germinating after dispersal in late summer and

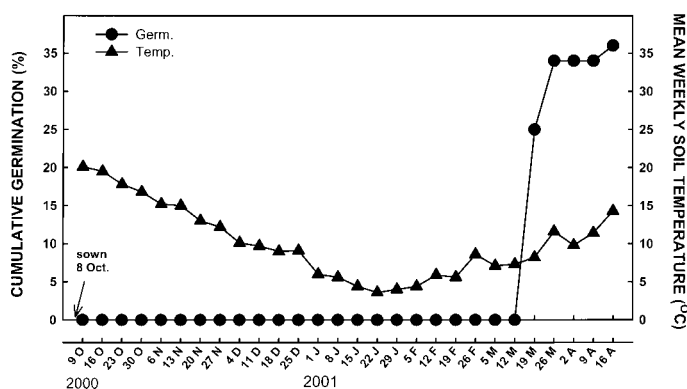


Fig. 4. Cumulative germination percentages of *Osmorhiza aristata* seeds collected on 22 August 2000 and sown on 8 October 2000 in a nursery. Mean weekly soil temperatures are shown for the duration of the study. Numbers and letters on the x-axis represent days and months of the year.

autumn because they are dormant. In our study, dormancy break and embryo growth took place under the natural (low) temperatures of winter, and germination in nature (i.e., in the nursery) occurred in late winter and early spring. However, only a small percentage of the seeds germinated during the first spring germination season. The rest (ca. 64%) of the seeds remained ungerminated but viable in the flat of soil. In laboratory experiments, seeds collected in Miyagi Prefecture (the same source for seeds used in the germination phenology study) required ≥ 12 wk at 5°C for moderate to high germination, and none germinated at temperatures $\geq 15^\circ/6^\circ\text{C}$ (mean = 10.5°C). In the nursery, seeds germinated during a narrow period between breaking of dormancy at low temperatures (ca. 5°C) and before mean weekly temperatures warmed to above ca. 10°C. We speculate that if the seeds remained viable in a soil seed bank, additional germination in this cohort of seeds could occur the following spring(s).

Stasis in morphological features, which is commonly observed in disjunct eastern North American–Asian congeners (Wen, 1999), does not necessarily imply that other traits have remained unchanged since the divergence of the taxa. While seeds of the Asian species *O. aristata* have deep complex MPD, those of the morphologically similar eastern North American species *O. claytonii* and *O. longistylis* exhibit non-deep complex MPD (Baskin and Baskin, 1984, 1991). A similar situation occurs in shrubs of the genus *Sambucus* (Hidayati, Baskin, and Baskin, 2000). Seeds of the Eurasian species *S. racemosa* L. have intermediate complex MPD, but those of the eastern North American species *S. pubens* Michx., sometimes recognized as an infraspecific taxon of *S. racemosa* or included in *S. racemosa* *sensu lato*, have deep simple. Two other genera with disjunct eastern North American–Asian members, however, show stasis with regard to seed dormancy. The North American–Asian herbaceous species pairs *Jeffersonia diphylla* (L.) Pers.–*J. dubia* (Maxim.) Benth. & Hook. ex Baker & Moore, which are morphologically distinct, and *Panax quinquefolius* L.–*P. ginseng* C.A. Meyer, which are morphologically similar, have deep simple MPD (cf. Baskin and Baskin, 1998).

Wake, Roth, and Wake (1983) thought that changes (plasticity) in some traits, including physiological responses, would allow an organism to compensate for maintaining a stable morphology over a long period. The situation they suggested apparently applies to the eastern North American–(Eur)Asian

congeners of *Osmorhiza* and *Sambucus*. In contrast, Ricklefs and Latham (1992) thought that stasis had occurred in traits related to ecological distribution because disjunct eastern North American–Asian taxa of herbaceous perennials had a significant correlation in area of geographic range. The timing of dormancy break and subsequent germination are important traits that influence the ecological (Allen and Meyer, 1998) and geographic (Hacker et al., 1984) distributions of plants. Thus, we would expect that North American–Asian disjunct herbs would share the same type of dormancy, a situation that is true for the congeners of *Jeffersonia* and *Panax* but not for those of *Osmorhiza*.

Baskin, Meyer, and Baskin (1995) compared the germination of western and eastern North American species of *Osmorhiza* and *Erythronium* and hypothesized that deep complex MPD originated from nondeep complex MPD. Seeds of the western species of both genera required cold temperatures to germinate (deep complex MPD), whereas those of the eastern species needed warm plus cold temperatures (nondeep complex MPD). They reasoned that seeds with deep complex MPD lost the warm stratification requirement, but retained the cold one, so that germination could take place in spring in habitats either too cold or too dry for effective warm stratification during summer. When Wen et al. (in press) placed the types of dormancy found in *Osmorhiza* in a phylogenetic framework, they concluded that the plesiomorphic condition was deep complex MPD and the derived one was nondeep complex MPD. Moreover, the evolution of nondeep complex MPD apparently occurred in the common ancestor of the closely related eastern North American sister species, *O. claytonii* and *O. longistylis*, and the occurrence of deep complex MPD in populations of the two western species is a symplesiomorphic condition.

The relative antiquity of *O. aristata* and the requirement in disjunct *Osmorhiza* species for cold stratification to overcome seed dormancy support the contention of Terui and Okagami (1993) that this requirement was present in the Tertiary before the Northern Hemisphere floristic elements separated. *Osmorhiza aristata* in Asia is thought to have diverged from some species in each of the three clades (i.e., eastern North American, western North American, and central Andean) in the middle to late Miocene, ca. 7–14 million years ago (mya) (Wen et al., in press). Thus, nondeep complex MPD may have originated during the diversification of *Osmorhiza* in North America in the Miocene. Apparently, the need for warm stratification requirement to break dormancy was added to a preexisting need for cold stratification in seeds of the two eastern North American *Osmorhiza* species. Baskin and Baskin (1995; see also Baskin and Baskin, 1998), however, suggested that the ancestors of species that require warm plus cold stratification for dormancy break may have needed only warm stratification, and the cold requirement was added to a preexisting warm requirement. This situation does not appear to be the case in *Osmorhiza*.

Seeds of many other species in eastern North America that exhibit MPD require only cold stratification to break dormancy (e.g., Baskin and Baskin, 1988; Baskin, Chester, and Baskin, 1992). However, the geographic pattern for types of MPD in *Osmorhiza* is similar to that observed in two other genera; i.e., seeds of eastern North American taxa require warm plus cold stratification to overcome dormancy, whereas their disjunct congeners in western North America or Eurasia require only cold stratification. Seeds from the eastern North American spe-

cies *Erythronium albidum* Nutt., *E. americanum* Ker-Gawl., and *E. rostratum* W. Wolf have nondeep complex MPD (i.e., require warm plus cold stratification), whereas those from the western North American species *E. grandiflorum* Pursh exhibit deep complex MPD (i.e., require only cold stratification) (Baskin, Meyer, and Baskin, 1995; Baskin and Baskin, 1998). In addition, seeds of the eastern North American species *S. canadensis* L. and *S. pubens* have deep simple MPD and require warm plus cold stratification to overcome dormancy, whereas those of the Eurasian species *S. racemosa* have intermediate complex MPD and require only cold stratification (Hidayati, Baskin, and Baskin, 2000).

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