

Contrasting seed germination patterns for intracontinental disjuncts of *Heuchera* (Saxifragaceae) in North America: the eastern temperate *H. parviflora* and western montane *H. cylindrica*

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Abstract: *Heuchera parviflora* var. *parviflora* grows primarily in southeastern North America and *Heuchera cylindrica* var. *cylindrica* in northwestern North America. Seeds of *H. parviflora* were collected in Kentucky in November 1995 and 1996 during the dispersal stage of the life cycle, and those of *H. cylindrica* were collected in Washington in August 1996. The purpose of the study was to compare the germination patterns of these two intracontinental disjunct species of *Heuchera*. During 2–12 weeks of incubation over a range of thermoperiods (15:6, 20:10, 25:15, 30:15, 35:20°C (12 h : 12 h)) that simulated habitat temperatures, fresh *H. parviflora* seeds germinated to 62–87% at 20:10 – 30:15°C in light. Cold stratification at 5°C effectively broke conditional dormancy in *H. parviflora* seeds: 76–96% germination took place during 2 weeks of incubation at 15:6 – 35:20°C in light following 12 weeks of cold stratification in light. In contrast, fresh *H. cylindrica* seeds germinated to 69–94% at 15:6 – 25:15°C in light during 2–12 weeks of incubation in light, but cold stratification at 5°C did not improve germination. Peak germination of *H. parviflora* seeds sown in November 1996 in an ambient-temperature greenhouse occurred in early March 1997, whereas that of *H. cylindrica* seeds sown in September 1996 occurred in late October 1996. Adaptive divergence of these species apparently has occurred with respect to their seed germination ecology. Their dormancy and germination characteristics are similar to the many other species growing in the same vegetation types.

Key words: adaptive divergence, *Heuchera cylindrica*, *Heuchera parviflora*, physiological dormancy, Saxifragaceae, seed germination.

Résumé : L'*Heuchera parviflora* var. *parviflora* pousse principalement dans le sud-est de l'Amérique du nord et l'*H. cylindrica* var. *cylindrica* dans le nord-ouest de l'Amérique du nord. Les auteurs ont récolté des graines de l'*H. parviflora* au Kentucky en novembre 1995 et 1996 au stade de dispersion des graines du cycle vital, et celles de l'*H. cylindrica* dans l'état de Washington en août 1996. Le but de l'étude est de comparer les patrons de germination de ces espèces d'*Heuchera* à aires intra-continentales disjointes. Au cours des 2–12 semaines d'incubation sous un ensemble de thermopériodes (15:6, 20:10, 25:15, 30:15, 35:20°C (12 h : 12 h)) qui simulent les températures des habitats, les graines fraîches de l'*H. parviflora* germent à 62–87 % à 20:10 – 30:15°C, à la lumière. Une stratification au froid à 5°C brise efficacement la dormance conditionnelle des graines, chez l'*H. parviflora* : 76–96 % des graines germent au cours de 2 semaines d'incubation à 15:6 – 35:20°C à la lumière, suite à 12 semaines de stratification au froid à la lumière. Au contraire les graines fraîches de l'*H. cylindrica* germent à 64–94 % à 15:6 – 25:15°C à la lumière au cours de 2–12 semaines d'incubation à la lumière, mais la stratification au froid à 5°C n'améliore pas la germination. Le maximum de germination chez des graines de l'*H. parviflora* semées en novembre 1996, dans une serre à température ambiante, est survenu en mars 1997, alors que les graines de l'*H. cylindrica* semées en septembre 1996 n'ont germé qu'en octobre 1997. Les divergences adaptatives de ces espèces se sont apparemment développées en relation avec l'écologie de la germination des graines. Leurs caractéristiques de dormance et germination sont similaires à celles de nombreuses autres espèces venant dans les mêmes types de végétation.

Mots clés : divergences adaptatives, *Heuchera cylindrica*, *Heuchera parviflora*, dormance physiologique, Saxifragaceae, germination des graines.

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Introduction

When seeds are dispersed, their level of dormancy could be classified into one of three categories. Seeds may germinate over a broad range of environmental conditions and be considered nondormant, germinate in a limited set of conditions and be conditionally dormant (*sensu* Baskin and Baskin 1985a), or not immediately germinate in any set of conditions and be dormant. Five general kinds of dormancy are known to occur in seeds: physiological, physical, combinational (physiological + physical), morphological, and morphophysiological. These are distinguished on whether the seed or fruit coat is permeable (or impermeable) to water and on whether the embryo is fully developed (or underdeveloped) and is physiologically dormant (or nondormant) (Nikolaeva 1977; Baskin and Baskin 1998).

Nondormancy and the various kinds of dormancy vary in occurrence among the vegetation types of the world (*sensu* Eyre 1963; Walter 1979). The proportion of species with nondormant seeds is highest in tropical rainforests (Baskin and Baskin 1998). However, nondormancy also is relatively abundant in the following vegetation types: semievergreen forest, temperate broad-leaved evergreen forest, woodland, montane, boreal-subalpine, and tundra. Physiological dormancy has maximum distribution in tropical deciduous forests, savannas, and hot deserts and in all temperate and arctic vegetation types. Physical dormancy is common in tropical deciduous forests, savannas, hot deserts, steppes, and matorral. Morphophysiological dormancy peaks in temperate broad-leaved evergreen, temperate deciduous, and boreal forests. Combinational and morphological dormancies are not very common in any vegetation type (Baskin and Baskin 1998).

The sharing of seed dormancy and germination characteristics among species with different lineages in a particular vegetation type suggests that ecophysiological traits are well adapted to environmental conditions (Ackerly et al. 2000). Yet, multiple solutions to a given environment often exist (Garland and Carter 1994). On the other hand, species growing in different vegetation types might be expected to exhibit adaptive divergence in their physiological traits (Ackerly et al. 2000). However, trait similarities of species in different vegetation can occur as a result of phylogenetic relatedness or of common selection forces (Westoby 1999). These various evolutionary processes undoubtedly explain why several kinds of dormancy are found in one vegetation type and, at the same time, why one kind of dormancy may be more common than others.

One approach to understanding physiological diversity is to survey taxa separated naturally in different environments (Feder et al. 2000). Most plant genera have species that grow in more than one type of vegetation. These congeneric species may have disjunctions in their geographical ranges corresponding to the spatial distribution of various vegetation types. Disjunctions can occur because many plants occur in discrete populations corresponding to the patchiness of vegetation rather than being distributed continuously. Disjunctions between congeners also can involve geographic areas sufficiently distant from each other, such as between two or more areas within a single continent or between continents (Thorne 1972; Crawford et al. 1992). Intra- and intercontinental disjunctions offer distinctive systems for studying

the evolutionary ecology of ecophysiological traits such as seed dormancy and germination (Baskin et al. 1995; Hidayati et al. 2000; Walck et al. 2002).

The genus *Heuchera* (Saxifragaceae) provides an opportunity to explore dormancy and germination patterns of intra-continental disjunctions growing in different vegetation types. The ca. 55 members of this genus are perennial herbs primarily confined to eastern and western North America, with very few species occurring in central North America (Rosendahl et al. 1936; Wells 1984; Mabblerley 1997). Two members of *Heuchera* were included in the present study. *Heuchera parviflora* Bartling var. *parviflora* grows on cliffs and in sandstone rockhouses of the temperate deciduous forest. It occurs from southern West Virginia and south-central Ohio; to southern Indiana, southern Illinois, and southeastern Missouri; and south to northern Georgia and Alabama (Walck et al. 1996). *Heuchera cylindrica* Dougl. var. *cylindrica* grows in rocky terrain and on gravelly slopes of the montane coniferous forest (St. John 1963; Abrams 1964; Rydberg 1969). It occurs from British Columbia and Washington; to Montana; and south to western Wyoming, Idaho, and Oregon (Hitchcock and Cronquist 1973). *Heuchera parviflora* belongs to the *Euheuchera* section of *Heuchera*, whereas *H. cylindrica* belongs to the *Holochloa* section (Rosendahl et al. 1936).

Among herbaceous plants, physiological dormancy is most common in the temperate deciduous forest (Baskin and Baskin 1998). In contrast, nondormancy is slightly more prevalent in montane herbaceous plants than physiological dormancy. Studies on other species of *Heuchera* suggest that, if *H. parviflora* and *H. cylindrica* seeds have dormancy, it should be of the physiological kind. This conclusion is based on the facts that (i) seeds readily imbibe water, (ii) embryos are fully developed, and (iii) embryos have a physiological inhibiting mechanism that prevents radicle emergence (Martin 1946; Greene and Curtis 1950; Bonde 1965; Deno 1994; Baskin and Baskin 1998).

We hypothesized that the seed dormancy or germination characteristics of both species would be similar to the prevailing vegetation: *H. parviflora* seeds would have physiological dormancy and *H. cylindrica* seeds would be nondormant. To determine whether nondormancy or physiological dormancy was present, we examined the (i) light and temperature requirements for germination of freshly matured seeds, (ii) effects of cold stratification and dry storage at ambient laboratory conditions on seed germination responses under various light and temperature conditions, and (iii) germination phenology of seeds sown in an ambient-temperature greenhouse.

Materials and methods

Plant material and seed size

Freshly matured seeds were collected from about 50 plants of *H. parviflora* var. *parviflora* in Wolfe County, Kentucky, on 24 November 1995 and 15 November 1996, and those of *H. cylindrica* var. *cylindrica* in Chelan County, Washington, on 4 August 1996. At the time of collection, some seeds already had dispersed from plants of both species. Seeds of *H. parviflora* and *H. cylindrica* were stored dry for 8 and 13 days, respectively, between collection and

the initiation of studies. Fifty seeds of each species collected in 1996 and stored dry at laboratory conditions were weighed separately to the nearest 0.001 mg (Cohn® C-33 Microbalance, Fisher Scientific, Pittsburgh, Penn.).

Germination tests

Germination studies were done in temperature- and light-controlled incubators and in a refrigerator equipped with a light and a time clock. The five incubators were set at 12 h : 12 h daily thermoperiods of 15:6, 20:10, 25:15, 30:15, and 35:20°C. The alternating thermoperiods approximate mean daily maximum and minimum monthly air temperatures near the collection site of *H. parviflora* seeds during March and November, 15:6; April and October, 20:10; May, 25:15; June and September, 30:15; and July and August, 35:20°C and near that of *H. cylindrica* seeds during April and October, 15:6; May, 20:10; June and September, 25:15; and July and August, 30:15°C (Climate Diagnostic Center 2001). The daily photoperiod in the incubators was 14 h light : 10 h dark, with the light period extending 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 5°C and a 14 h light : 10 h dark photoperiod. Seeds were cold-stratified at 5°C, since this temperature is near optimal for seeds of many species that require moist, low-temperature conditions to come out of dormancy (Stokes 1965; Nikolaeva 1969). Cool white fluorescent tubes, which produced a 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.

Seeds were placed on white quartz sand, moistened with distilled water, in 6.0-cm (diameter) plastic Petri dishes. Water was added to the dishes incubated or stratified in light as needed to keep the substrate moist. Three replications of 50 seeds per dish were used in each treatment. Dishes containing seeds undergoing light treatments were wrapped with plastic film, and those in dark treatments also were wrapped in aluminum foil.

Protrusion of the radicle was the criterion used for germination. Ungerminated seeds were checked under a dissecting microscope to see if they contained firm, white (viable) embryos or soft, brown (nonviable) ones. A tetrazolium test (Grabe 1970) performed on a subset of seeds confirmed that white embryos were alive and that brown ones were not.

Germination of freshly matured seeds and effect of cold stratification on dormancy break

Germination responses for nonstratified seeds of *H. parviflora* (1995 collection) and of *H. cylindrica* were determined. They were placed into the five thermoperiods, incubated in light for 12 weeks, and examined every 2 weeks. If seedlings were present, they were counted and removed from the dishes. In addition, seeds of both species were incubated in darkness for 2 weeks and checked only at the end of the 2-week period. Seeds incubated for 2 weeks are referred to as “fresh”. The control for the cold-stratification experiment on *H. parviflora* and *H. cylindrica* would be nonstratified seeds incubated for 14 weeks (i.e., 12 weeks cold stratification + 2 weeks incubation) and 11 weeks (i.e., 9 weeks cold stratification + 2 weeks incubation), respec-

tively. However, since percent germination of control seeds did not differ between 12 and 14 weeks for *H. parviflora* (data not shown) and 11 and 12 weeks for *H. cylindrica*, we standardized the length of incubation to 12 weeks for both species.

The effect of cold stratification was tested on seeds of both species. Seeds of *H. parviflora* (1995 collection) were (i) cold-stratified for 2, 4, 6, 8, 10, and 12 weeks in light and then incubated in light at the five thermoperiods for 2 weeks; (ii) cold-stratified for 12 weeks in light and incubated in darkness; (iii) cold-stratified for 12 weeks in darkness and incubated in light; and (iv) cold-stratified for 12 weeks in darkness and incubated in darkness. Seeds of *H. cylindrica* were (i) cold-stratified for 3, 6, and 9 weeks in light and then incubated in light at the five thermoperiods for 2 weeks; (ii) cold-stratified for 9 weeks in light and incubated in darkness; (iii) cold-stratified for 9 weeks in darkness and incubated in light; and (iv) cold-stratified for 9 weeks in darkness and incubated in darkness. Seeds both stratified and incubated in darkness were not checked until the end of the incubation period. Seeds that germinated during stratification were counted and removed from the Petri dishes prior to the incubation period.

Effect of dry storage on afterripening

Seeds of *H. parviflora* (1995 collection) were tested for germination following 0 (freshly matured), 16, and 52 months, and those of *H. cylindrica* following 0, 18, and 52 months, of dry laboratory storage (ca. 23°C, relative humidity 50–60%). After each time interval, seeds were incubated in light or darkness at each thermoperiod for 2 weeks, after which they were checked for germination.

Phenology of seed germination

Two replications of 300 seeds each of *H. parviflora* (1996 collection) and *H. cylindrica* were sown on the top of soil in metal flats (20 (width) \times 30 (length) \times 8 cm (depth)) on 19 November 1996 and 1 September 1996, respectively. The substrate used for *H. parviflora* seeds was a 1:1 (v/v) mixture of a sandstone-derived soil collected in eastern Kentucky and river sand, and that for *H. cylindrica* seeds was a 3:1 (v/v) mixture of river sand and limestone-derived topsoil. The flats, which remained uncovered, were placed in an ambient-temperature greenhouse (no heating or air conditioning, windows open all year; Baskin and Baskin 1985b) on the campus of the University of Kentucky, Lexington, Ky. At weekly intervals, all germinated seeds were counted and removed from the flats. The soil in the flats was watered once each week from 1 May to 31 August, and it was watered daily, except when frozen, during the remainder of the year. This watering regime simulates soil moisture that might occur in the field, i.e., soil wet in autumn, winter, and early spring, and alternately wet and dry in late spring and summer (Climate Diagnostic Center 2001). Mean maximum and minimum daily temperatures for each week of the study were calculated from continuous thermograph records made inside a weatherhouse in the greenhouse.

Statistical analyses

Germination data were transformed to percentages based on the number of viable seeds, and means and standard

Table 1. Percent germination (mean \pm SE) of nonstratified and cold-stratified seeds of *Heuchera parviflora*.

Condition	Light regime		Incubation thermoperiod ($^{\circ}$ C)				
	Stratification	Incubation	15:6	20:10	25:15	30:15	35:20
Nonstratified							
Fresh		Light	2 \pm 1Cd	20 \pm 1Cc	64 \pm 4Da	38 \pm 2Eb	2 \pm 2CDd
		Dark	0Ca	0Da	0Ea	0Fa	0Da
Control		Light	40 \pm 7Bc	62 \pm 3Bb	87 \pm 2Ba	80 \pm 2Ba	3 \pm 1Cd
Cold-stratified	Light	Light	89 \pm 5Aa (46)	90 \pm 3Aa (49)	96 \pm 1Aa (60)	93 \pm 3Aa (51)	76 \pm 2Ab (52)
		Dark	77 \pm 7Aa (50)	67 \pm 3Bab (44)	69 \pm 2Dab (43)	73 \pm 3Cab (47)	61 \pm 1Bb (51)
	Dark	Light	29 \pm 6Bc (0)	69 \pm 6Ba (0)	82 \pm 4Ca (0)	53 \pm 4Db (0)	1 \pm 1Dd (0)
		Dark	1 \pm 1Ca	1 \pm 1Da	0Ea	0Fa	1 \pm 1Da

Note: Fresh seeds were incubated in light or darkness for 2 weeks, and control seeds were incubated in light for 12 weeks. Seeds were cold-stratified at 5 $^{\circ}$ C in light or darkness for 12 weeks and then incubated in light or darkness for 2 weeks. Values in parentheses are percentages of germination that occurred during cold stratification. Values followed by different uppercase letters within columns or different lowercase letters within rows are significantly different (protected least significant difference, $P = 0.05$).

errors were calculated. A t test was used to determine whether seed size differed between the two species. Means of percent germination were compared by analyses of variances (ANOVAs) and by protected least significant difference tests (PLSDs, $P = 0.05$) (SAS Institute Inc. 1996). A three-way ANOVA was used to examine the effects and interactions of species (two levels), thermoperiod (five levels), and condition (seven levels; fresh seeds incubated in two light regimes + seeds cold-stratified and incubated in four light regimes + control in one light regime) on percent germination. A four-way ANOVA analyzed the effects and interactions of species (two levels), length of dry storage (three levels), light regime (two levels), and thermoperiod (five levels). Percent germination data were arcsine square-root transformed for statistical tests (Scott et al. 1984).

Results

Seed size

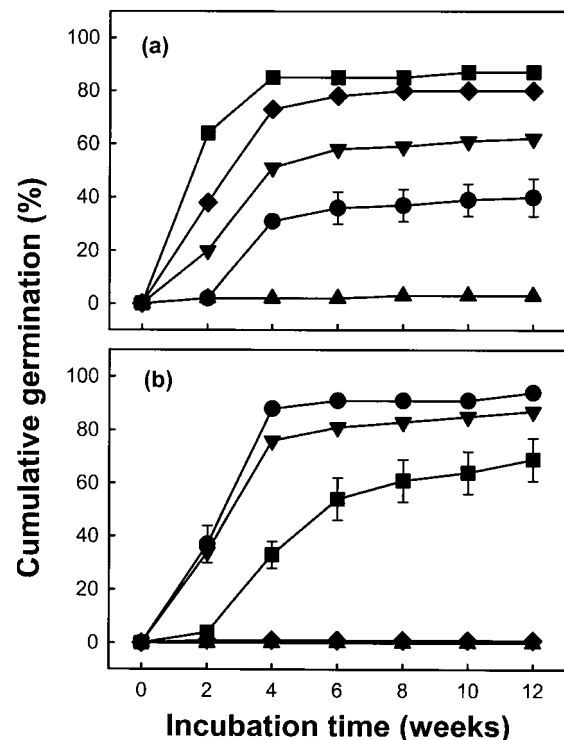
Seed weight differed significantly ($P = 0.0001$) between *H. parviflora* (0.020 ± 0.001 mg; mean \pm SE) and *H. cylindrica* (0.049 ± 0.001 mg).

Germination of freshly matured seeds and effect of cold stratification on dormancy break

Seed germination differed significantly between species and among thermoperiods and conditions ($P = 0.0001$). Germination responses within thermoperiods were highly dependent on the condition of the seed, and the responses for each thermoperiod and condition were dissimilar between species (two- and three-way interactions, $P = 0.0001$).

Fresh seeds of *H. parviflora* germinated to 64% at 25:15 $^{\circ}$ C and to \leq 38% at the other four thermoperiods with 2 weeks of incubation in light, and none germinated in darkness (Table 1). After 12 weeks of incubation in light, control seeds had germinated to 62–87% at 20:10, 25:15, and 30:15 $^{\circ}$ C and to \leq 40% at the other thermoperiods (Table 1, Fig. 1a). Percent germination increased at all thermoperiods in light with an increase in the length of the cold stratification period in light (Fig. 2a). Moderate percentages of seeds

Fig. 1. Cumulative percent germination (mean \pm SE, SE shown if \geq 5%) of freshly matured seeds of *Heuchera parviflora* (a) and *H. cylindrica* (b) that remained in light at five thermoperiods for 12 weeks. Symbols are as follows: 15:6 (\bullet), 20:10 (\blacktriangledown), 25:15 (\blacksquare), 30:15 (\blacklozenge), and 35:20 $^{\circ}$ C (\blacktriangle). The first (2 weeks) and final incubation times correspond to the fresh (in light) and control cases, respectively, in Tables 1 and 2.



germinated at 5 $^{\circ}$ C during stratification. Seeds that received light during 12 weeks of cold stratification and were incubated in light or darkness, germinated to 61–96% over the range of thermoperiods (Table 1). If seeds were in darkness during cold stratification, 1–82% of them germinated in light and 0–1% germinated in darkness over the range of thermoperiods.

Table 2. Percent germination (mean ± SE) of nonstratified and cold-stratified seeds of *Heuchera cylindrica*.

Condition	Light regime		Incubation thermoperiod (°C)				
	Stratification	Incubation	15:6	20:10	25:15	30:15	35:20
Nonstratified							
Fresh		Light	37±7Ca	34±1Ca	4±2Bb	0Bc	0Bc
		Dark	0Da	0Da	0Ca	0Ba	0Ba
Control		Light	94±2Aa	87±3Aa	69±8Ab	1±1Bc	0Ba
Cold-stratified	Light	Light	67±7Ba (57)	60±2Ba (53)	65±4Aa (63)	61±3Aa (60)	45±4Ab (45)
		Dark	65±2Ba (59)	65±5Ba (62)	57±2Aab (55)	50±6Abc (50)	43±2Ac (43)
	Dark	Light	0Da (0)	0Da (0)	0Ca (0)	0Ba (0)	0Ba (0)
		Dark	0Da	0Da	0Ca	0Ba	0Ba

Note: Fresh seeds were incubated in light or darkness for 2 weeks, and control seeds were incubated in light for 12 weeks. Seeds were cold-stratified in light or darkness for 9 weeks and then incubated in light or darkness for 2 weeks. Values in parentheses are percentages of germination that occurred during cold stratification. Values followed by different uppercase letters within columns or different lowercase letters within rows are significantly different (protected least significant difference, $P = 0.05$).

With 2 weeks of incubation, fresh seeds of *H. cylindrica* germinated to 34–37% at 15:6 and 20:10°C and to 0–4% at the other three thermoperiods in light, and none germinated in darkness (Table 2). After 12 weeks of incubation in light, control seeds had germinated to 69–94% at 15:6, 20:10, and 25:15°C and 0–1% at the other thermoperiods (Table 1, Fig. 1b). Seeds germinated at 5°C during stratification, and generally only a small percentage of seeds germinated once they were transferred to the incubation thermoperiods (Fig. 2b). If seeds received light during 9 weeks of cold stratification and then were incubated in light or darkness, 43–67% germinated over the range of thermoperiods, but 43–63% of this germination took place during the stratification period (Table 1). No seeds germinated in light or darkness that were in darkness during cold stratification.

Effect of dry storage on afterripening

Seed germination differed significantly between species and among storage times, light regimes, and thermoperiods ($P = 0.0001$). Germination responses of seeds in the different light regimes and thermoperiods differed for the various storage times, and these responses were dissimilar between the species (two-, three-, and four-way interactions, $P = 0.0001$).

Percent germination of *H. parviflora* seeds increased slightly from 2–64% (fresh) to 12–73% in light at 15:6–30:15°C and remained about the same (nearly zero) at 35:20°C, following 16 weeks of dry storage (Fig. 3a). In contrast, germination at 15:6, 20:10, and 25:15°C decreased between 16 and 52 weeks of dry storage but, at 30:15 and 35:20°C, remained relatively constant. Only 0–1% of the seeds stored dry for 16 and 52 weeks germinated in darkness (data not shown).

Percent germination of *H. cylindrica* seeds increased from 4–37% (fresh) to 28–70% in light at 15:6, 20:10, and 25:15°C following 18 weeks of dry storage but remained the same at 30:15 and 35:20°C (Fig. 3b). With the exception of germination at 20:10°C, little improvement in germination took place in light between 18 and 52 weeks of dry storage. None of the seeds stored dry for 18 and 52 weeks germinated in darkness (data not shown).

Fig. 2. Percent germination (mean ± SE, SE shown if ≥ 5%) of seeds of *Heuchera parviflora* (a) and *H. cylindrica* (b) following cold (5°C) stratification. Seeds were stratified in light for 0–12 weeks (a) or 0–9 weeks (b) and then incubated in light for 2 weeks at five thermoperiods: 15:6 (●), 20:10 (▼), 25:15 (■), 30:15 (◆), and 35:20°C (▲). Also shown is the percentage of seeds that germinated during stratification (●) before placement at the incubation thermoperiods. The first (zero week) and final stratification periods correspond to the fresh (in light) and cold stratification (stratified and incubated in light) cases, respectively, in Tables 1 and 2.

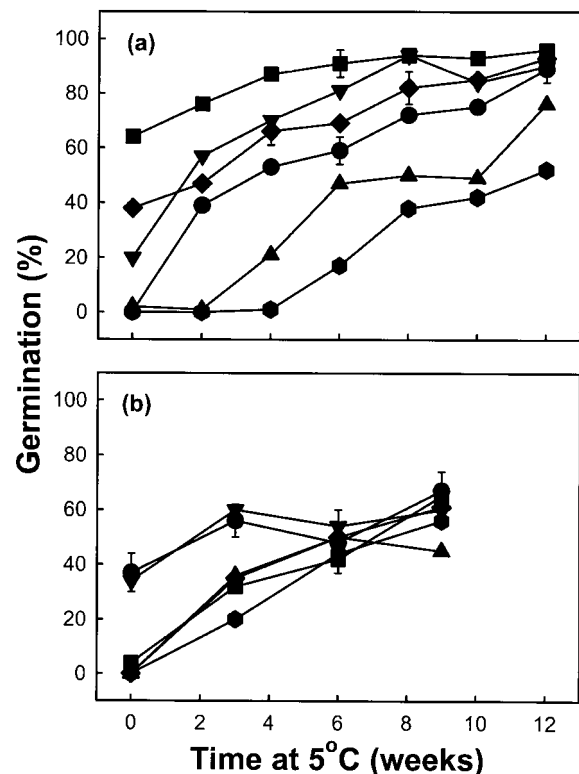
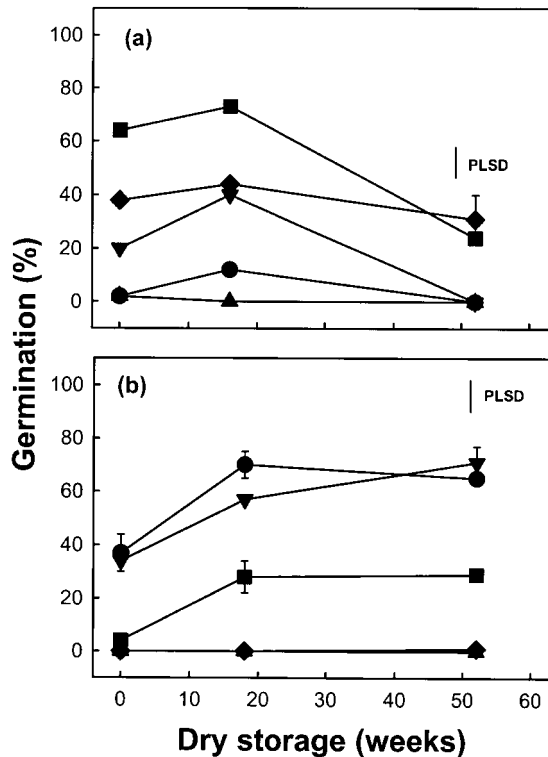


Fig. 3. Percent germination (mean \pm SE, SE shown if $\geq 5\%$) of *Heuchera parviflora* (a) and *H. cylindrica* (b) seeds after dry storage under ambient laboratory conditions for 0–52 weeks. Seeds were subsequently incubated in light at five thermoperiods for 2 weeks. Symbols are as in Fig. 1. The first set of dry storage values (zero week) corresponds to the fresh seeds (incubated in light) treatment in Tables 1 and 2. PLSD, minimum separation of values necessary for statistical significance ($P < 0.05$) by protected least significant difference test.

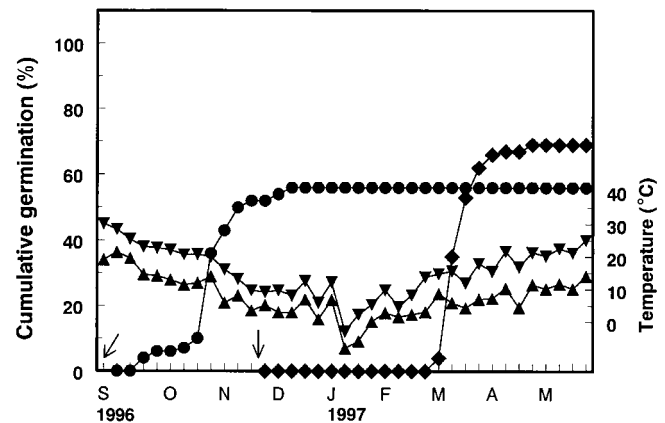


Phenology of seed germination

Seeds of *H. parviflora* sown on 19 November 1996 began germinating between 24 February and 3 March 1997, when mean weekly maximum and minimum temperatures were 13.8 and 3.0°C, respectively (Fig. 4). Peak germination occurred the following week (3–10 March 1997), when mean maximum and minimum temperatures were 14.8 and 8.7°C, respectively. Of the 600 seeds sown, 69% of them germinated in spring 1997. No additional seeds germinated after 21 April 1997 until 3 May 1999, when the study was terminated.

Heuchera cylindrica seeds sown on 1 September 1996 started to germinate between 16 and 23 September 1996, when mean weekly maximum and minimum temperatures were 23.0 and 14.5°C, respectively (Fig. 4). Peak germination occurred between 21 and 28 October 1996, when mean maximum and minimum temperatures were 20.7 and 11.9°C, respectively. Of the 600 seeds sown, 56% of them germinated in autumn 1996. After 9 December 1996, no seeds germinated until early March 1997 when an additional 1% did so. No additional seeds germinated after late March 1997 until 3 May 1999, when the study was terminated.

Fig. 4. Cumulative percent germination (mean \pm SE, all SEs $\leq 8\%$) of *Heuchera parviflora* (◆) and *H. cylindrica* (●) seeds collected in 1996 and sown on 19 November 1996 and on 1 September 1996 (arrows), respectively, in an ambient-temperature greenhouse. Mean weekly maximum (▼) and minimum (▲) temperatures are shown from 1 September 1996 to 30 May 1997. Letters on x axis are months from September 1996 to May 1997.



Discussion

When freshly matured, seeds of *H. parviflora* germinated to the highest percentage at 25:15°C during 2 weeks of incubation in light. Percent germination of nonstratified (control) seeds increased during 12 weeks of incubation in light, but high percentages of germination were achieved only at 25:15 and 30:15°C (Fig. 1a). On the other hand, following 12 weeks of cold stratification in light, seeds germinated to high percentages at all thermoperiods (15:6 to 35:20°C) during 2 weeks of incubation in light (Fig. 2a). In contrast, dry storage at ambient laboratory conditions did not cause an increase in the percentage of seeds germinating over the broad range of thermoperiods (Fig. 3a). Seeds of *H. parviflora* were conditionally dormant at maturity, and nontreated seeds germinated to high percentages over a narrow range of thermoperiods. With increased amounts of cold stratification, percent germination increased, and the range of temperatures over which high germination took place broadened. When nondormant, *H. parviflora* seeds germinated to high percentages over a wide range of temperatures.

Seeds, like those of *H. parviflora*, that germinate to high percentages first at intermediate temperatures and, upon additional stratification, extend both the minimum and maximum temperatures for germination, have a type 3 pattern for loss of nondeep physiological dormancy (Vegis 1964; Baskin and Baskin 1998). The probable adaptive significance of this type of dormancy loss in a species growing in a temperate region is the prevention of germination in middle to late autumn. If a seed germinated in middle to late autumn, the seedling would not have enough time to form a perennating structure necessary for persistence through winter. During late autumn – early winter, the temperature required for germination is lowered, and seeds can germinate in late winter – early spring at temperatures that were too low for germination the preceding autumn. Thus, seeds germinate at the beginning of the favorable period for seedling

growth (Baskin et al. 1993). Likewise, seeds of *H. parviflora* sown in the ambient-temperature greenhouse in mid-November germinated the following late February – early March (Fig. 4).

Seeds of *H. cylindrica* at maturity germinated to the highest percentage at 15:6°C during 2 weeks of incubation in light (Fig. 1b). Germination increased at 15:6 and 20:10°C and was completed mostly within 4 weeks of incubation in light and at 25:15°C within about 8 weeks. Neither cold stratification nor dry storage at ambient laboratory conditions was effective in widening the temperature range over which germination took place, and better germination was achieved without these treatments (Figs. 2b, 3b). Apparently, seeds of *H. cylindrica* were nondormant at maturity. They required about 4–8 weeks to complete germination without any dormancy-breaking treatment(s), and the optimum temperatures for germination were low to intermediate ($\leq 25:15^\circ\text{C}$).

At the time of dispersal, temperatures in late summer are too high (30:15°C) for seeds of *H. cylindrica* to germinate in nature. As temperatures decrease in autumn, seeds are able to germinate, indicating that the optimum temperatures for germination have been reached ($\leq 25:15^\circ\text{C}$). Seeds of this species sown in the ambient-temperature greenhouse in August started to germinate in mid-September and peaked in late October; a few seeds germinated the following March (Fig. 4). Although the germination phenology studies were conducted in Kentucky, temperature and precipitation (including snowfall) records (Climate Diagnostic Center 2001) indicate that suitable conditions in Washington State would allow germination to take place during autumn or the following spring.

Seeds of both species of *Heuchera* required light for germination (Tables 1 and 2). The light requirement could be fulfilled either during the stratification or incubation period for seeds of *H. parviflora*. In contrast, seeds of *H. cylindrica* did not germinate in light if they were cold-stratified in darkness. Generally, small-seeded species, like those of *Heuchera*, are more dependent on light for germination than large-seeded species (Milberg et al. 2000). Moreover, small seeds tend to persist in the soil for a longer time than large seeds (Thompson et al. 1993; Funes et al. 1999). Thus, seeds of both species of *Heuchera* should be capable of forming a persistent seed bank in the soil.

Physiological dormancy is very commonly found in seeds of herbaceous species that inhabit temperate deciduous forest. However, the proportion of herbaceous species with nondormant seeds is higher than that with physiologically dormant seeds in montane vegetation (Baskin and Baskin 1998). This observation at the vegetation level was reflected at the individual species level in the two intracontinental congeners of *Heuchera*. At maturity, seeds of *H. parviflora* were physiologically dormant, while those of *H. cylindrica* were nondormant. Having physiological dormancy in a temperate region would ensure that seed germination was prevented until plant survival and reproduction would be optimal (Allen and Meyer 1998).

The reason for the relatively high number of species in montane vegetation with nondormant seeds is unknown. Baskin and Baskin (1998) speculated that temperatures where this vegetation type occurs are below those required

for cold stratification during much of the winter, and thus, seeds do not receive enough cold stratification to come out of dormancy. If seeds were nondormant at the time of maturation in late summer and early autumn, low habitat temperatures would prevent germination. When temperatures increased in spring, seeds could germinate immediately without having to use up part of the short growing season for dormancy loss (Baskin and Baskin 1998). For the population of *H. cylindrica* in Chelan County, Washington, temperatures from December to February (mean -2.3°C ; Climate Diagnostic Center 2001) are below those that are optimal for cold stratification (0–10°C; Stokes 1965; Nikolaeva 1969). However, low habitat temperatures in autumn would promote germination of *H. cylindrica* seeds. Seedlings that emerged in early autumn would have enough time to grow into a small rosette stage and be capable of surviving overwinter, comparable with winter annuals (cf. Baskin and Baskin 1998). Indeed, some of the seedlings from seeds that germinated in the ambient-temperature greenhouse in Kentucky in October and November survived to the following spring (J.L. Walck, data not shown). Presumably, any seeds that did not germinate in autumn would do so early in the following spring.

The genus *Heuchera* may have arisen in western North America, where the diversity of species and of closely related genera is greatest (Wells 1984; Soltis et al. 1991). The separation and isolation of eastern *Heuchera* species from the western species probably took place during the middle Miocene to Pliocene ($\sim 15 \times 10^6$ to 2×10^6 years ago). During this time, uplift of the Cordilleras and accompanying aridity of the interior of the continent became effective barriers to plant migrations across North America (Wells 1984; Graham 1993). The differences in the dormancy and germination characteristics between *H. parviflora* and *H. cylindrica* (or their progenitors) may have been present in the Miocene–Pliocene or developed since the separation of the eastern and western members of this genus. The differences in the seed biologies of the two *Heuchera* species may represent adaptive divergence, largely reflective of the prevailing vegetation in which the species grow.

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