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Trait stasis versus adaptation in disjunct relict species: evolutionary changes in seed dormancy-breaking and germination requirements in a subclade of *Aristolochia* subgenus *Siphisia* (*Piperales*)

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Abstract

There are two ideas regarding changes in the physiological and ecological tolerances and requirements within plant lineages through geological time. One is that these attributes have changed very little, or not at all (trait stasis), and the other is that they have undergone considerable change (adaptation), as plants shifted to new climatic and vegetation zones. We tested these ideas for seed dormancy-breaking and germination requirements of four species in a subclade of *Aristolochia* subgenus *Siphisi*a: the three temperate species, *A. macrophylla* and *A. tomentosa* (the basal species in the subclade) of eastern USA and *A. manshuriensis* of East Asia, and the Mediterranean-climate species *A. californic*a endemic to California, USA. A long period at cold-stratifying temperatures was required for growth of the underdeveloped embryo and seed germination in *A. californica*, whereas embryos grew and seeds germinated in the other three species at warm temperatures, either before or after they were cold stratified. Thus, seeds of *A. californica* have either intermediate or deep complex morphophysiological dormancy (MPD), whereas those of the three temperate species have either morphological dormancy or non-deep simple MPD. Further, there were quantitative differences in temperature requirements for dormancy-break and germination between the Appalachian *A. macrophylla*, which did not differ from its sister species *A. manshuriensis*, and the lowland *A. tomentosa*. Thus, within this lineage there has been both trait stasis and divergence (adaptation) in the physiology and ecology of seed dormancy and germination.

Keywords: *Aristolochia*, embryo growth, evolutionary changes in a lineage, morphological seed dormancy, morphophysiological seed dormancy, trait stasis, underdeveloped embryo

Introduction

The idea that organisms change through time, whether it be slight modifications of an existing species or so dramatic that a new species arises, certainly is not a novel one. From Charles Darwin through the neo-Darwinian synthesis to modern-day molecular biology, the theory of evolution (from slight modifications to production of new taxa) by natural selection on heritable traits has become the underlying theme for the biological sciences. Rather than question whether species change over time, it seems more valid to ask: To what extent do particular species change through time? Closely related species of the same genus typically are differentiated based on morphological characters. Are these differences in morphology the only changes that separate extant species from their common ancestor(s)? Or, do substantial changes also occur in the physiological and ecological requirements and tolerances within a lineage of plant species from the ancient ancestor to the extant relative?

There are two ideas regarding physiological and ecological changes in a plant lineage. In his paper on oak biogeography from the Tertiary to the present, the late Daniel I. Axelrod (1983) proposed that despite moderate morphological differences the ecological tolerances and physiological requirements within

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lineages have changed very little, or not at all. Axelrod's conclusions undoubtedly were influenced by his adherence to the Arcto-Tertiary Geoflora concept of palaeobotanist Ralph Chaney (e.g. 1940, 1947, 1959), and thus his rejection of the idea that taxa move into plant associations significantly different from those of their ancestral communities. This concept refers to the temperate mixed forest with a characteristic assemblage of deciduous dicotyledonous angiosperms and deciduous and evergreen gymnosperms (Vankat, 1979) that evolved at high latitudes of the northern hemisphere during the late Cretaceous and early Tertiary and then moved, as an intact unit, to lower latitudes as climatic cooling of the Earth commenced in the late Eocene–early Oligocene. By the Miocene, there was a circumglobal band of forest composed of essentially the same taxa across the middle latitudes of the northern hemisphere. The taxa themselves were about the same as those present in the forests of higher latitudes in the early Tertiary. The key point here is that the individual taxa did not evolve in response to changing climate. They 'simply' moved to areas where the climate was similar to the one in which they had evolved and in which they already were adapted. Inherent in this concept, then, is the idea that there was little or no change (stasis) in the physiology or ecology of the taxa, since there was no need to adapt to new climatic regimes (Axelrod, 1983; Graham, 1999).

Even palaeobotanists who do not support the idea of an Arcto-Tertiary Geoflora have not ruled out the possibility that at least some taxa have basically retained the same physiological characteristics as those of their ancient ancestors. In a paper on evaluation of plant phylogeographic hypotheses, Tiffney and Manchester (2001) (see also Tiffney, 1994) discussed the idea of 'physiological uniformitarianism'. This term is used in reference to extant plant species that may have retained the majority of the same physiological adaptations present in their ancient ancestors in similar climates. These authors state that when faced with the general cooling trend of the Tertiary, taxa could have 'chosen' to migrate to areas that retained a climate to which they were already adapted, which Tiffney and Manchester suggest might have been 'easier' for plants than to evolve new physiological tolerances.

The idea that species within certain plant lineages change very little with regard to physiology and ecology has not been restricted to palaeobotanists. Based on a significant correlation in area of geographic range, ecologists Ricklefs and Latham (1992) and Qian and Ricklefs (2004) advocate the idea of evolutionary stasis, since the mid to late Tertiary (25 million to 5 million years ago), of traits related to the ecological distribution of perennial herbs relict to temperate forests of East Asia (eAs) and eastern North America (eNA). Qian and Ricklefs (2004, p. 262) state, 'The strong parallelism in area of geographical range for herbaceous genera [between eAs and eNA] is consistent with the hypothesis that the extant herbaceous disjunct genera have exhibited stable ecological requirements and tolerance over evolutionary time.' These authors suggest that the extant disjunct taxa of perennial herbs in the temperate forests of eAs and eNA have retained the ecological requirements and tolerances that allowed them to proliferate in these forests for millions of years. Thus, the ancestral species that adapted to the Tertiary forests gave rise to newer species that retained the ecological attributes of their ancient relatives, thereby preserving the ecological requirements and tolerances of the lineage. These authors seem convinced that the perennial herbs have maintained this long-term stasis of ecological attributes throughout the lineage, and that this has determined their distribution within the temperate forests of Asia and North America.

Although Ricklefs and Latham (1992) and Qian and Ricklefs (2004) advocated ecological trait stasis in perennial herbs relict to the temperate forests of eAs and eNA, they concluded that this evolutionary conservatism does not occur in the relict woody plants of the two regions. However, results of a recent study by Svenning (2003) are contrary to the suggestions of these two studies. Svenning found 'strong evolutionary conservatism' in climatic requirements for genera that went extinct in cool-temperate Europe in the Plio-Pleistocene, but still survive in Eurasia and North America. The conclusion of Svenning supports the argument of Axelrod (1983) that the ecological tolerances of woody taxa (and herbaceous ones too) of the 'Arcto-Tertiary' forest have not changed greatly since its break-up in the Neogene.

The other idea is that taxa within lineages have changed their physiological and ecological tolerances and requirements (adaptation) as they moved into new vegetation zones and were exposed to climatic changes through geological time. Jack A. Wolfe has been the foremost proponent of this view. He states that the fossil record does, in fact, support the idea that not only are plant lineages capable of adapting to new environmental conditions, but also that their physiological and ecological tolerances almost certainly have changed through time (Wolfe, 1969, 1972, 1975, 1977). Wolfe (1972, 1975, 1977) rejects the idea of the Arcto-Tertiary Geoflora concept and instead favours the Boreotropical hypothesis (Wolfe, 1975; Tiffney, 1985a, b). According to this hypothesis, forests that contained tropical, paratropical (near-tropical) and temperate genera developed in many different localities in the middle latitudes of the northern hemisphere during the Eocene (Wolfe, 1972; Tiffney, 1985a, b; Lavin and

Luckow, 1993; Lavin and Sousa S., 1995). Unlike the Arcto-Tertiary Geoflora, the Boreotropical flora was not a continuous band of forests around the globe *per se*, but rather an aggregate of similar (although not fully homogeneous) forest types found on several continents (Graham, 1999). Elements of these forests spread to different areas within the middle latitudes via the Bering and North Atlantic land bridges and along shores of the Tethys Seaway (Tiffney, 1985a, b). These Eocene forests experienced climatic changes and geographic disruptions later in the Tertiary that resulted in their isolation, after which they continued to develop independently of each other. Thus, the Boreotropical hypothesis allows for the idea that lineages of species could have changed their physiological and ecological characteristics in response to the changing climate that characterized much of the later Tertiary. Wolfe (1972) comments that the members of plant lineages likely have entered into and departed from the vegetation types in which they evolved as the tolerances of the individual species of the lineages have changed through time.

The purpose of our study was to test these ideas on a small subclade of *Aristolochia* subgenus *Siphisia* (González and Stevenson, 2002) by determining if requirements for dormancy-break and germination have diverged within the group. Thus, data were collected from a series of detailed experiments on physiological responses of seeds of four closely related, woody-vine species in this subgenus: the three US species, *A. californica* Torr., *A. macrophylla* Lamk. and *A. tomentosa* Sims, and the East Asian species *A. manshuriensis* Komarov (Fig. 1).

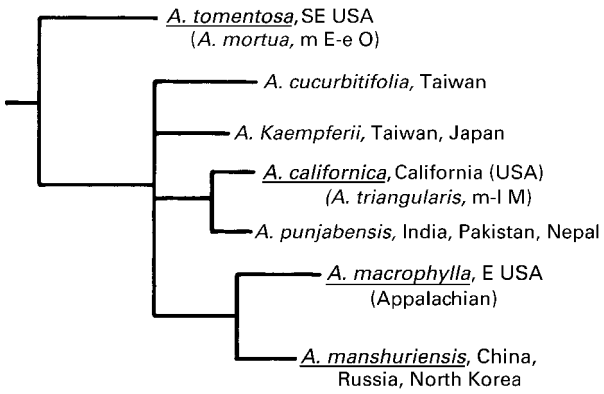

Figure 1. Cladogram of a subclade within the *Aristolochia* subgenus *Siphisia*, with geographical distribution indicated for each of the seven species. Fossil records are indicated for *A. mortua* (*A. tomentosa*) and *A. triangularis* (*A. californica*). m E-e O = middle Eocene–early Oligocene and m-l M = middle–late Miocene. Names of the study species are underlined. (Cladogram redrawn and modified from González and Stevenson, 2002.)

Dormancy-breaking and germination characteristics represent ecological attributes, as well as the underlying physiological controlling mechanisms, that (assuming correct identification of fossil leaves) have evolved in this group of *Aristolochia* species over their long evolutionary history, beginning at least as far back as the Eocene. MacGinitie (1937) discovered fossil leaves of *A. triangularis* in the Weaverville Beds of Trinity County, California, which, at that time, were thought to be middle–late Eocene in age. The beds are now known to be Miocene in age (Dr Jack A. Wolfe, Desert Laboratory, University of Arizona, personal communication, 2001; see Fig. 1). MacGinitie states that this plant most closely resembles *A. californica* with regard to leaf morphology. He also reported fossil leaves of *A. mortua* from the latest Eocene–earliest Oligocene (see Evanoff *et al.*, 2001) Florissant Beds of Colorado (MacGinitie, 1953) and from the late middle Eocene (see Graham, 1999) Green River flora of north-western Colorado and north-eastern Utah (MacGinitie, 1969). MacGinitie states that this species most closely resembles *A. tomentosa*.

This study is also the first one on seed dormancy and germination in the genus *Aristolochia*. Furthermore, we know of only two such reports in the literature on taxa within this large family of 350–500 species (Mabberley, 1997; González and Stevenson, 2002). *Asarum canadense* L. (Baskin and Baskin, 1986) and *Hexastylis heterophylla* Small (Adams *et al.*, 2003) were found to have deep, simple epicotyl MPD (*sensu* Baskin and Baskin, 2004).

Materials and methods

Seeds

Thirty to 40 mature fruits of *A. macrophylla* were collected from 10–12 vines on the north slope of Pine Mountain in Letcher County, Kentucky, in October 1999 and 2000. Numerous mature fruits of *A. tomentosa* were collected from 25–30 vines on the banks of the Cumberland River in Montgomery County, Tennessee, in November 2000 and in October 2001. Approximately 40 mature fruits of *A. californica* were collected from vines along Chico Creek in Butte County, California, in September 2000 and 2001. Seeds of these three species were removed from the fruits, mixed together in a metal pan, and allowed to dry on a bench in the laboratory for 3–5 d before germination studies were begun. Mature fruits of *A. manshuriensis* were collected in late October 2001 from uplands of Vladivostok, Russia, and seeds were sent by airmail to the University of Kentucky in mid-December 2001. Vladivostok is in a cool temperate climatic zone (see map by C. Troll/K.H. Paffen in Müller, 1982) and has deciduous forests (Wolfe, 1979; Breckle, 2002).

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The four *Aristolochia* species used in this study have an underdeveloped embryo and therefore either morphological dormancy (MD) or morphophysiological dormancy (MPD) (Nikolaeva, 1977; Baskin and Baskin, 1998, 2004; Geneve, 1998). Thus, there must be pregermination growth of the embryo before the radicle can emerge from the seed. Baskin and Baskin (1998) consider seeds to be morphologically dormant if embryo growth and radicle emergence are completed in suitable environmental conditions within 30 d. However, if seeds also require a cold- (0–10°C), warm- (≥15°C) or warm- plus cold-stratification pretreatment before germination can occur, then there is also a physiological component to dormancy (Baskin and Baskin, 1998). Thus, seeds would have MPD (Nikolaeva, 1969, 1977; Baskin and Baskin, 1998, 2004).

There are two major categories of MPD: simple and complex. In seeds with simple MPD, embryos grow only at warm-stratifying temperatures, while in those with complex MPD embryos grow only at cold-stratifying temperatures (Baskin and Baskin, 2004). Further, there are five levels of simple MPD and three of complex MPD. Levels are distinguished from one another based on depth (i.e. level) of physiological dormancy (PD) in the embryo (non-deep, intermediate and deep) or, in the case of deep, simple epicotyl MPD and deep, simple, double MPD, on the level of PD in the radicle and in the epicotyl (Nikolaeva, 2001; Baskin and Baskin, 2004).

Germination studies

Germination studies were carried out in temperature- and light-controlled incubators and a refrigerator equipped with a light and timer. Five incubators were set at 12/12 h daily alternating temperature regimes of 35/20, 30/15, 25/15, 20/10 and 15/6°C and two at constant 10 and 15°C. Seeds were cold stratified at 5°C. The light source for both incubation and stratification was cool white fluorescent tubes, and photon irradiance (400–700 nm) at seed level was about 40 $\mu mol\, m^{-2} s^{-1}$. The daily photoperiod was 14 h (hereafter 'light'), and it extended from 1 h before the beginning of the high-temperature period to 1 h after the beginning of the low-temperature period.

Seeds were placed in 5.5-cm-diameter Petri dishes on white quartz sand moistened with distilled water, which was added as needed. All dishes were wrapped with plastic film to reduce moisture loss during incubation and stratification. In the two experiments involving darkness, dishes were wrapped additionally with aluminium foil, and seeds were examined in the dark using a green 'safe' light (Walck *et al.*, 2000). There were three replications of 50 seeds per dish per treatment for *A. macrophylla* and *A. californica* and 30 seeds per dish per treatment for *A. tomentosa* and *A. manshuriensis*.

Emergence of radicle was the criterion for germination. Any seeds ungerminated at the end of a treatment were examined under a dissecting microscope to determine whether the embryo was firm and white, indicating they were viable, or soft and brown, indicating they were non-viable. Further, a firm endosperm indicated viability, and a soft, almost-liquid one indicated non-viability. More than 95% of seeds of the four species of *Aristolochia* used in this study were viable. For each experiment, germination percentages were based on number of viable seeds, and means (± SE) were calculated.

Aristolochia macrophylla

Freshly matured seeds collected in 1999 and in 2000 were incubated in light at the five alternating temperature regimes and at 5°C for 90 d to determine the effect of temperature on germination of non-stratified seeds. The effect of cold stratification was tested by keeping fresh seeds from 1999 on moist sand at 5°C for 0 (control), 4, 8, 10 or 12 weeks and then incubating them at 25/15°C in light for 60 d.

The effect of light versus darkness on germination of both stratified and non-stratified seeds was tested in a 'criss-cross' experiment. Fresh seeds collected in 2000 were cold stratified (S) in light (L) or in darkness (D) for 12 weeks and then incubated at the five alternating temperature regimes (I) in light or in darkness for 90 d. The treatments were as follows: (1) 12 weeks SL → 90 d IL; (2) 12 weeks SL → 90 d ID; (3) 12 weeks SD → 90 d ID; and (4) 12 weeks SD → 90 d IL. Controls were fresh (non-stratified) seeds incubated continuously for 174 d (12 weeks + 90 d) in L or in D.

Aristolochia manshuriensis

Approximately 2-month-old seeds, collected in 2001, were incubated in light at 35/20, 25/15 and 15/6°C for 90 d to determine the effect of temperature on germination. Only three regimes were used, due to a limited number of seeds. To test the effect of cold stratification, seeds were kept on moist sand at 5°C for 12 weeks and then moved to 25/15 and to 15/6°C in light. In a third ('move-along') experiment, seeds were kept at 25/15°C for 12 weeks, moved to 5°C for 12 weeks and finally to 15/6°C for 12 weeks, simulating the general field conditions seeds may be exposed to between dispersal in autumn and germination in spring.

Aristolochia tomentosa

Fresh seeds collected in 2000 and in 2001 were incubated in light at the five alternating temperature regimes and at 5°C for 90 d to determine the effect of

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temperature on germination. The effect of cold stratification was tested by keeping fresh seeds on moist sand at 5°C for 0, 4, 8, 10 or 12 weeks and then incubating them in light at the five alternating temperature regimes for 60 d. In a third ('criss-cross') experiment, fresh seeds collected in 2001 were subjected to the same combinations of temperature and light/dark conditions as described above for *A. macrophylla*.

Aristolochia californica

Two experiments were done to determine the effect of temperature on germination. In the first one, fresh seeds collected in 2000 were incubated in light at the five alternating temperature regimes for 16 weeks. However, very few seeds germinated. Thus, in a second experiment fresh 2001 seeds were incubated in light at 25/15 and 20/10°C (late summer–autumn temperatures) and at 15/6, 15, 10 and 5°C (winter and early spring temperatures) (Müller, 1982) for 16 weeks.

Depending on the time seeds of *A. californica* are dispersed (from mid-September to early November) and on the time cool-season rains begin, seeds in the field may be exposed to warm-stratifying temperatures in autumn before they are exposed to cold-stratifying temperatures in winter. Thus, we tested the effects of warm plus cold stratification and of cold stratification only on dormancy-break and germination. First, to evaluate the effect of a warm-stratification pretreatment on germination, seeds collected in 2001 were: (1) kept on moist sand at 25/15°C for 2, 4 or 6 weeks and then incubated at 15, 10 or 5°C for 18 weeks; and (2) warm stratified at 25/15°C for 4 weeks, cold stratified at 5°C for 0, 2, 4, 6, 8, 10 and 12 weeks and then incubated at 15°C for 60 d. Secondly, to determine the effect of cold stratification only on dormancy-break, seeds collected in 2001 were kept on moist sand at 5°C for (1) 8 and 12 weeks and then moved to 15°C; and (2) 8 weeks and then moved to 10 and 15°C.

Additionally, seeds from 2001 were subjected to three ('move-along') experiments designed to simulate the sequence of natural conditions in the habitat of *A. californica* between dispersal in September, October or November and germination in spring: (1) September, 4 weeks at 25/15 → 4 weeks at 20/10 → 4 weeks at 15/6 → 4 weeks at 10 → 4 weeks at 15/6°C; (2) October, 4 weeks at 15/6 → 4 weeks at 10 → 4 weeks at 5 → 4 weeks at 10 → 8 weeks at 15/6°C; and (3) November, 4 weeks at 10 → 4 weeks at 5 → 12 weeks at 15/6°C.

Embryo growth studies

The same temperature/light regimes described above under 'Germination studies' were used in embryo growth studies. Seeds were placed in 5.5-cm-diameter Petri dishes on white quartz sand moistened with distilled water. Water was added to dishes as needed; seeds were never allowed to dry out. All dishes were wrapped with plastic film to reduce water loss during incubation and stratification. At intervals during the growth studies, embryos were excised from seeds under a dissecting microscope using a single-edge razor blade, and lengths of whole embryos, cotyledons and embryo axes were measured with a micrometer.

Mean critical embryo length for germination

To be able to assign a length (size) to an embryo (and not to the seedling) in a seed that had germinated between monitoring intervals, it was necessary to determine the critical embryo length for radicle emergence in each species. Fifty seeds were selected randomly from seeds of *A. macrophylla* collected in 2000, *A. tomentosa* in 2001 and *A. californica* in 2001. Embryos were excised and measured as soon as the radicle tip could be observed emerging from the seed, i.e. an embryo had attained its maximum length. The 50 measurements for embryos in seeds of each species were averaged to determine mean critical length (± SE) that embryos must attain for germination (i.e. radicle emergence) to occur. Therefore, if a seed already had germinated when embryo measurements were made, the critical embryo length (for germination) was assigned to that embryo.

Aristolochia macrophylla

Rate of embryo growth was monitored for fresh seeds, collected in 2000, in light at 25/15°C, and for those collected in 2000 cold stratified for 12 weeks at 5°C and then incubated in light at 25/15°C. For seeds kept continuously at 25/15°C, embryos from a sample of 25 seeds were excised at time 0 and at 5-d intervals for 60 d. Lengths of embryos, cotyledons and axes were measured and means (± SEs) calculated. For seeds pretreated at 5°C and then moved to 25/15°C, lengths of embryos, cotyledons and axes for 25 seeds were measured at time 0, after 12 weeks of cold stratification at 5°C and daily for 18 d at 25/15°C, and means (± SEs) were calculated.

Aristolochia tomentosa

Rate of embryo growth was monitored for fresh seeds, collected in 2001, in light at 35/20°C and for those cold stratified for 12 weeks at 5°C and then incubated at 35/20°C. For seeds kept continuously at 35/20°C, embryos from a sample of 25 seeds were excised at time 0 and every 3 d for 33 d. Lengths of embryos, cotyledons and axes were measured and means (± SEs) calculated. For seeds cold stratified at 5°C and then moved to 35/20°C, lengths of embryos, cotyledons and axes were measured for 25 seeds at time 0,

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after 12 weeks of cold stratification at 5°C and daily for 10 d at 35/20°C, and means (± SEs) were calculated.

Aristolochia californica

Rate of embryo growth was monitored for seeds kept continuously at 10°C in light for 14 weeks and for seeds subjected to the following temperature regimes in light: 25/15 (4 weeks) → 5 (8 weeks) → 15°C (6 weeks). In both treatments, embryos from 25 seeds each were excised at weekly intervals. Lengths of embryos, cotyledons and embryo axes were measured, and means (± SEs) were calculated.

Results

Germination studies

Aristolochia macrophylla

For both 1999 and 2000, no seeds germinated at 5°C, and only about 10% of them did so at 35/20°C. At the other four temperature regimes, non-stratified seeds germinated to about 60–70% (1999, data not shown) and 50–70% (2000, Fig. 2), and they did not begin to germinate until after 10–20 d of incubation, depending on the temperature. The optimum temperature for germination (based on both rate and final percentage) was 25/15°C, with 30/15°C being a close second. Cold stratification for 4–12 weeks subsequently increased both rates and final germination percentages at 25/15°C (Fig. 3).

In the 'criss-cross' experiment, non-stratified (control) seeds germinated to 55–73% in light over the 15/6–30/15°C range but to only 10% at 35/20°C. In constant darkness, 40–61% of the seeds germinated at 15/6–30/15°C but only 13% at 35/20°C. Germination across the four light/dark treatments (SL → IL, SD → IL, SD → ID, SL → ID) was 76–95% at 15/6–30/15°C and 15–35% at 35/20°C (data not shown).

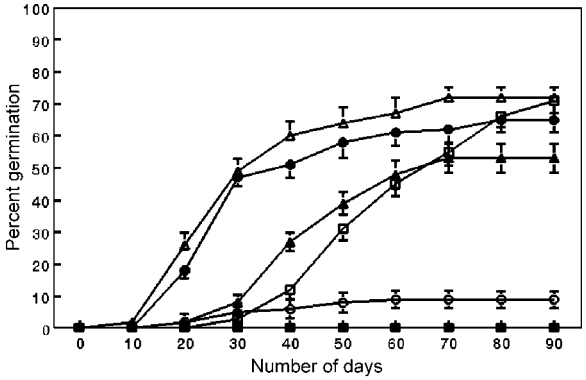

Figure 2. Mean (± SE) percent germination of fresh seeds of *Aristolochia macrophylla* collected in 2000 and incubated in light at 35/20°C (○), 30/15°C (●), 25/15°C (△), 20/10°C (▲), 15/6°C (□) and 5°C (■) for 90 d.

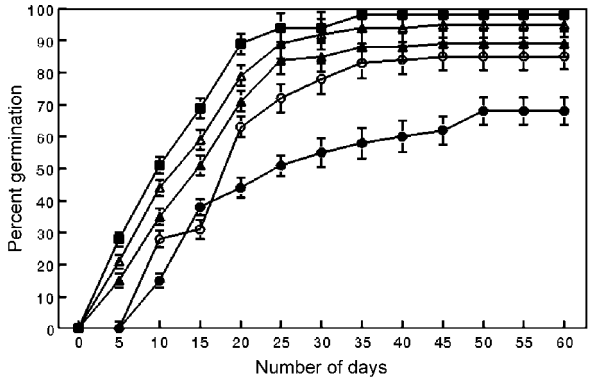

Figure 3. Mean (± SE) percent germination of fresh seeds of *Aristolochia macrophylla* collected in 1999 and incubated in light at 25/15°C for 60 d following 0 (●), 4 (○), 8 (▲), 10 (△) and 12 (■) weeks of cold stratification at 5°C, where no seeds germinated.

Aristolochia manshuriensis

Fresh seeds germinated at all three temperature regimes, with highest germination percentages at 25/15 and 15/6°C. However, these germination percentages were still relatively low, and none was higher than 53% (data not shown). In contrast, seeds cold stratified at 5°C for 12 weeks and then moved to 25/15 or 15/6°C germinated to high percentages (Fig. 4). However, the rate of germination was faster at 25/15°C than at 15/6°C. In the 'move-along' experiment, seeds had germinated to 39% after 12 weeks at 25/15°C, and then germination increased to 97% at 15/6°C following 12 weeks at 5°C, where no seeds germinated (data not shown).

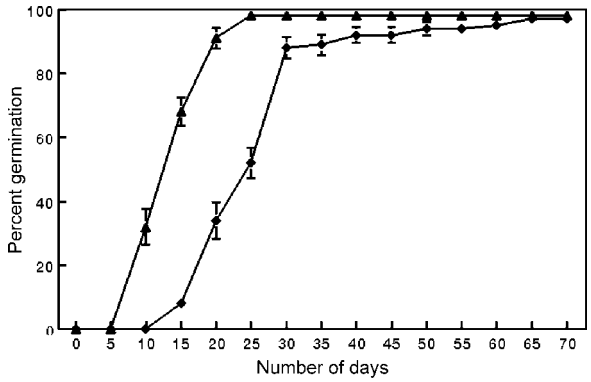

Figure 4. Mean (± SE) percent germination for seeds of *Aristolochia manshuriensis* collected in 2001, cold stratified at 5°C for 12 weeks and then incubated at 25/15°C (▲) and 15/6°C (◆) for 70 d. No seeds germinated at 5°C.

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Aristolochia tomentosa

Fresh seeds germinated at all five alternating temperature regimes, but not at constant 5°C, in 2000 (Fig. 5) and in 2001 (data not shown). Highest germination in both years was at 35/20°C, with 78% in 2000 and 79% in 2001. Seeds germinated to at least 60% in all other alternating temperature regimes in both years. Cold stratification increased both rates and final germination percentages at all temperature regimes in both years [Fig. 6 (25/15°C); data for other temperatures not shown]. Germination was ≥85% after 15–25 d at all temperature regimes following 4, 8, 10 or 12 weeks of cold stratification, except at 15/6°C, where seeds stratified for 4 weeks took 45–50 d to reach 80–90% (data not shown).

In the 'criss-cross' experiment, non-stratified (control) light- and dark-incubated seeds germinated to 58–79% and 51–78%, respectively, over the 15/6–35/20°C temperature range. Germination across the four light/dark treatments (SL → IL, SD → IL, SD → ID, SL → ID) ranged from 90 to 99% over the 15/6–35/20°C temperature range (data not shown).

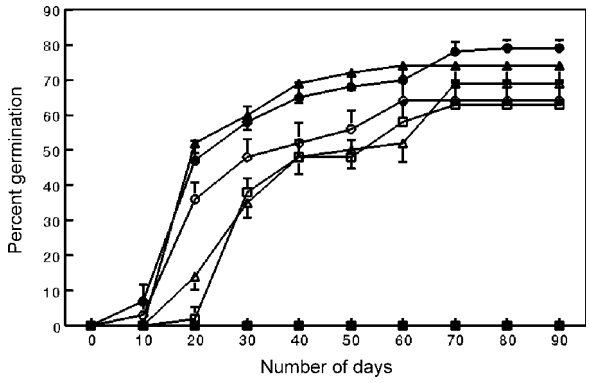

Figure 5. Mean (± SE) percent germination of fresh *Aristolochia tomentosa* seeds collected in 2000 and incubated in light at 35/20°C (•), 30/15°C (○), 25/15°C (▲), 20/10°C (△), 15/6°C (□) and 5°C (■) for 90 d.

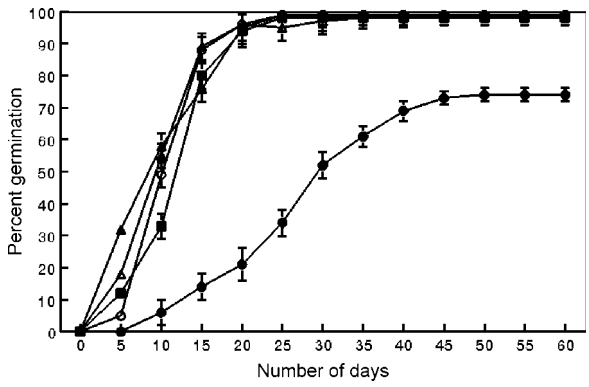

Figure 6. Mean (± SE) percent germination of fresh seeds of *Aristolochia tomentosa* collected in 2000 and incubated at 25/15°C for 60 d following 0 (•), 4 (○), 8 (▲), 10 (△) and 12 (■) weeks of cold stratification at 5°C, where no seeds germinated.

Aristolochia californica

After 16 weeks of incubation, seeds (collected in 2000) did not germinate at 35/20, 30/15, 25/15 or 5°C, and only to 1% at 20/10, 4% at 15/6 and 3% at 15°C. The best germination (seeds collected in 2001) was at 10°C, where seeds had germinated to 35% after 8 weeks, to 67% after 12 weeks and to 74% after 16 weeks (data not shown).

Whereas seeds warm stratified at 25/15°C for 2, 4 or 6 weeks and then moved to 5 or 15°C germinated to <10%, those moved to 10°C had germinated to more than 90% after 16 weeks (data not shown). Germination percentages of seeds given a warm plus cold treatment and then moved to 15°C increased with amount of cold stratification they received. Thus, seeds cold stratified for 10 and 12 weeks germinated to 97 and 98%, respectively, following warm + cold stratification (Fig. 7).

Dormancy was broken in a high percentage of the seeds by cold stratification without first exposing them to a period of warm stratification. In the first experiment, seeds pretreated at 5°C for 8 and 12 weeks germinated to 97 and 99%, respectively, after they were moved to 15°C, while those in the controls germinated to >90% at 10°C but to <20% at 5 and 15°C (data not shown). In the second experiment, seeds cold stratified for 8 weeks germinated to 87 and 90% after they were moved to 10 and 15°C,

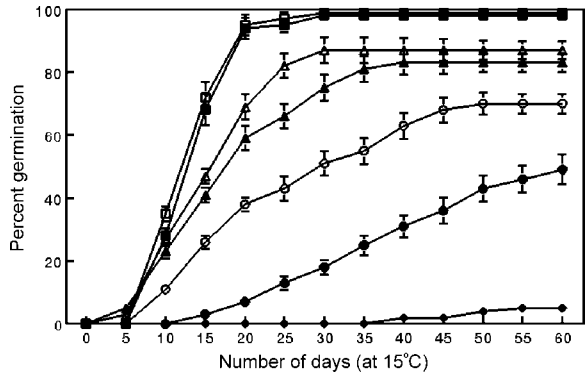

Figure 7. Mean (± SE) percent germination of fresh seeds of *Aristolochia californica* collected in 2001 and moved to 15°C following warm (25/15°C) + cold (5°C) stratification. Seeds were given warm stratification for 4 weeks followed by cold stratification for various periods: 0 (◆), 2 (•), 4 (○), 6 (▲), 8 (△), 10 (■) and 12 (□) weeks.

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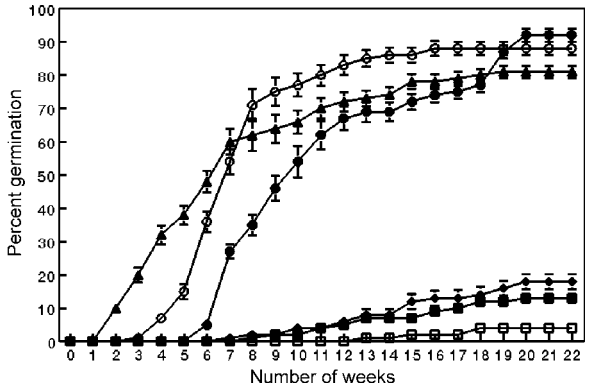

Figure 8. Mean (± SE) percent germination of seeds of *Aristolochia californica* collected in 2001 and incubated at 10 and 15°C following cold stratification. Seeds were cold stratified at 5°C for 8 weeks (no germination) and then moved to 15°C (○) or 10°C (▲). Controls were seeds kept at 15/6°C (◆), 15°C (□), 10°C (•) and 5°C (■).

respectively, while those in the controls germinated to 90% at 10°C but to <20% at 5, 15 and 15/6°C (Fig. 8).

Seeds germinated to high percentages in all three move-along experiments. Thus, seeds dispersed in September (4 weeks at 25/15 → 4 weeks at 20/10 → 4 weeks at 15/6 → 4 weeks at 10 → 4 weeks at 15/6°C) germinated to 96% (Fig. 9), those dispersed in October (4 weeks at 15/6 → 4 weeks at 10 → 4 weeks at 5 → 4 weeks at 10 → 8 weeks at 15/6°C) to 95% (data not shown), and those dispersed in November (4 weeks at 10 → 4 weeks at 5 → 12 weeks at 15/6°C) to 86% (data not shown).

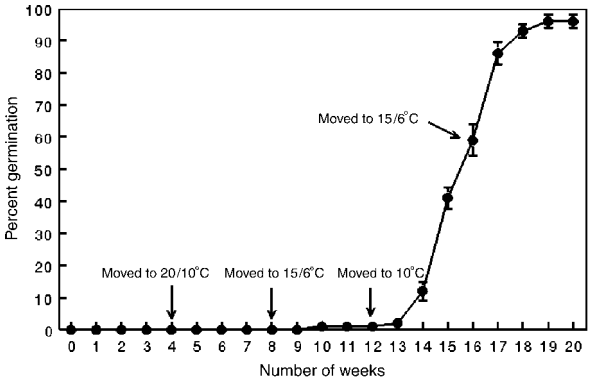

Figure 9. Mean (± SE) percent germination of fresh seeds of *Aristolochia californica* collected in 2001 and moved through a sequence of temperature regimes simulating field conditions between dispersal in September and germination in spring. Seeds were placed at 25/15°C (week 0) for 4 weeks, then moved to 20/10°C for 4 weeks, to 15/6°C for 4 weeks, to 10°C for 4 weeks and finally back to 15/6°C for 4 weeks.

Embryo growth studies

Mean critical embryo length for germination

Mean (± SE) lengths of embryos in fresh seeds were 1.92 ± 0.05, 2.49 ± 0.05 and 2.44 ± 0.05 mm for *A. macrophylla*, *A. tomentosa* and *A. californica*, respectively. For seeds to germinate, embryos must reach a mean critical length of 4.15 ± 0.08 mm in *A. macrophylla*, 6.12 ± 0.11 mm in *A. tomentosa* and 5.69 ± 0.10 mm in *A. californica*. The critical length for germination was not determined for *A. manshuriensis* due to an insufficient number of seeds, but it is assumed to be the same as that for *A. macrophylla* since seed size, embryo length and the embryo length:seed length ratio in fresh seeds are nearly identical (Adams *et al.*, 2005).

Aristolochia macrophylla

Embryos in seeds incubated at the optimal germination regime of 25/15°C increased from an initial mean length of 1.92 ± 0.05 mm to the mean required length for radicle emergence (4.15 ± 0.08 mm) in 55 d (Fig. 10). Growth began between days 15 and 20 [with some embryos reaching the critical length and seeds germinating by day 20 (see Fig. 2)], and continued steadily until all embryos had reached the critical length for germination by day 55. In fresh seeds, average length of cotyledons was 0.96 ± 0.05 mm and average length of axis 1.04 ± 0.03 mm. At the time of radicle emergence, the axis averaged 2.31 ± 0.08 mm in length and the cotyledons 1.84 ± 0.11 mm (Fig. 10). In contrast, embryos began to grow within about 5–6 d after the cold-stratified seeds were moved from 5 to 25/15°C. All embryos had reached the mean critical length for germination (4.15 mm) by day 17 (data not shown).

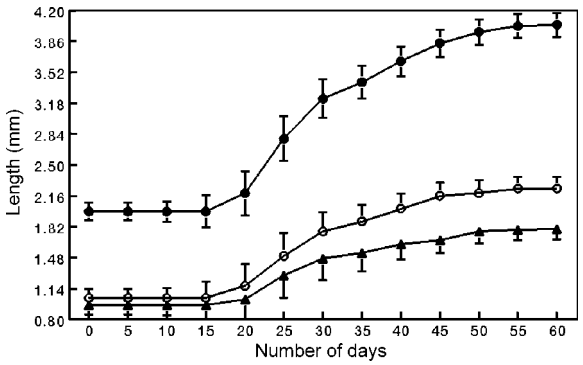

Figure 10. Embryo growth (mm, mean ± SE) in *Aristolochia macrophylla* seeds collected in 2000 and incubated at 25/15°C for 60 d. Embryos from 25 seeds were excised at time 0 and then every 5 d for 60 d, and lengths of the whole embryo (•), axis (○) and cotyledons (▲) measured.

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Thus, embryos grew much faster in cold-stratified seeds than they did in those that were not stratified. No embryo growth occurred in seeds during the 12-week cold stratification period.

Aristolochia tomentosa

Embryos in seeds incubated at the optimal germination regime of 35/20°C increased from an initial mean length of 2.42 ± 0.08 mm to the mean required length for radicle emergence (6.12 ± 0.12 mm) in 30 d (data not shown). Growth of embryos began almost immediately upon imbibition, with many embryos reaching critical length and seeds germinating by day 20 (see Fig. 5), and continued until all of them reached the mean critical length for germination by day 30. In fresh seeds, cotyledons and axis had an average length of 1.28 ± 0.06 mm and 1.16 ± 0.08 mm, respectively. At the time of radicle emergence, length of cotyledons and axis averaged 2.66 ± 0.06 mm and 3.46 ± 0.09 mm, respectively. For seeds cold stratified at 5°C, no embryo growth occurred during the 12-week stratification period. After seeds were moved to 35/20°C, embryos began to grow almost immediately (Fig. 11), and all of them had reached the mean critical length for germination (6.12 mm) by day 7.

Aristolochia californica

Growth of embryos in seeds incubated at 10°C began between weeks three and four [with many embryos reaching critical length and seeds germinating by week six (see Fig. 8)], and reached the mean critical length for germination (5.69 ± 0.10 mm) by week 12 (Fig. 12). Average length of cotyledons in freshly matured seeds was 1.06 ± 0.05 mm and that of axis

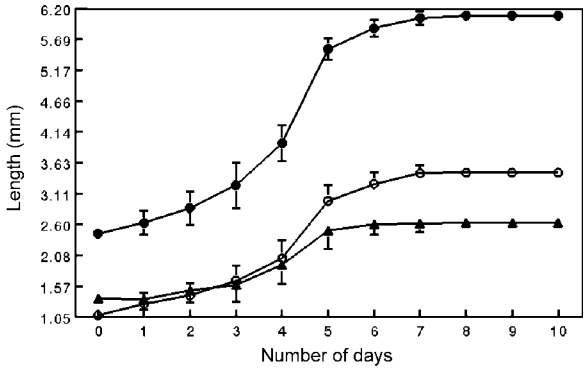

Figure 11. Embryo growth (mm, mean ± SE) in *Aristolochia tomentosa* seeds collected in 2001 and cold stratified at 5°C for 12 weeks, and then moved to 35/20°C for 10 d. Embryos from 25 seeds were excised at time 0 (following 12 weeks of cold stratification) and then every day for 10 d, and lengths of whole embryo (•), axis (○) and cotyledons (▲) measured. No growth occurred at 5°C.

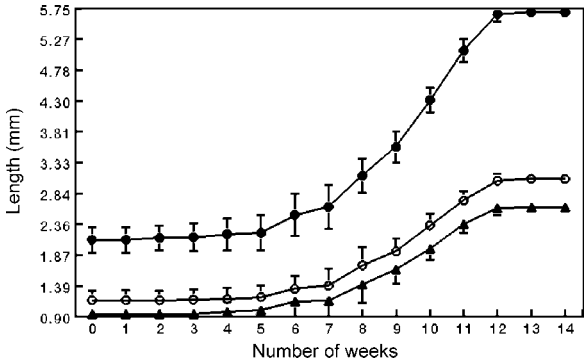

Figure 12. Embryo growth (mm, mean ± SE) in *Aristolochia californica* seeds collected in 2001 and incubated at 10°C. Embryos from 25 seeds were excised at time 0 and then once every week for 14 weeks, and lengths of the whole embryo (•), axis (○) and cotyledons (▲) measured.

1.24 ± 0.08 mm. These two embryo parts increased at about the same rate until week 10 or 11 of incubation, after which the axis grew slightly faster than the cotyledons until all embryos reached the critical length for germination (week 12). At this time, cotyledons had increased to a mean length of 2.62 ± 0.10 mm and axis to 3.07 ± 0.09 mm.

In the 'move-along' experiment, embryos in seeds incubated for 4 weeks at 25/15°C did not grow at all, and growth did not begin until after seeds were moved to 5°C. Some growth had occurred after 3 weeks at 5°C, and embryos grew very slowly for the next 5 weeks at this temperature. Embryos grew rapidly after seeds were moved to 15°C, and by week 17 they had reached the critical length for germination (data not shown).

Discussion

Using guidelines established by Baskin and Baskin (1998), fresh seeds of *A. macrophylla* that germinated within 30 d have only morphological dormancy (MD). By this cut-off period, the highest germination percentages in both years occurred at 25/15°C, where 47% of seeds germinated in 1999 and 50% in 2000. The remaining non-germinated seeds also had a physiological component of dormancy (Nikolaeva, 1977; Baskin and Baskin, 1998) and thus morpho-physiological dormancy (MPD). Between days 30 and 90, additional seeds of *A. macrophylla* germinated, but even at 25/15°C, the optimal regime, 30% of seeds in 1999 (data not shown) and 28% of those in 2000 had not germinated after 90 d (Fig. 2), or even after 150 d of incubation (C.A. Adams, unpublished). Further, at 25/15°C in the 'criss-cross' experiment, 28% of the seeds in the light control and 39% of those in the dark

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control remained viable and ungerminated at the end of the 174 d incubation period (data not shown). Thus, these seeds were in a deeper state of physiological dormancy than those that germinated between days 30 and 90. Light was not a strong promoter of germination in either stratified or non-stratified seeds. Thus, although the embryos of seeds collected in 1999 and 2000 had grown to their critical length for germination by 55 d, physiological dormancy prevented them from germinating. This would account for the fact that even though embryos grew to full length in all the seeds in 55 d (Fig. 10), only about 65% of the seeds had germinated by this time (Fig. 2). [This explanation also applies to differences in embryo growth and germination curves for *A. tomentosa* and for *A. californica*.]

Cold stratification increased both rates and percentages of germination after seeds of *A. macrophylla* were moved to warmer temperature regimes. These responses help explain the germination ecology of this species. Seeds are dispersed in mid to late October into November, at which time a fairly high percentage of them can germinate after 50–60 d at prevailing temperatures (e.g. 15/6°C). However, temperatures do not remain at this regime long enough in autumn for the seeds to germinate, and they do not germinate at winter temperatures (e.g. 5°C), even after 3 months or longer. Thus, germination is delayed until temperatures increase to about 15/6 to 20/10°C in early spring. In support of this scenario: (1) no seedlings have been found in the field in autumn, whereas numerous seedlings have been observed in the field in early March; and (2) seeds sown in a non-heated greenhouse in autumn did not germinate until the following spring (Adams, 2003).

For the portion of the *A. macrophylla* seed population that has MPD, both germination and embryo growth data show that these seeds have the non-deep simple level (*sensu* Baskin and Baskin, 2004) of MPD. The physiological component of dormancy break in seeds with this kind of MPD occurs during either warm or cold stratification, depending on the species, but warm temperatures are required for embryo growth and germination (Baskin and Baskin, 2004). Although we assigned seeds of *A. macrophylla* to definite kinds of dormancy (i.e. MD or MPD), it is clear that there is a continuum of physiological states of dormancy within a seed population. Seeds that germinate within 30 d have MD only; embryos begin to grow soon after seeds are placed at favourable temperatures, e.g. 25/15°C. However, seeds in the population that continue to germinate over the next 60 d probably are in various states of non-deep simple MPD, thus needing different periods of time at suitable temperatures to come out of dormancy. Further, within a population, some seeds require cold stratification to come out of dormancy, and these seeds are in the deepest state of non-deep simple MPD. Thus, the continuum of dormancy states in a population of *A. macrophylla* seeds can be visualized as follows: MD → non-deep simple MPD_1 → non-deep simple MPD_2 → → → non-deep simple MPD_x.

Seeds of *A. manshuriensis* and *A. tomentosa* have dormancy-breaking and germination requirements qualitatively similar to those of *A. macrophylla*. Thus, for these two species: (1) seeds germinated best at the warmer incubation regimes but not at all during cold stratification; (2) cold stratification increased both rates and final percentages of germination at warmer temperatures; (3) some seeds in the population have MD and the rest non-deep simple MPD; and (4) embryos did not grow at 5°C, but treatment of seeds at this regime greatly enhanced the rate of embryo growth (inferred in *A. manshuriensis*) at warmer temperature. Light is not required for germination of *A. tomentosa* seeds, which were even less light sensitive than those of *A. macrophylla.* (Light versus dark was not tested on seeds of *A. manshuriensis*.)

In contrast to the other three species of *Aristolochia*, seeds of *A. californica* did not germinate at the warmer temperature regimes. Further, they do not require warm stratification for loss of dormancy. Rather, seeds require a long cold period to come out of dormancy, and they can germinate rapidly once temperatures increase to 10–15°C. Embryos in fresh seeds of *A. californica* can grow at 10°C, but they can grow at 15°C only after seeds have received a period of cold stratification. Thus, unlike the other three species in this study, for which some seeds in a population have MD and others non-deep simple MPD, all seeds within a population of *A. californica* have either intermediate or deep complex MPD (*sensu* Nikolaeva, 1977; Baskin and Baskin, 2004). These dormancy-breaking and germination responses seem well suited for a species that grows in a Mediterranean climate. Dispersal begins near the end of the dry summer season, dormancy is broken during the cool, wet winter and non-dormant seeds germinate in late winter/early spring, allowing seedlings to become well-established by the time the hot, dry season begins. Slow embryo growth at 15°C, as indicated by only 5–10% germination after 22 weeks, in seeds that have not been cold stratified may be important in preventing germination in autumn of seeds dispersed in September or October.

Even the two eastern US species that grow in upland (*A. macrophylla*) and lowland (*A. tomentosa*) temperate broadleaved deciduous forests have diverged in their dormancy-breaking and germination responses. Although both species have non-deep simple MPD, they differ quantitatively in dormancy-breaking and germination requirements. First, seeds of *A. macrophylla* require 12 weeks of cold stratification to germinate to maximum percentages after they are

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moved to warmer regimes, whereas those of *A. tomentosa* require only 4 weeks of cold stratification to do so. Secondly, seeds of *A. tomentosa* germinate best at 35/20°C, while those of *A. macrophylla* germinate best at 25/15°C and poorly at 35/20°C, even after they have been cold stratified. Temperature differences between the two species are also readily evident in the growth responses of embryos within the seeds. These differences in temperature responses in seeds and embryos of *A. macrophylla* and *A. tomentosa* presumably represent adaptations of these two species to upland and lowland habitats, respectively.

The dormancy-breaking and germination requirements of the eastern North American *A. macrophylla* and the East Asian *A. manshuriensis*, as well as both the external and internal morphology of their seeds (Adams *et al.*, 2005), are nearly identical, indicating stasis in seed morphological and physiological traits in these widely disjunct sister species since their geographic and taxonomic divergence, presumably in the Tertiary (see Wen, 1999; Xiang *et al.*, 2000). These findings on the geography of seed dormancy and germination are in agreement with results of some other studies on levels of MPD in closely related taxa disjunct between East Asia (eAs) and eastern North American (eNA). For example, *Jeffersonia* (*Plagiorhegma*) *dubia* (eAs) and *J. diphylla* (eNA), which are closely related phylogenetically (Kim and Jansen, 1998), and four eAs and two eNA *Panax* species, with no intercontinental sister species in the genus (Wen and Zimmer, 1996; Wen, 1999), have deep simple MPD (Nikolaeva *et al.*, 1985; Baskin and Baskin, 1998). However, this geographical pattern in level of MPD does not hold true for all species in genera with an 'Arcto-Tertiary' distribution. For example, the East Asian species *Osmorhiza aristata*, which is ancestral to other members of the genus (Wen *et al.*, 2002), has deep complex MPD, while its eNA relatives *O. longistylis* and *O. claytonii* (eastern North American clade) have non-deep complex MPD (Baskin and Baskin, 1998; Walck *et al.*, 2002; Wen *et al.*, 2002). Thus, physiological stasis (*sensu* Ricklefs and Latham, 1992) has not occurred in all traits of all species of perennial herbs relict to the temperate forests of East Asia and eastern North America.

Our results show that seeds of eastern and western US species in a subclade of *Aristolochia* subgenus *Siphisia* have different levels of MPD. This geographical pattern in kind of seed dormancy has also been reported for other genera of perennial herbs with an 'Arcto-Tertiary' distribution. For example, the two species of *Osmorhiza* in the eastern North American clade have non-deep complex MPD, whereas the three species in the western North American (wNA) clade for which information is available, *O. berteroi* (= *O. chilensis*), *O. occidentalis* (Baskin and Baskin, 1998) and O. *depauperata* (Walck and Hidayati, 2004), have deep complex MPD. Similarly, three eastern North American *Erythronium* (*E. albidum, E. americanum, E. rostratum*) species have non-deep complex MPD, and the only western North American species for which information is available, *E. grandiflorum*, has deep complex MPD (Baskin and Baskin, 1998). Interestingly, the eAs species, *E. japonica*, which represents a different lineage in the same clade as *E. albidum* and *E. americanum* (Allen *et al.*, 2003), is reported to have deep simple epicotyl MPD (Kondo *et al.*, 2002).

Most phylogenetic studies on disjunct taxa in East Asia and eastern North America suggest a closer relationship between species of eNA and wNA than between those of eAs and eNA. Thus, in contrast to earlier thinking, sister species disjunct between these three regions are between eNA and wNA, not intercontinental (Xiang *et al.*, 1998; Wen, 1999). Further, morphological and ecological stasis (i.e. little or no change in morphology or ecology in species over geological time periods) are also thought to be common between the disjuncts (Ricklefs and Latham, 1992; Wen, 1999; Qian and Ricklefs, 2004). However, in the subclade of *Aristolochia* subgenus *Siphisia* investigated in the present study, evolution has not proceeded as such. First, the Appalachian *A. macrophylla* and the East Asian *A. manshuriensis* are sister species [also supported by molecular data (Murata *et al.*, 2001)], and neither is sister to *A. californica* (not included in the molecular study), whose sister species is the Indian *A. punjabensis* (Fig. 1). Secondly, although *A. macrophylla* and *A. manshuriensis* exhibit stasis in seed morphological (Adams *et al.*, 2005) and physiological traits, this is not the case within the entire subclade.

Thus, it is clear that there has been both trait stasis and divergence (adaptation) in both seed morphological and seed physiological traits within a small subclade of *Aristolochia* subgenus *Siphisia*. As such, the opposing ideas of both Axelrod and Wolfe are supported in part by this study, and thus each idea also is not supported in part. Lack of differences in seed physiology in *A. macrophylla* and *A. manshuriensis* supports the idea of Axelrod (1983) that there has been little or no change in ecological and physiological requirements of species within a lineage over geological time, since their phytosociological relationships have not changed greatly from those of their ancestors in the 'Arcto-Tertiary' forest. On the other hand, the differences in seed physiology between *A. macrophylla/A. manshuriensis* and *A. californica,* between *A. macrophylla/A. manshuriensis* and *A. tomentosa* and between *A. tomentosa* and *A. californica* support the idea of Wolfe (1969, 1972, 1975, 1977) that the ecological and physiological characteristics of organisms have changed through geological time as lineages moved into new climatic and vegetation zones.

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References

Adams, C.A. (2003) A comparative study of dormancy-breaking and germination requirements in seeds of four closely-related *Aristolochia* subgenus *Siphisia* species (Aristolochiaceae, Piperales): A test of two hypotheses on ecological changes in species within a lineage through geological time. PhD thesis, University of Kentucky, Lexington, USA.

Adams, C.A., Baskin, J.M. and Baskin, C.C. (2003) Epicotyl dormancy in the mesic woodland herb *Hexastylis heterophylla* (Aristolochiaceae). *Journal of the Torrey Botanical Society* **130**, 11–15.

Adams, C.A., Baskin, J.M. and Baskin, C.C. (2005) Comparative morphology of four closely-related species of *Aristolochia* subgenus *Siphisia* (Aristolochiaceae, Piperales). *Botanical Journal of the Linnean Society* **148** (in press).

Allen, G.A., Soltis, D.E. and Soltis, P.S. (2003) Phylogeny and biogeography of *Erythronium* (Liliaceae) inferred from chloroplast matK and nuclear rDNA ITS sequences. *Systematic Botany* **28**, 512–523.

Axelrod, D.I. (1983) Biogeography of oaks in the Arcto-Tertiary Province. *Annals of the Missouri Botanical Garden* **70**, 629–657.

Baskin, C.C. and Baskin, J.M. (1998) *Seeds: Ecology, biogeography, and evolution of dormancy and germination*. San Diego, Academic Press.

Baskin, J.M. and Baskin, C.C. (1986) Seed germination ecophysiology of the woodland herb *Asarum canadense*. *The American Midland Naturalist* **116**, 132–139.

Baskin, J.M. and Baskin, C.C. (2004) A classification system for seed dormancy. *Seed Science Research* **14**, 1–16.

Breckle, S.-W. (2002) *Walter's vegetation of the earth: The ecological systems of the geo-biosphere* (4th edition). Berlin, Springer-Verlag. [Translated from the 7th, completely revised and enlarged German edition by Gudrun and D. Lawlor.]

Chaney, R.W. (1940) Tertiary forests and continental history. *Bulletin of the Geological Society of America* **51**, 469–488.

Chaney, R.W. (1947) Tertiary centers and migration routes. *Ecological Monographs* **17**, 140–148.

Chaney, R.W. (1959) Miocene floras of the Columbia Plateau. Part I. Composition and interpretation. *Carnegie Institution of Washington Publications. Contributions to Paleontology* **617**, 1–134.

Evanoff, E., McIntosh, W.C. and Murphey, P.C. (2001) Stratigraphic summary and $^{40}Ar/^{39}Ar$ geochronology of the Florissant Formation, Colorado. pp. 1–16 *in* Evanoff, E.; Gregory-Wodzicki, K.M.; Johnson, K.R. (Eds) *Fossil flora and stratigraphy of the Florissant formation, Colorado*. Proceedings of the Denver Museum of Nature and Science Series 4, No. 1.

Geneve, R.L. (1998) Seed dormancy in commercial vegetable and flower species. *Seed Technology* **20**, 236–250.

González, F. and Stevenson, D.W. (2002) A phylogenetic analysis of the subfamily Aristolochioideae (Aristolochiaceae). *Revista de la Academia Colombiana de Ciencias Exactas. Físicas y Naturales* **26(8)**, 25–60.

Graham, A.W. (1999) *Late Cretaceous and Cenozoic history of North American vegetation north of Mexico*. New York, Oxford University Press.

Kim, Y.-D. and Jansen, R.K. (1998) Chloroplast DNA restriction site variation and phylogeny of the Berberidaceae. *American Journal of Botany* **85**, 1766–1778.

Kondo, T., Okubo, N., Miura, T., Honda, K. and Ishikawa, Y. (2002) Ecophysiology of seed germination in *Erythronium japonicum* (Liliaceae) with underdeveloped embryos. *American Journal of Botany* **89**, 1779–1784.

Lavin, M. and Luckow, M. (1993) Origins and relationships of tropical North America in the context of the boreotropics hypothesis. *American Journal of Botany* **80**, 1–14.

Lavin, M. and Sousa S., M. (1995) Phylogenetic systematics and biogeography of the tribe Robinieae (Leguminosae). *Systematic Botany Monographs* **45**, 1–165.

Mabberley, D.J. (1997) *The plant-book. A portable dictionary of the vascular plants* (2nd edition). Cambridge, Cambridge University Press.

MacGinitie, H.D. (1937) The flora of the Weaverville beds of Trinity County, California with descriptions of the plant-bearing beds. *Carnegie Institution of Washington. Contributions to Paleontology* **465**, 1–137.

MacGinitie, H.D. (1953) Fossil plants of the Florissant beds, Colorado. *Carnegie Institution of Washington Publications. Contributions to Paleontology* **599**, 1–198.

MacGinitie, H.D. (1969) The Eocene Green River flora of northwestern Colorado and northeastern Utah. *University of California Publications in Geological Sciences* **83**, 1–203.

Müller, M.J. (1982) *Selected climatic data for a global set of standard stations for vegetation science*. The Hague, The Netherlands, Dr W. Junk Publishers.

Murata, J., Ohi, T., Wu, S.G., Darnaedi, D., Sugawara, T., Nakanishi, T. and Murata, H. (2001) Molecular phylogeny of *Aristolochia* (Aristolochiaceae) inferred from *mat*K sequences. *Acta Phytotaxonomica et Geobotanica* **52**, 75–83.

Nikolaeva, M.G. (1969) *Physiology of deep dormancy in seeds*. Leningrad, Russia, Izdatel'stvo 'Nauka', [Translated from Russian by Z. Shapiro, NSF, Washington, DC, USA.]

Nikolaeva, M.G. (1977) Factors controlling the seed dormancy pattern. pp. 54–74 *in* Khan, A.A. (Ed.) *The physiology and biochemistry of seed dormancy and germination*. Amsterdam, North-Holland.

Nikolaeva, M.G. (2001) Ecological and physiological aspects of seed dormancy and germination (review of

investigations for the last century). *Botanicheskii Zhurnal* **86**, 1–14 (in Russian with English summary).

Nikolaeva, M.G., Rasumova, M.V. and Gladkova, V.N. (1985) *Reference book on dormant seed germination*. Danilova, M.F. (Ed.). Leningrad, 'Nauka' Publishers (in Russian).

Qian, H. and Ricklefs, R.E. (2004) Geographical distribution and ecological conservatism of disjunct genera of vascular plants in eastern Asia and eastern North America. *Journal of Ecology* **92**, 253–265.

Ricklefs, R.F. and Latham, R.E. (1992) Intercontinental correlation of geographical ranges suggests stasis in ecological traits of relict genera of temperate perennial herbs. *The American Naturalist* **139**, 1305–1321.

Svenning, J.-C. (2003) Deterministic Plio-Pleistocene extinctions in the European cool-temperate tree flora. *Ecology Letters* **6**, 646–653.

Tiffney, B.H. (1985a) Perspectives on the origin of the floristic similarity between eastern Asia and eastern North America. *Journal of the Arnold Arboretum* **66**, 73–94.

Tiffney, B.H. (1985b) The Eocene North Atlantic land bridge: Its importance in Tertiary and modern phytogeography of the northern hemisphere. *Journal of the Arnold Arboretum* **66**, 243–273.

Tiffney, B.H. (1994) An estimate of the early Tertiary paleoclimate of the southern Arctic. pp. 267–295 *in* Boulter, M.C.; Fisher, H.V. (Eds) *Cenozoic plants and climate of the Arctic*, Vol. 27. NATO ASI series. Berlin, Springer-Verlag.

Tiffney, B.H. and Manchester, S.R. (2001) The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere. *International Journal of Plant Sciences* **162** (suppl.), S3–S17.

Vankat, J.L. (1979) *The natural vegetation of North America: An introduction*. New York, John Wiley & Sons.

Walck, J.L. and Hidayati, S.N. (2004) Germination ecophysiology of the western North American species *Osmorhiza depauperata* (*Apiaceae*): Implications of preadaptation and phylogenetic niche conservatism in seed dormancy evolution. *Seed Science Research* **14**, 387–394.

Walck, J.L., Baskin, J.M. and Baskin, C.C. (2000) Increased sensitivity to green light during transition from conditional dormancy to nondormancy in seeds of three species of *Solidago* (*Asteraceae*). *Seed Science Research* **10**, 495–499.

Walck, J.L., Hidayati, S.N. and Okagami, O. (2002) Seed germination ecophysiology of the Asian species *Osmorhiza aristata* (Apiaceae): Comparison with its North American congeners and implications for evolution of types of dormancy. *American Journal of Botany* **89**, 829–835.

Wen, J. (1999) Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. *Annual Review of Ecology and Systematics* **30**, 421–455.

Wen, J. and Zimmer, E.A. (1996) Phylogeny and biogeography of *Panax* L. (the ginseng Genus, Araliaceae): Inferences from ITS sequences of nuclear ribosomal DNA. *Molecular Phylogenetics and Evolution* **6**, 167–177.

Wen, J., Cowry, P.P., Walck, J.L. and Yoo, K.-O. (2002) Phylogenetic and biogeographic diversification in *Osmorhiza* (Apiaceae). *Annals of the Missouri Botanical Garden* **89**, 414–428.

Wolfe, J.A. (1969) Neogene floristic and vegetational history of the Pacific Northwest. *Madroño* **20**, 83–110.

Wolfe, J.A. (1972) An interpretation of Alaskan Tertiary floras. pp. 201–233 *in* Graham, A. (Ed.) *Floristics and paleofloristics of Asia and eastern North America*. Amsterdam, Elsevier Scientific Publishing Company.

Wolfe, J.A. (1975) Some aspects of plant geography of the northern hemisphere during the late Cretaceous and Tertiary. *Annals of the Missouri Botanical Garden* **62**, 264–279.

Wolfe, J.A. (1977) Paleogene flora from the Gulf of Alaska region. *US Geological Survey Professional Paper* **997**, 1–107.

Wolfe, J.A. (1979) Temperature parameters of humid to mesic forests of eastern Asia and relation to forests of other regions of the Northern Hemisphere and Australasia. *US Geological Survey Professional Paper* **1106**, iii + 1–37 + 3 plates.

Xiang, Q.-Y., Soltis, D.E. and Soltis, P.S. (1998) The eastern Asian and eastern and western North American floristic disjunction: Congruent phylogenetic patterns in seven diverse genera. *Molecular Phylogenetics and Evolution* **10**, 178–190.

Xiang, Q.-Y., Soltis, D.E., Soltis, P.S., Manchester, S.R. and Crawford, D.J. (2000) Timing the eastern Asian–eastern North American floristic disjunction: Molecular clock corroborates paleontological evidence. *Molecular Phylogenetics and Evolution* **15**, 462–472.

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