The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants

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Summary

- Seed dormancy, by controlling the timing of germination, can strongly affect plant survival. The kind of seed dormancy, therefore, can influence both population and species-level processes such as colonization, adaptation, speciation, and extinction.
- We used a dataset comprising over 14 000 taxa in 318 families across the seed plants to test hypotheses on the evolution of different kinds of seed dormancy and their association with lineage diversification.
- We found morphophysiological dormancy to be the most likely ancestral state of seed plants, suggesting that physiologically regulated dormancy in response to environmental cues was present at the origin of seed plants. Additionally, we found that physiological dormancy (PD), once disassociated from morphological dormancy, acted as an 'evolutionary hub' from which other dormancy classes evolved, and that it was associated with higher rates of lineage diversification via higher speciation rates.
- The environmental sensitivity provided by dormancy in general, and by PD in particular, appears to be a key trait in the diversification of seed plants.

Introduction

Dormancy is an innate state of arrested growth that occurs across all life forms (Finch-Savage & Leubner-Metzger, 2006; Footitt *et al.*, 2011). In plants, seed dormancy is defined as an innate constraint on germination under conditions that would otherwise promote germination in nondormant seeds (Simpson, 1990). Dormancy enables seeds to avoid germination during periods that are only ephemerally favorable. By having seeds with various degrees of dormancy, plants can also distribute their offspring across time and bet-hedge against unpredictably variable environments (Venable, 2007; Poisot *et al.*, 2011). By contrast, the lack

of dormancy (nondormancy) enables seedlings to start developing as soon as conditions become favorable for germination and thereby to maximize the growing season and minimize seed predation risk. Thus dormancy, or the lack thereof, fundamentally determines the environment a plant experiences after germination and is an important component of plant life histories (Donohue *et al.*, 2005a).

As one of the earliest traits expressed in the life cycle of plants, seed dormancy can be a critical determinant of colonization and establishment success. The degree and kind of dormancy strongly regulate the timing of germination, which has been shown to be under strong natural selection, especially in colonizing populations (Donohue *et al.*, 2005b; Huang *et al.*, 2010). Dormancy may be a major determinant of species' distributions because seeds must first be able to germinate and establish in a habitat in

order for post-germination traits to adapt to it (reviewed in Donohue *et al.*, 2010).

As a consequence, dormancy may also influence evolutionary diversification. Species with nondormant seeds might be better able to explore novel environments because their germination is independent of specific dormancy-breaking cues that might be absent in that new environment. This, in turn, might promote diversification by fostering divergence and allopatric speciation. Alternatively, dormancy and germination cueing may enable colonization of new locations with different seasonality by ensuring germination under appropriate seasonal conditions, thereby reducing extinction risk and providing the opportunity for subsequent adaptive divergence. To date, it is not known whether any given dormancy class is associated with higher diversification.

Seed dormancy is critical for mediating the interaction between the ecological environment and one of the most vulnerable life stages in plants: seedlings. This interaction occurs with various degrees of precision and by employing different physiological, morphological, and anatomical mechanisms (Linkies *et al.*, 2010). The anatomical and physiological diversity of seed dormancy suggests a complex evolutionary history and has led several authors to systematize the classification of the kinds of dormancy (Nikolaeva, 1999; Baskin & Baskin, 2004a). Dormancy classification is based on the developmental state of the embryo at the time of seed dispersal, physical traits of the seed, and physiological responses of seeds to environmental stimuli. It has been proposed (Fig. 1) that dormancy imposed by the underdevelopment of embryos at the time of dispersal (morphological dormancy (MD); i.e. the embryo is fully differentiated into cotyledon(s) and hypocotyl/radicle, but is still small and needs to grow before hypocotyl/radicle emergence) is the ancestral state of seed plants, and that mechanisms of environmentally cued dormancy via specialized physical structures (physical dormancy (PY)) or physiological responses to environmental or hormonal cues (physiological dormancy (PD)) evolved subsequently (Forbis *et al.*, 2002; Baskin & Baskin, 1998). Whether loss of dormancy

Fig. 1 Alternative hypothesized scenarios for the evolution of dormancy classes. (I) Morphological dormancy (MD) is the ancestral state. The addition of physiological dormancy and embryo development before dispersal, led to morphophysiological dormancy (MPD) and physiological dormancy (PD), respectively. Physical dormancy (PY) and nondormancy (ND) are derived from PD. (II) MPD is plesiomorphic. MD and PD are derived independently from MPD by, respectively, losing the need for specific PD breaking requirements or embryo development before dispersal. PY and ND are derived from PD. (III) As in I, except ND emerged independently from MD and PD.

(nondormancy (ND)) is an intermediate or highly derived state, and the dormancy class from which it evolved are questions under debate (Fig. 1, Supporting Information, Notes S1).

Physiological dormancy is the most frequent dormancy class (Baskin & Baskin, 2004a, 2014). This form of dormancy provides seasonal cueing, ensuring that germination occurs only after specific environmental events (Finch-Savage & Leubner-Metzger, 2006). The adjustment of PD seeds to their external environment is highly specific, and increased germination occurs in response to specific temperature, chemical, or light signals (Baskin & Baskin, 2014). PD can be a major determinant of life-history expression; for example, it can determine whether temperate species behave as winter annuals or summer annuals (Baskin & Baskin, 2014).

The dormancy-breaking requirements of different taxa can be highly specific, which seems to indicate that dormancy in general, and PD in particular, constitute complex adaptations (Donohue *et al.*, 2010; Linkies *et al.*, 2010). It has even been proposed that the adjustment to seasonality provided by seed dormancy might hinder extinction (Soltis *et al.*, 2013). Moreover, theory on the adaptive value of dormancy has provided insight into why some seeds have high germination percentages and do not persist beyond 1 yr, while others germinate over multiple years (Venable & Lawlor, 1980; Ellner, 1985; Venable, 1989). It does not, however, clarify the origin of different dormancy classes, their patterns of evolution, or how they might influence diversification rates. Although the various dormancy mechanisms have been proposed to be adaptations to specific environments inhabited by different plant groups, our understanding of the evolution of seed dormancy and germination remains largely superficial (Finch-Savage & Leubner-Metzger, 2006; Linkies *et al.*, 2010; Dalling *et al.*, 2011).

In particular, the ancestral dormancy state is not known with certainty. It is also unclear whether all dormancy classes can derive from each other, or, alternatively, whether certain classes necessarily precede others. This knowledge can reveal whether different developmental, physical, and physiological mechanisms of dormancy have evolved independently or in concert. Furthermore, analysis of the evolutionary lability of dormancy and its effect on diversification can provide insight into the adaptive importance of dormancy and whether it can facilitate or inhibit diversification. An explicit phylogenetic analysis of the relationships among the different dormancy classes has heretofore not been attempted.

Here we investigate the evolution of dormancy in the seed plants. We compiled a dataset, collected over decades, that contains the dormancy classes of over 14 000 taxa in 318 seed plant families (Baskin & Baskin, 2014). We use these data to address the following questions. What is the most probable ancestral state of dormancy in the seed plants, and when did the most prevalent dormancy class, PD, and environmental cueing of germination evolve? What is the evolutionary lability of dormancy classes: what was the sequence of evolutionary transitions leading to the current diversity of dormancy classes, and are there biases in the evolutionary transitions to and from certain dormancy classes? Does the rate of diversification differ among dormancy classes; in particular, is the evolution of physiological environmental cueing
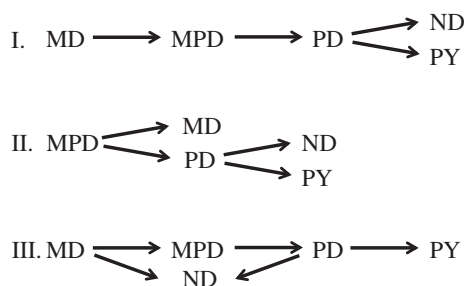
via PD associated with faster diversification rates, or does loss of dormancy altogether promote diversification?

Materials and Methods

Dormancy data collection

In this study, we recognized seven kinds of dormancy. Causes of dormancy include exogenous factors such as impermeable vs. permeable seed coat and germination inhibitors in the fruit coat, as well as endogenous factors such as hormone balance and under-developed vs. fully developed embryo (defined earlier). Conditions required for breaking dormancy include application of GAs or other hormones such as ethylene, dry storage (after-ripening), warm stratification, and cold stratification. Experiments that manipulated these factors and observations on embryos allow each taxon to be assigned to one of the seven kinds of dormancy (Baskin & Baskin, 2014; Notes S2).

Five of the seven kinds of dormancy are those recognized in Nikolaeva's seed dormancy classification system (1969), which is based on the causes of dormancy and the conditions required for breaking it. These five kinds of dormancy include: MD, when freshly matured embryos are underdeveloped upon dispersal and require time to grow before they can germinate (the dormancy period); PD, where dormancy is broken through specific physiological responses to environmental cues, such as warm and/or cold temperatures or through dry after-ripening; PY, where seeds are surrounded by a water-impermeable palisade layer, and dormancy is broken by physically making this layer water-permeable; morphophysiological dormancy (MPD), a combination of MD and PD; and finally, physiophysical dormancy (PYPD), a combination of PY and PD (Nikolaeva, 1969, 1999; Baskin & Baskin, 2004a, 2014).

In addition to the five kinds of dormancy defined by Nikolaeva, two other kinds of dormancy were recognized: ND, when freshly matured seeds have no dormancy; and dust seeds (DUST), which are small in size (mostly ≤ 1.0 mm in length) and have undifferentiated embryos with as few as two to three cells (Leake, 1994; Eriksson & Kainulainen, 2011). Nondormant seeds categorically differ from dormant seeds because they lack primary dormancy at seed maturity and germinate under the widest range of conditions possible immediately after dispersal without any dormancy-breaking treatments (Baskin & Baskin, 2004a, 2014). Genetic variation for the presence of dormancy has been documented in some species, so it is possible that a species classified here as nondormant may contain some genotypes that are capable of exhibiting dormancy when grown under certain conditions. However, if there was no available evidence to support that these taxa can exhibit dormancy, we classified them as ND. Dust seeds are distinguished more by the unique germination morphology and ecology of the seeds, and generally have very specialized MD or MPD requirements for germination (Leake, 1994; Baskin & Baskin, 2004b; Eriksson & Kainulainen, 2011).

Combined, these seven kinds of dormancy encompass the full range of diversity of dormancy in the seed plants and represent discrete categories that have been delineated through decades of comparative and experimental studies of seed dormancy and germination (see Baskin & Baskin, 2004a, 2014).

Dormancy data for each seed plant family were aggregated from data collected for more than 14 000 species (see Table S1). These data were compiled over the last several decades from several thousand published papers that contained information on seed dormancy and germination from all parts of the world and for species from all vegetation zones on earth, life forms, and specialized life cycles and habitats, such as parasites, carnivorous plants, orchids, aquatics and halophytes (Baskin & Baskin, 2014). In recording data on dormancy state, great care was taken to determine the state for freshly matured seeds and not to confuse the loss of PD via after-ripening with ND in fresh seeds. If the information in a paper was too incomplete to assign a species to a dormancy class, then the dormancy class was inferred based on germination data present in the publication and on the kind of embryo (Martin, 1946; Finch-Savage & Leubner-Metzger, 2006) and seed coat anatomy (as described in numerous publications) known to occur in the genus or family.

Three dormancy datasets were created with different combinations of dormancy classes (Table S1). The first dataset included all seven states of dormancy (dorm 7). However, estimating evolutionary parameters for an unordered discrete character with seven polymorphic states, several of which are rare, is a challenge. Thus, we also performed analyses across two reduced datasets in which the least common dormancy classes, PYPD and DUST, were either recoded as other dormancy states (dataset 5A) or removed from the analysis (dorm 5B). To recode the data, PYPD was classified as PY, while DUST was classified as MPD (dorm 5A). When PYPD and DUST families were removed, it reduced the overall number of families by 10 (dataset 5B).

Families with multiple dormancy states were dealt with in one of two ways. For most analyses, dormancy was treated as polymorphic, with taxa scored for multiple states of dormancy or proportionally for each dormancy state based on species-level data. For methods that could not accommodate polymorphic data, families were split into polytomies, with each taxon representing a single dormancy class. Analyses that used the polytomic tree included parsimony transition number estimation and trait-dependent diversification models (see later).

Phylogeny

Taxonomic information was standardized against the Plant List Database (http://www.theplantlist.org/). A composite phylogeny of seed plants families was constructed using Phylomatic v. 3 (Webb & Donoghue, 2005) and revised based on the most recent information from APweb v. 13 (Stevens, 2001). Branch lengths were corrected using the function 'bladj' in the Phylocom v. 4.2 (Webb *et al.*, 2008) and on 59 divergence time estimates based on the fossil record (Bell *et al.*, 2010; Smith *et al.*, 2010).

To account for phylogenetic uncertainty as a result of limited phylogenetic resolution, 100 trees with randomly resolved polytomies were created using Mesquite v. 2.75 (Maddison & Maddison, 2011). Branch-length adjustments were performed subsequent to

the resolution of polytomies. All subsequent analyses were run and averaged across all 100 trees (Notes S3–S6).

Ancestral state reconstruction

To estimate the basal state of dormancy in seed plants, we used ancestral state reconstruction of dormancy classes using maximum parsimony in Mesquite v. 2.75 (Maddison & Maddison, 2011), maximum likelihood (ML) and Bayesian Markov chain Monte Carlo (MCMC) in BayesTraits v. 2.0 (Pagel & Meade, 2006; http://www.evolution.rdg.ac.uk/), and stochastic character mapping (SIMMAP; Bollback, 2006) using the package 'phytools' (Revell, 2012) implemented in R (R Core Development Team, 2013). Given the large number of parameters to estimate and the difficulty of estimating transition rates for rare states, we restricted the transition rates to be equal for ML, MCMC, and SIMMAP analyses. MCMC analyses were run for 1.1×10^7 iterations with the first 1×10^6 iterations discarded as a burn-in, and a reversible-jump hyperprior with an exponential distribution (Pagel & Meade, 2006). For SIMMAP analyses, we ran 10 simulations per tree. Ancestral state reconstructions for all additional nodes were estimated with parsimony and SIMMAP methods only.

To test the significance of our estimates for the basal state of dormancy, we tested alternative hypotheses with ML and MCMC models by fixing the dormancy class at the basal node for the four major dormancy states: MPD, MD, PD, and ND. Nodes were fixed using the 'fossil' command in BayesTraits v. 2.0. We then compared harmonic mean log-likelihood scores across the constrained models to determine which dormancy class was the most likely at each node.

Character retention, transition numbers, transition bias

Character retention refers to the degree of synapomorphy in a given character. We estimated the retention of dormancy classes using the retention index (RI) in Mesquite v. 2.75 (Maddison & Maddison, 2011). An RI of 1 indicates maximum synapomorphy, such that the character (in this case the dormancy class) originated only once, in the common ancestor of all the extant taxa that exhibit it, while an RI of 0 indicates maximum homoplasy, such that every appearance of the character on the tree is as a result of a new, independent event.

Transition numbers were based on parsimony and SIMMAP estimates of ancestral states. For parsimony analyses, transition numbers were estimated using the 'Summarize State Changes Over Trees' function in Mesquite v. 2.75 (Maddison & Maddison, 2011). One hundred maximum parsimony reconstructions were sampled per tree and averaged across all 100 trees. For SIMMAP analyses, transition numbers were obtained from the 'describe.simmap' function in the package 'phytools' (Revell, 2012), with results averaged across all 100 trees.

We tested whether there was a bias among dormancy classes to act as either a net source or sink for transitions (see Crisp *et al.*, 2009). The null model was that transitions to and from a given class were equally probable. This null was tested using a binomial test. A specific dormancy state may act as a source of other states simply because it is more abundant than other states. We therefore repeated this test, correcting for the potential correlation between the number of transitions and the frequency of each dormancy class in the dataset. The number of transitions to or from a class was multiplied by one minus the fraction of species with that class and rescaled to the total number of transitions.

Diversification rates

We compared diversification rates among dormancy states using the multistate speciation and extinction model (MuSSE; FitzJohn *et al.*, 2009), implemented in the R package 'diversitree' v. 0.9–6 (FitzJohn, 2012). Given the difficulty of estimating all of the possible parameters in the MuSSE model with our full dataset, we restricted our analysis to the dorm 5A and dorm 5B datasets. Furthermore, we restricted transition rates to be equal for all analyses. MCMC chains were run for 1×10^3 iterations with the first 10% discarded as burn-in. Analyses were run across all 100 trees and combined.

Results

Ancestral state of dormancy

Environmentally cued physiological regulation of germination appears to have been present from the beginning of seed plants (Table 1). We found MPD to be the most probable ancestral state for seed plants, based on all ancestral state estimates analyzed (Table 1, Fig. 2). Furthermore, comparison of MCMC and ML models with different states of dormancy fixed as the most basal found MPD as the basal state to be the best-fit model (Table S2). Dormancy imposed by underdevelopment of embryos (MD) appears to be more easily lost than environmentally regulated dormancy: all early shifts from MPD were to PD and none to MD (Figs 2, S1, Tables S3, S4). There were three major shifts from MPD to PD in early seed plants. The first major shift from MPD to PD occurred early in the Acrogymnospermae (i.e. extant gymnosperms) during the origin of the Gnetophyta-Pinophyta clade (Figs 2, S1, Tables S3, S4). The second major MPD–PD shift occurred in the Magnoliids with the origin of Laurales (Figs 2, S1, Tables S3, S4). PD also arose more recently at least two more times within the Magnoliids: in the Piperales and Myristicaceae. The third major MPD–PD shift occurred with the Monocotyledonae–Eudicotyledoneae split (Figs 2, S1, Tables S3, S4).

In contrast to MPD and PD, dormancy imposed by impermeable seed coats (PY) and loss of dormancy altogether (ND) appear to be highly derived states, present primarily at the tips of the phylogeny (Fig. S1).

Evolutionary lability of dormancy and evolutionary hub

The distribution of dormancy classes across the seed plants is not random – certain dormancy classes occur more frequently in certain lineages (i.e. physiophysical dormancy occurs

Table 1 Ancestral state estimates for dormancy classes at the root of seed plants

Dormancy class	Parsimony			Maximum likelihood			MCMC			SIMMAP		
	Dorm 7	Dorm 5A	Dorm 5B	Dorm 7	Dorm 5A	Dorm 5B	Dorm 7	Dorm 5A	Dorm 5B	Dorm 7	Dorm 5A	Dorm 5B
ND	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01
PD	0.00	0.00	0.00	0.18	0.17	0.25	0.15	0.17	0.23	0.14	0.17	0.18
PY	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01
MD	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01
MPD	1.00	1.00	1.00	0.79	0.79	0.71	0.83	0.79	0.73	0.85	0.80	0.79
PYPD	0.00			0.00			0.01			0.01		
DUST	0.00			0.00			0.01			0.00		

ND, nondormancy; PD, physiological dormancy; MD, morphological dormancy; MPD, morphophysiological dormancy; PY, physical dormancy; PYPD, physiophysical dormancy; DUST, dust seeds; MCMC, Markov chain Monte Carlo; SIMMAP, stochastic character mapping.
Values provided represent the estimated probability of each state at the root, averaged across 100 trees. Ancestral states were estimated using four methods: maximum parsimony, maximum likelihood, Bayesian MCMC, and SIMMAP. The three datasets presented include: a dataset with all seven states (Dorm7) and two reduced datasets with five states (Dorm5A and 5B, see the Materials and Methods section). All three datasets were analyzed when computationally possible.

disproportionately in the Rosids). Dormancy was also evolutionarily labile, however, as evidenced by a large degree of homoplasy (i.e. convergent evolution) in dormancy classes across seed plants.
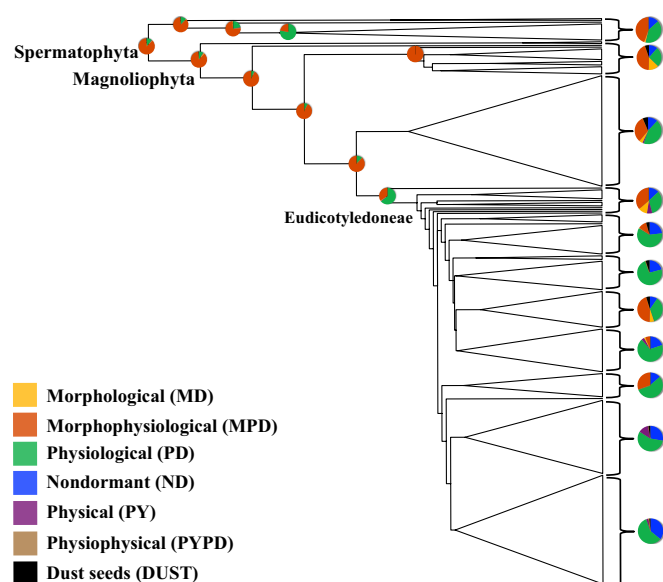

Fig. 2 Phylogeny of the seed plants (Spermatophyta), with stochastic character mapping ancestral-state reconstructions of dormancy classes for major nodes. Pie charts at nodes represent the posterior probabilities of each dormancy class, averaged across 100 trees. Pie charts at the tips of the tree represent the proportion of dormancy classes (by species) within each delineated group. Groups do not necessarily represent traditional taxonomic clades, but were chosen to highlight the diversity and distribution of dormancy classes across the seed plants. The groups are, from top to bottom: Gymnosperms; Magnoliids + Nymphaeales; Monocotyledoneae; Ceratophyllaceae + Basal Eudicots (e.g. Ranunculales) + Basal Core Eudicots (e.g. Gunnerales); Caryophyllales + Santalales; Cornales + Ericales; Campanulidae (e.g. Asteraceae, Apiaceae, Campanulaceae); Lamiidae (e.g. Solanaceae, Lamiaceae, Rubiaceae); Saxifragales + Vitales; Malvidae (e.g. Malvaceae; Brassicaceae, Myrtaceae); and Fabidae (e.g. Fabaceae, Rosaceae, Euphorbiaceae). Additional major clades indicated in the tree are Magnoliophyta (Angiosperms) and Eudicotyledoneae (Eudicots). The color key indicates the specific dormancy class. See Fig. S2 and Tables S3 and S4 for complete ancestral node estimates and family-level distribution of dormancy classes across the seed plants.

The observed RI, a measure of homoplasy scaled from 0 (maximum homoplasy) to 1 (no homoplasy), was significantly lower than null expectations (Table 2). The high degree of homoplasy is also evident in the number of transitions between dormancy classes (Tables S5, S6, Fig. 3).

Further analysis of transition numbers revealed that PD acted as an 'evolutionary hub', to which and from which the other dormancy states evolved (Fig. 3, Tables S5, S6). PD was the only significant evolutionary 'source' (i.e. the number of transitions from PD was significantly greater than the number of transitions to PD) for both parsimony and stochastic character mapping analyses (Tables 3, S7). The other tentative source was MPD, which was a significant 'source' based on analysis of the reduced datasets (5A and 5B) with parsimony, but not stochastic character mapping (Table S7). Correcting for frequency of the different dormancy classes, by scaling the number of transitions to and from a given dormancy class by the proportion of taxa with that class, did not have a significant effect on the overall pattern (Tables 3, S7). The dormancy classes that do not employ physiological environmental sensitivity (MD, PY and ND) behaved as 'sinks', indicating that gaining the ability to respond physiologically to specific environmental cues is more difficult than losing it. However, results concerning most of these and the other classes varied more between being 'sinks' or equilibria depending on the method and dataset.

Nondormancy was consistently a net evolutionary 'sink', (Tables 3, S7), indicating that other states evolved to ND and either remained in that state or became extinct. PD was by far the most common state from which ND evolved (Fig. 3), but all other dormancy classes (except PYPD, which had only seven taxa) were able to evolve to ND.

Associations between dormancy class and diversification rates

Dormancy class was significantly associated with diversification rates. An analysis of diversification rates comparing the five major dormancy classes simultaneously found that taxa with PD had

Table 2 Evolutionary lability of dormancy

Dataset	Median observed RI	Median null RI	95% CI lower null RI	95% CI upper null RI
Dorm 7	0.05	0.14	0.11	0.17
Dorm 5a	0.09	0.15	0.12	0.18
Dorm 5b	0.07	0.14	0.11	0.18

Retention index (RI) measures the relative proportion of observed parsimony steps to the maximum number of steps and ranges from 0 (maximum homoplasy) to 1 (maximum synapomorphy). An observed RI less or greater than the 95% confidence interval (CI) of the null distribution indicates a significant pattern. Here, the median observed RI was calculated across 100 trees, as were the null distribution statistics. The analysis was performed on the full (dorm7) and reduced (dorm5A, 5B) datasets (see the Materials and Methods section for details).
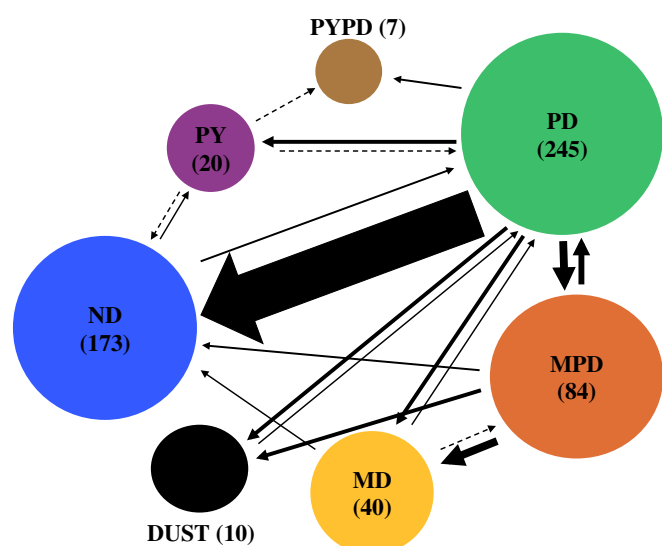

Fig. 3 Number of evolutionary transitions between dormancy classes. Arrows indicate the direction and relative proportion (given by line thickness) of transitions between classes. The circle size is proportional to the number of extant families with the given class (also included in parentheses). Dormancy classes are coded as follows: ND, nondormant; PD, physiological; MD, morphological; MPD, morphophysiological; PY, physical; PYPD, physiophysical; DUST, dust seeds. Transition numbers are based on parsimony analysis. See Table S6 for additional results.

Table 3 Analysis of transition number bias

	Sink	Source	Total	*P*
Uncorrected				
ND	**122.5**	**2.4**	**124.8**	**0.0000**
PD	**10.5**	**153.9**	**164.4**	**0.0000**
PY	**6.3**	**0.7**	**6.9**	**0.0156**
MD	**14.2**	**0.4**	**14.5**	**0.0001**
MPD	19.3	24.5	43.9	0.0910
PYPD	1.7	0.1	1.7	0.5000
DUST	**7.5**	**0.0**	**7.5**	**0.0078**
Corrected by the frequency of each dormancy class				
ND	**52.2**	**1.0**	**53.2**	**0.0000**
PD	**4.5**	**65.6**	**70.0**	**0.0000**
PY	2.7	0.3	3.0	0.2500
MD	**6.0**	**0.2**	**6.2**	**0.0156**
MPD	8.2	10.4	18.7	0.1669
PYPD	0.7	0.0	0.7	1.0000
DUST	3.2	0.0	3.2	0.1250

ND, nondormancy; PD, physiological dormancy; MD, morphological dormancy; MPD, morphophysiological dormancy; PY, physical dormancy; PYPD, physiophysical dormancy; DUST, dust seeds. See Tables S4 and S5 for additional results.

Sink, the mean total number of transitions to a given dormancy class; source, the mean total number of transitions from a given dormancy class. Transition numbers are based on parsimony analysis and were averaged across 100 trees. *P*-values were calculated based on the binomial expectation of equal rates of transitions to and from a dormancy class. Significant deviations from equal transitions rates, either toward being an evolutionary sink or source, are in shown bold.
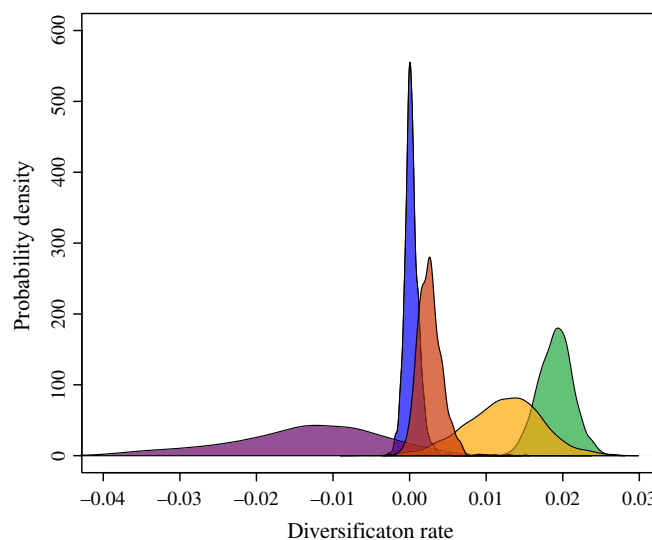

Fig. 4 Diversification rates for dormancy classes. Diversification rate (speciation − extinction rate) is given on the *x*-axis, and the probability density on the *y*-axis. Nondormancy (ND), blue; physiological dormancy (PD), green; morphological dormancy (MD), orange; morphophysiological dormancy (MPD), red; physical dormancy (PY), purple. Rate estimate distributions were aggregated from Markov chain Monte Carlo (MCMC) runs of the MuSSE model with 1×10^3 iterations, with a 10% burn-in for each of 100 trees. Results are for the Dorm5B dataset. See Table S8 and Fig. S2 for additional results.

the highest diversification rate, resulting from relatively high speciation rates (Figs 4, S2, Tables S8, S9). MD also had a high diversification rate as a result of a relatively high speciation rate. Since few taxa exhibited MD, however, estimates of speciation rates for MD may be less accurate than for PD because there is less branch-length time over which to estimate diversification parameters. The large range of estimates of diversification rates for MD support the idea that these results may be a statistical artifact. For PD, however, this is not likely to be a confounding issue, given the high abundance of PD in the data set. Nonetheless, speciation rates, and consequently overall diversification rates, did not significantly differ between MD and PD based on comparison of likelihood scores for constrained vs unconstrained models (Table S7). Thus PD – and possibly MD – appears to

accelerate lineage diversification rates. The rate of diversification estimated for ND was relatively low and not significantly different from 0. The lowest diversification rate was associated with

PY. Lineages with PY appear to have high extinction rates and negative diversification (Table S8, Figs 4, S2).

Discussion

We found that the most likely ancestral dormancy state is MPD, suggesting that the ability of seeds to sense and respond physiologically to the environment to regulate dormancy was present at the origin of seed plants. In addition, we found that the subsequent disassociation of PD from underdeveloped embryos (MD) was associated with increased diversification of dormancy classes and of the number of seed plant lineages.

The ancestral state of dormancy in seed plants has important implications for interpreting the adaptive and ecological significance of different dormancy classes. Baskin & Baskin (1998, 2004a) were the first to consider all the classes of dormancy from a phylogenetic perspective, which led them to posit that MD (underdeveloped embryos) was the ancestral dormancy state (Fig. 1 I, Notes S1). They hypothesized that PD evolved later, first in conjunction with MD (MPD), then independently as PD. Forbis *et al.* (2002) similarly favored MD as the more likely ancestral state based on comparative study of embryo morphology. They argued that the ancestral ecological conditions could have been continuously favorable for germination, and that PD evolved later as an adaptation to increased seasonality (Fig. 1 III, Notes S1). In contrast to the hypotheses proposed by Baskin & Baskin (1998) and Forbis *et al.* (2002), we found MPD (and hence physiologically regulated environmental cueing of dormancy) to be the most likely ancestral state of the seed plants (Figs 1 II, 2, Notes S1).

The finding that MPD is the ancestral state of seed plants has two major implications for understanding how dormancy evolved. First, full embryo development before dispersal appears to be a derived character, evolving at least three times independently in the seed plants. The independent evolution of a fully developed embryo in three major lineages of seed plants suggests that it was adaptive and raises the question of what environmental conditions favored its evolution. Secondly, it suggests that the genetic mechanisms that regulate physiological environmental sensitivity of dormancy either preceded or were contemporary with the evolution of seeds. Our understanding of the molecular underpinnings of dormancy remains incomplete (Graeber *et al.*, 2012), but there is growing evidence that at least some of the molecular pathways that control dormancy and germination are common among seed plants. Indeed, two major plant hormones, ABA and GA, are both common to all seed plants and play a central role in dormancy regulation. Recently, genes that regulate dormancy through their interaction with ABA, such as *DOG1* and MFT, appear to be homologous across the angiosperms (Ashikawa *et al.*, 2010, 2013; Graeber *et al.*, 2010, 2012). Furthermore, several environmentally sensitive genes that are homologous across the seed plants, such as phytochromes and *FLC*, have also been found to influence germination (Mathews & Sharrock, 1997; Casal & Sánchez, 1998; Mathews, 2006; Chiang *et al.*, 2013). These studies, in combination with our results, support the hypothesis that the origin of the molecular machinery

controlling physiologically regulated environmental responses of dormancy may be deep in the history of seed plants.

The evolution of fully developed embryos with environmental sensitivity – that is, PD – was followed by the subsequent diversification of dormancy classes and species lineages. While environmental sensitivity was present in ancestral seed plants, it was the uncoupling of environmentally sensitive dormancy from MD that appears to have enabled the diversification of dormancy classes and species lineages.

Physiological dormancy encompasses a wide range of physiological responses to environmental factors, such as temperature, light, gas concentrations (CO_2, O_2), and water (Finch-Savage & Leubner-Metzger, 2006). This diversity in the kinds of PD might very well underlie its role both as a facilitator of transitions between other dormancy classes (i.e. as an 'evolutionary hub') and as a driver of speciation. Theory predicts that phenotypic plasticity will facilitate adaptation to novel environments by allowing species to persist under less than optimal conditions (Lande, 2009). In this framework, PD may facilitate the colonization of novel environments by permitting species to adjust the timing of germination under different or novel seasonal regimes (Donohue *et al.*, 2005a). Given the ability to persist, plants could then be more likely to undergo directional selection toward a new class of dormancy that is better adapted to the new environment. Likewise, the increased ability to persist and adapt to novel environments would promote the process of speciation, independent of changes in dormancy state (Pfennig *et al.*, 2010; Thibert-Plante & Hendry, 2011). Other kinds of dormancy, such as MD and PY, also afford some degree of control in response to environmental conditions; that is, environmental conditions will influence the speed of embryo development and seed coat permeability (Baskin *et al.*, 2000). However, relative to PD, they are probably less flexible in adjusting dormancy behavior to new combinations of environmental conditions, which might limit their ability to promote adaptive divergence and speciation. The negative diversification rates of PY, in particular, suggest that PY is limited in its capacity to diversify. One hypothesis to explain this limitation is that PY is not simply an adaptation to control germination, but might also be under selection as a physical protection against pathogens and predators (Dalling *et al.*, 2011; Paulsen *et al.*, 2013). Multiple selective pressures might subsequently limit the ability of PY lineages to adapt in the face of a changing adaptive landscape (McPeek, 1996). Additionally, in some instances the water-impermeable seed coat might only be broken by a specific environmental cue such as fire (Moreira & Pausas, 2012), which could significantly limit the ecological range of PY lineages and increase their chance of going extinct.

The evolutionary patterns of ND were of particular note and provide insights into its ecological importance. ND is able to evolve from nearly every other dormancy state, but it is most likely to evolve from PD. Its repeated, independent evolution suggests that it may be adaptively important and favored under relatively common conditions. The evolution of ND (i.e. the loss of dormancy) in response to stable, constant environments has long been argued as a counterpoint to the evolution of bet-hedging (Ellner, 1985; Venable, 1989). By promoting rapid

germination, ND may increase competitive advantage, lengthen the growing period, or decrease generation time. ND may also be favored during colonization of novel environments because of the lack of restrictions on germination. Despite its adaptive potential, we did not find ND to be associated with increased lineage diversification. Being a significant evolutionary sink, combined with its relatively uncommon transitions to other states, its position at the tips of the phylogeny, and its comparatively low diversification rate all suggest that ND is likely a derived and ephemeral state and may be prone to extinction. Diversification analysis did not detect uncommonly high extinction rates of ND taxa, however. An alternative hypothesis is that ND may evolve back to PD (Fig. 3) or, less commonly, to other dormancy states. The observed evolutionary lability of ND is consistent with the documented existence of genetic variation for dormancy within certain species. For instance, ND can evolve quickly as a product of domestication (De Wet & Harlan, 1975; Gepts, 2010), but wild populations of these domesticated plants can just as easily re-evolve dormancy (Ellstrand *et al.*, 2010). Similarly, genetic variation for dormancy has been found to naturally occur within species (Kochánková & Mandák, 2009; Bentsink *et al.*, 2010; Fernández-Pascual *et al.*, 2013). Indeed, focusing on species that exhibit genetic variation for dormancy would be a promising avenue for investigating the underlying genetic basis of dormancy, as well as the selective conditions that favor its loss or gain. To better understand the evolutionary lability of ND, these alternative hypotheses should be tested with more fine-scale phylogenetic analysis as well as with field and genetic studies.

Summary and conclusion

The most probable ancestral dormancy state of seed plants is MPD, suggesting that the physiological environmental regulation of dormancy may be as ancient as seeds themselves. The subsequent dissociation of PD from MD, via the evolution of mature embryos at the time of dispersal, coincided with an increase in evolutionary lability and homoplasy. In particular, PD appears to be an 'evolutionary hub' from which other dormancy classes have repeatedly evolved, including ND. PY appears to be linked to high extinction, perhaps because it is only adaptive under very specific selective regimes and is restricted to only a few plant groups. Despite its prevalence among seed plants, ND seems to be a derived and evolutionarily ephemeral state. The evolution of PD was also associated with increased speciation rates. Thus, physiologically regulated environmental cueing of dormancy appears to have influenced major evolutionary patterns in the seed plants.

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Appendix 1

The NESCent Working Group 'Germination, Trait Coevolution, and Niche Limits in Changing Environments' was organized by K.D. and R.R.C. The participant list included all the co-authors plus K. Bradford (UC Davis, USA), L. Burghardt (Duke, USA), S. Kalisz (Pittsburgh, USA), S. Meyer (USDA), J. Schmitt (Brown/UC Davis, USA), S. Strauss (UC Davis, USA) and A. Wilczek (Brown/Deep Springs College, USA).

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Phylogeny of the seed plant families with current dormancy classes and ancestral state reconstructions.

Fig. S2 Speciation and extinction rates for dormancy classes.

Table S1 Dormancy data by plant family

Table S2 Log-likelihood scores comparing models of alternative ancestral dormancy states at the basal node

Table S3 Ancestral state reconstruction for dormancy types across the seed plants using stochastic character mapping

Table S4 Ancestral state reconstruction for dormancy types across the seed plants using parsimony

Table S5 Transition numbers between dormancy classes based on parsimony

Table S6 Transition numbers between dormancy classes based on stochastic character mapping

Table S7 Analysis of transition-number bias between dormancy classes

Table S8 Diversification results from MuSSE analysis for dormancy classes

Table S9 Comparison of unconstrained and constrained MuSSE models

Notes S1 Detailed discussion of the current hypotheses on the evolutionary origin of dormancy in seed plants.

Notes S2 Detailed explanation and dichotomous key for the classification of dormancy classes.

Notes S3 Phylogeny of seed plant families with unresolved polytomies with corresponding dormancy data being polymorphic by family.

Notes S4 One hundred phylogenies of seed plant families with polytomies randomly resolved with corresponding dormancy data being polymorphic by family.

Notes S5 Phylogeny of seed plant families with unresolved polytomies and families with corresponding dormancy data being polytomic by family.

Notes S6 One hundred phylogenies of seed plant families with polytomies randomly resolved with corresponding dormancy data being polytomic by family.

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