

# Seedling performance covaries with dormancy thresholds: maintaining cryptic seed heteromorphism in a fire-prone system

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**Abstract.** The production of morphologically different seeds or fruits by the same individual plant is known as seed heteromorphism. Such variation is expected to be selected for in disturbance-prone environments to allow germination into inherently variable regeneration niches. However, there are few demonstrations that heteromorphic seed characteristics should be favored by selection or how they may be maintained. In fire-prone ecosystems, seed heteromorphism is found in the temperatures needed to break physical dormancy, with seeds responding to high or low temperatures, ensuring emergence under variable fire-regime-related soil heating. Because of the relationship between dormancy-breaking temperature thresholds and fire severity, we hypothesize that different post-fire resource conditions have selected for covarying seedling traits, which contribute to maintenance of such heteromorphism. Seeds with low thresholds emerge into competitive conditions, either after low-severity fire or in vegetation gaps, and are therefore likely to experience selection for seedling characteristics that make them good competitors. On the other hand, high-temperature-threshold seeds would emerge into less competitive environments, indicative of stand-clearing high-severity fires, and would not experience the same selective forces. We identified high and low-threshold seed morphs via dormancy-breaking heat treatments and germination trials for two study species and compared seed mass and other morphological characteristics between morphs. We then grew seedlings from the two different morphs, with and without competition, and measured growth and biomass allocation as indicators of seedling performance. Seedlings from low-threshold seeds of both species performed better than their high-threshold counterparts, growing more quickly under competitive conditions, confirming that different performance can result from this seed characteristic. Seed mass or appearance did not differ between morphs, indicating that dormancy-breaking temperature threshold variation is a form of cryptic heteromorphism. The potential shown for the selective influence of different post-fire environmental conditions on seedling performance provides evidence of a mechanism for the maintenance of heteromorphic variation in dormancy-breaking temperature thresholds.

**Key words:** *bet-hedging; competition; Fabaceae; germination; heteromorphism; physical seed dormancy; seed ecology; seedling growth.*

## INTRODUCTION

The production of morphologically or physiologically different types of seeds or fruits within an individual plant, known as seed heteromorphism, is a phenomenon that occurs in both annuals and perennials from a wide range of ecosystems (Lloyd 1984, Venable 1985, Imbert 2002). The most widely studied heteromorphic character is seed size (e.g., Flint and Palmblad 1978, Venable and Brown 1988, Augspurger and Franson 1993, Turnbull et al. 1999); however, numerous other types of morphological heteromorphism including color, shape, and some of those not related to visible morphological differences (cryptic heteromorphism), including germination rate and dormancy, are also important sources of variation

(Imbert 2002). A key question in ecology is how such heteromorphism is maintained. While many studies document heteromorphic seed characteristics, there are few demonstrations that they should be favored by selection. Such variations become adaptive when the species fitness becomes higher in response to different environmental conditions as a result of different seed morphs within the population (Imbert 2002, Coomes and Grubb 2003). Furthermore, the mechanisms by which heteromorphism is maintained are likely to be dependent on the type of habitat and drivers of population dynamics in which each plant community occurs.

Plants that produce heteromorphic seeds have a greater chance of successfully producing recruits when environmental conditions vary over space and time (Lloyd 1984, Imbert 2002). Different seed morphs with distinct ecological properties can also increase fitness by contributing to a bet-hedging strategy in such environments

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(Venable 1985). However, while the variation in seed characteristics, such as dormancy or seed size, can contribute to recruitment success by allowing seedlings to emerge across a broad regeneration niche (Grubb 1977, Fenner 1991, Rees 1995, Bond et al. 1999, Coomes and Grubb 2003, Ooi et al. 2014), positive ecological benefits for the population are dependent on the ability of seedlings to succeed under the environmental conditions they emerge into. For example, *Suaeda corniculata* from semiarid Inner Mongolia produces non-dormant large brown and dormant small black seeds. The dormant, and therefore later germinating black seeds, provide a bet-hedging strategy against failure of the non-dormant brown seeds (Cao et al. 2012, Yang et al. 2015). However, the riskier strategy for brown seeds, which emerge earlier when moisture availability is less certain during spring, is offset by their seedlings' ability to grow larger than those of black seeds, and produce correspondingly higher numbers of seeds by the end of the growing season. Positive fitness outcomes from variation in dormancy and germination behavior related to seed heteromorphism is therefore strongly influenced by seedling characteristics and recruitment success (Simons and Johnston 2000, Mandák and Pyšek 2005, Leverett and Jolls 2014).

In ecosystems where fire is the primary disturbance, post-fire environmental conditions are highly variable as a result of the heterogeneity of the fire regime. In these ecosystems, post-fire seedling establishment is critical (Keeley 1991, Bond and van Wilgen 1996). The flush of seedling emergence and establishment immediately after fire is indicative of disturbance-induced dormancy loss or seed germination cues, and contributes to seedling establishment in post-fire conditions (Bond and van Wilgen 1996, Carrington and Keeley 1999, Fenner and Thompson 2005). The importance of heteromorphic and polymorphic (i.e., more than two morphs) seeds in post-disturbance recruitment has been investigated primarily in dryland and agricultural environments (Venable 1985, Fenner 1991, Beckstead et al. 1996, Gutterman 2000, Yang et al. 2015). Despite the importance of the fire regime as a driver of population dynamics across the world, only a few studies have investigated seed heteromorphism in fire-prone habitats (e.g., Hanley and Fenner 1998, Hanley et al. 2003, Moles and Westoby 2004) and none have investigated how such heteromorphism is maintained.

In fire-prone environments, physically dormant species represent a major component of the flora, and typically depend on heat-related dormancy-breaking cues for germination, with an increasing germination response related to increasing treatment temperature (Auld and O'Connell 1991, Santana et al. 2010, Hudson et al. 2015). The impermeable seed coat, which prevents the germination of physically dormant species, is ruptured primarily by elevated soil temperatures during fire, facilitating the gas and water exchange necessary for seed germination (Baskin and Baskin 2014). Liyanage and Ooi (2015) found that the temperatures required to rupture the hard seed coats (i.e., the dormancy-breaking threshold temperature) varied within

individual plants, indicating that, in any particular year, a plant produced seeds with both low and high thresholds. Variation in dormancy-breaking temperature thresholds could therefore be described as a form of heteromorphism. Variation in dormancy-breaking temperature thresholds also occurs both at the population and inter-species level, a characteristic that has been recorded in fire-prone regions around the world (e.g., Jeffery et al. 1988, Auld and O'Connell 1991, Keeley and Bond 1997, Pérez-García 1997, Moreira et al. 2012). This is presumably a strategy to ensure that at least some germination occurs across a range of fire-generated soil temperatures (Trabaud and Casal 1989, Auld and O'Connell 1991, Liyanage and Ooi 2015). Soil temperature is determined by the amount of fine litter burned (Bradstock and Auld 1995) and fire severity is a measure of the amount of organic material consumed during a fire event (Keeley 2009). Higher soil temperatures can therefore result from higher severity fire. At either the individual or population scale, this variation means that seeds with low thresholds can have their dormancy broken by soil temperatures experienced during low to high-severity fires and within canopy gaps (where soil heating results by direct insolation), while seeds with high dormancy-breaking temperature thresholds are restricted to germinating as a result of temperatures only reached during high-severity fires.

Variation in fire severity not only affects seed germination, but also the biotic and abiotic conditions of the post-fire environment into which seedling establishment occurs (Auld and Bradstock 1996, Ooi 2010). Such factors determine the competition levels for resources that seedlings experience (Wellington and Noble 1985, Taylor and Aarssen 1989, Carrington and Keeley 1999, Fenner and Thompson 2005). For example, a high-severity fire causes greater adult mortality of plants, which reduces water demand in the soil and increases soil nutrient availability by burning greater biomass. In contrast, low-severity fires result in low resource availability in post-fire environments, due to reduced combustion of wood and litter, and a higher soil water demand is maintained by higher numbers of surviving adult plants. Subsequently, as a result of either high- or low-severity fires, seeds experience correspondingly different germination cues from variable soil heating, while seedlings experience different competition levels due to different post-fire resource availabilities. Variation in seed dormancy traits therefore need to be offset by seedling traits if they are to be maintained, with faster seedling growth and leaf production beneficial for low-threshold seeds, which emerge into more competitive conditions.

Seedling performance differences have previously been correlated with heteromorphic seed traits, and also have been invoked to describe within- and between-species abilities to cope with variation in competitive resources, but not in fire-prone environments. Differences in early competitive ability of seedlings have often been attributed to variation in seed size, with larger seeds having an advantage over smaller seeds when competing for

resources, by producing larger seedlings (Wedin and Tilman 1993, Turnbull et al. 1999, Kozovits et al. 2005, Sharma et al. 2010). However, while a correlation between seed size and dormancy-breaking temperature thresholds has been identified between species (Hanley et al. 2003), no strong relationship has been found within species (Delgado et al. 2001, Tavşanoğlu and Çatav 2012). In this study, based on the strong relationship between dormancy-breaking thresholds and fire severity, we hypothesize that variation in dormancy represents a heteromorphic seed trait and that seedling performance offsets contribute to maintaining such heteromorphism. Different post-fire resource conditions resulting from high- and low-severity fires could therefore have selected for differences in either (1) seed size, (2) the ability of seedlings to establish in different resource environments, or (3) both of these traits. At present, there are no accounts in the literature that detail whether seeds with different dormancy-breaking thresholds have different ecological adaptations as seedlings. This is important, both for understanding the persistence of species in fire-prone environments, and the consequences of changes to the fire regime due to anthropogenic management practices and climate change (Ooi et al. 2014).

To address our hypothesis, we experimentally identify seed lots within population, based on their dormancy-breaking temperature thresholds, and categorize them as either having high thresholds, and can therefore only have dormancy broken by the passage of relatively high-severity fire, or low thresholds, where dormancy may be broken by low-severity fire or potentially during hot weather in the inter-fire period (see Ooi et al. 2014). We then investigate the relationship between threshold, seed size and seedling performance. The following specific questions are addressed: (1) Are there morphological differences between low and high dormancy-breaking temperature threshold seeds? (2) Is there a relationship between seed size and dormancy-breaking threshold temperatures within species, reflecting a difference in seed-stored resources, which could contribute to seedling establishment differences? (3) Do seedlings from different dormancy-breaking threshold seeds display different performances, including in their growth, resource allocation and competitive ability?

## MATERIALS AND METHODS

### *Study site and species*

Physical dormancy is one of two dominant seed dormancy types in southeastern Australia, present in over 40% of all dormant shrub species (Ooi 2007). The Fabaceae family represents the majority of native physically dormant species (Auld 1986). We conducted our experiment on two Fabaceae species, *Bossiaea heterophylla* and *Viminaria juncea*. Species were selected because both are common and widespread, and representative of physically dormant shrubs in drier (*B. heterophylla*) and

wetter (*V. juncea*) fire-prone sclerophyll vegetation types in eastern Australia. *Bossiaea heterophylla* is an obligate seeder, although it occasionally resprouts after low-severity fire (Kubiak 2009), and is commonly found in open woodlands in eastern Australia (distributional range over 1700 km). *Viminaria juncea* is also an obligate seeder (Kubiak 2009) and is commonly found in wetter heathland habitats with a dense shrub stratum and dominant layer of sedge species, such as *Gahnia sieberiana* and *Baumea teretifolia* (Keith and Myerscough 1993). It is widespread in such habitats around Australia, with a distributional range of over 4000 km. Seeds were randomly collected from 15 to 20 individuals within a single population of *V. juncea* from Royal National Park (34°03' S, 151°03' E) and *B. heterophylla*, from Heathcote (34°07' S, 150°58' E) National Park in southeastern Australia where fire is a ubiquitous disturbance (Keith et al. 2002).

### *Experiment 1: identification of dormancy breaking temperature thresholds and the relationship between seed mass and temperature thresholds within species*

The relationship between seed mass and temperature responses were determined by following the germination of weighed seeds. Three hundred seeds from each species were inspected for visible morphological differences including color and size. Individually weighed seeds were then divided into two samples and placed in separate labeled compartments of microtiter plates. Half the seeds from each species were then subjected to a low temperature treatment (10 minutes exposure to 60° and 40°C for *B. heterophylla* and *V. juncea*, respectively), and the other half to 10 min exposure to an 80°C temperature treatment. The low temperature treatments represented soil temperatures that had been recorded during low-severity fires or within canopy gaps at the study sites, and which had shown to be sufficient to promote some germination in the study species (Ooi et al. 2014, Liyanage and Ooi 2015). Temperatures of 80°C and above have only been recorded during relatively high-severity fires (Auld 1986, Ooi et al. 2014). The 80° temperature treatment therefore represented soil temperatures generated during relatively high-severity burns.

After the temperature treatment, seeds were allowed to cool and were subsequently placed on moistened filter papers in 9-cm Petri dishes. Filter papers were marked with a numbered 20-square grid, so that the dormancy-breaking temperature threshold and seed mass of each germinated seed could be identified. Seeds were incubated at 25°/18°C and light/dark conditions on a 12/12 h cycle, and checked at two-day intervals for germination, which was judged to occur when the radicle emerged.

Seeds with low dormancy-breaking temperature thresholds were identified by their germination in response to the low temperature treatments (60° or 40°C for *B. heterophylla* and *V. juncea*, respectively), and germinants were able to be used directly from these treatments. However, high dormancy-breaking threshold

seeds were identified as those viable seeds that did not germinate in response to the 80°C treatment. This was done to ensure that only high temperature threshold seeds were separated out, because using all seeds germinating in response to 80°C would also have included those with lower dormancy-breaking temperature thresholds. This is supported by previous experiments which have shown an increase in the proportion of seeds that germinate at heat treatments above 80°C (approximately 10% of seeds with thresholds above 80°C for *B. heterophylla*, and 42% for *V. juncea*; see Appendix S1). To identify the viable seeds from those remaining ungerminated after the 80°C temperature treatment, seeds were manually scarified and allowed to germinate in the same incubation conditions as above. Seeds that germinated after the low temperature treatment had dormancy-breaking thresholds at or below the treatment temperature (low-threshold morphs). Seeds that did not germinate after the high temperature treatment but were then able to germinate after scarification had dormancy-breaking thresholds above 80°C (high-threshold morphs).

The measured masses before the heat treatments of each of the seeds that germinated after the low temperature treatment were used to calculate the mean seed mass of low-threshold seeds. The seed masses of the seeds that only germinated after scarification were used to calculate the mean seed mass of high-threshold seeds.

#### *Experiment 2: performance of seedlings from low and high dormancy-breaking temperature threshold seeds*

Growth characteristics of seedlings from low and high-threshold seeds were measured under two levels of competition: seedlings grown alone in separate pots (no competition) or in pots already occupied by three heterospecific seedlings (competition). Competition occurs when neighboring plants reduce available resources for plant growth (Harper 1977, Casper and Jackson 1997). For both treatments, the same sized pots were used to plant the target seedlings alone and with three competitor seedlings, ensuring that competition for resources was induced. Previously established *A. linifolia* seedlings, approximately 5 cm tall and therefore much larger than the target species' individuals, were used as the competitor species. They represent a common co-occurring species in the target species habitat. It has been shown that there are no allelopathic impacts of *A. linifolia* after establishment (Quddus et al. 2014).

To set up the experiment, low and high-threshold morphs were first identified for each species as described in Experiment 1. For each species, 10 pots were used for each threshold (high and low) × treatment (no competition and competition) combination, meaning a total of 40 pots per species. Three germinated seeds were then planted into each pot, which measured 15 cm in diameter by 20 cm high and were filled with a 5:1 sand:vermiculite mixture and fertilized with Osmocote Native Gardens, Scotts Australia Pty Ltd, Auckland, New Zealand (10 g/pot). After

emergence, seedlings of the target species were thinned so that a single seedling remained in each pot. The number of days taken for cotyledons to open after emergence was then recorded during this period. By the end of the establishment phase, approximately 10% mortality meant that 35 seedlings per target species remained for growth measurements across all threshold × treatment combination. All pots were kept in an open area at the University of Wollongong's Ecological Research Centre from April to July 2014 throughout the experiment.

To control for differences in germination treatments (temperature treatments and scarification), growth characteristics were only measured after hypocotyl emerged from the soil. This controlled for the differences both in imbibition rate and potential initial mechanical resistance of the seed coat. After the cotyledons had opened, growth characters were measured for four months at three-week intervals, and included shoot height to the nearest 1 mm for both species, and number of leaves and number of branches for *B. heterophylla* only (due to the habit of the *V. juncea* seedlings).

At the end of four months, biomass allocation was determined for seedlings from both treatments. The number of seedlings available was determined by seedling survival after four months and the number of those that could be successfully extracted from pots. Six to eight plants from each of the low and high-threshold groups were carefully removed from the pots without damaging the roots and washed under running water. Plants were wiped dry. To determine dry mass, roots and shoots were put into paper bags separately and dried for 72 h at 60°C in a constant temperature oven. Dried roots and shoots were then measured separately using a digital balance to the nearest 0.01 mg and the Relative Interaction Index (RII; Armas et al. 2004) was calculated for both dry root and dry shoot masses for each species using the following equation:

$$RII = \frac{B_w - B_o}{B_w + B_o}$$

where  $B_o$  is the biomass potentially achieved by the target plant growing alone and  $B_w$  is the biomass achieved when growing in the competition treatment. For the calculation we used mean  $B_o$ . A greater absolute value of RII indicates a greater intensity of plant interaction.

#### *Data analysis*

Mean seed masses of low and high threshold seeds were compared using Welch's two-sample *t* tests. Data were tested for homogeneity of variances and normality prior to the statistical analysis. A one-way Generalized Linear Model (GLM) was used to compare time taken for cotyledon opening between low and high-threshold seeds, with a Poisson error structure and log link function.

We were interested in understanding seedling performance under either competitive or non-competitive environments, two post-fire conditions that occur independently (i.e., resulting from high-severity fire

[non-competitive] or unburned/low-severity fire [competitive]). As such, to analyze the growth characteristics of low and high-threshold seedlings, we used two-factor Linear Mixed-effects Models (LMMs) for both species for each competition treatment separately. Temperature threshold and seedling age were assigned as fixed factors and individual plants as the random factor. Shoot heights for both species and leaf and branch numbers for *B. heterophylla* were analyzed as the growth characters. When there was a significant interaction between threshold and age, a Tukey's multiple comparisons test was used to identify differences between levels. The normality of data were checked using histograms and residual graphs. Prior to analysis with GLMs, all data were examined and found to display homogeneity of variance and to not display overdispersion. The Relative Interaction Indices (RII) were analyzed between low and high-threshold seedlings separately for dry shoot and dry root biomasses using Welch's two-sample *t* tests. Data were tested for homogeneity of variances and normality prior to the statistical analysis. All analyses were conducted using the R statistical platform (R Core Development Team 2014). All graphs were plotted using untransformed data.

## RESULTS

### *Relationship between seed mass and dormancy breaking temperature thresholds within species*

There were no visible morphological differences of seed color or shape between seeds with different thresholds for either species. Additionally, mean seed masses of low and high-threshold seeds did not significantly differ for either *V. juncea*, where values ranged from  $5.60 \pm 0.62$  to  $6.05 \pm 1.12$  mg (Welch's *t* test,  $df = 18.17$ ,  $t = 1.52$ ,  $P = 0.146$ ), or *B. heterophylla*, where values ranged from  $16.44 \pm 2.51$  to  $17.24 \pm 2.07$  mg (Welch's *t* test,  $df = 17.3$ ,  $t = 1.10$ ,  $P = 0.286$ ) (Fig. 1).

### *Performance of seedlings from low and high dormancy-breaking temperature threshold seeds*

For both species, cotyledon opening was significantly faster for low-threshold seedlings than for high-threshold seedlings, with opening rates two days and two days faster for *B. heterophylla* (Poisson GLM,  $df = 1$ ,  $\chi^2 = 10.55$ ,  $P = 0.007$ ; Fig. 2a) and *V. juncea* (Poisson GLM,  $df = 1$ ,  $\chi^2 = 4.83$ ,  $P = 0.028$ ; Fig. 2b), respectively.

Measurement of later stage growth characteristics showed that growth is suppressed by competitive conditions for both species, irrespective of threshold. However, within the competition treatment, there were clear differences between low and high-threshold seedlings, although these differences were evident in different growth characteristics for each species.

For all *B. heterophylla* growth characteristics including shoot height (LMM,  $F_{1,66} = 24.002$ ,  $P < 0.0001$ ; Fig. 3a), leaf number (LMM,  $F_{1,66} = 27.826$ ,  $P < 0.001$ , Fig. 3c),

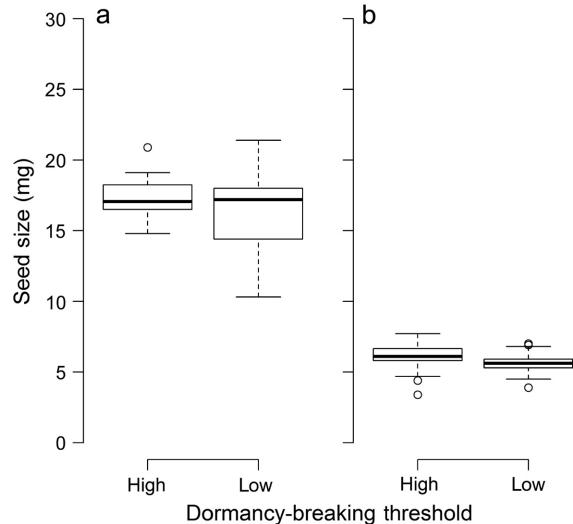


FIG. 1. Seed size as measured by mass for seeds with low or high dormancy-breaking threshold seeds for (a) *Bossiaea heterophylla* (low threshold  $n = 38$ , high threshold  $n = 17$ ) and (b) *Viminaria juncea* (low threshold  $n = 41$ , high threshold  $n = 20$ ) (box metrics: central line, median; box, inter-quartile range; whisker, 1.5 x inter-quartile range; white circles, outliers).

and branch number (LMM,  $F_{1,66} = 22.323$ ,  $P < 0.001$ , Fig. 3d), there was a significant interaction between threshold group and time. Growth was greater for low-threshold seedlings compared to high-threshold seedlings in the competition treatment. Differences began to differ significantly at 12 weeks for shoot heights (Tukey's test,  $df = 1$ ,  $Z = 4.520$ ,  $P < 0.01$ ), leaf number (Tukey's test,  $df = 1$ ,  $Z = 4.163$ ,  $P = 0.001$ ), and branch number (Tukey's test,  $df = 1$ ,  $Z = 3.177$ ,  $P = 0.044$ ). In the non-competitive conditions, only shoot height differed significantly, with

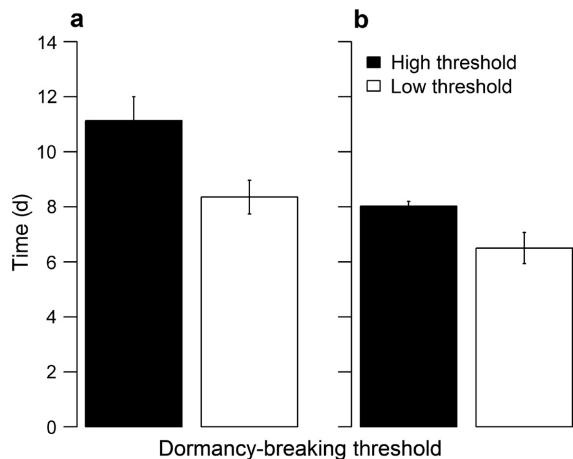


FIG. 2. Time taken for cotyledons to open for low and high dormancy-breaking threshold seeds (mean and SE) of (a) *Bossiaea heterophylla* (low threshold  $n = 18$ , high threshold  $n = 19$ ) and (b) *Viminaria juncea* (low threshold  $n = 19$ , high threshold  $n = 18$ ).

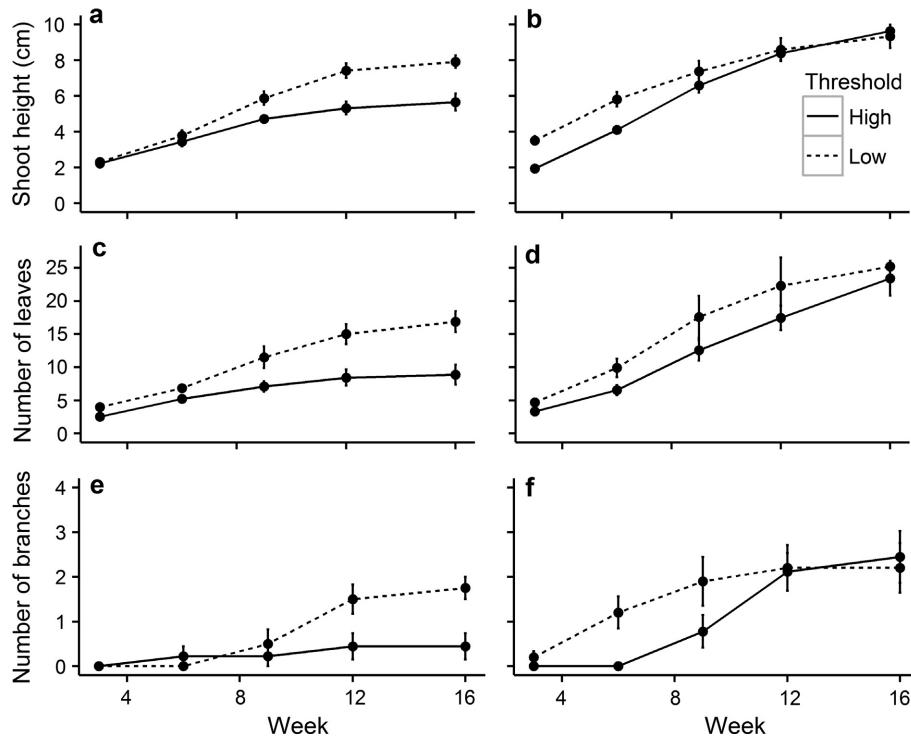


FIG. 3. Growth characteristics (mean  $\pm$  SE) of low and high dormancy-breaking threshold seedlings of *Bossiaea heterophylla* for (a) shoot height under competition (low threshold  $n = 10$ , high threshold  $n = 9$ ), (b) shoot height without competition (low threshold  $n = 7$ , high threshold  $n = 9$ ), (c) number of leaves under competition, (d) number of leaves without competition, (e) number of branches under competition, and (f) number of branches without competition treatments.

low-threshold seedlings taller than high-threshold seedlings (Tukey's test,  $df = 1$ ,  $F_{1,74} = 18.257$ ,  $P = 0.0001$ ) at early stages, a difference that became nonsignificant from week six (Tukey's test,  $df = 1$ ,  $Z = 2.706$ ,  $P = 0.134$ , Fig. 3a). The RIIs were significantly different between low and high-threshold seedlings dry shoot biomasses (Welch's  $t$  test,  $df = 11.62$ ,  $t = -3.6668$ ,  $P = 0.003396$ ), but not for dry root biomasses (Welch's  $t$  test,  $df = 11.155$ ,  $t = -1.7116$ ,  $P = 0.1146$ ) for *B. heterophylla* (Fig. 4a).

For *V. juncea*, significant differences between low and high-threshold seedlings were found only in biomass allocation. The same patterns were not evident for aboveground growth characteristics, with shoot heights attaining similar heights for both high and low-threshold seedlings under either competition (LMM,  $F_{1,78} = 1.095$ ,  $P = 0.299$ , data not shown) or non-competitive treatments (LMM,  $F_{1,66} = 1.900$ ,  $P = 0.173$ , data not shown). The RIIs were significantly different between low and high-threshold seedlings dry root biomasses (Welch's  $t$  test,  $df = 6.3313$ ,  $t = -2.6782$ ,  $P = 0.03477$ ), but not for dry shoot biomasses (Welch's  $t$  test,  $df = 8.6742$ ,  $t = 0.062876$ ,  $P = 0.9513$ ; Fig. 4b).

#### DISCUSSION

Seed heteromorphism is a mixed strategy for seed germination, to ensure offspring are spread over space

and time. Studies identifying variation in seed characteristics have contributed to the development of hypotheses for species coexistence (between-species variation) and bet-hedging strategies (within-species variation), when different morphs are able to occupy different regeneration niches (Lloyd 1984, Hanley and Fenner 1998, Imbert 2002, Coomes and Grubb 2003). However, to test these hypotheses, it is necessary to go beyond identification of such variation, and assess whether positive ecological outcomes result from the expression of different seed characteristics. Our study provides experimental evidence that intra-specific heteromorphic variation in dormancy-breaking temperature thresholds is related to subsequent seedling performance, with seedlings from low-threshold seeds performing better in competitive environments. This has implications for understanding the regeneration strategies of physically dormant species from fire-prone ecosystems, how variation in seed characteristics are maintained, and the potential for fitness differences of seedlings from different seed morphs.

A key reason for failure of seedling establishment in any ecosystem is competition with other emerging seedlings and surrounding vegetation (Gross 1980, Taylor and Aarssen 1989). Larger seeds are considered to have significant advantages over smaller seeds under competition by developing larger seedlings that perform better, particularly in low resource environments (Leishman

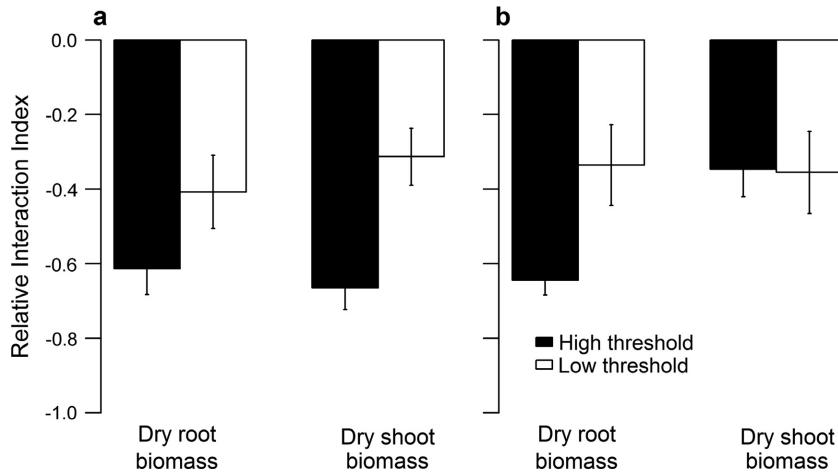


FIG. 4. Relative interaction index (RII; mean and SE) of dry root and dry shoot biomass for (a) *Bossiaea heterophylla* (low threshold  $n = 7$ , high threshold  $n = 8$ ) and (b) *Viminaria juncea* (low threshold  $n = 6$ , high threshold  $n = 6$ ). Bars represent seedlings from high or low temperature threshold seed morphs.

et al. 2000, Coomes and Grubb 2003). However, seed size of low- and high-threshold seeds did not differ significantly in either of our studied species. The differences in seedling response between the different threshold seeds that we observed therefore cannot be assigned to visible morphological characteristics such as size, but provide strong evidence that dormancy-breaking temperature thresholds are related to the physiology of seeds that lead to different seedling competitive abilities. This means that variation in dormancy thresholds is a form of cryptic heteromorphism (Imbert 2002).

Fire plays a significant role in plant regeneration by determining the soil temperatures experienced by dormant seeds to break their dormancy (Jeffery et al. 1988, Auld and O'Connell 1991), and influencing post-fire biotic and abiotic conditions that are important for seedling establishment (Wellington and Noble 1985, Taylor and Aarssen 1989, Carrington and Keeley 1999). For fire severity, there is a clear relationship between the level and duration of heating seeds experience in the soil during the passage of fire and the amount of vegetation consumed (Certini 2005), with lower soil temperatures associated with greater levels of remaining vegetation, and therefore more competitive conditions. Similar soil temperatures are experienced without fire, in gaps in unburned vegetation. For example, De Villalobos et al. (2007) showed that a reduction in vegetation cover and competition, and increased nutrients in burned sites, favored seedling establishment of *Prosopis caldenia* compared to unburned sites, where competition remained high due to existing vegetation. The seedlings germinating from low dormancy-breaking threshold seeds in our study have to overcome the negative effects of competition within canopy gaps or in the post-fire environment after low-severity fire, and both species produce seedlings that perform better under competitive conditions.

In our study, cotyledon opening was faster in seedlings produced from low-threshold seeds for both species. The early emergence of seedlings has been identified as beneficial for establishment in competitive environments, showing increased growth and fecundity over their late-emerging counterparts (Harper 1977, Verdú and Traveset 2005). Fast seedling emergence provides the opportunity for seedlings to absorb available nutrients quickly and to become more resilient to subsequent harsh environmental conditions. This was observed for four physically dormant Cistaceae and Fabaceae species from a Mediterranean gorse community, where early emergence resulted in higher fitness than late emergence, with differences remaining significant for at least nine years post-fire (De Luis et al. 2008).

One of the most striking results from our study was that differences in seedling performance between low and high-threshold seeds were only produced under the competition treatment. This suggests that the offset between dormancy threshold and seedling performance is likely to be selected for more in response to competition, rather than other factors such as ability to emerge from greater depth (e.g., see Hanley et al. 2003). For *B. heterophylla* this was highlighted at later stages, with the aboveground characteristics of shoot growth rate and numbers of leaves and branches all better performing for low dormancy-breaking threshold seeds under competitive but not non-competitive conditions. This was also confirmed by the significantly higher RII value of dry shoot biomass for low-threshold seedlings. Noticeably, the differences in performance increased with time, possibly reflecting increasing competition as the growth of competing heterospecific neighbors led to a greater depletion of resources later in the experiment. In our fire-prone study region, the soil seed bank can experience soil temperatures during higher severity fires that enable both low and high dormancy-breaking threshold seeds to

germinate into the post-fire environments. Competition after higher severity fires is relatively low because of the removal of standing vegetation and the increase in available nutrients (Lamont et al. 1993, Bradstock and Auld 1995, Penman and Towerton 2008). Seedling density and competition among seedlings is also low, with correspondingly little competition-related seedling mortality (Moles and Westoby 2004). This suggests that even though low-threshold seeds have been selected to produce better performing seedlings under competition typical of low-severity post-fire conditions, this advantage is not maintained in less competitive conditions produced after high-severity fire.

For *V. juncea*, a difference in later-stage seedling performance was found in the biomass allocation above- and belowground, rather than shoot growth characteristics. The significantly lower RII of dry root biomass for high-threshold seedlings showed the relative reduction of root biomass was related to the presence of competitive species. The high root biomass allocation found in low-threshold seedlings is considered to be advantageous for seedling survival under adverse conditions, especially in drought-prone or nutrient-poor environments. For example, Lloret et al. (1999) showed a positive correlation between seedling survival and higher root growth of 11 dominant species from Mediterranean shrublands. In our study region, *V. juncea* is commonly found in wet heath habitats with a dominant layer of sedges (Keith and Myerscough 1993). A high root mass allocation could be beneficial when growing in such environments, where large numbers of resprouting sedge species, which maintain a high density of roots and increase belowground competition, recover quickly after low-severity fires.

In summary, while seedlings from low-threshold seeds are consistently better performing, the way that this performance is expressed is likely to be dependent on the environment in which species occur. The development of early emergence and fast growth of seedlings has been found in other studies to primarily be a result of larger seed size, which produces a larger embryo, therefore providing greater ability for seedling survival (e.g., Leverett and Jolls 2014, Yang et al. 2015). The lack of size or mass differences between low and high dormancy-breaking threshold seeds in our study indicates that seed size or mass is not a factor that is related to the differences in seedling characteristics of low and high-threshold seeds. The mechanisms by which the different dormancy-breaking thresholds are maintained are therefore not clear. Variation in seed coat thickness or composition are possible factors that could underlie different dormancy-breaking thresholds, and if the thickness of seed coats vary, then equal-sized seeds may contain different embryo sizes. However, seed vigor appears to underpin variation in seedling performances for many agricultural species (e.g., Elliott et al. 2008, Xie et al. 2014), with higher temperatures during seed fill having strong negative effects on vigor in the Fabaceae (Tekrony and Egli 1991, Ellis 1992, Ooi 2015). The relative factors that determine

different dormancy-breaking thresholds are yet to be fully understood.

The inherent variability of the fire regime is likely to have selected for differences in dormancy-breaking thresholds within species. This ensures that populations persist, with dormancy variation ensuring that at least a portion of seeds germinate in any particular seed lot (Auld and O'Connell 1991, Liyanage and Ooi 2015). However, the variation in post-fire environmental conditions means that it is important to maintain different seedling characteristics for successful regeneration. Within the highly competitive environment that results after a low-severity fire or within a vegetation gap during the inter-fire period, the competitive ability of seedlings from low-threshold seeds is advantageous. While our experiment did not allow direct measurement of reproductive output, this advantage suggests greater expected fitness of low-threshold seed morphs under such competitive conditions. Conversely, in the low competitive environments after high-severity fire, being a seedling produced from high-threshold seeds is not disadvantageous. Subsequently, we conclude that because of the relationship between dormancy threshold and competitive ability, and the inherent variability of the fire regime on subsequent habitat modification, seed heteromorphism can be maintained. Future experiments growing seedlings from the two seed morphs through to maturity, under different levels of competition and ideally in post-fire conditions, would allow a thorough test of fitness outcomes.

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