Chapter 3

INTERACTION EFFECTS OF SEED MASS AND TEMPERATURE ON GERMINATION IN AUSTRALIAN SPECIES OF *FRANKENIA* L. (FRANKENIACEAE).

Lyndlee C. Easton & Sonia Kleindorfer

ABSTRACT

Easton, L.C. & Kleindorfer, S. (2008) Interaction effects of seed mass and temperature on germination in Australian species of *Frankenia* L. (Frankeniaceae). *Folia Geobotanica* 43(4).

Seed size and number theories have been proposed to explain the advantages of having many small versus a few large seeds in plants. In particular, seed germination is predicted to be shaped by temperature, and may differ for small and large seeds. In this study, we experimentally test germination at different temperatures in 12 species of arid zone plants in the genus *Frankenia* that differ in seed mass. Seed mass was categorized as 'smaller-seeded species' versus 'larger-seeded species' for analysis (six species per category). Many of these species co-occur geographically and hence experience similar abiotic conditions (unpredictable rainfall, extremes in temperature, poor soil conditions). The results demonstrated differences in germination as a result of the temperature*seed mass(species) interaction effect. There were significant differences in germination rates across seed mass categories during the first eight days of germination. Germination rates were higher in the larger-seeded species than the smaller-seeded species. Smaller-seeded species had lower germination success but had higher germination rates at lower temperatures, and had a more stringent temperature as a germination cue. These findings are discussed in the context of life history strategies in arid zone plants.

Key words: seed size and number, Australian arid zone plants, germination, seed mass*temperature interactions

INTRODUCTION

Seedlings are the most sensitive stage in a plant's life history to environmental conditions. The ambient temperature during periods of soil water availability is known to be an important cue for seed germination, and the interaction effects of temperature and moisture availability at seed germination substantially contribute to promoting germination during conditions that enhance the survival of the seedling stage (Thompson & Grime 1983; Baskin & Baskin 1988; Milberg *et al*. 1996).

Life history strategies, including seed germination cues, may be shaped by natural selection, and the distribution of alternative strategies between species along life history trait gradients is considered to be an adaptive solution to maximize fitness under different environmental conditions (Roffe 2002). Life history strategies also influence the allocation of resources by a parent to the size and number of offspring it produces (Lovett Doust 1989; Fenner & Thompson 2005). This reproductive allocation is theorized to represent a trade-off in quantity versus quality of offspring, and in plants is thought to be characterized in the size versus number of seeds produced by the plant (Smith & Fretwell 1974; Salisbury 1974).

In basic seed size and number theory models, which assume that all species occurring in a particular habitat are subjected to a similar set of environmental conditions, all plants in a given population should evolve seeds of only one size (and concomitantly seed number) because there is always (mathematically) a single best seed size that optimizes fitness (Smith & Fretwell 1974; Rees & Westoby 1997). However, seed size (mass) within species is one of the least plastic components of plant life history strategies (Harper *et al*. 1970), and seed numbers are dependent genetically on ovule number per flower (Wiens 1984). Flowering plants have (within limits) perpetual growth patterns, and meristems generally continue to grow as long as resources are available and conditions remain favourable (Wiens 1984). Thus, resource availability and environmental conditions determine the number of reproductive modules (flowers) produced. An increase in flower production, and therefore the potential for an increase in seed number per plant, does not necessarily correlate with a decrease in seed size. Consequently, the total number of seeds produced per plant (and concomitantly plant seed mass output) will depend on environmental conditions from year to year. For these reasons, Wiens (1984) has advocated the concept of 'brood' size in plants. Wiens (1984) defines brood size in plants as the mean number of seeds maturing per fruit – not per plant – arguing that in sessile organisms with multiple reproductive modules, natural selection acts primarily on the individual module (fruit) and not on plant seed output. Consequently, this

reasoning underlies the study of seed packaging strategies (seed size versus seed number) in relation to life histories strategies.

Large seeds have been demonstrated to have a competitive advantage over smaller seeds by having higher germination rates and having greater nutrient reserves for the young seedlings, which enable the seedlings to grow larger to tap resources earlier than their small-seeded counterparts (Fenner 1983, 1992; Marshall 1986; Stock *et al*. 1990; Milberg *et al*. 1996; Venable *et al*. 1998; Easton & Kleindorfer 2008a). Consequently, seedlings from large-seeded species should be able to establish under a wider range of environmental conditions that could not be tolerated by seedlings from small-seeded species. Drought conditions and arid environments are commonly cited hazards that create conditions that favour large seed mass among plants (Baker 1972; Salisbury 1974; Wulff 1986; Mazer 1990; Geritz *et al*. 1999). However, variation in seed mass in arid regions persists, despite the apparent advantages of being large-seeded (Leishman *et al*. 2000; Westoby *et al*. 2002).

A problem when studying seed size and number theories is the definition of 'large' and 'small' seeds. 'Large' seeds are generally identified as seeds whose larger size is the result of containing extra nutrient reserves for the growth of the resultant seedlings, and not by an absolute numerical interval. Similarly, 'small' seeds are identified as having few nutrient reserves for seedling growth. Seed size and number theories predict that plant reproductive strategies evolve to invest more resources into either fewer large seeds which contain extra nutrients per fruit, or alternately produce many smaller seeds that do not contain extra nutrients per fruit. Taxa evolving the strategy of packaging available resources into fewer larger seeds, rather than many smaller seeds, would arguably be producing the largest seeds possible within its phylogenetic constraints, despite the actual mass of the seed.

We compare seed germination in 12 Australian arid zone species within the genus *Frankenia*. Six species produce low numbers of larger seeds per fruit, and six species produce many smaller seeds per fruit. This study therefore addresses the problem of phylogenetic non-independence using a comparison across species within a single genus. Furthermore, as both seed-mass strategies occur within similar phytogeographic regions, we can address the ecological implications for each strategy that would otherwise be masked by the strong effects of region or habitat disparity (see Silvertown 1981).

There are few published studies on arid zone plants that compare germination across seed mass within a genus (but see Easton & Kleindorfer 2008a, in press). We test the effects of seed mass and ambient temperature on the germination in *Frankenia* to determine if these factors, or a combination of these factors, support seed size and number theories in arid zone

species. We test the hypothesis that larger-seeded species germinate under environmental conditions that are less favourable for seedling establishment in the smaller-seeded species. We predict (1) faster germination rates in the larger-seeded species at higher temperatures, (2) higher germination success in the larger-seeded species after 28 days at higher temperatures, and (3) more stringent temperature requirements as germination cues in the smaller-seeded species.

MATERIALS AND METHODS

Test species

This study uses the taxonomic relationships described by Summerhayes (1930) and revised by Barnsley (1982). Table 1 lists the 12 *Frankenia* species (with authorities) included in this study, six species with few ovules per fruit and larger seed mass, and six species with a high number of ovules per fruit and smaller seed mass. We include two varieties of *F. pauciflora*. A recent revision of the *F. pauciflora* group has demonstrated that South Australian *F. pauciflora* and Western Australian *F. pauciflora* are sufficiently molecularly unrelated to warrant their inclusion as two separate species in this study (Craigie 2007). Seeds were collected from naturally occurring populations in early autumn from 2001 to 2005 by one of the authors (L. Easton) [1], and stored at optimal storage conditions (i.e. <21°C, <30% humidity, away from sunlight – see Wrigley & Fagg 2003). All germination experiments were undertaken within one and two years from seed collection. Seeds from all species were periodically germinated over time to check for loss of viability. No degradation in seed germinability, or indication of seed dormancy was found during this time period. *Frankenia* seeds have been demonstrated to retain *in situ* viability for at least seven years (Easton & Kleindorfer 2008a).

A random selection of 150 individual seeds from each population was weighed using a Mettler Toledo MX/UMX microbalance, and mean seed mass per population and per species was recorded. The mean seed mass of the species with few ovules per fruit included in this study was 0.382 mg (±0.005 s.e.), and are henceforth designated 'larger-seeded species'(*sensu*

---

* Herbarium voucher specimens, collection details including month and year of seed collection, and number of plants sampled per population are available from the authors at Flinders University, School of Biological Sciences.[1]

Easton & Kleindorfer 2008a). The mean seed mass of the species with many ovules per fruit included in this study was 0.098 mg (±0.007 s.e.) and are henceforth designated 'smaller-seeded species' (*sensu* Easton & Kleindorfer 2008a).

Treatments

To examine the effects of temperature and seed mass on germination, replicates of 15 seeds were sown on Whatman Number 1 filter paper in 500 ml plastic containers. The filter paper was suspended on round perforated PVC disks with 10 mm legs. Distilled water was added to each plastic container to the level of the PVC disk, suspending the filter paper and seeds over a 200 ml reservoir of water. Consequently, the seeds were in constant contact with water (adapted from Zubrinich 1990). Containers were sealed to prevent evaporation and placed in growth cabinets of 17°C, 23°C, or 29°C, and illuminated with Silvanian Gro-lights (25 $\mu mol^{m_{-2}S_{-1}}$, 400–700 nm) on a 14–hour day and 10–hour night regime. All experiments were undertaken at constant temperatures. Previous studies have demonstrated that using a constant temperature adequately emulates the naturally occurring fluctuating ('alternating') diurnal temperatures for germination of arid zone species (Mott & Groves 1981; Washitani & Masuda 1990; Bell *et al.* 1993). The light requirements for germination in *Frankenia* are discussed in Chapters 2 and 4. *Frankenia* does not require pre-treatment to stimulate germination or to break seed dormancy (Brightmore 1975; Brochman *et al.* 1995; Easton & Kleindorfer 2008a).

Experimental design followed a completely randomized block design. The following arrangements of effects were tested; species effect (three populations with four replications each per each of the 12 species), seed mass effect ('larger-seeded species' and 'smaller-seeded species'), and treatment effect (17°C, 23°C, 29°C). Thus four replicates of each population of each species were subjected to 17°C, 23°C or 29°C. This experimental design produced a total of 432 units (containers).

Seeds were checked every second day at which time germinated seeds were removed. Seeds were considered germinated with the emergence of the radicle. The experiment was terminated at Day 28. No viability tests (i.e. with tetrazolium) were performed on ungerminated seeds at the end of the experiment, since most seeds showed clear signs of rotting or fungal attack, lacked firmness, and were presumably non-viable (see Verger *et al.* 2003; Ramírez-Padilla & Valverde 2005).

Statistical analysis

All statistical analyses were calculated using SPSS Version 14 (SPSS Inc. 2006). The mean number of seeds that germinated at each 2–day interval per species was recorded, and then transformed into percentages. This data were then angular transformed (arc-sine square root) to satisfy the requirements of normality for percentage data (Dytham 2003).

First, we looked for an interaction between germination, temperature, and seed mass by examining differences in germination success (defined as the percentage of the total number of seeds per species that germinated) between the seed mass categories at Day 8, and at the end of the experiment (Day 28). We then compared the germination rates (defined as the percentage of seeds that germinated at each 2–day interval) of the larger-seeded species and smaller-seeded species every two days over the experimental period. We used Nested (hierarchical) Design ANOVAs, using temperature and seed mass as fixed factors, and species as a random factor. The lowest model terms (temperature*species(seed mass category)) were computed using a modified custom hypothesis test to test for the interaction of species nested within seed mass category. Tukey (HSD) posthoc tests examined any significant differences to establish where the differences lay. Partial Eta Square (η_{p2}) values – which provide a measure of effect size – were examined to quantify the degree of association between the main effects or interactions (seed mass category*temperature) and the dependent variable (germination) (Becker 2000). Partial Eta Square values can be interpreted as the proportion of variance in the dependent variable that is attributed to each effect, and is calculated as;

$$\eta_{p2} = SS_{effect} / (SS_{effect} + SS_{error}).$$

Next we investigated differences in germination rates and germination success within each temperature. Data were separated into the three temperature categories (17°, 23°, 29°) and the Nested Design ANOVAs were repeated after removing temperature from the calculations. Thus we could compare the germination of larger- and smaller-seeded species to investigate how temperature affected germination rates and germination success.

Finally, time (in days) to 50% germination (the T_{50} index – Trudgill *et al.* 2000) was examined. A Single Factor ANOVA compared differences in T_{50} between temperatures at Days 8 and 28.

RESULTS

Germination for both seed mass categories was rapid during the first eight days of the experiment irrespective of temperature, although germination outcome was affected by temperature at each 2-day interval to Day 10. Larger-seeded species had higher germination than smaller-seeded species,

Table 2 lists the interaction effects, calculated by Nested Design ANOVAs, for germination rates at Day 2, Day 4, Day 6, and Day 8, and for germination success at Day 8 and Day 28. For germination rates, the temperature*seed mass category interaction terms were not significantly different, and the species(seed category) terms were only significantly different at Day 2. However, the temperature terms were statistically significant for germination rates at each 2-day interval to Day 10. This indicates that temperature effects were comparable across species and seed mass categories. The interaction of temperature*species(seed mass category) (henceforward called the TMS interaction effect) was only significantly different at Day 2, where germination rates for the larger-seeded species were three-fold higher than that of the smaller-seeded species. Conversely, the TMS interaction effect was significantly different for germination success at both Day 8 and Day 28. A Tukey (HSD) posthoc test revealed significant differences in the germination success of seed mass categories between 17°C and 29°C, and between 23°C and 29°C, but not between 17°C and 23°C.

Table 3 lists species(seed mass category) effects, calculated by Nested Design ANOVAs for germination rates at Day 2, Day 4, Day 6, and Day 8, and for germination success at Days 8 and Day 28 at each temperature (17°C, 23°C, 29°C). The effect of seed mass category *per se* was not significantly different for any tests, suggesting that germination between seed mass categories per temperature per time period were constant. However, most species(seed mass category) terms were significantly different, indicating an effect of species for the results.

Figure 1 shows germination success over the 28–day period of the experiment for the seeds of the larger-seeded and smaller-seeded species at 17°C, 23°C, and 29°C, and for all temperatures combined. Overall, there was consistently higher germination for larger-seeded species than smaller-seeded species. By Day 28, 76.3% (±1.61 s.e.) of seeds of larger-seeded species had germinated, but only 62.4% (±2.25 s.e.) of seeds of smaller-seeded-species had germinated (Single Factor ANOVA: $P < 0.001$, df=324, F=5.27). However, germination decreased as temperatures increased. At 17°C, germination success in larger- and smaller-

seeded species was similar (85% and 81% respectively). Time to 50% germination (T_{50}) was five days for both seed categories. At 23°C, germination success at Day 28 was still 85% for the larger-seeded species (T_{50} at Day 4), but only 66% for the smaller-seeded species (T_{50} at Day 6). At 29°C, germination success at Day 28 was 59% for the larger-seeded species (T_{50} at Day 14), and 40% for the smaller-seeded species (T_{50} not reached within 28 days).

Figure 2 shows the Partial Eta Squared values (the proportion of the germination rates accounted by the TMS interaction effect) for the first eight days of the experimental period. The TMS interaction effect increased as temperatures increased, although the lower temperatures (17°C and 23°C) followed similar trends over time. However, the significant differences in germination resulting from the TMS interaction effect varied between temperatures. At 17°C, there were no significant differences in the TMS interaction effect in germination success at Day 8 or Day 28. Germination rates were significantly different on Day 4 and on Day 8. At 23°C, the TMS interaction effect was significantly different for germination success on Day 8 and on Day 28. The TMS interaction effect strongly influenced germination rates, being significantly different at each 2–day interval. Figure 1 shows that there were also significant differences between the larger- and smaller-seeded species in actual germination rates and germination success. At 29°C, the TMS interaction effect on germination success was significantly different on Day 8 but not on Day 28. Germination rates were significantly lower for the smaller-seeded species and TMS interaction effects significantly different (except at Day 4) during the first eight days of the experiment, which explains the success outcome at Day 8. However, germination in both seed mass categories decreased proportionally over time and was not significantly different by Day 28.

DISCUSSION

Our results demonstrate an unequivocal temperature*species(seed mass category) interaction effect on germination in *Frankenia*. Interestingly, germination rates and germination success were always higher in larger-seeded than the smaller-seeded species, except at 17°C at Day 4. Temperature not only affected germination rates and germination success differently between larger-seeded species and smaller-seeded species, but also within seed mass category. Overall, higher temperatures resulted in slower germination rates and lower germination success. The magnitude of the decrease was more pronounced in the smaller-seeded species. The differences in germination rates and germination success between

seed mass categories explained by TMS interaction effects increased with increasing temperatures. Seed germination has been demonstrated to be delayed by non-optimal temperatures (see below). Increases in temperature delayed germination in both seed mass categories as shown by the increase in time to T_{50} with increasing temperatures. One interpretation of the TMS interaction effect is that in the presence of available water, as seed mass decreases and temperature increases, germination is less likely to occur. At the lower temperatures, germination was similar between the larger-seeded species and the smaller-seeded species. Partial Eta Squared values were also smaller than at the higher temperatures. Thus, differences in germination between larger-seeded species and smaller-seeded species at 17°C were the result of something other than the TMS interaction effect. For example, it may be the result of a physiological germination characteristic other than seed mass, or solely related to temperature irrespective of seed mass.

Temperature affects the timing of germination by inhibiting germination under unsuitable environmental conditions and subsequently promoting germination under suitable conditions (Baskin & Baskin 1988; Meyer *et al.* 1998; Marone *et al.* 2000). While germination rates in both larger-seed and smaller-seed categories generally decreased as temperature increased, the magnitude of the decrease in germination rates was more substantial in the smaller-seeded species. Germination success for the smaller-seeded species at Day 28 was 82% at 17°C, 66% at 23°C, and only 40% at 29°C, compared with 85% at both 17°C and 23°C, and a decrease to 59% at 29°C for the larger-seeded species. This supports many studies on seed germination that have demonstrated lower optimal temperature for germination in smaller versus larger seeds (e.g. Van Tooren & Pons 1988; Keeley 1991; Bell *et al.* 1993; Bell *et al.* 1995; Bell 1999).

In regions of unpredictable rainfall there are two strategies that plant taxa may adopt for successful seed germination (Jurado & Westoby 1992a, 1992b). One strategy is for seeds to germinate after any rainfall event thus exploiting any available soil moisture. Alternately, seeds could have mechanisms that delay germination until they have had several days of contact with soil-water, which should signify the onset of a rainy season (Frasier *et al.* 1985; Jurado & Westoby 1992a). Our results suggest that both of these germination strategies have been adopted within the genus *Frankenia*. Larger-seeded species had rapid initial germination rates (i.e. imbibed promptly within the first two days of water availability), while smaller-seeded species had low initial germination. In this study water was not a constraint to germination as seeds were in constant contact with water. If water accessibility was the only stimulus for germination then all viable seeds should have had comparable germination rates

irrespective of seed mass.

The weather conditions immediately after germination are important for successful seedling establishment (Gurvich *et al.* 2004; Marone *et al.* 2000). In arid regions this post-germination period is also unpredictable (Mott 1972; Hastwell & Facelli 2003). A wet period consisting of intermittent brief showers would favour the faster germinating larger-seeded species (Elberse & Breman 1990). Fast germinating species, however, gamble on the frequency of these brief showers before the return of the ensuing drought conditions (Freas & Kemp 1983; Frasier *et al.* 1985; Gurvich *et al.* 2004). If the showers cease before successful seedling establishment, seedlings will die. In a wet period of intermittent brief showers the slower germinating seeds may never fully imbibe before the drought conditions return. Slower germinating taxa would only achieve successful seedling establishment with prolonged rainfall events or constant high soil-water content (Frasier *et al.* 1985). The latter would occur after rare but intense flooding events.

Arguments for larger seeds in arid regions generally incorporate three major predictions; (1) large seeds contain energy reserves for more rapid seed germination, (2) large seeds produce larger seedlings that grow longer roots to track receding soil-water levels after rainfall events, and to establish quickly before the onset of the ensuing drought conditions, and (3) large seeds contain essential nutrients for seedlings germinating in nutrient poor soils. Larger-seeded *Frankenia* species did have faster germination rates, thus supporting prediction (1) for large-seededness in arid regions. Partial support for prediction (2) is provided by L. Easton (unpublished data) who found evidence for rapid root extension in *F. serpyllifolia*. Many studies, including those by Lamont and Witkowski (1995), and Milberg and Lamont (1997) have demonstrated that root growth is more rapid in large-seeded species. Rapid vertical root growth is required to ensure that contact with the receding soil-water levels is maintained in drying soil (Richards & Lamont 1996). Rapid seedling growth has also been associated with conditions where fast emergence from sand cover is vital for survival (Maun & Lapierre 1986; Facelli & Ladd 1996). In regions dominated by sand, substantial sudden changes in microtopography can occur from aeolian actions. Therefore arguably, large-seeded species would occur in microsites which are predisposed to sand accretions (Maun & Lapierre 1986). Prediction (3) is discussed in Chapter 7.

The higher germination in the larger- than the smaller-seeded *Frankenia* species at 17°C, 23°C and 29°C implies that stringent temperature requirements are a more important germination cue in the smaller-seeded species. Studies by Milberg *et al.* (1996) support this argument. In their studies on the effects of seed mass on germination, germination varied

between 40–90% at sub-optimal temperatures and only the heaviest seeds exceeded 80% germination. In contrast to larger seeds, small seeds have minimal nutrient reserves for seedling growth and are more reliant on nutrients in the soil of their immediate surrounds. Note that soil nutrients and minerals – being water soluble – are more freely available to plants after rains. Also, smaller seeds produce smaller seedlings with shorter roots. We predict that small seeds have more stringent temperature regulated germination cues that only allow germination at the time of greatest resource availability for seedling establishment. Perez-Garcia and Gonzalez-Benito (2006) observed germination inhibition at temperatures greater than 25°C in plant species native to Mediterranean climates and interpreted this as an adaptation against germination following chance summer rains.

We have demonstrated that larger-seeded species of *Frankenia* have faster germination rates and higher germination success, which supports hypotheses for large-seededness in drought prone areas. However, no *Frankenia* seeds could be described as 'large'. Even the largest-seeded Australian *Frankenia* species, *F. setosa,* have mean seed weights of only 0.7 mg (±0.02 s.e.). The smallest-seeded Australian *Frankenia* species*, F. laxiflora* and *F. fecunda,* have mean seed weights of 0.06 mg (±0.001 s.e.). Thus, we provide evidence that even a small increase in seed mass affects germination in arid zone plant taxa.

ACKNOWLEDGEMENTS

REFERENCES

Baker, H.G. (1972) Seed weight in relation to environmental conditions in California. *Ecology* **53**: 997–1010.

Barnsley, B. (1982) Frankeniaceae. *In:* George A.S. (ed.) **Flora of Australia Volume 8**. Australian Government Publishing, Canberra. pp 112-146.

Baskin, C.C. & Baskin, J.M. (1988) Germination ecophysiology of herbaceous plant species in a temperate region. *American Journal of Botany* **75**: 286–305.

Becker, L.A. (2000) S**PSS Course Content – Fall, 2000.** At <http://www.uccs.edu/lbecker/SPSS/content.htm>. [Accessed 4th October 2008.]

Bell, D.T. (1999) Turner Review No 1. The process of germination in Australian species. *Australian Journal of Botany* **47**: 475–517.

Bell, D.T., Plummer J.A. & Taylor S.K. (1993) Seed germination ecology in southwestern Western Australia. *Botanical Review* **59**: 24–53.

Bell, D.T., Rokich D.P., McChesney C.J. & Plummer J.A. (1995) Effects of temperature, light and gibberellic acid on the germination of seeds of 43 species native to Western Australia. *Journal of Vegetation Science* **5**: 797–806.

Brightmore, D. (1979) Biological flora of the British Isles. *Frankenia laevis* L. *Journal of Ecology* **67**: 1097–1197.

Brochmann, C., Lobin, W., Sinding, P. & Stabbetorp, O. (1995) Parallel ecoclinal evolution and taxonomy of *Frankenia* (Frankeniaceae) in the Cape Verde Islands, W. Africa. *Nordic Journal of Botany* **15**: 603–623.

Craigie, A.I. (2007) **Systematic studies of the Australian species of *Frankenia* L. (Frankeniaceae).** PhD thesis, Flinders University, Adelaide, Australia.

Dytham, C. (2003): **Choosing and using statistics: a biologist's guide (2nd ed.).** Blackwell Publishing.

Easton, L.C. & Kleindorfer, S. (2008a) Germination in two Australian species of *Frankenia* L., *F. serpyllifolia* Lindl. and *F. foliosa* J.M.Black (Frankeniaceae) – effect of seed age, seed mass, light, and temperature. *Transactions of the Royal Society of South Australia* **132**: 29–40.

Easton, L.C. & Kleindorfer, S. (in press) Effects of salinity levels and seed mass on germination in Australian species of *Frankenia* L. (Frankeniaceae). *Environmental and Experimental Botany.*

Elberse, W.T. & Breman, H. (1990) Germination and establishment of Sahelian rangeland species. II. Effects of water availability. *Oecologia* **85**: 32–40.

Facelli, J.M. & Ladd, B. (1996) Germination requirements and responses to leaf litter of four species of eucalypt. *Oecologia.* **107**: 441–445.

Fenner, M. (1983) Relationships between seed weight, ash content and seedling growth in twenty four species of Compositae. *New Phytologist* **95**: 697–706.

Fenner, M. (1992) Environmental influences on seed size and composition. *Horticultural Review* **13**: 183–213.

Fenner, M. & Thompson, M. (2005) **The ecology of seeds.** Cambridge University Press, UK.

Frasier, G.W., Cox, J.R. & Woolhiser, D.A. (1985) Emergence and survival responses of seven grasses for six wet-dry sequences. *Journal of Range Management* **38**: 372–377.

Freas, K.E. & Kemp, P.R. (1983) Some relationships between environmental reliability and seed dormancy in

desert annual plants. *Journal of Ecology* **71**: 211–217.

Geritz, S.A.H., van der Meijden, E. & Metz, J.J. (1999) Evolutionary dynamics of seed size and seedling competitive ability. *Theoretical Population Biology* **55**: 324–343.

Gurvich, D.E., Enrico, L., Funes, G. & Zak, M.R. (2004) Seed mass, seed production, germination and seedling traits in two phenological types of *Bidens pilosa* (Asteraceae). *Australian Journal of Botany* **52**: 647–652.

Harper, J.L., Lovell, P.H. & Moore, K.G. (1970) The shapes and sizes of seeds. *Annual Review of Ecology and Systematics* **1:** 27–355.

Hastwell, G.T. & Facelli, J.M. (2003) Differing effects of shade-induced facilitation on growth and survival during the establishment of a chenopod shrub. *Journal of Ecology* **91**: 941–950.

Jurado, E. & Westoby, M. (1992a) Germination biology of selected central Australian plants. *Australian Journal of Ecology* **17**: 341–348.

Jurado, E. & Westoby, M. (1992b) Seedling growth in relation to seed size among species of arid Australia. *Journal of Ecology* **80:** 407–416.

Keeley, J.E. (1991) Seed germination and life history syndromes in the Californian Chaparral. *Botanical Review* **57**: 81–116.

Lamont, B.B. & Witkowski, E.T.F. (1995) A test for lottery recruitment among four *Banksia* species based on their demography and biological attributes. *Oecologia* **101**: 299–308.

Leishman, M.J., Wright, I.J, Moles, A.T. & Westoby, M. (2000) The evolutionary ecology of seed size. *In:* Fenner M. (ed) **Seeds, the ecology of regeneration in plant communities**. 2nd ed. CAB International, UK. pp 31-58.

Lovett Doust, J. (1989) Plant reproductive strategies and resource allocation. *TREE* **4**: 230–234.

Marone, L., Horno, M.E. & Solar, R.G.D. (2000) Post-dispersal fate of seeds in the Monte desert of Argentina: patterns of germination in successive wet and dry years. *Journal of Ecology* **88**: 940–949.

Marshall, D.L. (1986) Effects of seed size on seedling success in three species of *Sesbania* (Fabaceae). *American Journal of Botany* **73**: 457–464.

Maun, M.A. & Lapierre, J. (1986) Effects of burial by sand on seed germination and seedling emergence of four dune species. *American Journal of Botany* **73**: 450–455.

Mazer, S.J. (1990) Seed mass of Indiana Dune genera and families: taxonomic and ecological correlates. *Evolutionary Ecology* **4**: 326–357.

Meyer, S.E., Carlson, S.L. & Garvin, S.C. (1998) Seed germination regulation and field seed bank carryover in shadescale (*Atriplex confertifolia*: Chenopodiaceae). *Journal of Arid Environments* **38**: 255–267.

Milberg, P. & Lamont, B.B. (1997) Seed/cotyledon size and nutrient content play a major role in early performance of species on nutrient poor soils. *New Phytologist* **137:** 665–672.

Milberg, P., Anderson, L., Elfverson, C. & Regnér, S. (1996) Germination characteristics of seeds differing in mass. *Seed Science Research* **6**: 191–197.

Mott, J.J. (1972) Germination studies on some annual species from an arid region of Western Australia. *Journal of Ecology* **60**: 293–304.

Pérez-García, F. & González-Benito, M.E. (2006) Seed germination of five *Heliathemum* species: effect of temperature and pre-sowing treatments. *Journal of Arid Environment* **65**: 688–693.

Ramirez-Padilla, C.A. & Valverde, T. (2005) Germination responses of three congeneric cactus species (*Neobuxbaumia*) with differing degrees of rarity. *Journal of Arid Environment* **61**: 333–343.

Rees, M. & Westoby, M. (1997) Game-theoretical evolution of seed mass in multi-species ecological model. *OIKOS* **78**: 116–126.

Richards, M.B. & Lamont, B.B. (1996) Post-fire mortality and water relations of three congeneric shrub species under extreme water stress – a trade-off with fecundity? *Oecologia* **107**: 53-60.

Roffe, D.A. (2002) **Life history evolution.** Sinauer Associated Inc., USA.

Salisbury, E. (1974) Seed size and mass in relation to environment. *Proceedings of the Royal Society London B* **186**: 83-88.

Silvertown, J. (1981) Seed size, life span and germination date as coadapted features of plant life history. *The American Naturalist* **118**: 860–864.

Smith, C.C. & Fretwell, S.D. (1974) The optimal balance between size and number of offspring. *The American Naturalist* **108**: 499–506.

SPSS Inc. (2006) **SPSS Programming and Data Management (3rd ed.): A Guide for SPSS and SAS Users.** SPSS Inc., Chicago.

Stock, W.D., Pate, J.S. & Delfs, J. (1990) Influences on seed size and quality in seedling development under low nutrient conditions in five Australian and South African members of Proteaceae. *Journal of Ecology* **78**: 1005–1020.

Summerhayes, V.S. (1930) A revision of the Australian species of *Frankenia. Linnean Journal of Botany* **48**: 337–387.

Thompson, K. & Grime, J.P. (1983) A comparative study of germinating responses to diurnally fluctuating temperatures. *Journal of Applied Ecology* **20**: 141–156.

Trudgill, D.L., Squire, G.R. & Thompson, K. (2000) A thermal basis for comparing the germination requirements of some British herbaceous plants. *New Phytologist* **145**: 107–114.

Van Tooren, B.F. & Pons, T.L. (1988) Effects of temperature and light on the germination in chalk grassland species. *Functional Ecology* **2**: 303–310.

Venable, D.L., Dyreson, E., Piñero, D. & Becerra, J.X (1998) Seed morphometrics and adaptive geographic differentiation. *Evolution* **52**: 344–354.

Verger, P., Rengelink,R., Copal, A. & Ouberg, N.J. (2003) The interacting effects of genetic variation, habitat quality and population size on performance of *Succisa pratensis. Journal of Ecology* **91**: 18–26.

Washitani, I. & Masuda, M. (1990) A comparative study of the germination characteristics of seeds from a moist tall grassland community. *Functional Ecology* **4**: 543–557.

Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. & Wright, I.J. (2002) Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* 33: 125–159.

Wiens, D. (1984) Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. *Oecologia* **64:** 47–53.

Wrigley, J.W. & Fagg, M. (2003) **Australian native plants: Cultivation, use in landscaping, and propagation** (5th ed.). Reed New Holland Publishing, Australia.

Wulff, R.D. (1986) Seed size variation in *Desmodium paniculatum.* II. Effects on seedling growth and

physiological performance. *Journal of Ecology* **74:** 99–114.

Zubrinich, T.M. (1990) **An experimental examination of factors influencing zonation of the halophyte plant species,** *Frankenia pauciflora* **in a Goolwa salt marsh.** BSc. Honours thesis, Flinders University, Adelaide, South Australia.

Species	Voucher #	Population site	GPS	Seed mass (±se mg)
Few ovules				
F. cordata J.M.Black	LE05007	Rainbow Valley, NT	S24°19'57" E133°37'53"	0.295±0.02
	LE05006	Ormiston Gorge, NT	S23°40'45" E132°42'42"	0.391±0.02
	LE05011	Curtin Springs, NT	S25°21'01" E131°50'47"	0.375±0.009
F. interioris Ostenf.	LE03011	Kalgoorlie–Menzies road, WA	S30°32'03" E121°24'45"	0.302±0.007
	LE03012	Lake Googarrie, WA	S30°00'56" E121°09'46"	0.320±0.09
	LE03065	Mundrabilla,WA	S31°54'37" E127°21'26"	0.286±0.007
F. serpyllifolia Lindl.	LE02003	Birdsville Track, SA	S27°57'30" E138°39'36"	0.930±0.02
	LE02007	Bungadillina Creek, Oodnadatta Track, SA	S28°16'33" E135°50'16"	0.724±0.02
	LE02013	Mt Barry Station, SA	S28°16'33" E135°50'16"	0.537±0.02
F. sessilis Summerh.	LE01008	Fowlers Bay, SA	NR	0.159±0.004
	LE03066	Eucla, WA	S31°42'49" E128°53'06"	0.138±0.003
	LE03068	Cactus Beach, SA	S32°03'56" E132°59'37"	0.235±0.02
F. setosa W.Fitzg.	LE01041	Carnarvon Range, WA	S25°08'44" E120°14'20"	0.688±0.02
	LE03035	Gascoyne Junction, WA	S24°51'18" E115°18'20"	1.214±0.03
	LE03045	Kalbarri, WA	S27°45'23" E114°08'21"	0.935±0.03
F. tetrapetala Labill.	LE03058	Newdegate, WA	S33°11'29" E119°12'49"	0.122±0.003
	LE03060	Esperence, WA	S33°34'46" E121°45'44"	0.202±0.01
	LE03061	Grasspatch, WA	S33°25'09" E121°42'32"	0.185±0.01
Many ovules				
F. cinerea DC.	LE03037	Shark Bay, WA	S26°01'18" E113°35'07"	0.062±0.002
	LE03057	Lake King, WA	S35°05'24" E119°36'37"	0.095±0.003
	LE03062	Scadden, WA	S33°24'32" E121°42'15"	0.115±0.002
F. fecunda Summrh.	LE03013	Malcolm–Leonora road, WA	S29°01'59" E121°29'13"	0.052±0.002
	LE03018	Lake Miranda, WA	S27°41'58" E120°32'32"	0.048±0.002
	LE03023	Lake Austin, WA	S27°35'15" E117°53'55"	0.082±0.002
F. foliosa J.M.Black	LE01004	Finnis Springs, Oodnadatta Track, SA	S29°30'05" E137°24'29"	0.115±0.004
	LE01005	Oodnadatta Track, SA	NR	0.072±0.001
	LE02006	Blanche Cup, Oodnadatta Track, SA	S29°27'17" E136°51'25"	NR
F. laxiflora Summerh.	LE03003	Ponton River, WA	S31°02'22" E123°47'04"	0.059±0.002
	LE03021	Lake Annean, WA	S26°53'15" E118°17'21"	0.067±0.002
	LE03029	Pindar,WA	S28°28'29" E115°47'27"	0.040±0.002
F. pauciflora DC. (SA varieties)	LE01010	Thevernard, SA	S32°08'33" E133°40'35"	0.147±0.003
	LE05024	Corney Point, SA	S34°35'53" E137°00'00"	0.159±0.009
	LE05025	Port Rickaby, SA	S34°40'50" E137°29'37"	0.145±0.007
F. pauciflora DC. (WA varieties)	LE03038	Shark Bay, WA	S26°10'10" E113°40'55"	0.059±0.005
	LE03039	Leeman, WA	S29°41'23" E114°57'50"	0.059±0.002
	LE03041	Port Gregory, WA	S28°41'23" E114°57'50"	0.067±0.002

Table 1. Population sites for *Frankenia* species (with authorities) included in this study, listing GPS co-ordinates where known, and mean seed mass (±se) in milligrams. 'NR' indicates information that was not recorded.

	df	Mean Squares	F value	Significance level
GERMINATION RATES				
Day 2				
Seed category	1	0.209	1.424	
Species(seed category)	10	0.209	8.135	***
Temperature	2	0.104	4.048	*
Seed category*temperature	2	0.015	0.598	
Temperature*species(seed category)	10	0.209	8.135	***
Day 4				
Seed category	1	0.030	0.226	
Species(seed category)	10	0.134	1.145	
Temperature	2	0.553	4.710	*
Seed category*temperature	2	0.082	0.696	
Temperature*species(seed category)	10	0.117	1.145	
Day 6				
Seed category	1	0.013	0.276	
Species(seed category)	10	0.046	1.727	
Temperature	2	0.825	31.060	***
Seed category*temperature	2	0.004	0.161	
Temperature*species(seed category)	10	0.027	1.727	
Day 8				
Seed category	1	0.010	0.703	
Species(seed category)	10	0.015	1.148	
Temperature	2	0.048	3.765	*
Seed category*temperature	20	0.001	0.095	
Temperature*species(seed category)	10	0.015	1.148	
GERMINATION SUCCESS				
Day 8				
Seed category	1	0.447	0.959	
Species(seed category)	10	0.466	5.438	**
Temperature	2	3.664	42.745	***
Seed category*temperature	2	0.133	1.552	
Temperature*species(seed category)	10	0.466	5.438	**
Day 28				
Seed category	1	0.997	2.116	
Species(seed category)	10	0.471	6.042	***
Temperature	2	3.001	38.461	***
Seed category*temperature	20	0.079	0.742	
Temperature*species(seed category)	10	0.471	6.042	***

Table 2. Summary of the interaction effects of temperature, seed mass category and species on germination rates, calculated using Nested Design ANOVAs, at Day 2, Day 4, Day 6 and Day 8, and for germination success at Day 8 and Day 28. (*) indicates a significance level of $P<0.05$, (**) indicates a significance level of $P<0.01$, and (***) indicates a significance level of $P<0.001$.

Temperature	Day	Effect	df	Mean squares	F- value	Significance level
17°C						
Rates						
	2	Seed category	1	0.036	1.079	
		Species(seed category)	10	0.033	0.844	
	4	Seed category	1	0.177	0.678	
		Species(seed category)	10	0.261	2.957	*
	6	Seed category	1	0.071	0.277	
		Species(seed category)	10	0.071	1.034	
	8	Seed category	1	0.009	0.341	
		Species(seed category)	10	0.028	2.310	*
Success						
	8	Seed category	1	0.001	0.005	
		Species(seed category)	10	0.257	0.072	
	28	Seed category	1	0.060	0.344	
23°C		Species(seed category)				
Rates						
	2	Seed category	1	0.192	1.524	
		Species(seed category)	10	0.126	3.415	**
	4	Seed category	1	0.017	0.182	
		Species(seed category)	10	0.021	4.503	**
	6	Seed category	1	0.001	0.071	
		Species(seed category)	10	0.018	3.442	**
	8	Seed category	1	0.001	0.108	
		Species(seed category)	10	0.009	5.565	***
Success						
	8	Seed category	1	0.581	2.362	
		Species(seed category)	10	0.246	2.349	*
	28	Seed category	1	0.757	2.749	
		Species(seed category)	10	0.275	2.678	*
29°C						
Rates						
	2	Seed category	1	0.100	0.993	
		Species(seed category)	10	0.101	8.040	***
	4	Seed category	1	0.000	0.009	
		Species(seed category)	10	0.015	1.452	
	6	Seed category	1	0.000	0.036	
		Species(seed category)	10	0.010	3.502	**
	8	Seed category	1	0.002	0.629	
		Species(seed category)	10	0.004	3.333	**
Success						
	8	Seed category	1	0.131	0.974	
		Species(seed category)	10	0.134	2.376	*
	28	Seed category	1	0.376	2.135	
		Species(seed category)	10	0.176	1.713	

Table 3. Summary of the interaction effect of seed mass and species on germination rates, calculated using Nested Design ANOVAs, at Day 2, Day 4, Day 6 and Day 8, and for germination success at Day 8 and Day 28, at 17°C, 23°C, and 29°C. (*) indicates a significance level of $P<0.05$, (**) indicates a significance level of $P<0.01$, and (***) indicates a significance level of $P<0.001$.
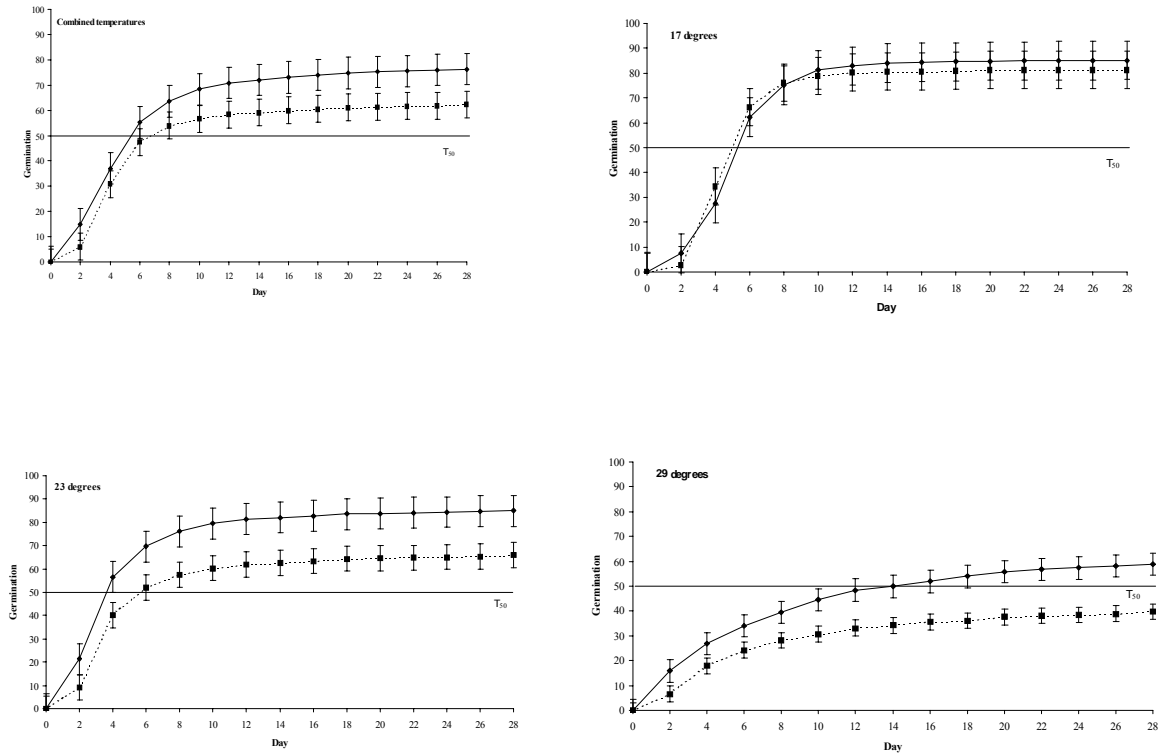
Figure 1. Germination success of larger- and smaller-seeded species. Solid lines denote larger-seeded species and hatched lines denote smaller-seeded species. Time to 50% germination indicated by the T_{50} line.
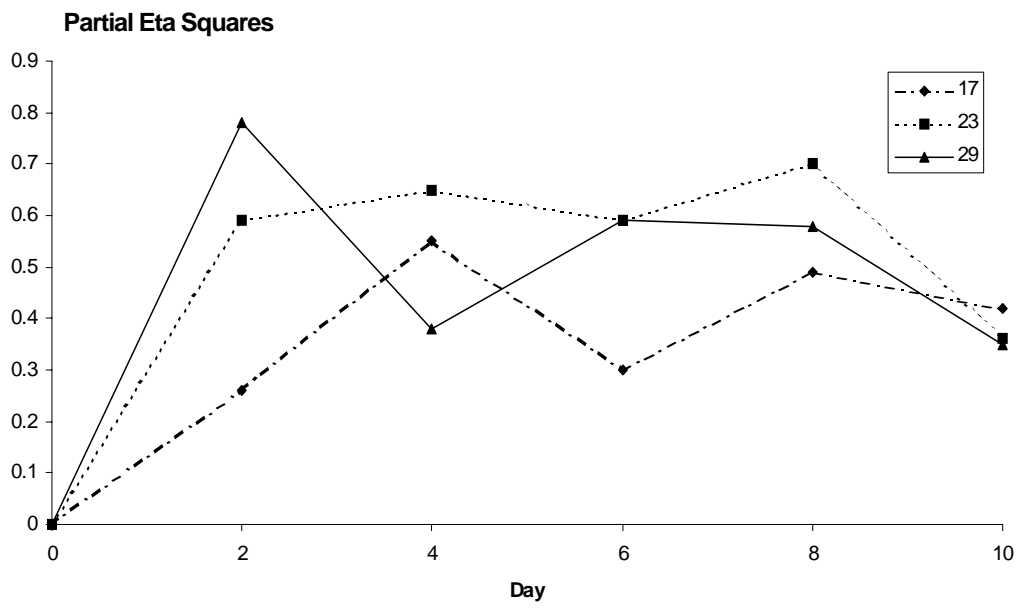
Partial Eta Squares

Figure 2. Partial Eta Square values for each 2-day time interval showing the proportion of the germination rates accounted for by the interaction of seed mass and species at 17°C, 23°C, and 29°C.