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Seed mass and elevation explain variation in seed longevity of Australian alpine species

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Research Paper

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Abstract

Conserving alpine ecosystems and the plant communities they contain using *ex situ* conservation requires an understanding of seed longevity. Knowledge of seed longevity may determine the effectiveness of *ex situ* seed banking for alpine plant conservation, and may provide insight into plant recruitment *in situ*. We sought to determine the influence of elevation and climatic variables, as well as plant and seed traits, on the seed longevity of 57 species inhabiting a unique biome, (sub-)alpine regions of mainland Australia. Seed longevity was estimated using controlled accelerated ageing tests to determine the time taken for seed viability to fall by 50%. We found that, across the study species, like alpine seeds elsewhere in the world, Australian alpine seeds are relatively short-lived and overall shorter-lived than Australian plants in general. Seed mass and elevation explained most of the variation in seed longevity among the Australian alpine species considered. Species with larger seed mass, and collections made at higher elevations, were found to have relatively short-lived seeds. Phylogeny, however, explained very little of the variation in longevity. Our results suggest that viability testing for Australian alpine seeds in *ex situ* seed banks should be conducted with shorter intervals than for the non-alpine flora. This study highlights how seed longevity in the Australian Alps is not dictated primarily by evolutionary lineage but rather by a complex combination of environmental variables and intrinsic seed characteristics. Potential implications for conservation *ex situ* and *in situ* in the context of climate change are discussed.

Introduction

The potential for many plant species to respond to climate change and other disturbances depends heavily on seeds. Seeds enable plants to regenerate and re-establish either in their current site or elsewhere and also to disperse germination over time. Seeds can persist in the soil for thousands of years (Shen-Miller *et al.*, 1995; Sallon *et al.*, 2008; Yashina *et al.*, 2012) and this dispersal over time assists plants to spread the risk of recruitment failure (Thompson, 2000; Ooi *et al.*, 2009). This realized persistence *in situ* is a result of intrinsic seed longevity in concert with other biotic and abiotic factors (e.g. microclimatic conditions, granivory, disturbance). Measures of seed longevity therefore describe the potential lifespan of a mature seed underlain by complex interactions of physiological and physical traits (Long *et al.*, 2015).

Understanding variation in and drivers of longevity is important for effective *ex situ* seed banking because it underpins the selection of viability re-test intervals and re-collection strategies (Probert *et al.*, 2009). Direct measures of seed longevity and persistence, however, are hard to obtain in real time as it may take decades or even centuries (Walters *et al.*, 2005). The p_{50} value, which is the time taken for seed viability to decline to 50% in artificial ageing, is now a well-established proxy for seed longevity in storage (Newton *et al.*, 2009) and in particular in conservation many studies have been undertaken at 45°C and 60% relative humidity (RH) to allow comparative ranking of species (Probert *et al.*, 2009; Merrit *et al.*, 2014). P_{50} serves as an index, although an approximate storage lifespan/longevity can be calculated using viability equations (if storage conditions and species constants are available) (http://data.kew.org/sid/viability/). A p_{50} value might also be somewhat related to persistence *in situ*, for example Long *et al.* (2008) demonstrate that a p_{50} of 20 days in their study species and conditions is likely to indicate persistence of several seasons *in situ*. However, multiple factors can interact with longevity to affect persistence *in situ* (see Long *et al.*, 2015). The controlled accelerated ageing test is a valuable technique because by applying a standard set of protocols it yields comparable results for understanding relative longevity across multiple species and biomes.

The longest-lived seeds tested thus far are Australian, with a maximum p_{50} of 771 days and an average of 202 days under accelerated ageing conditions, which is much greater than longevity of seed from other biomes and regions (Probert *et al.*, 2009). Merritt *et al.* (2014) confirm the relatively long lifespan of Australian species with an investigation of 172 species,

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which shows that p_{50} ranges from 3 up to 588 days revealing that athough high on average, not all Australian species have long-lived seeds. Alpine seeds in general are known to be short-lived. Mondoni *et al.* (2010) report a mean p_{50} of 20.5 days across 36 Italian alpine species. From the 36 species, six accessions were compared with lowland accessions of the same species and alpine seed were significantly shorter-lived than the lowland pairs, with mean p_{50} values of 28.2 and 56.1, respectively (Mondoni *et al.*, 2010).

Previous studies (Probert *et al.*, 2009; Mondoni *et al.*, 2010; Merritt *et al.*, 2014; Long *et al.*, 2015) show that p_{50} is associated with other seed or plant traits including seed mass, dormancy, embryo type, endospermy and life-form, and with environmental variables such as elevation and climatic variables. Maternal conditions can also affect seed longevity (Kochanek *et al.*, 2010, 2011; He *et al.*, 2014; Righetti *et al.*, 2015; Bernareggi *et al.*, 2016). However, correlations or significant factors determining seed longevity vary depending on the species, biomes, the scale of study, as well as the combinations of variables included. Indeed, the only consistent pattern found multiple times across these studies is that non-endospermic seeds tend to be longer-lived. From a plant evolutionary biology perspective, endospermy, seed mass, embryo, dormancy type (Finch-Savage and Leubner-Metzger, 2006), and the environment of origin (Feild and Arens, 2005) would be expected to be related to longevity, but intriguingly, the variation of p_{50} often seems to be independent of these traits and to change across environments and biomes. P_{50} might also be expected to be correlated with phylogeny, as more closely related species could share mechanisms of longevity (Walters *et al.*, 2005; Probert *et al.*, 2009; Mondoni *et al.*, 2010; Merritt *et al.*, 2014). Lastly, given the significance of regeneration by seed for the persistence of species, p_{50} might reflect differences in germination strategy but this is yet to be investigated. Thus in a search of predictors of p_{50}, one must consider a broad range of seed, plant and environmental traits.

Alpine ecosystems and alpine plant communities worldwide have been identified as particularly vulnerable to global warming (Coyne, 2001; Parolo and Rossi, 2008; Thuiller *et al.*, 2008). Local extinction of plant taxa formerly restricted to high elevations has already been reported (IPCC, 2001). In Australia, the alpine region is distributed across just 0.15% of the continent's landmass but because of its high level of endemism is recognized as an alpine biodiversity hotspot, and is distinctive in many ways in relation to mountain areas worldwide (Myers *et al.*, 2000; Kirkpatrick, 2002; Hopper and Gioia, 2004). The Australian Alps region maintains an assemblage of vegetation communities found nowhere else in the world (Costin *et al.*, 2002), harbouring unique ecological communities (Department of Environment, 2015) and 212 plant species that include 30 exclusively alpine and 21 species endemic to Mt Kosciuszko (Costin *et al.*, 2000). In the face of climate change, the limited capacity of alpine plants to migrate to higher elevations may increase their extinction risk.

To ensure meaningful *ex situ* conservation of Australian alpine seeds, seed conservation programmes must be underpinned by data on seed longevity. This study aims to investigate whether Australian alpine seeds perform like other alpine species and are short-lived, or are more like other Australian species and are relatively long-lived. Secondly, we aim to understand important variables that underlie the variation of p_{50} for Australian alpine seeds. Because previous studies have shown that correlations between p_{50} and seed or plant traits and environmental variables vary, we took a comprehensive approach, taking account of as many plausible intrinsic and extrinsic variables as we could. In addition, ours is the first study to examine potential associations between germination strategy and p_{50}. The germination strategy of Australian alpine plants is associated with seed mass and endospermy (Hoyle *et al.*, 2015), hence germination strategy (or the associated traits mass and endospermy) could be expected to be related to longevity as well. We set out to determine the relative importance of both seed/plant traits and environmental variables in explaining variation in p_{50} values, and predicted that Australian alpine seeds would be short-lived (mean p_{50} values ~20 days) like alpine seeds elsewhere.

Materials and methods

Study species

Species of Australian native plants that occur predominantly in the alpine bioregion on the mainland (from 1330–2228 m elevation), and in any alpine vegetation communities, were selected for this study. The Australian Alps, based on the bioregion's physiographic elements, can be categorized as alpine (>1850 m), subalpine (1400–1850 m), montane (1100–1400 m), and tableland (<1100 m) (NSW NPWS, 1988). In this study, we considered alpine broadly, therefore seeds that were collected at elevations above 1330 m were termed alpine. Most seeds were collected from Kosciuszko National Park between 2007 and 2013, and were retrieved from the National Seed Bank, Australian National Botanic Gardens (ANBG) Canberra, Australia. These seeds were stored according to international gene bank standards at approximately –20°C after drying at 15°C and 15% RH (Table 1, supplementary Fig. 1).

Germination assays

Assays were carried out to determine whether a species had high enough germination (>75% viability adjusted) to be included in the controlled ageing tests. The assays were also used to determine the species germination strategy. Germination of 121 accessions of 96 species was investigated following the methodology published by Hoyle *et al.* (2015). Species known to have immediate germination (Hoyle *et al.*, 2015) were germinated on 1% water agar at 25/15°C. Species identified as postponed, staggered, or not listed in Hoyle *et al.* (2015) were germinated on both 1% water agar and 1% water agar infused with gibberrelic acid (GA_3) to achieve a final concentration of 200 mg l^{-1} GA_3 at 25/15°C. If germination was low in both agar and GA_3 assays under summer temperatures (25/15°C) then stratification was applied to mimic the natural progression of seasons. For the stratification steps, a further three replicates of 25 seeds each were exposed to 6 weeks at 25/15°C, followed by 8 weeks at 5/5°C, and returned to 25/15°C for at least 6 weeks, to mimic seasonal change in the field (see Hoyle *et al.*, 2015). These temperatures mimic *in situ* soil temperatures and the main seasonal changes alpine seeds are exposed to, and respond to, in Australia (Hoyle *et al.*, 2015).

Germination assays were also used to determine the germination substrate and dormancy alleviation methods. Following controlled seed ageing, each species was germinated either on water agar, water agar with GA_3 infusion, or with stratification. The selection was based on the substrate/treatment that gave the highest germination percentage for each species. For staggered and postponed strategies, if GA_3 or stratification increased germination compared with germination on water agar then the most

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Table 1. Details of 56 species analysed for their seed longevity (p_{50}) and the correlates used in the analysis

Species	Family	Locality	p_{50}	Germination strategy	Life form	Endosperm presence	Embryo type	Seed mass (mg)	Collection elevation (m)	Elevation range (m)	Precipitation: annual (mm)	Radiation–annual mean (MJ m^{-2} day^{-1})	Temperature: annual mean (°C)	Temperature: seasonality
*Gingidia algens**	Apiaceae	KNP	2,28	Postponed	Herb	Yes	Axile	3,189	1799	642	1764	14,3	5,4	1,71
Poa costiniana	Poaceae	KNP	3,85	Immediate	Monocot	Yes	Basal	0,246	2109	1578	2282	13,7	3,7	1,76
*Cardamine robusta**	Brassicaceae	KNP	4,1	Immediate	Herb	No	Axile	0,463	2083	632	2326	13,7	3,5	1,76
Ranunculus clivicola	Ranunculaceae	KNP	4,16	Staggered	Herb	Yes	Basal	2,838	1742	530	1890	14,2	5,2	1,71
Astelia alpina var. *novae-hollandiae*	Liliaceae	KNP	4,94	Postponed	Herb	Yes	Axile	1,067	2008	733	2170	13,9	4,2	1,76
*Ranunculus acrophilus**	Ranunculaceae	KNP	5,3	Staggered	Herb	Yes	Basal	1,514	2039	184	1419	15,2	7,3	1,76
Australopyrum velutinum	Poaceae	KNP	5,45	Immediate	Monocot	Yes	Basal	3,875	1401	1244	1420	15,1	8,2	1,77
Ranunculus lappaceus	Ranunculaceae	NNP	6,48	Staggered	Herb	Yes	Basal	2,895	1592	1853	2073	14	4,6	1,76
Gingidia harveyana	Apiaceae	KNP	6,63	Postponed	Herb	Yes	Axile	6,288	1704	743	2287	13,8	3,7	1,69
*Craspedia leucantha**	Asteraceae	KNP	7,15	Immediate	Herb	No	Axile	0,829	1996	512	2143	13,9	4,2	1,75
Carpha nivicola	Cyperaceae	KNP	7,51	Postponed	Herb	Yes	Basal	3,407	1924	1646	2073	14	4,6	1,75
Carex cephalotes	Cyperaceae	KNP	7,55	Postponed	Monocot	Yes	Basal	0,628	2095	1001	1074	15,8	9	1,76
Celmisia costiniana	Asteraceae	KNP	7,95	Staggered	Herb	No	Axile	3,086	2046	2019	2196	13,9	4,1	1,77
Richea continentis	Ericaceae	NNP	9,59	Staggered	Herb	Yes	Axile	0,06	1588	754	1419	15,2	7,3	1,76
Psychrophila introloba	Ranunculaceae	KNP	9,61	Postponed	Herb	Yes	Basal	0,707	1941	2126	1863	14,2	5,3	1,75
Wahlenbergia ceracea	Campanulaceae	KNP	9,77	Immediate	Herb	Yes	Axile	0,032	1974	1354	680	16,9	13,3	1,76
Aciphylla glacialis	Apiaceae	KNP	9,84	Postponed	Herb	Yes	Axile	5,761	2164	954	2368	13,7	3,4	1,76
Plantago glacialis	Plantaginaceae	KNP	9,99	Immediate	Herb	Yes	Axile	0,279	1957	487	2196	13,9	4,1	1,75
Poa hiemata	Poaceae	KNP	10,44	Immediate	Monocot	Yes	Basal	0,314	2042	1762	1363	15,2	8,5	1,77
Ranunculus muelleri	Ranunculaceae	KNP	10,82	Staggered	Herb	Yes	Basal	1,356	1955	1758	2167	13,9	4,2	1,75
*Erigeron setosus**	Asteraceae	KNP	10,94	Immediate	Herb	No	Axile	0,229	1928	1062	1831	14,3	5,5	1,75
Oreomyrrhis ciliata	Apiaceae	KNP	11,95	Staggered	Herb	Yes	Axile	1,926	1748	942	1903	14,2	5,1	1,71
Erigeron paludicola	Asteraceae	KNP	11,96	Immediate	Herb	No	Axile	0,022	1989	1204	2105	14	4,4	1,76
Luzula acutifolia subsp. *nana**	Juncaceae	KNP	12,02	Immediate	Monocot	Yes	Basal	0,285	1957	668	1074	15,8	9	1,75
Aciphylla simplicifolia	Apiaceae	KNP	12,28	Postponed	Herb	Yes	Axile	4,188	1743	1135	1863	14,2	5,3	1,71

(*Continued*)

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Table 1. (*Continued.*)

Species	Family	Locality	p_{50}	Germination strategy	Life form	Endosperm presence	Embryo type	Seed mass (mg)	Collection elevation (m)	Elevation range (m)	Precipitation: annual (mm)	Radiation–annual mean (MJ m^{-2} day^{-1})	Temperature: annual mean (°C)	Temperature: seasonality
Austrodanthonia alpicola	Poaceae	KNP	12,43	Immediate	Monocot	Yes	Basal	0,793	2052	1387	2190	13,9	4	1,76
Stylidium graminifolium	Stylidiaceae	KNP	15,74	Postponed	Herb	Yes	Axile	0,137	1745	2006	1850	14,2	5,4	1,71
Olearia algida	Asteraceae	NNP	16,41	Staggered	Shrub	No	Axile	0,192	1636	1935	1890	14,2	5,2	1,76
*Ranunculus dissectifolius**	Ranunculaceae	KNP	17,17	Postponed	Herb	Yes	Basal	2,346	1752	338	2000	14	4,8	1,71
Ranunculus graniticola	Ranunculaceae	KNP	18	Staggered	Herb	Yes	Basal	2,766	1734	1946	1419	15,2	7,3	1,73
Ozothamnus cupressoides	Asteraceae	NNP	18,53	Staggered	Shrub	No	Axile	0,163	1599	1084	2105	14	4,4	1,76
Oreomyrrhis eriopoda	Apiaceae	KNP	20,36	Postponed	Herb	Yes	Axile	3,969	1743	1766	1419	15,2	7,3	1,72
Erigeron bellidioides	Asteraceae	KNP	20,63	Immediate	Herb	No	Axile	0,022	1730	919	2170	13,9	4,2	1,71
Carex hebes	Cyperaceae	KNP	21,24	Postponed	Monocot	Yes	Basal	1,098	2153	1941	2282	13,7	3,7	1,76
Craspedia alba	Asteraceae	KNP	21,46	Immediate	Herb	No	Axile	0,705	1855	495	2073	14	4,6	1,75
Rytidosperma pumilum	Poaceae	KNP	21,58	Immediate	Monocot	Yes	Basal	0,165	2115	960	2215	13,8	4	1,76
Rytidosperma nudiflorum	Poaceae	KNP	21,9	Immediate	Monocot	Yes	Basal	0,709	2105	2147	2190	13,9	4	1,76
Epacris paludosa	Ericaceae	NNP	22,43	Postponed	Shrub	Yes	Axile	0,039	1636	2089	1456	15,1	7,2	1,76
Scleranthus singuliflorus	Caryophyllaceae	KNP	22,86	Staggered	Herb	Yes	Peripheral	1,248	2042	480	2199	13,9	4,1	1,76
*Brachyscome stolonifera**	Asteraceae	KNP	24,32	Staggered	Herb	No	Axile	0,264	1941	406	2287	13,8	3,7	1,76
Deschampsia cespitosa	Poaceae	KNP	24,43	Staggered	Monocot	Yes	Basal	0,3	1743	2069	1903	14,2	5,1	1,72
Baeckea utilis	Myrtaceae	NNP	27,08	Staggered	Shrub	No	Axile	0,091	1603	2082	1419	15,2	7,3	1,76
Leucochrysum alpinum	Asteraceae	KNP	27,42	Immediate	Herb	No	Axile	1,275	2131	1651	2105	14	4,4	1,76
Luzula modesta	Juncaceae	KNP	27,72	Immediate	Monocot	Yes	Basal	0,435	1340	1451	680	16,9	13,3	1,74
Senecio gunnii	Asteraceae	KNP	27,79	Immediate	Herb	Yes	Axile	0,328	1735	1638	2167	13,9	4,2	1,71
Brachyscome spathulata	Asteraceae	KNP	29,08	Immediate	Herb	No	Axile	0,322	2090	2110	2237	13,8	3,9	1,77
Epilobium gunnianum	Onagraceae	KNP	29,27	Immediate	Herb	No	Axile	0,039	1339	2147	1863	14,2	5,3	1,74

Neopaxia australasica	Portulacaceae	KNP	29,3	Postponed	Herb	No	Peripheral	0,533	2028	2137	1456	15,1	7,2	1,76	
Craspedia lamicola	Asteraceae	KNP	32,71	Immediate	Herb	No	Axile	0,623	1986	877	2215	13,8	4	1,76	
Colobanthus affinis	Caryophyllaceae	KNP	33,17	Immediate	Herb	Yes	Peripheral	0,149	1736	1562	2014	14	4,7	1,74	
*Craspedia costiniana**	Asteraceae	KNP	34,7	Immediate	Herb	No	Axile	0,66	1986	705	2215	13,8	4	1,76	
Carex gaudichaudiana	Cyperaceae	KNP	39,3	Postponed	Monocot	Yes	Basal	2,193	1330	2110	2368	13,7	3,4	1,74	
Baeckea gunniana	Myrtaceae	NNP	45,34	Staggered	Shrub	No	Axile	0,077	1592	2034	1419	15,2	7,3	1,76	
Carex blakei	Cyperaceae	KNP	50,35	Postponed	Monocot	Yes	Basal	2,329	1683	1349	1831	14,3	5,5	1,71	
Poa phillipsiana	Poaceae	KNP	71,2	Immediate	Monocot	Yes	Basal	0,346	1399	1342	2073	14	4,6	1,77	
Epacris celata	Ericaceae	KNP	81,59	Postponed	Shrub	Yes	Axile	0,022	1604	941	1419	15,2	7,3	1,76	

Further details, including geographic coordinates, dates of collection, germination treatment and categorization of germination strategy is presented in supplementary Table S1.

successful treatment was selected to be applied to that species throughout the ageing experiment (supplementary Table S1). If GA_3 and stratification produced similar results then stratification was selected. At the end of the germination assay, we performed cut-tests to determine seed fill and death for ungerminated seeds. There were 57 species that had germination >75% and thus were amenable to Probit analyses (see below) and were included in the controlled ageing test (Table 1, supplementary Table S1).

Controlled ageing test

Seed longevity (represented by a p_{50} value) is determined using a controlled artificial ageing test (also called a standard rapid ageing protocol; Newton *et al.*, 2009; Probert *et al.*, 2009). The basic principle of the controlled ageing protocol is to expose seeds to warm and humid conditions in order to decrease seed viability rapidly. The controlled artificial ageing test consists of two steps: rehydration and ageing. To first hydrate the seeds, 11 samples of 50 seeds for each species were placed in open glass vials for 14 days in airtight polycarbonate electrical enclosure boxes (28 × 28 × 14 cm; NHP Fibox, Australia) above a non-saturated solution of LiCl (370 g l^{-1}, anhydrous; Sigma, Australia) in an illuminated incubator (TRIL 396-1-SD; Thermoline Scientific, NSW, Australia), creating a RH of 47% at 20°C (Hay *et al.*, 2008). Overall, the design consisted of six boxes of seed vials. Each box contained a stratified random sample of multiple species with representatives of each of the different germination strategies. The 11 vials of a given species were kept in a single box.

To ensure seeds had equilibrated to 47% RH by the end of seed rehydration period their relative humidity was checked using a reference sample of *Eucalyptus macrorhyncha* seeds that accompanied the study species (supplementary Table S2). Unlike the study species, *E. macrorhyncha* seeds are relatively large and were available in sufficient quantity to fill in a hygrometer chamber for measurement of humidity. The seed equilibrium relative humidity was measured using a water activity measuring instrument that consisted of a hygrometer sensor housed in an AW VC-DI0 water activity probe, used in conjunction with a HygroPalm PD 2 dock-station display unit (Rotronic, Bassersdorf, Switzerland).

Following rehydration, the solution in the polycarbonate electrical enclosure boxes was replaced with a non-saturated solution of LiCl (this time 300 g l^{-1}) at 45°C, which results in 60% RH in the incubator (Oven model TO-500 F, Thermoline Scientific) for seed ageing. The relative humidity was checked at 6-week intervals using a combined temperature and humidity data logger (T-TEC 7-1C, Temperature Technology, Adelaide, Australia) and the bulk solution adjusted as necessary to maintain the 60% RH, by adding distilled water (Hay *et al.*, 2008).

One vial of 50 seeds for each species was removed for germination testing after each of 11 times (1, 7, 14, 21, 28, 35, 42, 50, 75, 156 and 250 days). Seeds were sown on agar in 90 mm diameter Petri dishes and placed in an incubator (TRIL 396-1-SD, Thermoline Scientific) under the germination treatment previously demonstrated in the germination assays to be suitable for germination of each species (supplementary Table S1). Species that required stratification to germinate were subjected to stratification steps following removal from the electrical boxes. Germination was checked weekly and seeds scored as germinated once the radicle was visible, approximately >1 mm. Observation for each plate was considered completed when there were no further germinants for 6 weeks.

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To ensure that conditions were homogenous across boxes over the course of the experiment we placed seeds of *Ficinia nodosa* in each box and retrieved and germinated them at the same ageing time as the study species (supplementary Table S2). The variation of p_{50} values of the *Ficinia nodosa* were small across boxes (mean $p_{50} = 60.61 \pm 3.56$ days). To ensure the experimental procedures were comparable to other longevity studies we included seeds from four of the same accessions used in the Merritt *et al.* (2014) longevity study: *Brachyscome tenuiscapa* var. *pubescens*, *Poa hiemata*, *Streptoglossa macrocephala* and *Wurmbeia dioica* (supplementary Table S2). There were no significant differences in mean p_{50} values for these species (paired *t*-test, d.f. = 3, $P = 0.442$), indicating that the conditions and thus results of the two studies, are comparable.

Potential correlates of seed longevity

Seed and plant traits and environmental data were gathered for the 57 study species (Table 1, supplementary Table S1). The plant and seed trait variables were: seed mass, embryo type, endosperm presence, germination strategy and plant life-form. Environmental data were: long-term mean annual temperature, temperature seasonality, mean annual precipitation and mean radiation. We also assessed elevation of collection site, average elevation for the species, and species elevation range. Specifics of each variable are discussed below.

Seed mass was measured on three replicates of 50 seeds per species, and average individual seed mass was calculated from this. Embryo type and endosperm presence were determined by dissecting the seeds, assessing presence/absence of endosperm, and categorizing embryo type. There were 38 species with endospermic seed and 18 species with non-endospermic seed. Embryo type was categorized as axile (31 species), basal (22 species), or peripheral (3 species) following the classification system of Martin (1946).

Previously, three germination strategies (immediate, staggered and postponed) were identified among Australian alpine species (Hoyle *et al.*, 2015). Germination strategy represents germination timing and pattern over time and seasons (Hoyle *et al.*, 2015). Based on the germination results we categorize species as having a particular germination strategy under the conditions tested (see germination assays above). Non-dormant seeds germinate immediately once the germination requirement is met. Seeds with staggered and postponed strategies require certain dormancy alleviation cues (e.g. cold stratification) and the presence of favourable germination conditions before germination will occur. Staggered and postponed strategies differ in the timing of germination. Species with a staggered strategy have seeds that germinate both before and after winter, whereas seeds of postponed species germinate almost entirely after winter. Of the 57 species, 28 species were also tested in Hoyle *et al.* (2015) and almost all of these exhibited the same germination strategy as in our assays. *Ranunculus acrophilus* and *Wahlenbergia ceracea* were exceptions to this (details in supplementary Table S1). In total, 24 species were categorized as immediate, 15 species as staggered, and 17 species as having a postponed strategy. Details on the germination strategy categorization of the 57 species can be found in supplementary Table S1. Finally, plant life-form was categorized as monocotyledonous herbs (14 species), other herbs (36 species), and woody shrubs (6 species) to follow the plant functional types described by Costin (2000) for the alpine flora (Table 1, supplementary Table S1).

For seed collection and environmental variables, we retrieved the collection elevation data from the ANBG living collection database. Mean, minimum and maximum elevation of the species in Australia were gathered from the Australian National Herbarium Specimen Information Register (http://www.anbg.gov.au/cgi-bin/anhsir) or from Australia's Virtual Herbarium (http://avh.ala.org.au) to determine the elevation range spanned by each species. Mean temperature, temperature seasonality, precipitation and mean radiation were bioclimatic values estimated from interpolation of long-term climate data, 75 years of data for temperature and precipitation, and 25 years for solar radiation, at a resolution of 0.01 degrees, and were imported from the Atlas of Living Australia (http://spatial.ala.org.au).

Data analyses

Probit analysis of seed longevity

Probit analysis was carried out on the germination data for the study species as a function of ageing days using GenStat Release 17.1 (VSN International Ltd, Oxford, UK). P_{50} was estimated by fitting a viability equations (Ellis and Robert, 1980):

$$v = K_i - \left(p/\sigma\right), \quad (1)$$

where v is the viability in normal equivalent deviates (NED) at time p days in storage, K_i is the estimated initial viability, and σ is the standard deviation of the normal distribution of seed deaths over time.

Drivers of seed longevity

First, we checked for collinearity among explanatory variables using correlation tests, ANOVAs or χ^2 tests, depending on the nature of the data (supplementary Table S3). Some variables were very strongly correlated ($r > 0.85$; supplementary Table S3), so we kept only one from each group. We kept collection elevation and elevation range but removed mean elevation. We kept mean temperature and temperature seasonality but removed mean precipitation and radiation. Finally, we kept endospermy type but removed embryo type and life form. These selections were made because mean annual temperature has previously been shown to be positively related to p_{50} (Probert *et al.*, 2009), and longer-lived seeds to be non-endospermic (Probert *et al.*, 2009; Merritt *et al.*, 2014). After removing those variables, we performed preliminary explorations with all-subset regressions and found that seed mass and collection elevation were consistently significant predictors of p_{50} (results not shown). We also tested family and genus as fixed factors and confirmed that the variation in p_{50} was not related to family ($P = 0.13$) or genus ($P = 0.37$). Similarly, family and genus did not explain much variation of p_{50} when assigned as random factor (supplementary Table S4).

To formally test which explanatory variable(s) best explained p_{50}, we performed model selection based on the goodness-of-fit of the data quantified by the corrected or second order Akaike Information Criterion (AICc) (Burnham and Anderson, 2003; Johnson and Omland, 2004; Bolker *et al.*, 2009; Grueber *et al.*, 2011) in R 3.2.4 (R Core Team, 2016). All continuous and binary explanatory variables were standardized to facilitate model convergence and comparison of effect sizes between models (Gelman, 2008; Merkling *et al.*, 2017). Standardization was achieved by subtracting the mean of the all data points and dividing by 2 standard deviations (Gelman, 2008). Standardization does not influence the correlations of variables with the response

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variable and is undertaken to facilitate comparisons between the effects of variables that are on different scales. The p_{50} values were log-transformed in all analyses to meet model assumptions. Following our data exploration (above), we also included the interaction term between seed mass and each of the two elevation variables in our list of models (supplementary Table S5). To avoid over-fitting and to limit the number of models in our list, each model contained a maximum of two explanatory variables and we did not consider models containing weakly correlated explanatory variables (supplementary Table S5).

We did not have access to a phylogeny of the 56 species considered, so we accounted for the effect of relatedness by running linear mixed models with family or genus as a random effect. There were no significant effects of the random terms, therefore showing that phylogeny explained a negligible amount of the variance in p_{50} (supplementary Table S5). Although we could probably have discarded the random effects and run linear models (supplementary Table S5), we decided to retain genus as a random effect to get more conservative estimates of the factors influencing p_{50}. Genus was selected as random because the AICcs were smaller compared with when we used family, or genus nested in family, as the random factors (supplementary Table S5).

Model selection was carried out using the *lme4* package (Bates *et al.*, 2015; R Core Team, 2016). For each model, we calculated its AICc and its AICc difference (ΔAICc). ΔAICc provides a measure of information lost between the best model and a given model of a candidate set. We selected models with ΔAICc < 5 following Bolker *et al.* (2009) and calculated a weight (ωAICc) for each model using the *AICcmodavg* package (Mazerolle, 2016) based on maximum likelihood estimation (supplementary Table S6). The calculated weight (ωAICc) represents the probability that a given model is the best of the subset of models. We then computed model-averaged parameter estimates, standard errors and confidence intervals to take model selection uncertainty into account.

Comparisons of seed longevity within Australia and between alpine regions

In order to determine whether Australian alpine longevity is similar to other alpine seeds or more similar to the seed longevity of Australian plants, we did two comparisons: Australian alpine versus alpine elsewhere (European species), and Australian alpine versus Australian non-alpine. To enable international and continental scale comparisons we selected families that were shared across the different studies. We compared the p_{50} for the available paired families, i.e. families that were included in the present study as well as in the Italian alpine study of Mondoni *et al.* (2010). Likewise, we paired among families of Australian non-alpine species using data from Merritt *et al.* (2014). The Italian alpine p_{50} values were selected from those species specifically indicated as alpine in Mondoni *et al.* (2010). For Australian seed longevity comparison we excluded five species from the Merritt study (*Brachyscome tenuiscapa* subsp. *pubescens*, *Epilobium gunnianum*, *Poa hiemata*, *Podolepis robusta* and *Xerochrysum subundulatum*) to ensure the analysed data were exclusively non-alpine accessions (collected at elevations <1300 m) (Merritt *et al.*, 2014). We used linear mixed models, assigning p_{50} as a response variable, the region of origin (either alpine versus non-alpine in Australia, or Australian alpine versus Italian alpine) as an explanatory variable, and paired family (six families for Australian alpine versus Italian alpine and 10 families for Australian alpine versus non-alpine comparisons) to control for potential effects of relatedness among species.

Results

Australian alpine seed longevity

Across the 57 species considered, all but one (*Melaleuca pityoides*) declined in viability such that p_{50} could be calculated (supplementary Table S1). Hereafter, we will discuss the 56 species for which p_{50} could be calculated. Our index of longevity (p_{50}) ranged from 2.28 days for *Gingidia algens* (Apiaceae) to 81.59 days for *Epacris celata* (Ericaceae), with a mean of 19.8 ± 15.5 days. Nearly 60% of the species tested had seeds with p_{50} values <13.7 days (<13.7 days = short-lived; Newton, 2009, Probert *et al.*, 2009), and so can be considered to be relatively short-lived. Four species had $p_{50} > 50$ days, relatively long lived compared with other floras. The seeds of seven of the nine Kosciuszko alpine zone endemic plants tested were short-lived (p_{50} values <13.7 days), whereas *Ranunculus dissectifolius*, *Brachyscome stolonifera* and *Craspedia costiniana* had relatively intermediate p_{50} values of 17.17, 24.3 and 34.7 days, respectively.

Among Australian alpine species, the variation of seed longevity among species and across families was large (Fig. 1). Sometimes, the variation within a single genus was larger than variation between genera within a family (Fig. 2). For example, within Ericaceae, the p_{50} values of two *Epacris* species differed by 59.19 days (*Epacris celata* and *E. paludosa*; Fig. 2). Among the ecologically dominant Poaceae species we found most were short- to intermediate-lived (seven species), with the exception of one, *Poa phillipsiana*. Apiaceae (five of six species short-lived, one intermediate) and Ranunculaceae (five of seven species short-lived, two intermediate) were shown to have consistently short-lived seeds compared with other Australian alpine families (Fig. 2). Conversely, Myrtaceae had relatively intermediate- to long-lived seeds (p_{50} 45.34 and 27.08 for *Baeckea gunniana* and *B. utilis*, respectively); germination of *Melaleuca pityoides* did not decline sufficiently over the 250 days of ageing time and therefore we could not calculate a p_{50} value for this species (supplementary Table S1).

Correlates of Australian alpine seed longevity

Seed mass and elevation were the only two of the ten variables tested in this study that were in all the top-ranked models of our model list and therefore explained most of the variance of

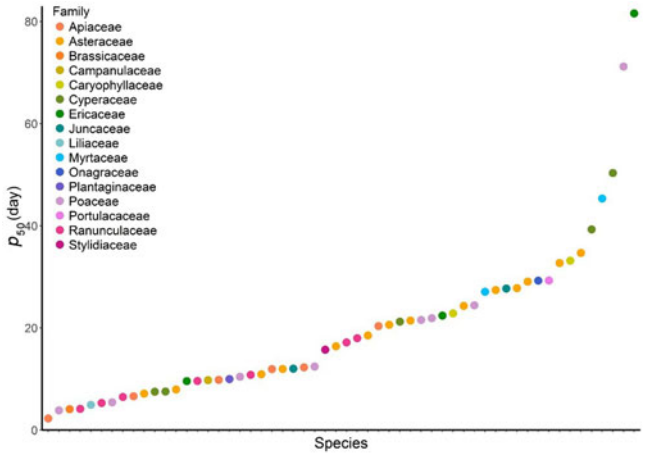


Figure 1. Variation in seed longevity index (p_{50}) across 56 Australian alpine species from 16 families. Each dot represents one species, colours indicate family.

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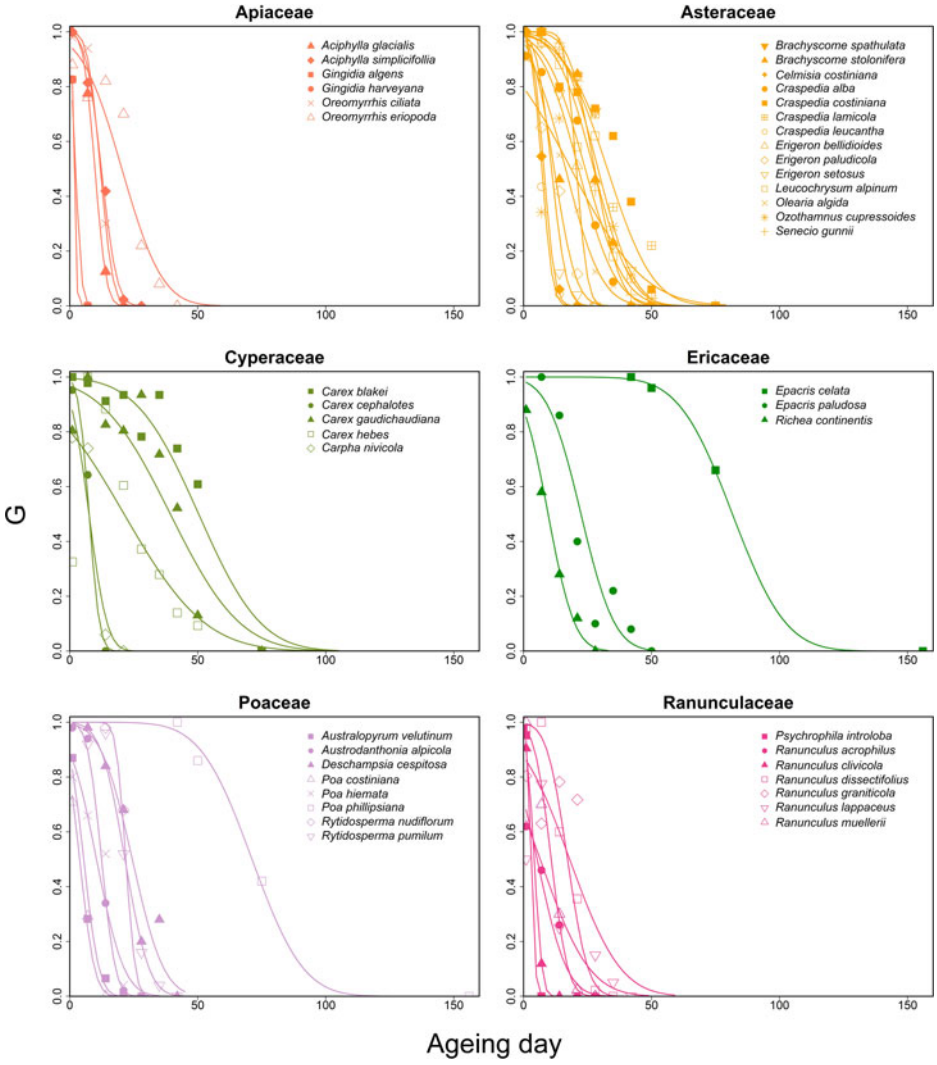


Figure 2. Seed longevity varies among families and among genera within a family. G (*y*-axis) is germination proportion at an ageing day (*x*-axis). For every species (each represented by a different line), p_{50} is the day when G was 0.5. Families shown here are those for which more than two species were included in our study, family colour-matched to Fig. 1.

p_{50} for the Australian alpine species considered (supplementary Table S6). Across species, seeds with lower seed mass were longer-lived than seeds with higher seed mass (Fig. 3a, Table 2), and seeds collected at lower elevation were longer-lived than those from higher elevation (Fig. 3b). The model with the interaction between seed mass and elevation was the second best of the models considered (supplementary Table S6). However, its AICc was only 0.59 higher than the best (additive) model, indicating that the interaction had a very low explanatory power. The interaction suggests that if anything, at higher elevation seed mass was more negatively correlated with p_{50} than at lower elevation (Fig. 3d). Also, species elevation range had a positive effect on p_{50}, but the relationship was much weaker compared with the effects of seed mass and collection elevation, as the confidence interval did slightly overlap with zero (Table 2, Fig. 3c).

The climatic variables and other seed traits such as germination strategy, endospermy and embryo type, did not explain significant variation in p_{50}; these other variables only appeared in a top model also containing seed mass and elevation or they did not appear in any of the best models (supplementary Table S6). Most of the models containing just one of these variables were actually worse than the null model (supplementary Table S5).

Comparisons of the Australian alpine flora with other floras

The longevity of Australian alpine seeds was relatively similar to alpine seeds in Italy, across the six families, i.e. 19.8 and 19 days, respectively ($P = 0.741$; Fig. 4a). In contrast, Australian alpine seeds were relatively short-lived compared with non-alpine Australian flora ($P < 0.000$; Fig. 4b). The difference in p_{50} between alpine and non-alpine families was large and varied across the ten families. For example, the difference between mean p_{50} values for alpine and non-alpine Australian Poaceae was 9 days, whereas for Myrtaceae the difference was 139.8 days. Despite large geographic separation there was a greater similarity among species and families from the same bioregion (i.e. alpine) than there was among species that occur within Australia in different bioregions (alpine or not).

Discussion

We aimed to understand patterns of seed longevity among Australian alpine flora and the important variables influencing it. Our index of seed longevity (p_{50}) for 56 Australian alpine species ranged from ~2 days to more than 81 days with a mean of

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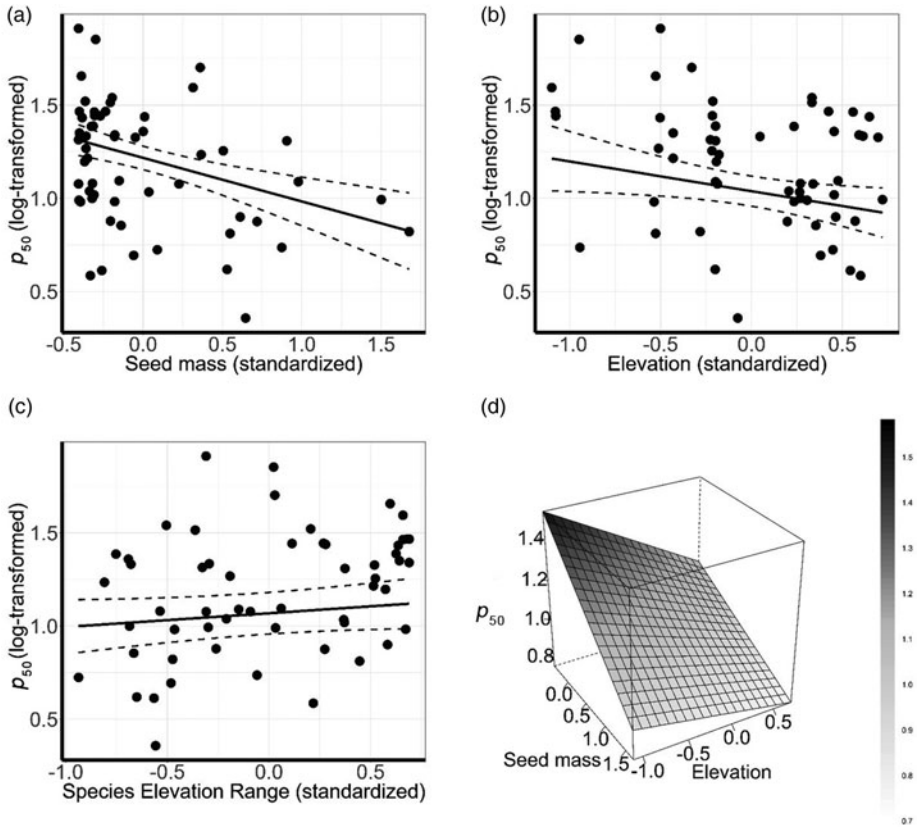


Figure 3. (a) Seeds with lower seed mass were longer lived than seeds with higher seed mass. (b) Seeds collected at higher elevation are shorter lived than seeds collected from lower elevation. (c) Species with wide elevation range are longer lived, but the significance was borderline. For panels a, b, and c, the continuous line represents model-averaged predictions from the model, dashed lines are the model-averaged standard errors, and points are standardized raw data. (d) Three-dimensional graph depicting the interaction of seed mass (standardized) and collection elevation (standardized); the plane represents the p_{50} value predictions from the interaction model.

19.8 days; these values were relatively small compared with the wider Australian flora in which the p_{50} can exceed 750 days and averages 105 days, and comparable to other alpine flora. The present results indicate that seed mass and collection elevation are important drivers of seed longevity in the Australian Alps. Germination strategy and endospermy, although predicted to be important intrinsic traits, were not associated with longevity of Australian alpine seeds. Here we explore intrinsic traits and environmental drivers of Australian alpine seed longevity, as well as the significance longevity may hold for *ex situ* plant conservation and *in situ* seed bank persistence, particularly in the context of a changing climate.

Table 2. Model-averaged estimates of the variables explaining the seed longevity of 56 Australian alpine species (p_{50}, log-transformed)

Parameter	Estimate	Standard error	Lower CI	Upper CI
Intercept	1,178	0,0392	1,1011	1,2548
Elevation of collection	**−0,2145**	**0,0777**	**−0,3668**	**−0,0621**
Seed mass	**−0,2313**	**0,078**	**−0,3843**	**−0,0784**
Species elevation range	0,1527	0,0785	−0,0011	0,3065
Seed mass × elevation of collection	0,2633	0,1513	−0,0332	0,5598

Continuous and binary input variables were standardized to facilitate model comparisons. Bold type indicates estimates in which CI (confidence interval) does not overlap with zero and are therefore significant.

Comparative longevity and drivers thereof

The p_{50} variation across the 56 species considered here is rather large (2.28 to 81.59 days), and twice that reported for another alpine region (Mondoni *et al.*, 2010). This might be attributed to the diverse combinations of plant and seed traits, and collection locations or habitats that we sampled in our study. In contrast to some previous studies (Probert *et al.*, 2009; Mondoni *et al.*, 2010; Merritt *et al.*, 2014), we found that the p_{50} of Australian alpine species was positively associated with seed mass. Merritt *et al.* (2014) also found that Australian seed longevity generally increased with seed mass, but no correlation was found across 195 species worldwide (Probert *et al.*, 2009). Although seed mass is often a significant factor in longevity studies, the direction of the relationship seems to vary across biomes and geographic scales. Seed mass is dependent on environmental variables. For instance, larger seeds are generally found at lower latitudes and this relationship co-varies with higher long-term temperature (Moles *et al.*, 2005, 2007; Soper Gorden *et al.*, 2016). Furthermore, within the Australian Alps, the seed mass of *Aciphylla glacialis*, *Oreomyrrhis eriopoda*, *Ranunculus gunnianus*, *Richea continentis* and *Wahlenbergia ceracea* increased with elevation (Segal et al., unpublished data). Such within-species variation may potentially have a counter-balancing effect on the p_{50} decline among species at higher elevation. Some of the species in our study (e.g. *Carex gaudichaudiana*, *Neopaxia australasica*, *Oreomyrrhis eriopoda*, *Ranunculus lappaceus* and *Stylidium graminifolium*) have cosmopolitan distributions, and thus one could examine whether the effect of elevation and seed mass on seed longevity is consistent across regions.

Endosperm has been shown to have a consistent positive association with p_{50} in other species elsewhere, but was not directly

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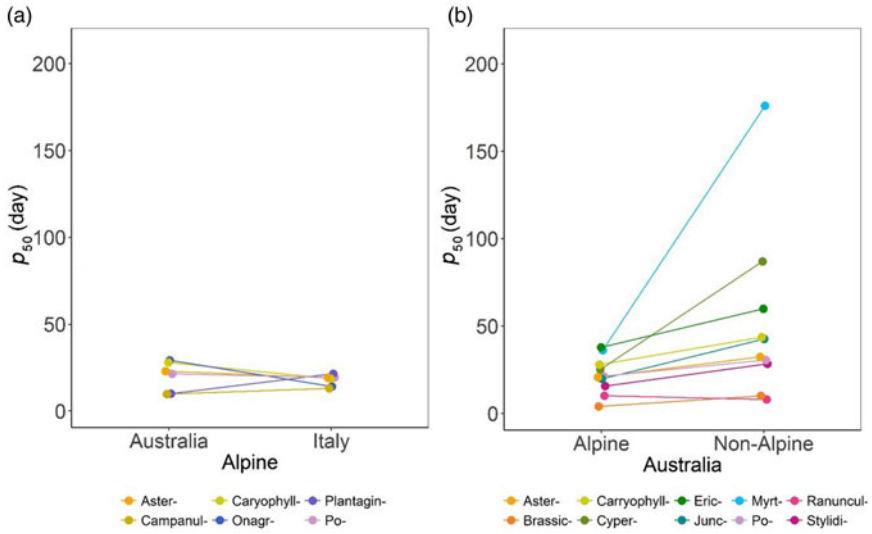


Figure 4. Comparison of longevity (p_{50}) of Australian alpine seeds with (a) Italian alpine species (Mondoni *et al.*, 2010) and (b) Australian non-alpine seeds (Merritt *et al.*, 2014). The comparisons were made across paired families; family colour-matched to Fig. 1.

related to the p_{50} of the species tested here. Across Australian alpine plants, non-endospermic species have a low seed mass, are non-dormant at dispersal, and thus have an immediate germination strategy, but endospermy has not been shown to be associated with SLA (Specific Leaf Area), plant height or elevation (Sommerville *et al.*, 2013; Hoyle *et al.*, 2015). We found the same correlation patterns among non-endospermy, seed mass, dormancy class and germination strategy as in the previous studies (Sommerville *et al.*, 2013; Hoyle *et al.*, 2015), yet endospermy and germination strategy were not associated with longevity. Endospermic seeds in Australian alpine species are generally associated with dormant seed and a postponed germination strategy (Hoyle *et al.*, 2015). One can expect that for a dormant seed, high longevity is more relevant than for a non-endospermic seed with an immediate germination strategy that, if the required conditions are met, presumably germinates soon after natural dispersal. These patterns may indicate that across Australian alpine plants seed longevity, though influenced by some of the same traits as germination strategy, may be an ecologically important trait that evolves somewhat independently of germination strategy.

Seed longevity has also previously been shown to be associated with phylogeny (Probert *et al.*, 2009; Merritt *et al.*, 2014), particularly when a given family possesses a distinctive character, e.g. hard-seededness (physical dormancy) in Fabaceae. However, this pattern was not detected among the alpine species studied here. A more thorough analysis applying actual phylogenetic data would be needed to formally assess the relationship between p_{50} and phylogeny of Australian alpine seeds. Variation in seed longevity at family and genus level has previously been demonstrated in the Asteraceae (Walters *et al.*, 2005), Australian Cyperaceae (Merritt *et al.*, 2014), and the genus *Plantago* (Plantaginaceae; Mondoni *et al.*, 2010). We suggest that the results presented here, in conjunction with the results of other studies that show high levels of variation within phylogenetic groups (and even species), phylogenetic data seem unlikely to reveal strong associations. Rather, longevity appears to be a fairly labile trait.

Mondoni *et al.* (2010) demonstrated that seeds of alpine plants are short-lived compared with seeds of the same species collected from lowland plants. Our study is the first to provide evidence that even within the higher elevation (sub-alpine and alpine), elevation still affects seed longevity; even though the climate gradient was relatively narrow and did not itself directly explain variation in p_{50}. Interestingly, climate was a significant correlate of seed longevity in other studies (Probert *et al.*, 2009; Mondoni *et al.*, 2010; Merritt *et al.*, 2014) but was not significant in this study, possibly indicating that climate affects longevity at bioregional scales but that other drivers may be more important within a region. That elevation matters for p_{50} but climate variables do not in our study may seem contradictory, but most likely indicates that a narrow amplitude of long-term climate variability recorded by alpine climate stations may not reflect conditions at plant or soil level. The long-term temperature (used to model bioclimate values), for instance, are calculated from temperature data measured at 1.2 m above the ground and there could be as much as a 7°C discrepancy between the temperatures measured at this height and at the ground (www.bom.gov.au). Seed development (post-zygotic and pre-zygotic) conditions, instead of long-term mean climate variables, were the significant factor explaining p_{50} variation in Italian flora (Mondoni *et al.*, 2010), and maternal conditions may increase and decrease the p_{50}, again depending on the species and origin (Kochanek *et al.*, 2010, 2011; Bernareggi *et al.*, 2015). Hence in addition to broad-scale elevation patterns, microclimatic and maternal conditions may explain subtler patterns of variation in p_{50} and deserve further investigation.

Ex situ and in situ conservation implications in a climate change context

Of the species assessed here, nearly half had seeds with p_{50} values lower than 13.7 days, a commonly applied threshold to define seeds to be short-lived (Newton *et al.*, 2009; Probert *et al.*, 2009), or if we refer to the classification by Mondoni *et al.* (2010), 18 species would be considered short-lived ($p_{50} < 10$ days). The low p_{50} values might mean years of viability under *ex situ* conditions, where relative humidity and temperature are kept low, but this still needs to be empirically proven.

Climate model studies have demonstrated that alpine plants are unlikely to be able to adapt or disperse quickly enough to keep up with rapid climate change, and thus there is a high risk of local extinction (Cotto *et al.*, 2017). Our data provide some indication that longevity was higher for species with wide elevation ranges than for species with short elevation ranges; such

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patterns could indicate an advantage for these species compared with those with restricted alpine distribution, and this effect thus may warrant further consideration.

The Australian alpine region is relatively low-lying and permanent snow is absent, leading to an even more constrained potential for upward migration. Thus, *ex situ* plant conservation might be the best option to provide a safeguard for many alpine plants, particularly in the Australian context. From an *ex situ* conservation perspective, p_{50} values provide a convenient and reliable proxy to estimate the storage life. For native species where seed availability is often limited, determination of species constants and calculation of a full viability equation to determine storage lifespan, which requires many more seeds, cannot be performed. Thus p_{50} derived from controlled ageing is the most practical approach to determine seed management in *ex situ* storage. *Ex situ* seed bank collections consist of 'active' collection (10–20 years term storage) for research use, and planting materials and 'base' collections (long-term storage; FAO/ IPGRI, 1994). The original FAO/ IPGRI (1994) genebank standards recommend for 5 to 10 years interval for viability testing but the more recent FAO (2013) protocol advised for an interval one third of the time predicted for viability to fall from its initial value. Our finding advocates for a more considered handling of alpine seeds in *ex situ* seed conservation – alpine seeds should be stored and utilized relatively quickly (i.e. base may be only 10–20 years) with ~3 year intervals of viability monitoring (see Hay and Probert, 2013).

The relatively short longevity of Australian alpine seeds could also influence *in situ* persistence. Australian alpine soils contain a substantial seed bank of viable seeds that germinate well (Venn and Morgan, 2010; Hoyle *et al.*, 2013). Alpine communities elsewhere also have sizeable soil seed banks (Arroyo *et al.*, 1999, 2004), and can survive 5 years in a burial experiment *in situ* (Schwienbacher *et al.*, 2010). More than 50% of alpine species present in the standing vegetation may exist in the soil seed bank (McGraw and Vavrek, 1989). Australian alpine seeds in contrast are relatively small (1.25 ± 0.2 mg across our study species, which was small compared with Australian seed mass tested in Merritt *et al.* (2014): 8.84 ± 1.29 mg). Small size makes them more likely to be buried and incorporated into the soil. Seeds that are small and round underlie the strategy for longer persistence and a form of seed predator avoidance (Moles *et al.*, 2000). Further, Mondoni *et al.* (2010) suggested that seeds buried in the substrate at alpine sites are likely to deteriorate more slowly in alpine compared with lowland sites because of the low temperatures. Small-seeded species usually persist longer (e.g. Thompson *et al.*, 1993; Cerabolini *et al.*, 2003; Funes *et al.*, 2003; Peco *et al.*, 2003).

Soil seed banks are likely to be important in maintaining the alpine communities in changing and variable climatic conditions and can potentially act as a buffer against variable weather conditions, and extreme fluctuations plants are not adapted to. Our data at least indicate that the seed longevity of many Australian alpine species may facilitate soil seed bank formation and persistence, but how seed longevity may interact with other biotic and abiotic factors to affect soil seed bank dynamics remains unknown. Actual persistence in the soil depends not only upon potential longevity, but also on other factors (Long *et al.*, 2015). In the Australian Alps, soil warming reduces germination from the soil seed bank, but increases the diversity of species and thus alters species composition of germinants (Hoyle *et al.*, 2013). Nevertheless, Long *et al.* (2008) show that p_{50} values are positively correlated to realized seed persistence for 27 northwestern European species. Long *et al.* (2008) also estimate the association between p_{50} and seed persistence for 13 Australian weed species, and suggest that p_{50} values <20 days are equivalent to approximately 1 year persistence *in situ* and p_{50} values of 20–50 days correspond to 1–3 years field persistence in their study region. Further efforts to refine estimates of *in situ* seed persistence from p_{50} in different regions will further assist in developing approaches for habitat management and species conservation.

Conclusions

Recent comparative longevity studies using controlled accelerated ageing protocols show that the Australian flora generally has long-lived seed. Our study reveals that Australian alpine seeds are relatively short-lived just like alpine seeds elsewhere, and also demonstrated that elevation combined with seed mass were the strongest determinants for Australian alpine seed longevity (p_{50}) and suggest that even within the relatively narrow topographic range, the elevation of collection location is a strong determinant of seed longevity, although, at this scale we found little indication that specific climate variables were directly associated with elevation. This study is also the first study of longevity, globally, to examine the patterns and drivers of seed longevity by including regeneration processes such as germination strategy as explanatory variables. Germination strategy is particularly important for alpine plant recruitment. Interestingly, we found that seed longevity was not associated with germination strategies or with other seed traits known to be ecologically important for alpine plants. This suggests that although longevity may be influenced by some of the same seed traits that influence germination strategy, longevity may be an ecologically important process that operates independently from germination. The variables shown to be important for p_{50} in Australian alpine species differed from other comparative longevity studies. In part, this reflects differences in approach. For example, we analysed 56 species which all had relatively low seed mass in a global context and were collected in one bioregion, and we took into account 12 potential correlates (including five seed/plant traits, see supplementary Table S1). Other studies have included more species with broader variation in seed mass and climatic gradients or bioregions, but often fewer correlates. Because the controlled ageing protocol has been shown to be robust and is a standardized method, a study looking at global p_{50} is highly feasible and would bring even more comprehensive insights into the role of plant traits, seed structure and climate in explaining the evolution of seed longevity. Seed persistence and seed burial studies in the field, to complement the p_{50} data, would also increase our confidence to use p_{50} to predict seed persistence in various habitats and may be useful for informing *in situ* and *ex situ* conservation of priority species. Australian alpine regions are valuable yet vulnerable, and the success of *ex situ* as well as *in situ* plant conservation efforts will in part rely on information on seed longevity, soil seed bank persistence and germination of the alpine flora; the present study is an important step towards that knowledge.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0960258518000090.

Details of Australian alpine species collection, p_{50} values, plant and seed traits as well as climate variable used in the analysis, and model averaging results can be found in the supplementary material online.

Supplementary Information. **Supplementary Table S1**. Collection information, details of ageing test (box number and germination treatment), p_{50}

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result and intrinsic and environmental variables for each of the 57 accessions, ordered by p_{50} values. Asterisk (*) indicates species endemic to the Australian Alps. Accession numbers are preceded by herbarium code CANB. KNP, Kosciuszko National Park, NSW; NNP, Namadgi National Park, ACT. During the controlled ageing each box contained at least one representative of each germination strategy [Immediate, Staggered, Postponed (Hoyle *et al.*, 2015)]. Treatment is the germination condition used for each germination screen where S = stratification (12 h/12 h photoperiod, 6 weeks 25/15°C, 8 weeks 5/5°C, 6 weeks 25/15°C), G = 1% water agar with 200 mg l^{-1} GA_3 (12 h/12 h photoperiod 25/15°C), and A= 1% water-agar (12 h/12 h photoperiod 25/15°C). Embryo type follows Martin (1946). Details on climate data can be accessed from http://spatial.ala.org.au/layers. *Melaleuca pityoides* was not included in the probit or further analyses and hence the environmental correlates were not shown.

Supplementary Table S2. Additional seed accessions used as box-effect controls for rehydration and ageing and to ensure results of this study were comparable with Merritt *et al.* (2014).

Supplementary Table S3. Correlations among the variables considered (seed/plant traits, climate variables and elevations).

Supplementary Table S4. Family ($n = 16$), genus ($n = 33$), and genus nested within family were assigned as either fixed term or random term, with p_{50} (log-transformed) as response variable to tease apart the effect of phylogeny on p_{50} values. *F*-statistics (fixed term) and estimated variance component (random term) values indicate the absence of phylogeny signal in the variation of our alpine seed longevity.

Supplementary Table S5. List of model selections ordered by second-order Akaike Information Criterion (AICc) values – better models are indicated by smaller AICc. Response variable p_{50} was log-transformed, explanatory variables were standardized, and random variable was genus. Within a model, explanatory variables were independent from one another.

Supplementary Table S6. AICc model selection tables for models explaining p_{50} in relation to the variables. 'AICc' is the AIC corrected for finite sample size, 'ΔAICc' is the difference between AICc of a given model to that of the best model, 'ωAICc' is the probability of each model being the best model given the data and the model set. These are models with the lowest ΔAICc (ΔAICc < 5) among all possible models listed in supplementary Table S5.

Supplementary Fig. S1. Map of the collection sites of the 56 species.

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