Can seed characteristics or species distribution be used to predict the stratification requirements of herbs in the Australian Alps?

KAREN D. SOMMERVILLE, AMELIA J. MARTYN and CATHERINE A. OFFORD*

The Australian Botanic Garden, Mount Annan Drive, Mount Annan, NSW 2567, Australia

The germination requirements of 19 herbs in the Australian Alps were investigated to determine which species may be sensitive to predicted climate changes. Seeds were subjected to factorial treatments of cold stratification for 0, 4, 8 and 12 weeks, followed by incubation at constant temperatures of 10, 15, 20 and 25 °C and alternating temperatures of 20/5 and 20/10 °C. Germination responses were used to identify stratification-dependent species, to classify dormancy and to determine optimum conditions for laboratory germination. Ordinal logistic regression was used to determine whether the duration of stratification required for $\geq 50\%$ germination could be predicted by seed weight, seed length, embryo : seed ratio or species distribution (latitudinal range, altitudinal range and maximum altitude). The Kruskal–Wallis test was used to determine any significant differences in stratification requirement between endospermic and non-endospermic seeds. Species varied considerably in their response to the treatment combinations, and therefore their dormancy class. No significant predictors of stratification requirement were identified by ordinal logistic regression ($P > 0.9$); however, there was a significant difference in stratification requirement between endospermic and non-endospermic seeds ($P = 0.003$). Species with non-endospermic seeds did not require any stratification to germinate well over a range of temperatures, and appear most likely to remain stable or expand in range in response to climate warming. Conversely, the need for ≥ 8 weeks of cold stratification was associated with the presence of endosperm and either a restricted distribution or upland ecotypes of widely distributed species. Alpine species with endospermic seed and a restricted distribution are most likely to contract in range under climate change and would be appropriate to prioritize for *ex situ* conservation. © 2013 The Linnean Society of London,, *Botanical Journal of the Linnean Society*, 2013, **172**, 187–204.

ADDITIONAL KEYWORDS: alpine – climate – dormancy – germination – plant conservation – seed structure – temperature requirement.

INTRODUCTION

Physical and biological systems on all continents are already being affected by recent climate changes, particularly by regional temperature increases (Rosenzweig *et al*., 2007). Alpine ecosystems are considered to be among the most sensitive to these changes (Hughes, 2003; Fischlin *et al*., 2007; Laurance *et al*., 2011), and recent declines in average snow depth have been observed in alpine areas in both the Northern and Southern Hemispheres (Hughes, 2003; Nicholls, 2005; Hennessy *et al*., 2007;

Rosenzweig *et al*., 2007). Further increases in temperature and associated declines in snow cover and duration are expected. In Australia and New Zealand, for example, it has been predicted that, by 2050, the duration of snow cover may be reduced by 15–100 days and the total area of snow cover may shrink by 20–85% (Hennessy *et al*., 2007).

The duration and depth of snow cover are key factors affecting species distributions in alpine ecosystems (Korner, 2003; Daimaru & Taoda, 2004; McDougall & Walsh, 2007). Changes in the snow regime are thus likely to have significant impacts on the alpine flora and fauna, as a lack of snow cover during winter results in their exposure to extreme temperature fluctuations, strong winds, frost and

*Corresponding author. E-mail: cathy.offord@rbgsyd.nsw.gov.au

frost heave (Good, 1998), and influences the availability of water in spring (Keller, Goyette & Beniston, 2005). The projected rates of climate change are thought likely to exceed the rates of evolutionary adaptation in many species, while habitat fragmentation caused by direct habitat loss and the installation of physical barriers, such as roads and dams, may limit species migration in response to the shifting climatic zones (Hennessy *et al*., 2007). Community-level changes have already been observed in alpine regions of Europe, with cold-adapted species decreasing in range and warm-adapted species increasing (Gottfried *et al*., 2012). Similar changes are expected in Australia (Pickering, Good & Green, 2004).

The rising temperatures affecting snow cover may also have more direct impacts on alpine vegetation as temperature is a critical driver of plant regeneration, directly influencing seed dormancy, germination (Baskin & Baskin, 1998; Probert, 2000; Walck *et al*., 2011) and vegetative reproduction. In spite of this, the impact of climate change on plant regeneration has been neglected until recently, and there are few data on the response of individual species to forecast climate changes (Cochrane, Daws & Hay, 2011; Walck *et al*., 2011; Ooi, 2012). Although clonal growth is thought to be a more important mode of regeneration than seed production in some alpine regions (Bliss, 1971; Welling, Tolvanen & Laine, 2004), recent research has shown that many species in the Australian Alps regenerate from seed (Walsh & McDougall, 2008; Venn & Morgan, 2009). The germination–emergence stage is a high-risk phase of the plant life cycle, and therefore seed-based research can be useful in identifying species at risk of extinction from climate change, i.e. species with a narrow germination niche in terms of temperature range and/or stratification requirement (Cochrane *et al*., 2011; Walck *et al*., 2011). Information of this type provides a link between environmental change and the mechanisms that control population processes (Ooi, Auld & Denham, 2009; Cochrane *et al*., 2011; Walck *et al*., 2011; Ooi, 2012), and can thus help to improve the accuracy of models predicting plant response to climate change (Ooi, 2012).

Given that the duration of snow cover is predicted to decrease with increasing temperatures, species that require protracted cold stratification to initiate germination may be expected to contract in range or (if they have the capacity for vegetative reproduction) may become more reliant on clonal growth to survive. Species with no cold stratification requirement may become more competitive and potentially expand in range. The germination requirements of herb species in the Australian Alps have not been studied extensively (with the exception of work by MacPhee, 1998;

Wardlaw, 1998; Venn, 2007), and therefore our first objective was to determine which of 19 characteristic herbs from the region have an obligate requirement for cold stratification in order to germinate and the minimum duration of stratification required. We also aimed to determine the range of temperatures over which germination can occur once any need for stratification has been met. Many species germinate at a wide range of temperatures following stratification (Baskin & Baskin, 1998), but the exceptions may be severely impacted in a warmer climate (Walck *et al*., 2011).

For those species likely to be negatively affected by global warming, and which do not have the potential to migrate upwards in response, *ex situ* seed storage may provide a safety net to ensure species survival (Cochrane & Daws, 2008). The seed resource in conservation seedbanks can only be effectively utilized when germination requirements are understood, and therefore our second objective was to investigate each species to determine the class and depth of seed dormancy present following storage under standard seedbank conditions and the optimum conditions required for laboratory germination. Alpine seeds have recently been found to be shorter lived in storage than their lowland counterparts, and therefore require more frequent testing to monitor viability (Mondoni *et al*., 2011); information on optimal conditions for germination ensures that testing can be carried out effectively using the minimum number of seeds. Developing an understanding of germination needs can also provide information that is useful to restoration practitioners, enabling them to select species that are matched sufficiently well to site conditions to enable future recruitment from seed and the development of self-sustaining populations (Vitt *et al*., 2010), and to select appropriate pretreatments for 'on-demand' release of dormancy (Merritt & Dixon, 2011).

The area covered by alpine vegetation in Australia is narrow, bound by the treeline at altitudes of 1800–1830 m above sea-level (a.s.l.) in New South Wales (Good, 2006) and the peak of Mount Kosciuszko at 2229 m. This narrow range severely limits the potential for upwards migration in response to climate change, and places the few strictly alpine species of this region at most risk. The majority of species in the Australian Alps, however, have wider distributions (Good, 1998), in some cases extending to coastal areas and/or much lower latitudes (Royal Botanic Gardens and Domain Trust, 2012). Venn & Morgan (2009) demonstrated that altitude had no discernible influence on seedling emergence and establishment for several species in the Australian Alps (at least within the alpine region); however, other studies have shown differences in germination response between upland
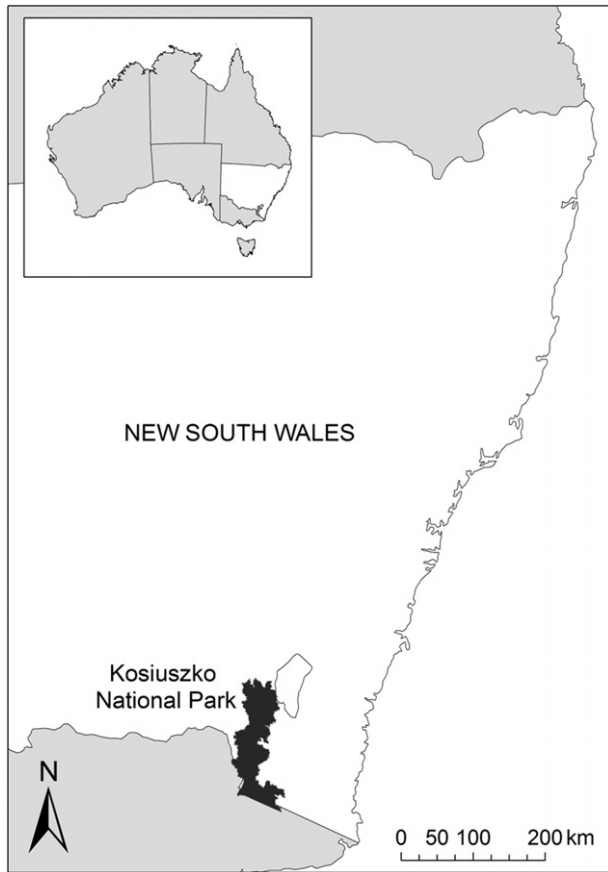
Figure 1. Seed for 19 native herb species was collected from alpine and subalpine areas in Kosciuszko National Park, New South Wales, Australia.

and lowland ecotypes of some species (Sayers & Ward, 1966; Beardsell & Mullett, 1984; Meyer, Kitchen & Carlson, 1995; Mondoni *et al.*, 2008). Our final objective was thus to determine whether the current distribution of the 19 herb species gives any indication of their requirement for cold stratification, and therefore of their sensitivity to climate change.

MATERIAL AND METHODS
STUDY SPECIES

Seed samples and herbarium specimens for a number of Australian native species were collected from various locations in Kosciuszko National Park (Fig. 1), in February 2004 and 2007, as part of the New South Wales (NSW) Seedbank collection programme. Seeds were collected at the point of natural dispersal and then dried and held at 15 °C and 15% relative humidity for up to 9 months, with the exception of *Euchiton involucratus* (G.Forst.) Holub (14 months) and *Coronidium scorpioides* (Labill.) Paul.G.Wilson (28 months). Seeds were then vacuum sealed in foil

packets and stored in the NSW Seedbank at −20 °C. Accompanying herbarium specimens were lodged, and species identities were confirmed, at the National Herbarium of NSW. From these collections, 19 high-altitude herb species from ten families were selected for use in this study (Table 1). Fifteen of the selected species were among the diagnostic taxa listed for one or more of the treeless vegetation communities of the Australian Alps (as described by McDougall & Walsh, 2007). All species had known ranges that extended into alpine and/or subalpine regions as defined by Good (2006), with the exception of *C. scorpioides*, which extended into montane regions only. Germination tests conducted prior to cold storage (average of 210 days after collection) indicated that all species had high initial levels of viability (84–100%, Table 1).

SEED CHARACTERISTICS

A subsample of 30 dried seeds per species was used to examine seed size, the presence/absence of endosperm, embryo type (following Martin, 1946) and the ratio of embryo length to seed length (E:S). E:S values were measured on ten seeds previously placed on water agar for 24 h to allow the imbibition of water. Seeds were photographed using an Olympus Colorview Soft Imaging System camera mounted on a binocular microscope; measurements were made on the digital images using AnalySIS LS Starter Version 2.6 Life Science software (Olympus Soft Imaging Solutions GmbH, Münster, Germany). Mean seed air-dried weight was measured on three replicates of 50 seeds (equilibrated to 15% relative humidity and 15 °C) and then converted to individual seed weights. For very small seeds, the number of seeds per gram was estimated by counting the number of seeds in three subsamples of known weight.

GERMINATION REQUIREMENTS

Prior to germination testing, the foil packets holding each seed collection were withdrawn from cold storage, held at room temperature for ≥ 24 h and opened to allow seeds to equilibrate to room humidity for ≥ 12 h. Random subsamples of seed were then withdrawn from each packet and sown on water agar (0.7% w/v) in five replicates of 20 or 25 seeds per treatment. Seeds were stratified at 3 or 5 °C for 0, 4, 8 or 12 weeks, then incubated at constant temperatures of 10, 15, 20 and 25 °C, alternating temperatures of 20/10 °C and, for 14 species, 20/5 °C. A diurnal cycle of 12 h light and 12 h dark was used in all cases. An additional set of replicates was maintained at the stratification temperature for the duration of the experiment. Nonstratified seeds were also incubated at 20/10 °C on agar incorporating

Table 1. Collection location and distributional range for 19 herb species collected from alpine and subalpine regions in Kosciuszko National Park, Australia

| Voucher | Family | Species | Collection | | Range | |
			Location†	Altitude‡	Latitude§	Altitude¶
NSW617457	Apiaceae	*Aciphylla glacialis** (F.Muell.) Benth.	36 25 52 S, 148 19 47 E	1850	36–38°S	1450–2210
NSW742480		*Aciphylla simplicifolia** (F.Muell.) Benth.	35 54 33 S, 148 33 37 E	1360	36–38°S	920–2100
NSW617522		*Oreomyrrhis eriopoda** (DC.) Hook.f.	36 29 28 S, 148 17 6 E	1960	29–44°S	20–2190
NSW617511	Asparagaceae	*Arthropodium* sp. B *sensu* Conran *et al.* in Harden (1993)	36 22 15 S, 148 28 42 E	1620	28–36°S	124–1620
NSW617534	Asteraceae	*Brachyscome* sp. 1 *sensu* P.S.Short (1999)*	36 29 23 S, 148 17 3 E	1950	32–42°S	1710–2000
NSW617506		*B. tenuiscapa* var. *pubescens* (Benth.) G.L.Davis	36 22 16 S, 148 28 47 E	1630	28–38°S	850–2050
NSW617560		*Coronidium scorpioides** (Labill.) Paul G.Wilson	36 31 41 S, 148 11 35 E	1120	28–44°S	10–1940
NSW617603		*Coronidium waddelliae* (J.H.Willis) Paul G.Wilson	36 2 58 S, 148 17 3 E	1250	33–38°S	20–1645
NSW742495		*Euchiton involucratus** (G.Forst.) Holub	35 53 7 S, 148 30 49 E	1345	23–44°S	10–1680
NSW742460		*Podolepis robusta** (Maiden & Betch) J.H.Willis	35 54 49 S, 148 34 22 E	1380	35–38°S	1130–1900
NSW617586		*Xerochrysum subundulatum** (Sch.Bip.) R.J.Bayer	36 3 13 S, 148 14 53 E	1310	29–42°S	850–1960
NSW617499	Campanulaceae	*Wahlenbergia ceracea** Lothian	36 22 15 S, 148 28 42 E	1620	29–42°S	480–2220
NSW617594		*Wahlenbergia gloriosa** Lothian	36 3 8 S, 148 15 27 E	1300	30–38°S	1120–2180
NSW617502	Onagraceae	*Epilobium gunnianum** Hausskn.	36 22 15 S, 148 28 42 E	1620	30–43°S	60–1950
NSW742466	Orobanchaceae	*Euphrasia collina* R.Br. subsp. *diversicolor** W.R.Barker	36 29 23 S, 148 17 3 E	1950	35–37°S	1420–2200
NSW617545	Plantaginaceae	*Veronica perfoliata** R.Br.	35 57 57 S, 148 23 21 E	1170	30–38°S	200–1830
NSW617525	Poaceae	*Poa hiemata** Vickery	36 29 23 S, 148 17 3 E	1950	35–42°S	1200–2220
NSW742469	Rosaceae	*Geum urbanum* L.	35 53 12 S, 148 22 25 E	860	29–43°S	20–1500
NSW617572	Violaceae	*Viola betonicifolia** Sm.	36 3 13 S, 148 14 53 E	1310	24–43°S	10–2190

*One of a group of diagnostic taxa for one or more treeless vegetation communities in the Australian Alps (McDougall & Walsh, 2007).

†Map datum WGS 84.

‡Metres above sea level (± 20 m).

§Data derived from Australia's Virtual Herbarium, with permission of the Council of Heads of Australasian Herbaria Inc., and rounded to the nearest degree.

¶Metres above sea level. Data derived from the combined ranges of communities for which the species is a diagnostic taxon (McDougall & Walsh, 2007) and/or from NSW Herbarium specimens.

250 mg L^{-1} of gibberellic acid (GA$_3$; Sigma, St Louis, MO, USA) to determine whether this plant growth regulator could be used as a substitute for cold stratification and to assist in dormancy classification.

Incubator temperatures were accurate to ± 1 °C. Initial cold stratification (for the germination of seven species at 20/10 °C only) took place in an incubator with a mean temperature of 5 °C. Stratification for all subsequent experiments took place in a cool room with a mean temperature of 3 °C. Germination (defined as radicle emergence) was monitored weekly and germinants were removed as observed. Germination tests were terminated after 3–4 months when germination had ceased or become sporadic (as recommended by Milbau et al., 2009). Any nongerminated seeds were then dissected to determine viability. Seeds were considered to be viable if the contents were still firm and creamy-white in colour. Viability was expressed as the percentage of germinated + viable seeds out of the total number of seeds per replicate.

For several species, insufficient seeds were available to apply the full range of treatments. *Brachyscome* sp. 1 *sensu* P.S.Short (1999) was tested at a more limited range of temperatures (10, 15, 20/5 °C and 20/10 °C), and germination following stratification for this species and two others (*Epilobium gunnianum* Hausskn. and *Euphrasia collina* R.Br. subsp. *diversicolor* W.R.Barker) was tested at 20/10 °C only.

The mean time to germination (MTG) for seed subjected to cold stratification was determined using the equation:

$$MTG = \Sigma(n \times d)/N$$

where n is the number of seeds germinated between scoring intervals, d is the incubation period in days and N is the total number of seeds germinated (Cochrane & Probert, 2006). Note: germination during the stratification period was excluded from this calculation.

As data could not be normalized by transformation for all species, the effects of stratification and temperature on total germination and MTG of individual species were compared using the nonparametric Kruskal–Wallis test in GenStat v.10 (VSN International, 2007). The Kruskal–Wallis test was also used to determine any significant differences in stratification requirement between endospermic and non-endospermic seeds. Optimal conditions for laboratory germination were determined on the basis of the treatments generating maximum germination percentages in the least amount of time (incorporating both the time for stratification and MTG following stratification).

DORMANCY CLASSIFICATION

Dormancy was classified for each species following Baskin & Baskin (2004a, b), using seed characteristics (E:S, embryo type), ability to imbibe water and germination responses. The ability to imbibe water was determined by evidence of seed coat softening and swelling following 24 h on water agar (in preparation for dissection to examine E:S). The germination responses used included MTG and the timing of germination following dormancy-breaking treatments of cold stratification and GA$_3$. The germination response to GA$_3$ is a key criterion in evaluating the depth of physiological seed dormancy (Baskin & Baskin, 2004a). Treatment with GA$_3$ can also substitute for a period of cold stratification in some species (Wardlaw, Moncur & Totterdell, 1989; Walck, Baskin & Baskin, 2000; Vandelook, Bolle & Van Assche, 2007; Mondoni et al., 2008).

STRATIFICATION REQUIREMENTS VS. DISTRIBUTION AND SEED CHARACTERISTICS

Species were given a stratification rating of 0, 4, 8 or 12 based on: (1) the minimum number of weeks of stratification required to achieve ≥ 50% germination at one or more temperatures; and (2) the minimum number of weeks of stratification required to achieve maximum germination across a range of temperatures. The latitudinal range and a distribution map for each species were derived from Australia's Virtual Herbarium (AVH, 2012) and reproduced with permission of the Council of Heads of Australasian Herbaria. The altitudinal range for each species was derived from McDougall & Walsh (2007) and herbarium records held at the National Herbarium of NSW. Seed characteristics (E:S, seed weight and seed length) were determined as described above. Ordinal logistic regression (Minitab v16) was used to determine whether the stratification ratings bore any relationship to distribution data (minimum and maximum latitude, latitudinal range, minimum and maximum altitude and altitudinal range) or seed characteristics. Simple correlations were used to identify any autocorrelated variables prior to the regression analysis. Pearson and Deviance goodness-of-fit tests were used to determine how well the models fitted the data.

RESULTS

SEED CHARACTERISTICS

Seeds ranged in size from 0.6 ± 0.1 mm to 5.4 ± 0.1 mm and in weight from 0.03 mg to 3.80 ± 0.10 mg [*Wahlenbergia ceracea* Lothian and *Aciphylla glacialis* (F.Muell) Benth., respectively]. Embryo types included dwarf, linear, spathulate, lateral and invest-

Table 2. Seed characteristics of 19 herb species collected from alpine and subalpine regions in Kosciusko National Park

| Family | Species | Seed characteristics | | | |
		Weight (mg)	Length (mm)	Embryo type	E:S
Apiaceae	*Aciphylla glacialis*	3.81 ± 0.12	5.4 ± 0.13	Linear*	0.16
	Aciphylla simplicifolia	4.34 ± 0.18	4.7 ± 0.18	Linear*	0.14
	Oreomyrrhis eriopoda	3.70 ± 0.11	5.1 ± 0.08	Linear*	0.16
Asparagaceae	*Arthropodium* sp. B	1.21 ± 0.06	1.8 ± 0.33	Linear (coiled)*	2.11
Asteraceae	*Brachyscome* sp. 1	0.30 ± 0.01	1.6 ± 0.03	Investing	1.00
	Brachyscome tenuiscapa var. *pubescens*	0.56 ± 0.02	2.5 ± 0.10	Spathulate	1.00
	Coronidium scorpioides	0.15 ± 0.00	2.0 ± 0.05	Investing	0.80
	Coronidium waddelliae	0.46 ± 0.01	1.8 ± 0.08	Spathulate	1.00
	Euchiton involucratus	0.03	0.6 ± 0.03	Linear	1.00
	Podolepis robusta	0.69 ± 0.01	2.3 ± 0.16	Spathulate	1.00
	Xerochrysum subundulatum	0.39 ± 0.01	2.2 ± 0.11	Spathulate	1.00
Campanulaceae	*Wahlenbergia ceracea*	0.03	0.6 ± 0.01	Linear underdeveloped*	0.47
	Wahlenbergia gloriosa	0.04 ± 0.00	0.8 ± 0.02	Linear underdeveloped*	0.45
Onagraceae	*Epilobium gunnianum*	0.07 ± 0.00	0.9 ± 0.03	Investing	1.00
Orobanchaceae	*Euphrasia collina* subsp. *diversicolor*	0.24 ± 0.00	1.2 ± 0.03	Linear fully developed*	0.79
Plantaginaceae	*Veronica perfoliata*	0.27 ± 0.02	0.8 ± 0.05	Linear	1.00
Poaceae	*Poa hiemata*	0.33 ± 0.04	1.6 ± 0.03	Lateral*	0.23
Rosaceae	*Geum urbanum*	1.32 ± 0.02	3.4 ± 0.07	Spathulate	1.00
Violaceae	*Viola betonicifolia*	1.23 ± 0.03	1.9 ± 0.03	Linear-spathulate*	0.80

Embryo type according to Martin (1946) and Baskin & Baskin (2007).
*Endosperm present.
E:S, embryo length to seed length ratio.

ing. Dwarf embryos (as defined by Martin, 1946) were further classified as linear fully developed or linear underdeveloped using the system of Baskin & Baskin (2007). Endosperm was present in seeds of nine species, but was absent from all seven species of Asteraceae, *Ep. gunnianum*, *Geum urbanum* L. and *Veronica perfoliata* R.Br. E:S varied from 0.14 for *Aciphylla simplicifolia* (F.Muell) Benth. to 2.11 for *Arthropodium* sp. B *sensu* Conran *et al*. in Harden (1993) (Table 2).

GERMINATION REQUIREMENTS

Two species, *A. simplicifolia* and *Eu. collina* subsp. *diversicolor*, failed to germinate without prior stratification. The remaining 17 species germinated to some degree without prior stratification at one or more temperature regimes, although the germination percentages for two of these species were low (0–9% for *A. glacialis* and 0–2% for *Viola betonicifolia* Sm.). Optimal temperatures for germination without stratification varied greatly (Table 2); however, in terms of total germination percentage, species could be placed into one of six categories:

1. Germinated poorly (< 10%), or not at all, at all temperatures (*A. glacialis*, *A. simplicifolia*, *Eu. collina* subsp. *diversicolor* and *Vi. betonicifolia*).
2. Germinated optimally at temperatures of 10 °C or less [*Arthropodium* sp. B and *Oreomyrrhis eriopoda* (DC.) Hook.f.].
3. Germinated equally well at all temperature regimes ≥ 10 °C [*Brachyscome tenuiscapa* var. *pubescens* (Benth.) G.L.Davis, *C. scorpioides*, *G. urbanum*, *Podolepis robusta* (Maiden & Betche) J.H.Willis and *Xerochrysum subundulatum* (Sch.Bip.) R.J.Bayer].
4. Germinated equally well at all temperature regimes ≥ 15 °C [*Coronidium waddelliae* (J.H.Willis) Paul.G.Wilson and *Poa hiemata* Vickery].
5. Germinated optimally at moderate temperatures of 15–20 °C and/or alternating temperatures of 20/5 and 20/10 °C (*E. involucratus*, *Ep. gunnianum*, *Brachyscome* sp. 1 and *V. perfoliata*).
6. Germinated optimally at 25 °C (*W. ceracea* and *Wahlenbergia gloriosa* Lothian).

Seven species showed a significant increase in germination percentage following stratification (Table 3),

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Table 3. Treatments resulting in optimum germination on 0.7% agar for 19 herbs collected from alpine and subalpine regions in Kosciusko National Park

Family	Species	Optimum treatment			Response	
		Stratification (weeks)	Temperature (°C)	GA$_3$ (mg L^{-1})	Total (%)	MTG (weeks)
Apiaceae	*Aciphylla glacialis*	Cont.	3	NR	85 ± 2.2	11
	Aciphylla simplicifolia	12	10, 15, 20/5	NR	≥ 60 ± 6.5	≤ 1
	Oreomyrrhis eriopoda	Cont.	5	NR	92 ± 3.0	9
Asparagaceae	*Arthropodium* sp. B	8	All temps ≥ 10	NR	≥ 59 ± 8.3	≤ 2
Asteraceae	*Brachyscome* sp. 1	NR	20/5, 20/10	NR	≥ 67 ± 3.7	≤ 5
	Brachyscome tenuiscapa var. *pubescens*	NR	All temps ≥ 10	NR	≥ 97 ± 1.2	≤ 1
	Coronidium scorpioides	NR	All temps ≥ 10	NR	≥ 73 ± 4.1	≤ 3
	Coronidium waddelliae	NR	15, 20, 25	NR	≥ 58 ± 4.6	≤ 4
	Euchiton involucratus	NR	15	NR	77 ± 7.8	2
	Podolepis robusta	NR	All temps ≥ 10	NR	≥ 74 ± 5.3	≤ 2
	Xerochrysum subundulatum	NR	All temps ≥ 10	NR	≥ 93 ± 3.0	≤ 2
Campanulaceae	*Wahlenbergia ceracea*	4	25	NR	93 ± 3.7	2
	Wahlenbergia gloriosa	NR	20/10	250	77 ± 3.4	3
Onagraceae	*Epilobium gunnianum*	NR	15, 20/5, 20/10	NR	≥ 90 ± 3.5	≤ 3
Orobanchaceae	*Euphrasia collina* subsp. *diversicolor*	*	*	*	–	–
Plantaginaceae	*Veronica perfoliata*	NR	20/10	250	96 ± 2.9	6
Poaceae	*Poa hiemata*	NR	All temps ≥ 15	NR	≥ 61 ± 2.5	≤ 4
Rosaceae	*Geum urbanum*	NR	All temps ≥ 15	NR	≥ 98 ± 1.2	≤ 3
Violaceae	*Viola betonicifolia*	12	All temps ≥ 15	NR	≥ 7 ± 2.0	≤ 1

*Not determined.

Stratification, duration of cold stratification; Cont., continuous; Temperature, incubation temperature following stratification; GA$_3$, gibberellic acid, incorporated into the germination medium at the concentration shown; NR, treatment not required for optimum germination; Total, final germination percentage; MTG, mean time to germination.

whereas the remainder showed either no response or a negative response as follows:

1. Significantly improved germination across all temperature regimes ($P ≤ 0.05$; *A. glacialis*, *A. simplicifolia*, *Arthropodium* sp. B, *Vi. betonicifolia*, *W. ceracea*; Fig. 2).
2. Significantly improved germination for some temperature regimes ($P ≤ 0.05$; *O. eriopoda*, *C. waddelliae*).
3. No significant effect, species germinated equally well with or without stratification ($P > 0.05$; *Brachyscome* sp. 1, *B. tenuiscapa* var. *pubescens*, *C. scorpioides*, *Ep. gunnianum*, *G. urbanum*, *P. robusta*, *W. gloriosa*, *X. subundulatum*; Fig. 3).
4. Significantly reduced germination across some or all temperature regimes ($P ≤ 0.05$; *V. perfoliata*, *E. involucratus*, *Po. hiemata*; Fig. 4).

Note: although the germination of *Eu. collina* subsp. *diversicolor* improved from 0 to 4% following

12 weeks of stratification, the increase was not significant. Viability remained high following stratification for all species, except *Po. hiemata*. For this species, stratification resulted in a significant reduction in viability ($P = 0.000$) and an associated reduction in germination percentage.

For eight of the above species, including some for which stratification was not beneficial to the overall germination percentage, considerable amounts of germination occurred during the stratification period. For example, 87–93% of the total germination occurred during weeks 8–12 of the stratification period for *A. glacialis*, 48–61% occurred during weeks 10–12 for *A. simplicifolia*, 83–88% occurred during weeks 7–12 for *O. eriopoda*, 59–76% occurred in weeks 4–12 for *B. tenuiscapa* var. *pubescens* and 37–66% occurred in weeks 7–12 for *C. scorpioides*.

In most cases, alternating temperatures were not required for optimal germination, i.e. species germi-
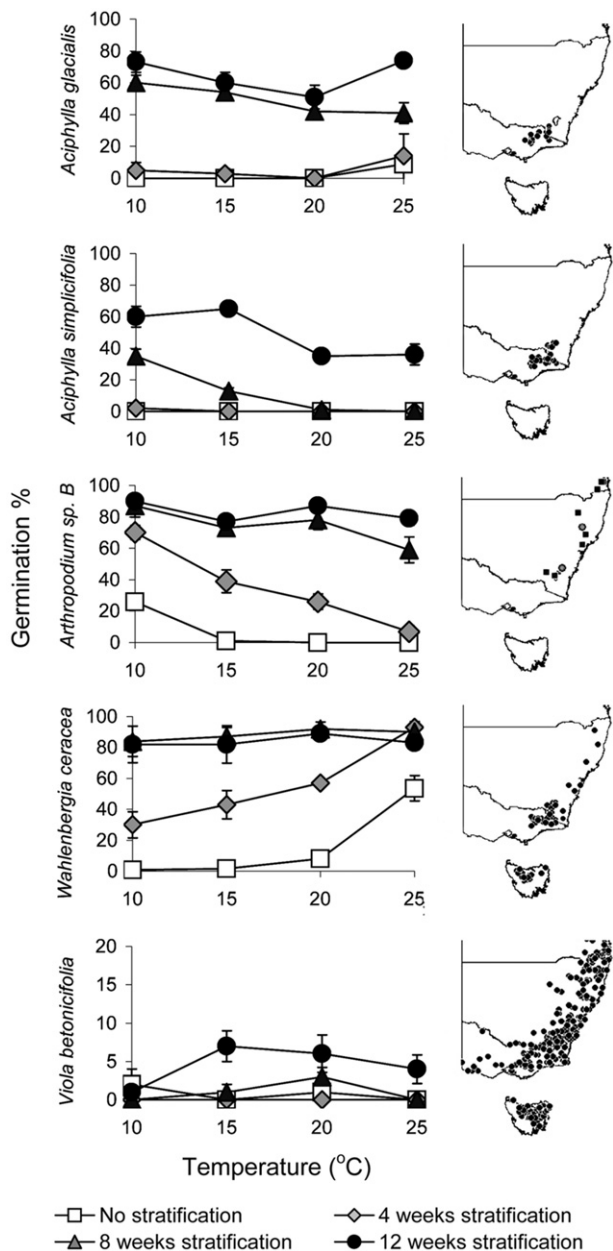
Figure 2. The effect of cold, moist stratification for 0, 4, 8 or 12 weeks on subsequent germination of *Aciphylla glacialis*, *A. simplicifolia*, *Arthropodium* sp. B, *Wahlenbergia ceracea* and *Viola betonicifolia* at temperatures ranging from 10 to 25 °C. Maps indicate the relative distribution of each species in south-east Australia.

Figure 3. The effect of cold, moist stratification for 0, 4, 8 or 12 weeks on subsequent germination of *Brachyscome tenuiscapa* var. *pubescens*, *Coronidium waddelliae*, *Geum urbanum*, *Oreomyrrhis eriopoda* and *Wahlenbergia gloriosa* at temperatures ranging from 10 to 25 °C. Maps indicate the relative distribution of each species in south-east Australia.

nated equally well at one or more of the constant temperatures and alternating temperatures of 20/5 and/or 20/10 °C (Table 3). *Veronica perfoliata* and *Brachyscome* sp. 1, however, germinated significantly better at alternating temperatures of 20/5 and 20/10 °C than at any of the constant temperatures (73% and 52%, respectively, vs. 0–17% for *V.*
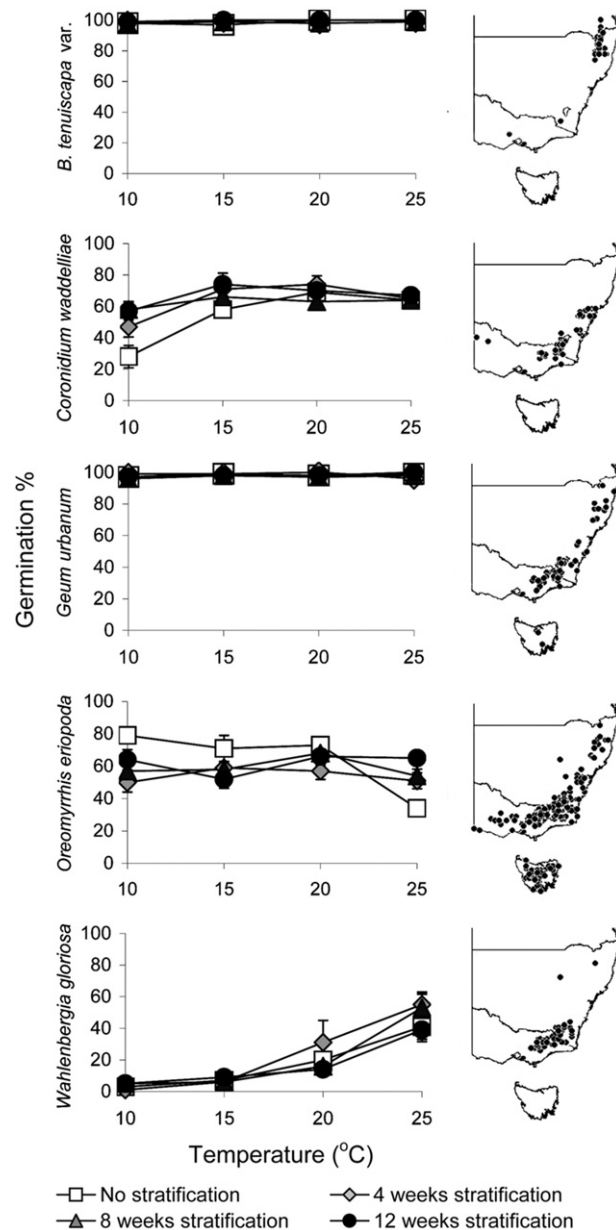
perfoliata; 57% and 67%, respectively, vs. 6–35% for *Brachyscome* sp. 1).

Although total germination was improved by stratification for only seven of the 19 species tested, stratification significantly reduced MTG, at some or all of the incubation temperatures, for 15 species ($P < 0.05$, Table 4).
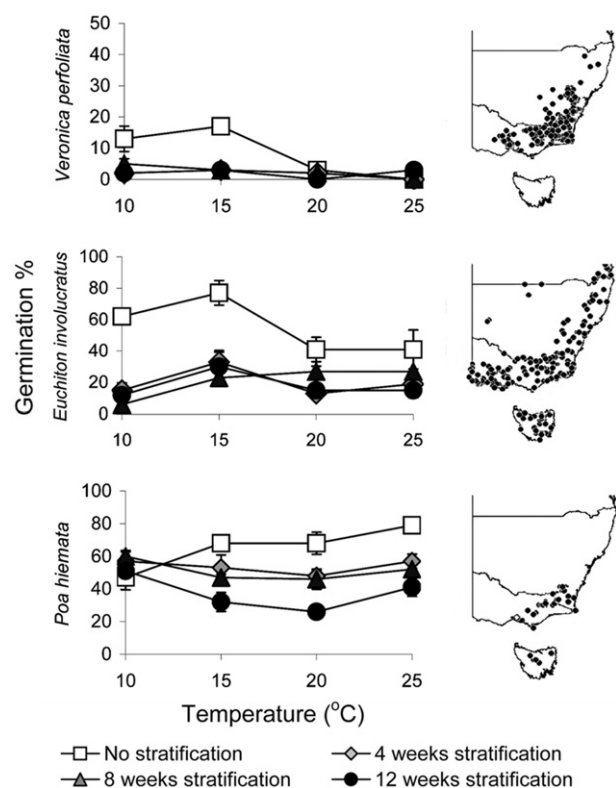
Figure 4. The effect of cold, moist stratification for 0, 4, 8 or 12 weeks on subsequent germination of *Veronica perfoliata*, *Euchiton involucratus* and *Poa hiemata* at temperatures ranging from 10 to 25 °C. Maps indicate the relative distribution of each species in south-east Australia.

DORMANCY CLASSIFICATION

All species readily imbibed water and thus did not exhibit physical dormancy. Based on E:S, MTG and response to dormancy-breaking treatments, eight species were classified as nondormant, whereas the remaining species exhibited either physiological dormancy (PD; six species) or morphophysiological dormancy (MPD; five species, Table 4). Five species of Asteraceae (*B. tenuiscapa* var. *pubescens*, *C. scorpioides*, *E. involucratus*, *P. robusta* and *X. subundulatum*) were nondormant; the remaining two exhibited PD (nondeep PD, type 3 for *C. waddelliae*; PD was not classified further for *Brachyscome* sp. 1 as germination after stratification was only tested at one temperature). All three species of Apiaceae (*A. glacialis*, *A. simplicifolia* and *O. eriopoda*) and both species in the Campanulaceae (*W. ceracea* and *W. gloriosa*) exhibited MPD, which was inferred on the basis of E:S ≤ 0.5 (denoting an underdeveloped embryo; Baskin & Baskin, 2007). The level and type of MPD

was difficult to assign precisely for most of these species because of incomplete dormancy break, i.e. total germination improved following the application of dormancy-breaking treatments, but was still less than total viability. Two species (*Eu. collina* subsp. *diversifolia* and *Vi. betonicifolia*) exhibited deep PD that could not be satisfactorily broken with any of the treatments used in this study. Dormancy in these two species was assumed to be physiological in nature, rather than morphophysiological, as the embryos were well developed (E:S of 0.79 and 0.80, respectively).

STRATIFICATION REQUIREMENTS VS. SEED CHARACTERISTICS AND DISTRIBUTION

A positive stratification response (increased germination percentage and/or reduced MTG) was observed for species with low, medium and high E:S, species with a variety of embryo types and species with and without endosperm. In terms of germination percentage alone, however, non-endospermic seeds ($n = 10$) required significantly less stratification than seeds with endosperm ($n = 9$; $P = 0.003$), i.e. non-endospermic seeds did not require any stratification to germinate optimally, whereas six of the nine species with endospermic seeds required at least 8 weeks of stratification to achieve maximal germination.

The majority of species with relatively broad distributions did not require stratification to germinate well (Figs 3, 4). However, three species with distributions that extended to coastal areas (*Arthropodium* sp. B, *Vi. betonicifolia* and *W. ceracea*) germinated far better at a wider range of temperatures following 8–12 weeks of stratification (Fig. 2). For three species restricted to subalpine and alpine regions (*A. glacialis*, *A. simplicifolia*, *Eu. collina* subsp. *diversicolor*), stratification for at least 8 weeks was essential to initiate any germination at all or to achieve germination percentages > 20%. For the fourth species restricted to this region (*Po. hiemata*), stratification resulted in a reduction in viability and total germination.

Analysis of correlations among seed characteristics and distributional data identified strong autocorrelation between seed weight and seed length, between altitudinal range and minimum altitude and between latitudinal range and maximum and minimum latitudes. Ordinal logistic regression of stratification requirements was thus conducted using the variables E:S, seed weight, maximum altitude, altitudinal range and latitudinal range. No significant relationships were detected between stratification ratings and any of these variables ($P > 0.9$ in all cases).

Table 4. Response to dormancy-breaking treatments and dormancy classification according to Baskin & Baskin (2004a, b) for 19 species collected from alpine and subalpine regions in Kosciusko National Park

Family	Species	Cold stratification response		GA$_3$ response	Dormancy	
		Germination (%)	MTG		Class	Level, type
Apiaceae	*Aciphylla glacialis*	Inc. at all temps	Red. at all temps	= 8 weeks strat.	*MPD	Int. + deep complex
	Aciphylla simplicifolia	Inc. at all temps	Red. at all temps	> 8, < 12 weeks strat.	*MPD	Int. + deep complex
	Oreomyrrhis eriopoda	Inc. at 25, 20/10 °C	Red. at all temps	= no strat.	*MPD	Deep complex?
Asparagaceae	*Arthropodium* sp. B	Inc. at all temps	Red. at all temps	= no strat.	PD	ND, type 1
Asteraceae	*Brachyscome* sp. 1	No change	Red. at 20/10 °C	= 0, 4, 8, 12 weeks strat.	PD	Not tested
	Brachyscome tenuiscapa var. pubescens	No change	Red. at 10, 20/5 °C	= 0, 4, 8, 12 weeks strat.	ND	N/A
	Coronidium scorpioides	No change	Red. at all temps	= 0, 4, 8 weeks strat.	ND	N/A
	Coronidium waddelliae	Inc. at 10, 20/5 °C	Red. at all temps	= 0, 4, 8, 12 weeks strat.	PD	Non-deep, type 3
	Euchiton involucratus	Red. at most temps	No change	= 4, 8, 12 weeks strat.	ND	N/A
	Podolepis robusta	Red. at 10 °C	Red. at 10, 15, 20/5 °C	= 0, 4, 8, 12 weeks strat.	ND	N/A
	Xerochrysum subundulatum	No change	Red. at 10 and 20/5 °C	= 0, 4, 8, 12 weeks strat.	ND	N/A
Campanulaceae	*Wahlenbergia ceracea*	Inc. at all temps	Red. at all temps	= 12 weeks strat.	*MPD	Int. complex
	Wahlenbergia gloriosa	No change	Red. at all temps	> 0, 4, 8, 12 weeks strat.	*MPD	Nondeep simple + ?
Onagraceae	*Epilobium gunnianum*	No change	Red. at 20/10 °C	= 0, 4, 8 weeks strat.	ND	N/A
Orobanchaceae	*Euphrasia collina subsp. diversicolor*	No change	†	= 0, 4, 8, 12 weeks strat.	PD	Deep
Plantaginaceae	*Veronica perfoliata*	Red. at most temps	Red. at 10 °C	> 0, 4, 8, 12 weeks strat.	PD	ND
Poaceae	*Poa hiemata*	Red. at 15, 20, 25 °C	Inc. at 10, 25, 20/5 °C	= no strat.	ND	N/A
Rosaceae	*Geum urbanum*	No change	Red. at most temps	= 0, 4, 8, 12 weeks strat.	ND	N/A
Violaceae	*Viola betonicifolia*	Inc. at most temps	†	< 12 weeks strat.	PD	Deep

*MPD was inferred by embryo type, E:S and germination response; data not yet supported by embryo growth studies.
†Insufficient germinating replicates to analyse.
MTG, mean time to germination; Inc., increased; Red., reduced; PD, physiological dormancy; MPD, morphophysiological dormancy; ND, nondormant; Int., intermediate; N/A, not applicable; strat., stratification.

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DISCUSSION

GERMINATION REQUIREMENTS: STRATIFICATION

The response to cold stratification was positive, to some extent, for the majority of species tested here. The main effects of the treatment included an increase in total germination, a widening of the range of temperatures at which germination occurred and/or a decrease in the time required for germination, as has been observed in previous studies of alpine species (Shimono & Kudo, 2005). In some cases, the benefits were minimal; for three species of Asteraceae (*B. tenuiscapa* var. *pubescens*, *P. robusta* and *X. subundulatum*), for example, cold stratification simply resulted in the reduction of MTG by less than 1 week. In other cases, stratification was essential to achieve high rates of germination or to initiate germination at all.

For six species in this study (*A. glacialis*, *A. simplicifolia*, *Arthropodium* sp. B, *Eu. collina* subsp. *diversicolor*, *Vi. betonicifolia* and *W. ceracea*), a strong dependence on stratification to initiate or achieve high rates of germination suggests that these species would be greatly disadvantaged in an environment without winter snow cover (Fig. 2). Species requiring stratification for periods of 8 weeks or more for optimal germination may be particularly sensitive to climate change, given that the duration of snow cover in the Australian Alps may be reduced by as much as 100 days (Hennessy *et al.*, 2007). Currently, snow cover in most years in the Australian Alps lasts a few weeks at elevations of 1200–1400 m, and up to 4 months at elevations of 1800–2200 m (Hughes, 2003). A reduction in duration of 100 days could therefore mean no cover at all at the lower elevations by 2050, and cover for 4 weeks or less in the alpine zone. A period of 4 weeks of stratification may be sufficient for > 50% germination of *Arthropodium* sp. B at the range of temperatures expected in spring and for *W. ceracea* at the range of temperatures expected in summer (Fig. 2), but would not be sufficient for germination of the remaining four species. As none of the four species has the capacity for clonal growth, their distributions may eventually shrink to pockets on south- and east-facing slopes where snow tends to accumulate in drifts and currently persists through summer because of the protection from solar radiation afforded by aspect (McDougall & Walsh, 2007).

Snow provides insulation for soil and seeds, protecting them from extreme cold. In the Australian Alps, for example, the minimum soil temperature recorded under snow cover at five alpine summits in 2005 (1729–2114 m a.s.l.) was −0.2 °C, whereas the minimum temperature recorded under patchy snow cover was −5.2 °C (Pickering & Green, 2009). A reduction in snow cover is thus likely to result in colder soils and deeper soil frost (Milbau *et al.*, 2009; Walck *et al.*, 2011). Milbau *et al.* (2009) found that stratifying the seed of subarctic species at temperatures representing greatly reduced snow cover (−5 and −10 °C) had no significant effect on the germination percentages of four tree, two shrub and four grass species compared with stratification at a temperature representing thick snow cover (0.5 °C). Similarly, Mondoni *et al.* (2012) found that stratification at −7 °C had no significant effect on final germination percentages of six alpine forbs, one rush and one grass species compared with stratification at 0 °C. In the experiment conducted by Milbau *et al.*, (2009), however, stratification at temperatures below zero did result in a significant reduction in germination for two of six forb species and two of four dwarf shrubs. The authors concluded that, although most of the species tested would not be negatively impacted by reduced snow cover, the distribution of species sensitive to very low temperatures could be reduced to small pockets where the insulating effect of snow allows for stratification at milder temperatures.

Although the seed of some species may be able to tolerate winter temperatures in the absence of snow, a reduction in snow cover may also mean a reduction in the amount of available water. Predictions regarding changes in total precipitation in winter and spring in the Australian Alps range from a decrease of 40% to an increase of 20% by 2050 (CSIRO & Bureau of Meteorology, 2012). As the level of seed hydration plays a role in breaking seed dormancy (Baskin & Baskin, 1998; Hoyle *et al.*, 2008a; Walck *et al.*, 2011), relative drought during winter and spring may prove to be more important in limiting the germination of these species than the lack of snow cover *per se* (Liu *et al.*, 2011).

The species in this study that germinated well without cold stratification have the greatest potential to remain stable or increase in range in the absence of snow cover, providing that rainfall is sufficient to meet hydration needs and their seeds are able to tolerate temperatures below zero. Thus, species in Asteraceae and Poaceae, and species for which cold stratification reduced germination (*V. perfoliata*, *E. involucratus* and *Po. hiemata*, Fig. 4), may be most likely to benefit from climate change in the Australian Alps. Asteraceae and Poaceae are already among the most common families in Australian alpine soil seed-banks (Venn & Morgan, 2010) and standing vegetation (Venn, 2007). Species in these families have been found to germinate well without stratification in other studies (Venn, 2007; Liu *et al.*, 2011; Schwienbacher *et al.*, 2011), and so have the potential to become even more common as snowfall declines. By contrast, species in Apiaceae depend on cold moist conditions (wet stratification) to break dormancy; reduced snow

cover during winter may threaten the survival of these species, even if subsequent temperatures are suitable for germination (Liu *et al.*, 2011).

GERMINATION REQUIREMENTS: TEMPERATURE

In a review of seed dormancy in alpine plants, Amen (1966) stated that there was no evidence to suggest that any alpine seeds can germinate at temperatures below 10 °C. In this study, seven of 19 species (*A. glacialis*, *A. simplicifolia*, *B. tenuiscapa* var. *pubescens*, *C. scorpioides*, *C. waddelliae*, *Ep. gunnianum* and *O. eriopoda*) germinated to ≥ 60%, albeit very slowly, at temperatures of ≤ 5 °C. In fact, *A. glacialis* and *O. eriopoda* germinated optimally at 3 and 5 °C, respectively (Table 3), a temperature range that reflects the onset of thaw (at 3.2 °C) in the Australian Alps (Pickering & Green, 2009).

The ability to germinate at very low temperatures has been observed in a few other alpine and high-mountain species, including the Australian *Caltha introloba* F.Muell. (Wardlaw *et al.*, 1989) and Asian and North American species of *Osmorhiza* Raf. (Apiaceae; Baskin, Meyer & Baskin, 1995; Walck, Hidayati & Okagami, 2002; Walck & Hidayati, 2004). This trait provides an advantage to species growing in environments with a short growing season by allowing the germination process to begin under snow banks (Meyer *et al.*, 1995; Forbis & Diggle, 2001; Walck & Hidayati, 2004). As germination is dependent on the availability of water, however, germination may only occur under snow with the onset of snow melt, and may not occur at all at such low temperatures in dry habitats where snow cover is absent (Liu *et al.*, 2011).

Germination in response to incubation temperatures of ≥ 10 °C was quite variable among the species tested here. Some showed considerable plasticity, germinating well over the full range of temperatures used, whereas a few only germinated well within a narrow range. For those species that germinated well at a range of temperatures without prior cold stratification (all Asteraceae, *Ep. gunnianum*, *Po. hiemata*, *G. urbanum* and *V. perfoliata*), warmer temperatures in the Alps may result in a higher proportion of seeds germinating in autumn soon after dispersal (as observed by Mondoni *et al.*, 2012) or earlier germination in spring.

Although climate warming might also be expected to lead to earlier seed dispersal and thereby extend the time available for the establishment of autumn-emerging seedlings, experimental warming of six Australian subalpine species produced little difference in the timing of fruit maturation, despite earlier budding and flowering (Hoffmann *et al.*, 2010). A shift to germination in autumn could therefore lead to a higher proportion of seedlings dying as a result of the exposure of unhardened tissues to winter temperatures (Mondoni *et al.*, 2012). The magnitude of this effect will depend strongly on the timing of seed dispersal and any need for a period of after-ripening. In the Australian Alps, seed dispersed in February and germinating immediately will produce seedlings that have 2–3 months to develop cold hardiness prior to the onset of winter. Seed dispersed in February, but requiring a period of after-ripening to break PD, may be more susceptible to deleterious effects resulting from autumn germination. An investigation of the time (and conditions) required to develop cold hardiness in these seedlings would be useful to predict the consequences of autumn emergence. Earlier spring emergence may also increase the likelihood of seedling death as a result of exposure to frost events (Mondoni *et al.*, 2012); however, surviving seedlings will have a longer growing season than they currently experience and a greater chance of developing cold hardiness prior to the onset of winter.

Warmer temperatures during the growing season are likely to be particularly beneficial to *W. gloriosa*, which required temperatures above 20 °C to germinate well, whether or not it was previously cold stratified (Fig. 3). For other species that germinated to some degree without cold stratification, but at a limited range of temperatures, germination may still occur in the absence of snow cover, but may occur more slowly and for a reduced period of time. Without stratification, for example, germination of *W. ceracea* in the alpine zone may be limited to a short period of summer when temperatures exceed 20 °C (Fig. 2), whereas germination of *Arthropodium* sp. B may be limited to a short period in spring or autumn when temperatures are around 10 °C (Fig. 2). For those species with the capacity for vegetative reproduction (e.g. *W. ceracea*), however, any reduction in germination may potentially be offset by enhanced clonal growth as a result of warmer soil temperatures during the growing season.

The incubation temperatures used in this study (10–25 °C) encompassed mean maximum air temperatures from October to February at Charlottes Pass (1755 m a.s.l.) over the period 1971–2000 (9–17 °C; Bureau of Meteorology, 2012) and temperatures that may be expected in the area by 2050 under a high emissions scenario (13–21 °C, based on an increase of up to 4 °C by 2050; CSIRO & Bureau of Meteorology, 2012). How these temperatures will relate to soil temperature in the region (i.e. the temperature of the germination substrate) is more difficult to determine. Mean soil temperatures at a depth of 50 cm in south-eastern Australia are generally 1–2 °C higher than mean air temperatures (up to an altitude of 600 m; Watson, 1980), but the relationship

between soil and air temperature at shallower depths, where seed germination occurs, can be very different. In the arid north-west NSW, for example, mean maximum summer air temperatures of 34 °C resulted in mean maximum soil surface temperatures (< 1 cm depth) of 60 °C (Ooi *et al*., 2009). In temperate heathland in eastern NSW, mean maximum summer air temperatures of 27 °C resulted in mean maximum soil surface temperatures (at 1–2 cm depth) of 40 °C on dry days (Ooi, Auld & Denham, 2012). Every 1 °C rise in maximum air temperature at this site resulted in a 1.5 °C rise in soil temperature.

In contrast, the maximum soil temperature recorded on one summit of Mount Clarke (1729 m a.s.l.) in the Australian Alps (13 °C at 10 cm depth; Pickering & Green, 2009) was 6 °C lower than the highest mean monthly maximum air temperature recorded in the area at a similar altitude in the same year (19 °C at Charlottes Pass, 1755 m a.s.l.; Bureau of Meteorology, 2012). If we take the maximum soil temperatures (13–16 °C) recorded at 10 cm depth on five summits of Mount Clarke (1729–2114 m a.s.l.) as a baseline, assume a 4 °C rise in air temperature by 2050 and apply the relationship between soil and air temperature found in temperate heathland (1.5 °C rise in soil temperature for every 1 °C rise in air temperature), we might expect mean maximum soil temperatures in the region to rise to 19–22 °C during the summer. These values are within the range of incubation temperatures used in this study; however, the temperature near the soil surface can be higher than at 10 cm depth (Ooi *et al*., 2009) and possibly affected to a greater extent by variation in vegetative cover, aspect, wind exposure and soil moisture content. A study of near-surface soil temperature in relation to air temperature in the Australian Alps may therefore allow predictions of germination response to be made with greater confidence.

DORMANCY CLASSIFICATION

There is limited information on the germination ecology of southern temperate alpine herbs, but we observed the same types of dormancy in this study as have been observed in alpine herbs from the Northern Hemisphere (Baskin & Baskin, 1998; Schwienbacher *et al*., 2011). That is, a combination of dormant and nondormant species and, among dormant species, a similar proportion displaying PD and MPD. The dormancy classes described here were determined following long periods of storage at 15% relative humidity (initially at 15 °C and subsequently at −18 °C), which may have affected the depth of dormancy (Kaye, 1997; Andersson & Milberg, 1998; Baskin & Baskin, 1998; Wang *et al*., 2010). Baskin & Baskin (1998), noted that the relative humidity must be at least 30–40%

for species to come out of dormancy during storage, and Hoyle *et al*. (2008b) found that dry storage of seeds under the conditions used here (15 °C and 15% relative humidity) had no effect on dormancy and subsequent germination of *Goodenia fascicularis* F.Muell. & Tate. As a result of the potential for after-ripening, however, it should be noted that some of the species classified here as nondormant may be found to display PD when fresh. This could be clarified in future studies. Regardless, most of the species with nondormant seeds in this study lacked endosperm and showed little response to cold stratification in terms of germination percentage (Table 4). These might make useful criteria for determining those species *least* likely to be negatively impacted by a warming climate.

The determination of the precise level and type of MPD can be quite difficult for some species (Schwienbacher *et al*., 2011), as was the case in this study. *Aciphylla glacialis*, for example, germinated best at 3 °C, although cold stratification resulted in germination at a greater range of higher temperatures (including spring temperatures, as specified in the key of Baskin & Baskin, 2004b). GA$_3$ substituted for cold stratification for only a proportion of the viable seeds, suggesting that some seeds had intermediate complex MPD and others had deep complex MPD. *Oreomyrrhis eriopoda*, a member of the same family (Apiaceae), germinated best at 5 °C and appeared to fit into the category of deep complex MPD, as cold stratification increased germination at 25 °C and 20/10 °C and reduced MTG at all temperatures, but there was no response to GA$_3$. As this species germinated to some extent at all temperatures, the collection may have been close to the nondormant end of the dormancy continuum at the time of testing. An alternative explanation may be that these species do not have a physiological component to their dormancy and simply require low temperatures (such as those experienced at the end of winter) to germinate well once the embryo has grown sufficiently. This was recently reported to be the case for the alpine species *Fritillaria tubiformis* Gren. & Godr. subsp. *moggridgei* (Boiss. & Reut. ex Planchon) Rix following embryo growth and germination studies (Carasso *et al*., 2011).

Although *W. ceracea* and *W. gloriosa* are congeneric and have the same embryo type, they displayed different dormancy classes. Positive responses to cold stratification and GA$_3$ placed *W. ceracea* in intermediate complex MPD. Cold stratification was ineffective for *W. gloriosa* and germination of a proportion of seeds occurred at 25 °C, suggesting nondeep simple MPD. Further classification for this species requires embryo growth studies at a range of temperature and/or moisture conditions, as the maximum germi-

nation achieved was only 55% at 25 °C. Other cues or higher temperatures may be required for complete germination. Multiple dormancy classes and/or levels within a collection have been observed in other Australian alpine species, such as *Caltha introloba* F.Muell. (Wardlaw *et al*., 1989), which exhibited nondeep simple and deep complex MPD (Baskin & Baskin, 2004a). Ecologically, this is a strategy to ensure that germination is distributed over time, preventing the loss of an entire seed cohort if subsequent conditions are unfavourable for seedling growth.

The deep PD exhibited by *Eu. collina* subsp. *diversicolor* requires further investigation. This species germinated poorly under all test conditions in this study, despite the apparently high viability of the seed lot used. Two European alpine species of *Euphrasia* L. showed a similar depth of dormancy in a study conducted by Liebst & Schneller (2008). In that study, the species germinated at 5 °C or varying low temperatures (3–10 °C), but took up to 3 years to do so. Two other Australian subspecies of *Eu. collina* [subsp. *tetragona* (R.Br.) W.R.Barker and subsp. 'NW Tasmania'] germinated quickly ($T_{50} < 40$ days) when incubated at 5 °C and 15 °C, respectively; however, these collections were made at lower altitudes (66 m and 37 m, respectively; Wood, 2009). Higher altitude collections of Australian *Euphrasia* taxa have been found similarly difficult to germinate, with *Eu*. *collina* subsp. *diemenica* (Spreng.) W.R.Barker (altitude, 960 m) germinating slowly at 5 °C and *Eu*. *gibbsiae* Du Rietz (altitude, 1255 m) germinating very slowly with warm stratification followed by low temperatures (8 weeks at 20 °C, then 5 °C; Wood, 2009). *Euphrasia collina* subsp. *diversicolor* may therefore require periods of stratification > 12 weeks, a longer incubation period and/or other treatments, such as alternating cold and warm stratification, to break dormancy.

STRATIFICATION REQUIREMENTS VS. DISTRIBUTION AND SEED CHARACTERISTICS

Although most of the broadly distributed species tested here did not require cold stratification in order to germinate, for three species (*Arthropodium* sp. B, *W. ceracea* and *Vi. betonicifolia*) stratification was essential. This was interesting given that all three species have distributions that extend to much lower elevations and much warmer latitudes (Fig. 2). *Wahlenbergia ceracea* is a rhizomatous species, and thus at least part of its broad distribution might be explained by vegetative reproduction; however, neither *Arthropodium* sp. B nor *Vi. betonicifolia* is known to have the capacity for clonal growth.

Seed from lowland populations of *Arthropodium* sp. B and *Vi. betonicifolia* apparently germinates readily without cold stratification (L. von Richter, The Australian Botanic Garden, Mount Annan, pers. comm. and J. Wilde, Greening Australia, Brisbane, pers. comm., respectively), and therefore ecotypic variation may be an explanation for their presence in both warm and cold environments. Differences in stratification requirements between upland and lowland ecotypes of some species have been observed in several other studies (e.g. Nichols, 1934; Sayers & Ward, 1966; Beardsell & Mullett, 1984; Meyer *et al*., 1995; Mondoni *et al*., 2008); it would be interesting to determine whether these differences resulted from a fixed genetic adaptation to alpine conditions or from a phenotypically plastic trait induced by the local environment. That is, would the need for stratification persist if the progeny of upland ecotypes were grown to maturity in lowland conditions or if the upland ecotypes themselves were subjected to milder winters as a result of climate change?

The depth of dormancy is known to be influenced by external factors, such as the maternal environment during seed maturation. In a glasshouse study of *Goodenia fascicularis*, for example, Hoyle *et al*. (2008b) found that subjecting plants to cooler temperatures during seed development resulted in a deeper level of dormancy in the mature seed than those subjected to warmer temperatures. In this experiment, seed that developed on plants grown at a mean temperature of 17 °C had significantly deeper dormancy than seed that developed at a mean temperature of 26 °C. Although the authors commented that this difference, although significant, was comparatively minor, the effect is likely to be amplified as the temperature gap between environments widens.

Andersson & Milberg (1998) also studied the depth of dormancy in four annual weed species, and attributed significant differences among populations, and among individuals within a population, to the mother plant environment. If the maternal environment also explains some of the differences between upland and lowland ecotypes of high-altitude species, it would have important implications for the conservation of alpine populations and for the source of material used for re-vegetation. If the need for lengthy stratification is a fixed trait, and climate change is taken into consideration in restoration planning, it may be better to re-vegetate alpine areas using plants grown from seed collected in lowland populations that are adapted to germination in warmer conditions. If the trait is plastic, material for translocation could be confidently sourced from nearby upland populations.

An alternative explanation for the broad distribution of species requiring cold stratification is that the

need for stratification may be satisfied at higher temperatures than those tested here. Baskin & Baskin (1998) defined cold stratification as a period of exposure to temperatures below 10 °C, whereas the stratification temperatures commonly used in studies of alpine and high-mountain species are between 0 and 5 °C (Wardlaw *et al*., 1989; Meyer *et al.,* 1995; Kaye, 1997; Cavieres & Arroyo, 2000; Forbis & Diggle, 2001; Giménez-Benavides, Escudero & Pérez-García, 2005; Shimono & Kudo, 2005; Mondoni *et al*., 2008). The stratification temperatures used in this study were within the range of soil temperatures likely to be encountered in the field at high elevation in Australia (average annual temperatures of around 5 °C at 100 mm depth and minimum temperatures under snow cover of –0.2 °C: Pickering & Green, 2009), but are not likely to be representative of minimum soil temperatures encountered by these species in warmer climes.

IMPLICATIONS FOR CONSERVATION

Limited upslope refugia for high-altitude species and the limited rate of natural migration through seed dispersal mean that climate change is likely to impact significantly on alpine and subalpine plant species in Australia. Even some species restricted to low-elevation mountains in Australia may be at risk of extinction because of their narrow temperature range for germination (Cochrane *et al*., 2011). A number of species in this study, however, appeared to be sufficiently plastic in their germination response to ensure their persistence in the future. Species in Asteraceae and Poaceae and species with no endosperm would seem to be the least likely to suffer under climate change and the most likely to either remain stable or expand in range. Such species may be useful for inclusion in alpine restoration sites because of their ability to germinate at a range of temperatures, with or without cold stratification. Given that it is not possible to anticipate all combinations of climate and soil microclimate as warming occurs, the inclusion of native species with high phenotypic plasticity may be the most effective strategy for ensuring the ongoing success of restoration programmes in the face of climate change (Vitt *et al*., 2010).

For the remaining taxa in this study (*A. glacialis*, *A. simplicifolia*, *Arthropodium* sp. B, *Eu. collina* subsp. *diversicolor*, *Vi. betonicifolia*, *W. ceracea*, *W. simplicifolia*), climate change may limit the capacity to regenerate via seed and contractions in range may result, particularly for those species that do not have the capacity for clonal growth. The continued regeneration of these species in the wild will depend on the temperature and moisture status of the soil during winter and the maintenance of conditions suitable for stratification for an appropriate length of time. The changing climate is also likely to impact on the germination response of these species through maternal effects on the developing seed (Giménez-Benavides *et al*., 2005; Mondoni *et al*., 2008). Species with similar characteristics to those in this group, i.e. with endospermic seeds and restricted to alpine or subalpine environments (or with known upland ecotypes), could be targeted for conservation in *ex situ* collections, whilst monitoring their response in the field.

Whether alpine species are included in restoration programmes or conserved *ex situ* by seed banking, understanding their germination requirements is essential to monitoring viability, improving seed use and minimizing the waste of valuable seeds (Martyn, Merritt & Turner, 2009; Merritt & Dixon, 2011). Effective viability testing using minimal seed numbers is particularly important when banking seed of species from cold, wet environments (as in alpine regions), as these have been found to be shorter lived in storage and thus require more frequent monitoring than species from hot, dry environments (Probert, Daws & Hay, 2009; Mondoni *et al*., 2011). This study identified the most appropriate germination conditions for 17 of 19 high-altitude herb species and also identified those for which GA$_3$ could be used as a substitute for cold stratification or to maximize germination.

We conclude that variability in optimal germination temperature, dormancy class and timing of germination should be the expectation, rather than the exception, for seeds collected from high altitudes. However, it appears that a combination of species distribution and seed characteristics may be used to predict the stratification needs of high-altitude herbs and therefore their sensitivity to climate change.

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