Conditional cold avoidance drives between-population variation in germination behaviour in *Calluna vulgaris*

Joachim P. Spindelböck[1,2,*], Zoë Cook[3], Matthew I. Daws[3], Einar Heegaard[2,4], Inger E. Måren[5] and Vigdis Vandvik[2]

[1]*Faculty of Engineering and Science, Sogn og Fjordane University College, PO Box 133, 6851 Sogndal, Norway,* [2]*Department of Biology, University of Bergen, Allégaten 41, 5007 Bergen, Norway,* [3]*Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, West Sussex RH17 6TN, UK,* [4]*Department of Geography, University of Bergen, Fosswinckelsgt 6, 5007 Bergen, Norway and* [5]*Norwegian Forest and Landscape Institute, Fanaflaten 4, 5244 Fana, Norway*
* For correspondence. E-mail joachim.spindelbock@hisf.no

• *Background and Aims* Across their range, widely distributed species are exposed to a variety of climatic and other environmental conditions, and accordingly may display variation in life history strategies. For seed germination in cold climates, two contrasting responses to variation in winter temperature have been documented: first, an increased ability to germinate at low temperatures (cold tolerance) as winter temperatures decrease, and secondly a reduced ability to germinate at low temperatures (cold avoidance) that concentrates germination towards the warmer parts of the season.

• *Methods* Germination responses were tested for *Calluna vulgaris*, the dominant species of European heathlands, from ten populations collected along broad-scale bioclimatic gradients (latitude, altitude) in Norway, covering a substantial fraction of the species' climatic range. Incubation treatments varied from 10 to 25 °C, and germination performance across populations was analysed in relation to temperature conditions at the seed collection locations.

• *Key Results* Seeds from all populations germinated rapidly and to high final percentages under the warmer incubation temperatures. Under low incubation temperatures, cold-climate populations had significantly lower germination rates and percentages than warm-climate populations. While germination rates and percentages also increased with seed mass, seed mass did not vary along the climatic gradients, and therefore did not explain the variation in germination responses.

• *Conclusions* Variation in germination responses among *Calluna* populations was consistent with increased temperature requirements for germination towards colder climates, indicating a cold-avoidance germination strategy conditional on the temperature at the seeds' origin. Along a gradient of increasing temperatures this suggests a shift in selection pressures on germination from climatic adversity (i.e. low temperatures and potential frost risk in early or late season) to competitive performance and better exploitation of the entire growing season.

Key words: *Calluna vulgaris*, germination, temperature, frost, conditional, cold avoidance, incubation, gradient, heathland, cold stratification.

INTRODUCTION

Seedlings are more sensitive to severe environmental conditions than both adult plants and seeds of the same species (Marcante *et al.*, 2012), rendering germination a risky one-way transition in the plant life cycle (Daws *et al.*, 2007). Consequently, strong selection pressures act on germination responses, resulting in a range of strategies to time germination appropriately to places or times that are suitable for seedling survival and onward growth. A range of environmental conditions tell seeds whether or not they are in a place suitable for germination, such as light conditions (Pons, 1989; Bell *et al.*, 1995), smoke after burning (Brown *et al.*, 2003; Måren *et al.*, 2010) and moisture (Perez-Fernandez *et al.*, 2000); timing strategies for germination are often cued by temperature regime (Grime *et al.*, 1981; Cavieres and Arroyo, 2000) and cold stratification (Meyer *et al.*, 1989; Meyer and Monsen, 1991; Cavieres and Arroyo, 2001). Variation in germination behaviour response to such cues has been documented both between (Grime *et al.*, 1981;

Daws *et al.*, 2002) and within species (Billings and Mooney, 1968; Meyer and Monsen, 1991; Philippi, 1993; Schütz and Milberg, 1997; Cavieres and Arroyo, 2001).

It is often argued that life under severe and unfavourable climatic conditions will select for increased environmental tolerance in local populations. Accordingly, Bevington (1986) using incubation temperatures of both 14 and 18 °C, and Benowicz *et al.* (2001) using ambient temperatures between 20 and 30 °C found generally higher and faster germination for seeds of paper birch from populations with colder climates. Mondoni *et al.* (2008) showed that seeds of *Anemone nemorosa* from cool locations display increased germination under incubation at 4 °C when compared with seeds from populations at warmer sites. In addition, Hawkins *et al.* (2003) reported increased frost resistance in seedlings from cold-climate populations for *Picea glauca × engelmannii* and *Thuja plicata*. Such germination strategies may confer a selective advantage by enabling earlier germination and thus more effective exploitation of a relatively short growth season. Alternatively, a high risk of recruitment failure under

adverse climates may pose strong selective pressure for timing germination to periods of optimal survival probability, while avoiding potentially severe periods, which would result in a cold-avoidance strategy, i.e. higher temperature requirements for germination in populations from cold climates. While this has been reported at the between-species scale, when comparing cold-climate with warm-climate species (Chabot and Billings, 1972; Grime *et al.*, 1981; Baskin and Baskin, 1998), it is rarely documented in studies of within-species variation in germination strategies (but see Meyer *et al.*, 1989; Meyer and Monsen, 1991; Cavieres and Arroyo, 2000).

In this study, we investigate germination behaviour along climatic gradients in the monotypic species *Calluna vulgaris* (Fig. 1). *Calluna* is the keystone species of Europe's heathland systems (Gimingham, 1960; Vandvik *et al.*, 2005) and occurs throughout a broad geographical and climatic range, being found along Europe's western coast from the Strait of Gibraltar to northern Norway, from sea level into the alpine zone (Pyrenees, Alps, Scottish Highlands and Scandinavian Mountains) and even in continental Western Russia (Beijerinck, 1940; Gimingham, 1960). *Calluna* has been tested for germination responses both at different thermal regimes (Thomas and Davies, 2002) and along climatic gradients (Lavorel, 1987; Miller and Cummins, 1987; Vera, 1997; Miller and Cummins, 2001), but never for both simultaneously. Thermal regime studies have found that germination around 20 °C is generally high (Gimingham, 1960; Thomas and Davies, 2002), but is reduced by 50 % when approaching 10 or 28 °C, respectively (Grime *et al.*, 1981). In previous gradient studies, seeds were germinated at or close to this reported optimum germination temperature of 20 °C. When combined, these gradient studies cover a substantial part of the species' North–South distribution but they do not provide consistent results: germination was reported to decrease with altitude in Scotland (Miller and Cummins, 2001), whereas Vera (1997) and Lavorel (1987) found it to increase with altitude in Northern Spain and Southern France, respectively. It remains unknown whether the reduction in germination towards sub-optimal incubation temperatures as indicated in the thermal regime studies varies systematically along climatic gradients occurring within the species' range, i.e. if there are potentially adaptive germination responses.

We assess germination responses of *Calluna* seeds to a range of incubation temperatures. The seeds were sampled along two geographical gradients in Norway that cover comparable ranges in temperature conditions: (1) latitudinal along the coast from the temperate south to the sub-arctic north; and (2) altitudinal from the temperate coast into the alpine. Using two gradients rather than one breaks up the space–environment correlation and helps avoid confounding factors arising from co-variation between temperature and other climatic attributes, geographical attributes and historical land use, and should provide a robust picture of whether any emerging patterns are general or local phenomena. As any systematic variation in germination response among populations could also be due to variation in seed mass (Baskin and Baskin, 1998), which in itself may be related to climate (Moles and Westoby, 2003), we also assessed variation in seed mass and any impact on germination.

Based on Thomas and Davies (2002) and Grime *et al.* (1981), we expect the germination of *Calluna* seeds to decrease with falling incubation temperature and we specifically ask whether this pattern is (1) consistent among the populations sampled; or (2) varying systematically along climatic gradients in ways indicating either cold tolerance or cold avoidance; and (3) to what extent any climate effect acts independently of seed mass.

Fig. 1. Images of flowering *Calluna vulgaris* (top) and close-ups of an inflorescence (lower, left), a seed (lower, middle) and a seedling (lower, right).

MATERIALS AND METHODS

Sample sites and sampling

Calluna seed material was collected between 20 October and 4 November 2007 from 11 heathland sites ($i = 1, \ldots, m$) along two major geographical and climatic gradients in Norway (Fig. 2, Table 1). The altitudinal gradient (1–1000 m a.s.l.) comprised the sites of Lygra, Gulbotn, Kvamskogen, Vøringsfossen and Ustaoset, and the latitudinal gradient (58°4′N to 69°38′N) comprised the coastal sites Lista, Fedje, Møre, Smøla, Bodø and Tromsø.
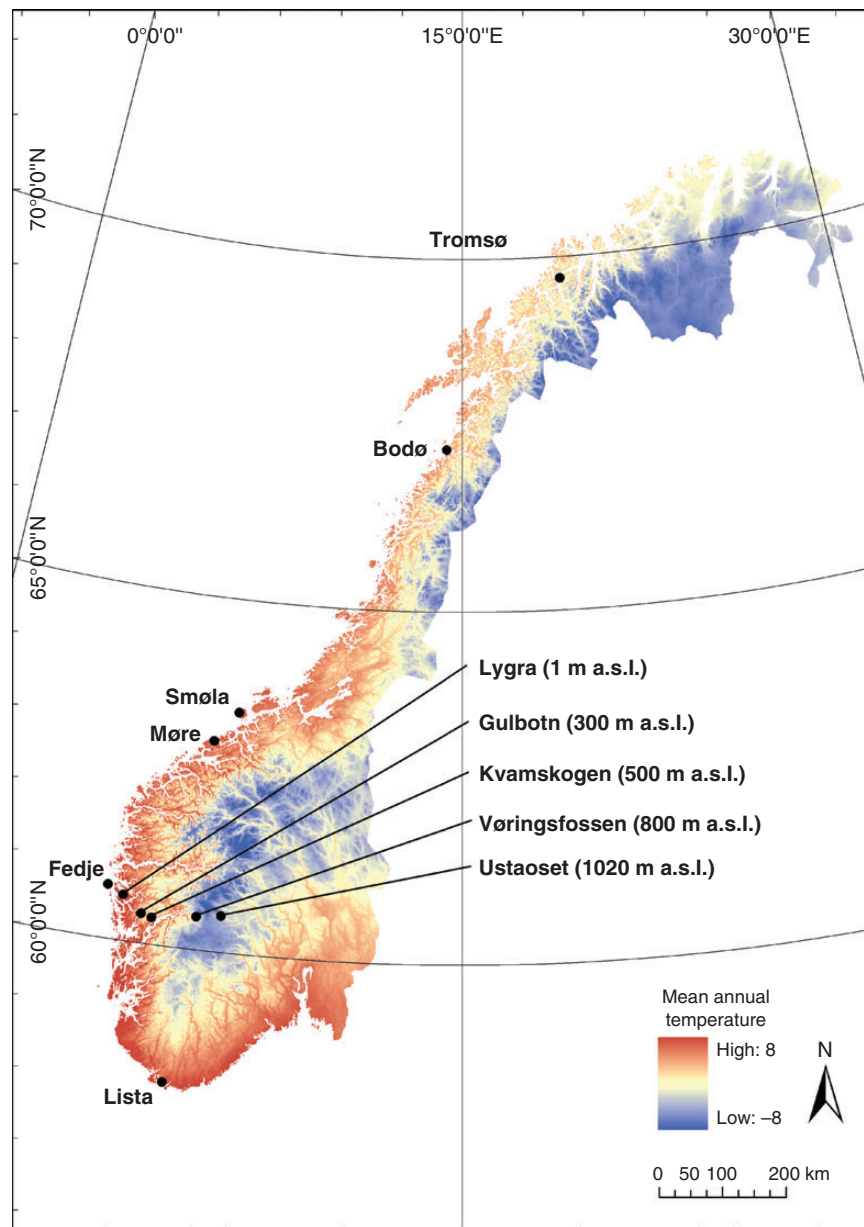
FIG. 2. Map of Norway indicating mean annual temperature and the sampled populations as well as the geographical extent of the sampled gradients. Source: Norwegian Meteorological Institute (www.met.no).

Infructescences of 15 individual plants, spatially separated by at least 15 m, were harvested and thereafter dried at 20 °C for 2 d before the seeds were shaken out and separated from the remaining debris. Unfortunately, the samples from the Vøringsfossen site (800 m a.s.l.) did not contain any intact seeds due to arthropod damage. The seeds from the other ten sites were stored in paper bags at 15 % relative humidity and 15 °C before being used in the germination experiments 5 months later. Generally, low humidity during seed storage should minimize the rate of any after-ripening (Baskin and Baskin, 1979) and, according to Grime *et al.* (1981), 6 months dry storage does not affect *Calluna* germinability. For each individual plant, three replicates of 20 seeds were weighed to the nearest 0.1 µg and used to calculate average seed mass of mother plants and populations.

Climate data

As a measure of the variation in 'climatic adversity' among the *Calluna* populations, we used the mean annual temperature of each population, calculated from monthly mean interpolations from the 'normal period' 1960–1990 (as defined by the World Meteorological Organization). It could be argued that it is seasonal temperatures, rather than annual means, that constitute the main limiting factors for plant recruitment and growth. In our study, however, correlations between mean annual temperature and seasonal means (spring, summer, autumn and winter) were very high ($r > 0.95$), and it was thus not possible to distinguish statistically between the effects of the different seasonal temperatures. Consequently, we chose to use the annual mean

TABLE 1. *Geographical and climatic characteristics of the sampled* Calluna vulgaris *populations*

Population	Longitude	Latitude	Altitude m a.s.l.	Temperature (°C)	Frost events (no. year^{-1})	Precipitation (mm)	Seed mass (mg seed^{-1})
Lista	6°48′E	58°4′N	1	7·3	13	1200	0·030
Fedje	4°49′E	60°47′N	3	7·2	9	1680	0·037
Møre	6°1′E	62°20′N	20	6·5	13	1770	0·033
Smøla	8°4′E	63°17′N	1	6·1	15	1290	0·036
Bodø	14°24′E	67°17′N	20	4·5	14	1040	0·032
Tromsø	18°55′E	69°38′N	1	2·7	13	1020	0·030
Lygra	5°5′E	60°42′N	1	7·3	9	1890	0·032
Gulbotn	5°44′E	60°26′N	300	4·9	14	2960	0·037
Kvamskogen	5°58′E	60°23′N	500	3·6	14	3010	0·031
Vøringsfossen	7°16′E	60°25′N	800	–	–	–	–
Ustaoset	8°2′E	60°29′N	1020	−0·6	10	750	0·020

Latitudinal gradient above the line and altitudinal gradient below. M a.s.l, metres above sea level; temperature, mean annual temperature; Frost events, mean annual number of frost events per year, no. year^{-1}, number of events per year, Precipitation, mean annual precipitation.

as a general measure of the climatic adversity of the sampled populations.

Daily minimum temperature records for the nearest accessible climate station (period 1950–2009) were used to calculate the occurrence and frequency of 'warm spell followed by frost' (events, from here on called 'frost events', which we defined as days with minimum temperature <0 °C after three consecutive days with minimum temperature >0 °C). The annual pattern of frost events seems closely connected to mean annual temperature within the sites of our populations (Fig. 1). The coldest sites are characterized by low frost event probability during winter as temperatures stay consistently below 0 °C and warm spells are rare. With increasing mean annual temperature, the risk of winter frost events also increases. In spring, the coldest sites reach peak frost event probability in April/May, the intermediate ones in April and the warmest ones in March. From the spring peak onwards, the risk of frost events then falls to zero or near-zero in the warmer sites first, resulting in an earlier and longer frost-free season than in the colder sites. In autumn, frost events start to occur from September/October onwards in the warmer sites but already from August in the coldest ones (for Ustaoset there even remains a tiny risk of frost in July). In the warmer sites, the autumn frost event probability stays high compared with in the colder sites where it gradually decreases with decreasing mean annual temperature.

All climate data were provided by the Norwegian Institute of Meteorology (met.no).

Seed germination

Seeds from ten individual plants per population/site ($j = 1, \ldots, n_i$) were germinated at four different temperatures (10, 15, 20 and 25 °C). All of the *Calluna* studies to which we relate our results have used constant incubation temperatures; therefore, we also used constant temperatures. For the Møre and the Kvamskogen populations, seeds of several individuals were either dead or eaten, resulting in only five and eight individuals from these populations, respectively. For each individual–treatment combination, three replicates ($k = 1, \ldots, n_{ij}$) of 22 seeds ($l = 1, \ldots, n_{ijk}$) were plated on approx. 5 mm deep 1 % water agar in Petri dishes (Sterilin 50 mm diameter, shallow Petri

dishes, single vent). For germination, the plates were kept in four independent incubators at the above-mentioned temperatures with 16 h of light and 8 h of darkness per day. Germination was defined as radicle emergence of ≥0·5 mm and was scored at least weekly for a maximum of 117 d. Both germinated and decomposing seeds were removed from the dishes.

In a preliminary germination trial, we assessed whether cold stratification was required for the main temperature experiment. *Calluna* seeds from five of the Norwegian populations in this study (Tromsø, Smøla, Fedje, Lista and Gulbotn; three replicate batches of 20 seeds from one individual per population) were imbibed at 5 °C for both 4 and 8 weeks prior to incubation at 20 °C. Neither of the stratification treatments showed a significant difference in germination after 2 months when compared with a control group incubated at 20 °C without any pretreatment (see the Results). The seeds used in the temperature experiment described above were therefore not cold stratified prior to the main germination trial.

Statistical analysis

For the analysis of germination, we used generalized additive mixed models, hereafter referred to as 'GAMM' (Woods, 2004; Pinheiro *et al.*, 2010). These models consist of a fixed effects part and a nested random contribution. By accounting for the nested sampling structure in the random effects part, these models handle dependencies provided by pseudo-replication as a potential problem in this study.

The effects of mean annual temperature and seed mass on germination along time in the ten populations across incubation treatments were assessed by models with the following structure:

$$\text{Logit(germination}_{ijkl}) = \beta_0 + f(\text{time}_{ijk} \times \text{incubation}$$

$$\text{treatment}_{ijk} \times \text{variable}_{ijk}) + b_{0i} + b_{0ij} + b_{0ijk}$$

were β and $f(.)$ are fixed effects to be estimated for interpretation, time is number of days since the incubator experiment was initiated and the bs are nested random contributions specific for replicates of individuals from populations. The latter was implemented here to allow the experimental design to influence the estimation of the fixed effects. The response variable

'germination' is recorded as the number of germinated seeds (success) until that time; hence we assume a binomial distribution with number of seeds in total per replicate as trials. Non-significant fixed effects parameters were removed in a step-wise backward selection if possible. The output statistics of this type of model provide (1) effects on intercepts, which translates into effects on the onset of germination in a seed lot; and (2) effects on curve slopes, which translates into effects on a seed lot's germination rate. The germination rate reflects the uniformity of a seed lot's germination in time. In the seed mass model, all interactions between seed mass and incubation treatment were not significant and hence six parameters could be removed from the model. However, we included the two interaction variables 'seed mass × mean annual temperature' and 'time × seed mass × mean annual temperature' (with the obligatory main factor 'mean annual temperature' giving three more model parameters) in a forward selection process to evaluate the dependency of the seed mass effect on climate.

For the analysis of final germination percentages across population temperature and incubation treatments, we used the same model structure but without the time parameter in the fixed effects. The same accounts for the analysis of the effect of individual seed mass on final germination percentages; we again included mean annual temperature as a covariate but we had to use one model per incubation treatment instead of having incubation treatment as a fixed effect in order to promote model convergence. Final germination percentages were obtained at day 92 after sowing for the 25 °C incubation treatment, day 95 for the 15 and 20 °C incubation treatments, and day 96 for the 10 °C incubation treatment. By these times, germination had ceased in all but the 10 °C incubation treatment.

For the analysis of final germination percentages in the preliminary stratification screening we used a generalized linear mixed effects model (GLMM; Bates *et al.*, 2012) since the GAMM described above would not converge with this smaller data set. Like GAMM, this model consists of a fixed effects part and a random contribution where pre-treatment (4 weeks and 8 weeks of stratification and control) was specified as the fixed effect and 'replicate nested within population' as the random effect.

A jackknife cross-validation procedure on the replicate level was used to assess the relative performance of the germination models of the two gradients with an overall model on all populations; here we used a log-likelihood as summary statistics. The log-likelihood values were compared by paired Wilcoxon rank sum tests to identify the model with the highest predictive performance. The correlation between mean annual temperature and seed mass was assessed by non-parametric Spearman's rank correlation tests as not all variables were normally distributed. The assumption of normality of errors was tested by the Shapiro–Wilk test. All analyses were performed in R 2.12.2. (R Development Core Team, 2010).

RESULTS

Germination responses: incubation temperature vs. climatic severity gradient

Calluna germination performance decreased with falling incubation temperature, showing a delayed onset and a lower rate of germination in the coldest incubation treatment (Table 2). Further, this pattern varied along the climatic severity gradients as indicated by significant interactions between mean annual temperature and incubation treatment on both intercept and slope. The positive effects of increasing incubation temperature on the intercept were significantly reduced with increasing mean annual temperature; for the positive effects on the slope, however, this was only the case for the 20 °C incubation treatment (Table 2). In total, incubation temperature has progressively weaker effects on germination dynamics as the home climate gets warmer, and seeds from colder climates displayed far greater differences in germination timing between the incubation treatments than seeds from populations with warmer climates (Fig. 4). Likewise, final germination percentages were positively affected by warmer incubation treatments, but the effect was most distinct at the cold end of the climatic gradient while showing progressively smaller differences in the warmer populations (Fig. 5).

Germination responded consistently to variation in incubation treatments and mean annual temperature along the two gradients (not shown). A comparison of log-likelihood values obtained for the statistical model on all the ten populations with log-likelihood values obtained for the two gradients separately gave no significant difference (*P*-value = 0·7, paired Wilcoxon rank sum tests). Hence, the separation of the two gradients did not yield higher predictive performance and we chose to present one model based on all the ten *Calluna* populations.

Cold stratification did not influence germination of *Calluna* seeds significantly (Table 3). Mean germination after 4 and 8 weeks of pre-chilling treatment was lower than in the control, but was associated with a considerable standard deviation.

Effects of seed mass

The average seed mass in each population ranged between 0·020 and 0·037 mg seed^{-1} (Table 1). Mean annual temperature

TABLE 2. *Fixed effect coefficients for the GAMM model on germination timing in the four incubation treatments along the mean annual temperature (m.a.t.) gradient*

	Intercept	Δintercept (m.a.t.)	Slope (time)	Δslope (time:m.a.t.)
10 °C incubation	**−6·12*****	*0·32****	**0·035*****	*0·0017****
Δ15 °C incubation	*3·07****	−0·20***	*0·014****	0·0026***
Δ20 °C incubation	*2·40****	−0·13**	*0·029****	−0·0022***
Δ25 °C incubation	*2·52****	−0·24***	*0·023****	−0·0003^{NS}

The two values in **bold** indicate the model's main effects (intercept and slope) for the 10 °C incubation treatment, whilst the values in *italic* show effect differences (Δ) from the **bold** values due to increasing m.a.t. or incubation temperature. The remaining values (interactions between incubation temperature and m.a.t.) show effect differences (Δ) from the additive effects of the *italic* value above and the value directly to the left of the cell in question; for example, under Δintercept, −0·13 relates to the values 0·32 and 2·40. *n* for each incubation treatment: 279 replicates within 93 individuals within ten populations.

Asterisks indicate level of statistical significance: ***P < 0·001, **P < 0·01, *P < 0·05, NS, *P* > 0·05.
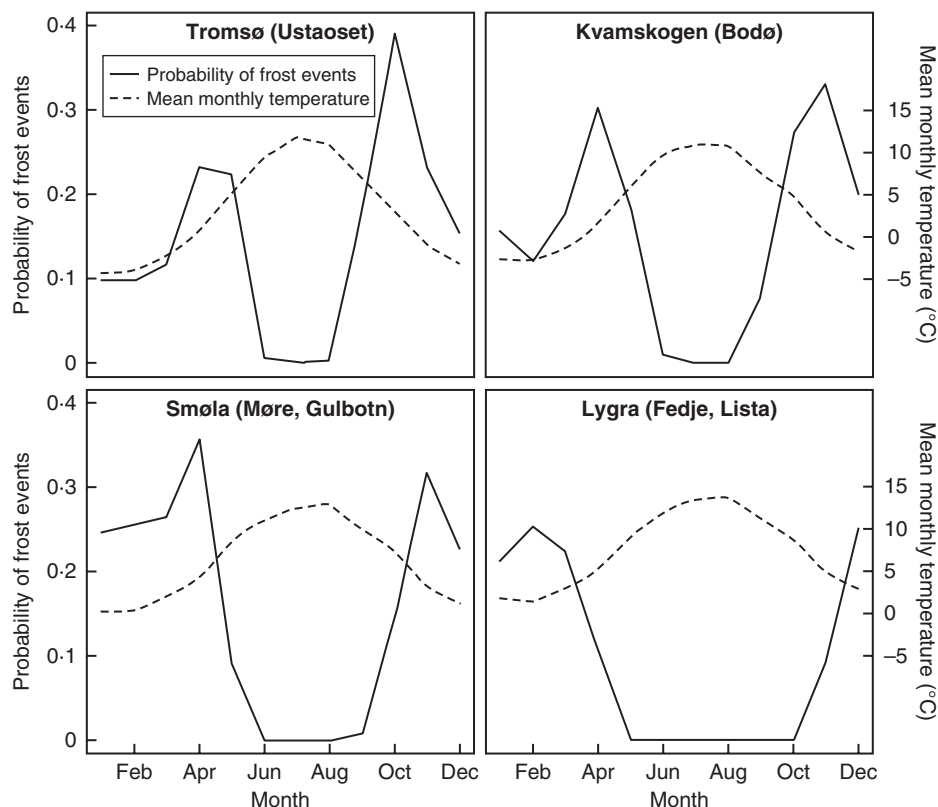
Fɪɢ. 3. Annual cycle of probability of frost events (solid lines) and mean monthly temperature (dashed lines) for the ten sites in this study. Displayed are the data from four sites experiencing progressively higher mean annual temperatures (from the cold Tromsø to the warm Lygra). The sites that show a similar annual pattern are indicated in parentheses.

and seed mass were not significantly correlated ($r_S = 0.5$, $P = 0.13$, Spearman's rank correlation test).

Seed mass had a significant effect on germination dynamics and final germination percentages; while it did not show any interactions with incubation treatment, the seed mass effects on germination dynamics were significantly related to mean annual temperature. The onset of germination was significantly later for heavier seeds (seed mass main effect on intercept = -41.56, $P = 0.017$); however, they then germinated at a higher rate (seed mass main effect on slope = 0.43, $P < 0.01$). Mean annual temperature had a positive effect on both the seed mass effect on the intercept (6.36, $P < 0.01$, i.e. reducing the seed mass-mediated delay with increasing mean annual temperature) and the seed mass effect on the slope (0.03, $P < 0.01$, i.e. increasing the seed mass-mediated germination rates with increasing mean annual temperature). Since the interaction parameters between seed mass and incubation treatment were not significant and hence removed from the model, this slope effect was consistent across all incubation treatments. Final germination percentages were positively affected by seed mass at 20 and 25 °C incubation temperature (the 10 and 15 °C treatments showed similar, but non-significant patterns; results not shown). However, the interaction term 'seed mass × mean annual temperature' was not found to be significant for any of the four incubation treatments and hence mean annual temperature did not alter the effect of seed mass on final germination percentages.

DISCUSSION

For plant species living under severe climatic conditions, seed germination can evolve either to tolerate the environmental stress conferred by the local environment or to be timed to periods of relatively low risk in terms of seedling exposure to potentially lethal conditions. Seedlings are generally considered to be very susceptible to both chilling and frost (Larcher, 2003; Neuner *et al.*, 2008; Marcante *et al.*, 2012), but several plant species have also been shown to develop seedlings with a certain tolerance towards cold (Baruah *et al.*, 2009) or frost (Aitken and Hannerz, 2001; Hawkins *et al.*, 2003). In our study, cool incubation temperatures led to later onset, lower rate and lower final percentage of germination, in accordance with previous findings (Grime *et al.*, 1981; Thomas and Davies, 2002), which indicates a general strategy of cold avoidance for germination. More specifically, our results show that this cold avoidance varies among populations (question 1) being systematically more pronounced in seed lots from cold climates (question 2). The cold avoidance germination strategy in *Calluna* should hence be considered 'conditional' on the climatic severity of the home site. Similar records of conditional cold avoidance within species are rare, but have been described for other widely distributed species such as *Chrysothamnus nauseosus* (Meyer *et al.*, 1989), *Artemisia tridentata* (Meyer and Monsen, 1991) and *Phacelia secunda* (Cavieres and Arroyo, 2000) in the Americas.
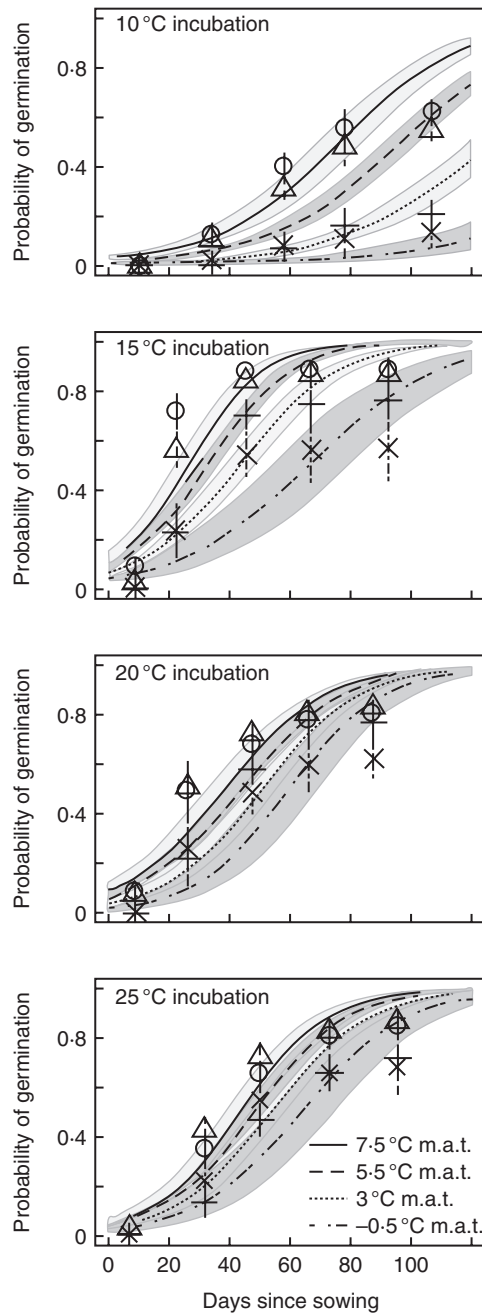
FIG. 4. GAMM model predictions of germination in *Calluna vulgaris* across time for four incubation temperatures and four levels of mean annual temperature (m.a.t.). Shaded areas constitute 95 % confidence intervals. Empirical probabilities ($\pm$ 2 s.e.) as means of the nearest populations are indicated by different symbols: circle, Lista, Lygra and Fedje for 7·5 °C; triangle, Møre, Smøla, Gulbotn and Bodø for 5·5 °C; plus sign, Kvamskogen and Tromsø for 3 °C; cross, Ustaoset for −0·5 °C.
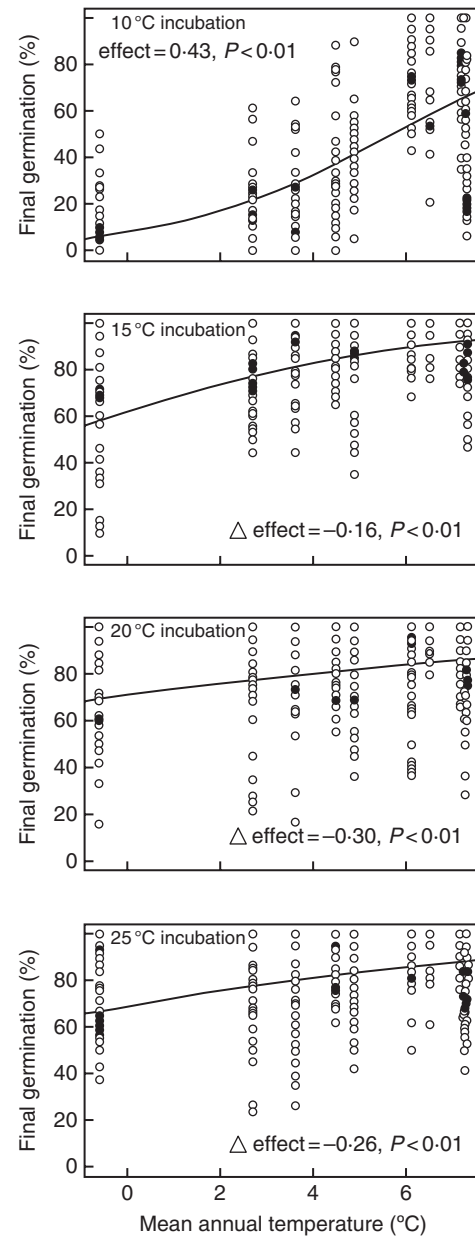

FIG. 5. Final germination rates of *Calluna vulgaris* in relation to mean annual temperature for each of four incubation temperatures. Lines represent the respective GAMM model. The *P*-value in the 10 °C plot indicates a significant temperature effect. *P*-values in the 15, 20 and 25 °C plots indicate significant differences in the temperature effect (Δeffect) compared with the 10 °C plot.

TABLE 3. *Mean germination and standard deviation (s.d.) after a germination period of 2 months in the preliminary stratification screening*

Stratification treatment	Germination mean (%)	$\pm$ s.d.	*P*-value
Control	75	16	
5° C for 4 weeks	65	26	0·63
5 °C for 8 weeks	57	19	0·09

P-values indicate the significance of the difference from the control group. *n* for all treatments: 15 replicates within five populations.

In many other species, cold-stratification germination requirements ensure that germination does not occur until after the winter season (Baskin and Baskin, 1998; Fenner and Thompson, 2005). Although Grimstad (1985) and Miller and Cummins (2001) report a slight positive effect of cold stratification on *Calluna* germinability, we found no such response, supporting Gimingham (1960) who stated that *Calluna* in the British

Isles germinates both in autumn, directly after seed set, and in spring. The conditional cold-avoidance strategy found in the Scandinavian *Calluna* populations appears to be an alternative strategy to achieve the same risk reduction outcome as cold stratification. Thus, by delaying germination at low autumn and early spring temperatures, seedling recruitment will be concentrated in the summer season, particularly for the populations from the coldest locations. Reduced autumn germination would enable seedlings to avoid the hazards imposed by long cold winters in the northern and alpine regions, as compared with warmer climates where *Calluna* seedling survival over the winter can be high (Gimingham, 1960).

Risk assessments of warm spells and spring frosts are available for many regions (Laughlin and Kalma, 1987; Avissar and Mahrer, 1988; Tait and Zheng, 2003; Rahimi *et al.*, 2007; Kaukoranta *et al.*, 2010) and, while all our study sites are prone to frost events, their seasonal pattern of occurrence varies among populations (Fig. 3). Our warmer sites are characterized by an earlier and more rapid decrease in frost event probability during spring, and consequently a longer frost event-free period during the growth season. Here, germinating later in the season may expose seedlings to competitive effects from already dense vegetation cover in late spring/early summer. Conversely, at the severe end of the environmental distribution (here, the cold North and the mountains), a cold-avoidance strategy would protect *Calluna* seedlings from the high frost risk in early spring, and this could outweigh the disadvantage conferred by higher competition and shorter growing season. In the arctic and alpine, vegetation cover is generally less dense, even later in the growing season, reducing the disadvantage imposed by late germination.

Fenner and Thompson (2005) describe a shift from cold (in the North) to drought (in the South) as the key limiting factor for germination and seedling survival across Europe. *Calluna* records from Spain (Vera, 1997) and Southern France (Lavorel, 1987), where periods of drought may be more common than in Scandinavia, do indeed show progressively higher germination success at higher elevations, indicating that drought might impose a greater threat to *Calluna* seedlings in these regions than cold temperatures and frost. In contrast, in Norway, we find faster and higher germination towards warmer climates, which we, in the absence of drought along our climate gradients, attribute to a shift from temperature to competition as the main limiting factor for seedling survival. This may explain why Miller and Cummins (2001), seemingly in contrast to the previous findings, found slightly decreased germination (germination trial conducted at $20\,^{\circ}\text{C}$) for seeds from high altitudes in Scotland, as this region resembles Southern Norway in terms of climate.

Seed mass had a significant positive impact on germination in all of the Norwegian *Calluna* populations tested here, which is in line with a range of species (Baskin and Baskin, 1998). Heavier seed lots germinated later but, once germination commenced, these seed lots germinated faster than lighter ones. Heavier seeds might simply need more time to imbibe and therefore show a delayed germination onset (Norden *et al.*, 2009), whereas lighter seed lots might potentially contain some seeds too small to germinate at all, which would bias the germination speed of the seed lot downwards. Variability in seed mass itself may be related to the pre-conditioning environment in which the maternal plants lived (Baskin and Baskin, 1998; Moles and Westoby, 2003). In our study, seed mass was not related to climate but we found a significant positive interaction between mean annual temperature and seed mass promoting both the germination onset and speed of heavier seed lots from warm populations. In a warmer climate, embryo development may benefit from higher nitrogen availability (Blödner *et al.*, 2007), and a more even seed development may result in both earlier and more uniform germination. However, seed mass did not show (1) any significant interactions with incubation temperature while it did with mean annual temperature, nor (2) any significant interactions with mean annual temperature in the analyses of final germination percentages, while (3) the interaction effects found in the germination dynamics model were very small compared wuith the main effects on intercept and slope. Therefore, we conclude that the climate-related germination patterns found here are largely independent of seed mass (question 3). Nonetheless, since a range of seed traits other than seed mass can also be affected by the maternal environment (Baskin and Baskin, 1998; Schmuths *et al.*, 2006; Blödner *et al.*, 2007; Hoyle *et al.*, 2008), our study cannot unequivocally address whether the patterns in germination behaviour in *Calluna* are based on genetic, long-term adaptations to climate or plastic responses to the annual weather conditions experienced by the mother plant. To distinguish between plastic and genetic responses would require second-generation seeds from populations that have been grown under common conditions (Wagner and Simons, 2008).

Alongside seed germination, vegetative regeneration has also been reported to play an important role for the sustaining and spreading of *Calluna* populations. From Spain to South-Western Norway (Mallik and Gimingham, 1983; Forgeard, 1990; Calvo *et al.*, 2002) *Calluna* shrubs frequently re-sprout after fire, but, further north, vegetative recruitment becomes rare, leaving seed germination as the only means of regeneration (Nilsen *et al.*, 2005). This reduction in ability to regenerate vegetatively might impose an even stronger selective pressure on seedling survival and hence germination timing in the North (and possibly in the mountains).

Our finding of a conditional cold-avoidance strategy for *Calluna* germination together with previous records from Scotland, France and Spain support a theory of gradual replacement of cold as the main hazard for seedlings as we move south in Europe by first competition and then, further south, possibly drought, that explains varying germination patterns in relation to temperature (Fenner and Thompson, 2005). Our main results suggest that *Calluna* in Northern Europe generally avoids hazards imposed by cold climates by cueing germination towards the relatively warm frost-free late spring to early summer season. In populations from less adverse climates, however, the species' cold-avoidance strategy seems to be weakened in favour of earlier germination, which would allow the species to address other limitations of, for example, light and space as a consequence of higher competition under warmer climates.

comments on the manuscript, and Eric Meineri was an indispensable source of discussion and mapping expertise. We thank two anonymous referees for their constructive and helpful feedback on this manuscript. This work was supported by the Olaf Grolle Olsens Legat and the Faculty of Mathematics and Natural Sciences at the University of Bergen, Norway.

LITERATURE CITED

Aitken SN, Hannerz M. 2001. Genecology and gene resource management strategies for conifer cold hardiness. In: Bigras FJ, Colombo SJ. eds. *Conifer cold hardiness.* Dordrecht, The Netherlands: Kluwer Academic Publishers, 23–53.

Avissar R, Mahrer Y. 1988. Mapping frost-sensitive areas with a 3-dimensional local-scale numerical model. 2. Comparison with observations. *Journal of Applied Meteorology* **27**: 414–426.

Baruah AR, Ishigo-Oka N, Adachi M, *et al*. 2009. Cold tolerance at the early growth stage in wild and cultivated rice. *Euphytica* **165**: 459–470.

Baskin CC, Baskin JM. 1979. Effect of relative humidity on after-ripening and viability in seeds of the winter annual *Draba verna*. *Botanical Gazzette* **47**: 475–517.

Baskin CC, Baskin JM. 1998. *Seeds. Ecology, biogeography, and evolution of dormancy and germination*. London: Academic Press.

Bates D, Maechler M, Bolker B. 2012. *lme4: linear mixed-effects models using S4 classes*. R package version 0.999999-0. http://lme4.r-forge.r-project.org/.

Beijerinck W. 1940. *Calluna*: a monograph on the Scotch heather. *Verhandelingen der Koninklijke Akademie van Wetenschappen (3rd Section)* **38**: 1–80.

Bell DT, Rokich DP, McChesney CJ, Plummer JA. 1995. Effects of temperature, light and gibberellic acid on the germination of seeds of 43 species native to Western Australia. *Journal of Vegetation Science* **6**: 797–806.

Benowicz A, Guy R, Carlson MR, El-Kassaby YA. 2001. Genetic variation among paper birch (*Betula papyrifera* MARSH.) populations in germination, frost hardiness, gas exchange and growth. *Silvae Genetica* **50**: 7–13.

Bevington J. 1986. Geographic differences in the seed germination of paper birch (*Betula papyrifera*). *American Journal of Botany* **73**: 564–573.

Billings WD, Mooney HA. 1968. The ecology of arctic and alpine plants. *Biological Reviews* **43**: 481–529.

Blödner C, Goebel C, Feussner I, Gatz C, Polle A. 2007. Warm and cold parental reproductive environments affect seed properties, fitness, and cold responsiveness in *Arabidopsis thaliana* progenies. *Plant, Cell and Environment* **30**: 165–175.

Brown N, van Staden J, Daws M, Johnson T. 2003. Patterns in the seed germination response to smoke in plants from the Cape Floristic Region, South Africa. *South African Journal of Botany* **69**: 514–525.

Calvo L, Tarrega R, Luis E. 2002. Regeneration patterns in a *Calluna vulgaris* heathland in the Cantabrian mountains (NW Spain): effects of burning, cutting and ploughing. *Acta Oecologica-International Journal of Ecology* **23**: 81–90.

Cavieres LA, Arroyo MTK. 2000. Seed germination response to cold stratification period and thermal regime in *Phacelia secunda* (Hydrophyllaceae) – altitudinal variation in the mediterranean Andes of central Chile. *Plant Ecology* **149**: 1–8.

Cavieres LA, Arroyo MTK. 2001. Persistent soil seed banks in *Phacelia secunda* (Hydrophyllaceae): experimental detection of variation along an altitudinal gradient in the Andes of central Chile (33 degrees S). *Journal of Ecology* **89**: 31–39.

Chabot BF, Billings WD. 1972. Origins and ecology of the Sierran alpine flora and vegetation. *Ecological Monographs* **42**: 163–199.

Daws MI, Burslem D, Crabtree LM, Kirkman P, Mullins CE, Dalling JW. 2002. Differences in seed germination responses may promote coexistence of four sympatric *Piper* species. *Functional Ecology* **16**: 258–267.

Daws MI, Bolton S, Burslem D, Garwood NC, Mullins CE. 2007. Loss of desiccation tolerance during germination in neo-tropical pioneer seeds: implications for seed mortality and germination characteristics. *Seed Science Research* **17**: 273–281.

Fenner M, Thompson K. 2005. *The ecology of seeds*. Cambridge: Cambridge University Press.

Forgeard F. 1990. Development, growth and species richness on Brittany heathlands after fire. *Acta Oecologica-International Journal of Ecology* **11**: 191–213.

Gimingham CH. 1960. Calluna *Salisb*. *Journal of Ecology* **48**: 455–483.

Grime JP, Mason G, Curtis AV, *et al*. 1981. A comparative study of germination characteristics in a local flora. *Journal of Ecology* **69**: 1017–1059.

Grimstad SO. 1985. Effect of gibberellic acid, temperature and storage on seed germination of heather (*Calluna vulgaris* L. Hull). *Agricultural University Norway, Meldinger fra Norges Landbrukshogskole* **64**: 11.

Hawkins BJ, Guest HJ, Kolotelo D. 2003. Freezing tolerance of conifer seeds and germinants. *Tree Physiology* **23**: 1237–1246.

Hoyle GL, Steadman KJ, Daws MI, Adkins SW. 2008. Pre- and post-harvest influences on seed dormancy status of an Australian Goodeniaceae species, *Goodenia fascicularis*. *Annals of Botany* **102**: 93–101.

Kaukoranta T, Tahvonen R, Ylamaki A. 2010. Climatic potential and risks for apple growing by 2040. *Agricultural and Food Science* **19**: 144–159.

Larcher W. 2003. *Physiological plant ecology*. Berlin, Springer.

Laughlin GP, Kalma JD. 1987. Frost hazard assessment from local weather and terrain data. *Agricultural and Forest Meteorology* **40**: 1–16.

Lavorel S. 1987. *Etude de la plasticite phenotypique chez* Calluna vulgaris *(L.) Hull*. Diplôme d'Etudes Approfondies en Sciences de l'Evolution et Ecologie, Université des Sciences et Techniques du Languedoc, Montpellier, France.

Mallik AU, Gimingham CH. 1983. Regeneration of heathland plants following burning. *Vegetatio* **53**: 45–58.

Marcante S, Sierra-Almeida A, Spindelböck J, Erschbamer B, Neuner G. 2012. Frost as a limiting factor for recruitment and establishment of early development stages in an alpine glacier foreland? *Journal of Vegetation Science* **23**: 858–868.

Måren IE, Janovsky Z, Spindelböck JP, Daws MI, Kaland PE, Vandvik V. 2010. Prescribed burning of northern heathlands: *Calluna vulgaris* germination cues and seed-bank dynamics. *Plant Ecology* **207**: 245–256.

Meyer SE, McArthur ED, Jorgensen GL. 1989. Variation in germination response to temperature in rubber rabbitbrush (*Chrysothamnus nauseosus*, Asteraceae) and its ecological implications. *American Journal of Botany* **76**: 981–991.

Meyer SE, Monsen SB. 1991. Habitat-correlated variation in mountain big sagebrush (*Artemisia Tridentata* ssp *vaseyana*) seed-germination patterns. *Ecology* **72**: 739–742.

Miller GR, Cummins RP. 1987. Role of buried viable seeds in the recolonization of disturbed ground by heather (*Calluna vulgaris* L. Hull) in the Cairngorm mountains, Scotland, UK. *Arctic and Alpine Research* **19**: 396–401.

Miller GR, Cummins RP. 2001. Geographic variation in seed-setting by heather (*Calluna vulgaris* (L.) Hull) in the Scottish Highlands. *Journal of Biogeography* **28**: 1023–1031.

Moles AT, Westoby M. 2003. Latitude, seed predation and seed mass. *Journal of Biogeography* **30**: 105–128.

Mondoni A, Probert R, Rossi G, Hay F, Bonomi C. 2008. Habitat-correlated seed germination behaviour in populations of wood anemone (*Anemone nemorosa* L.) from northern Italy. *Seed Science Research* **18**: 213–222.

Neuner G, Hacker J, Spindelböck J, Sierra-Almeida A. 2008. Frost resistance of glacier foreland species during germination and seedling establishment. *Berichte des naturwissenschaftlichen-medizinischen Vereins in Innsbruck* **Suppl. 18**: 24.

Nilsen LS, Johansen L, Velle LG. 2005. Early stages of *Calluna vulgaris* regeneration after burning of coastal heath in central Norway. *Applied Vegetation Science* **8**: 57–64.

Norden N, Daws MI, Antoine C, Gonzalez MA, Garwood NC, Chave J. 2009. The relationship between seed mass and mean time to germination for 1037 tree species across five tropical forests. *Functional Ecology* **23**: 203–210.

Perez-Fernandez MA, Lamont BB, Marwick AL, Lamont WG. 2000. Germination of seven exotic weeds and seven native species in south-western Australia under steady and fluctuating water supply. *Acta Oecologica-International Journal of Ecology* **21**: 323–336.

Philippi T. 1993. Bet-hedging germination of desert annuals – variation among populations and maternal effects in *Lepidium-Lasiocarpum*. *American Naturalist* **142**: 488–507.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team. 2010. *nlme: linear and nonlinear mixed effects models*. R package version 3.1-97. http://cran.r-project.org/web/packages/nlme/.

Pons TL. 1989. Dormancy and germination of *Calluna-vulgaris* (L) Hull and Erica-tetralix L seeds. *Acta Oecologica-Oecologia Plantarum,* **10**: 35–43.

Rahimi M, Hajjam S, Khalili A, Kamali GA, Stigter CJ. 2007. Risk analysis of first and last frost occurrences in the Central Alborz Region, Iran. *International Journal of Climatology* **27**: 349–356.

R Development Core Team. 2010. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org.

Schmuths H, Bachmann K, Weber WE, Horres R, Hoffmann MH. 2006. Effects of preconditioning and temperature during germination of 73 natural accessions of *Arabidopsis thaliana. Annals of Botany* **97**: 623–634.

Schütz W, Milberg P. 1997. Seed dormancy in *Carex canescens*: regional differences and ecological consequences. *Oikos* **78**: 420–428.

Tait A, Zheng XG. 2003. Mapping frost occurrence using satellite data. *Journal of Applied Meteorology* **42**: 193–203.

Thomas TH, Davies I. 2002. Responses of dormant heather (*Calluna vulgaris*) seeds to light, temperature, chemical and advancement treatments. *Plant Growth Regulation* **37**: 23–29.

Vandvik V, Heegaard E, Måren IE, Aarrestad PA. 2005. Managing heterogeneity: the importance of grazing and environmental variation on post-fire succession in heathlands. *Journal of Applied Ecology,* **42**: 139–149.

Vera ML. 1997. Effects of altitude and seed size on germination and seedling survival of heathland plants in North Spain. *Plant Ecology* **133**: 101–106.

Wagner I, Simons AM. 2008. Intraspecific divergence in seed germination traits between high- and low-latitude populations of the arctic–alpine annual *Koenigia islandica. Arctic Antarctic and Alpine Research* **40**: 233–239.

Woods SN. 2004. Stable and efficient multiple smoothing parameter estimation for generalized additive models. *Journal of the American Statistical Association* **99**: 673–686.