Environmental filtering drives the shape and breadth of the seed germination niche in coastal plant communities

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Abstract

Background and Aims A phylogenetic comparative analysis of the seed germination niche was conducted in coastal plant communities of western Europe. Two hypotheses were tested, that (a) the germination niche shape (i.e. the preference for a set of germination cues as opposed to another) would differ between beaches and cliffs to prevent seedling emergence in the less favourable season (winter and summer, respectively); and (b) the germination niche breadth (i.e. the amplitude of germination cues) would be narrower in the seawards communities, where environmental filtering is stronger.

Methods Seeds of 30 specialist species of coastal plant communities were collected in natural populations of northern Spain. Their germination was measured in six laboratory treatments based on field temperatures. Germination niche shape was estimated as the best germination temperature. Germination niche breadth was calculated using Pielou's evenness index. Differences between plant communities in their germination niche shape and breadth were tested using phylogenetic generalised least squares regression (PGLS).

Key Results Germination niche shape differed between communities, being warm-cued in beaches (best germination temperature = 20 ºC) and cold-cued in cliffs (14 ºC). Germination niche was narrowest in seawards beaches (Pielou's index = 0.89) and broadest in landwards beaches (0.99). Cliffs had an intermediate germination niche breadth (0.95). The relationship between niche and plant community had a positive phylogenetic signal for shape (Pagel's λ = 0.64) and a negative one for breadth (Pagel's λ = -1.71).

Conclusion Environmental filters shape the germination niche to prevent emergence in the season of highest threat for seedling establishment. The germination niche breadth is narrower in the communities with stronger environmental filters, but only in beaches. This study provides empirical support to a community-level generalization of the hypotheses about the environmental drivers of the germination niche. It highlights the role of germination traits in community assembly.

Keywords: Beach, cliff, community assembly, dune, environmental filters, germination ecology, maritime, PGLS, phylogenetic comparative methods, plant community specialists, regeneration, seed dormancy

Introduction

The seed germination niche is the range of environmental conditions in which a plant can complete a successful transition from seed to seedling (Grubb, 1977). Timing this critical transition allows plants to perform developmental niche construction, selecting the environments of subsequent sessile life stages (Donohue, 2014). Germination affects many aspects of plant biology including population dynamics (Huang et al., 2016), geographical distribution (Bykova et al., 2012) and climate change responses (Cochrane et al., 2015). Interest is now building around the incorporation of germination traits into vegetation science and especially community assembly studies (Jiménez-Alfaro et al., 2016). The community assembly framework proposes that the environment 'filters' certain species out of a regional species pool according to the functional traits they possess (Keddy, 1992). Germination traits need to

be accounted for, given that the filters for regeneration may differ from those for adult plant survival and growth (Grubb, 1977). A key step towards integrating germination traits into community assembly models requires to identify general rules that describe germination dynamics at the community level (Larson and Funk, 2016). One useful approach to do this is to assess variation in the germination niche across plant communities that are known to vary in their environmental filters.

The germination niche is constructed by a set of environmental germination cues that include temperature, moisture, light and chemical signals (Baskin and Baskin, 2014). For the purposes of this article we shall define the germination niche shape as the preference for some specific set of germination cues as opposed to another. The preference of many Mediterranean species for low germination temperatures

is an example of cold-cued niche shape (Thanos et al., 1989). We shall also define the germination niche breadth as the amplitude of germination cues to which the seed is responsive. Generalist bromeliads that germinate readily across different environments have a broad germination niche, while specialist bromeliads that respond only to a restricted set of cues have a narrow niche (Marques et al., 2014). Plant species can be thought of as having a maximum set or germination cues to which they are responsive (i.e. a maximum germination niche breadth). In practice their responsiveness may be regulated by physiological seed dormancy, an inner seed property that controls which conditions must be met to trigger germination (Vleeshouwers et al., 1995). Dormancy breaking cues gradually increase the sensitivity of the seed to its potential germination cues (Finch-Savage and Leubner-Metzger, 2006). Germination then occurs when a sensitive seed experiences these germination cues, which inform it of a current environment that is favourable for seedling establishment (Thompson and Ooi, 2013).

General ecological theory suggests two hypotheses about the relationship between the seed germination niche and the environment. A first hypothesis is that seasonally unfavourable environments favour germination niche shapes that prevent emergence during the season of highest seedling mortality (Baskin and Baskin, 2014). If different environments differ on when their seasons of seedling mortality are, then the cues that control germination timing should vary across these environments (Jurado and Flores, 2005). For example, plants from cold regions tend to have warm-cued germination to prevent emergence when frosts are more likely (Rosbakh and Poschlod, 2015). In regions with hot and dry summers, warm temperatures suppress germination to avoid emergence during periods of water stress (Mayfield et al., 2014). A second hypothesis is that strong environmental filters reduce the spread of functional trait values that are 'filtered in', reflecting shared strategies of stress tolerance (Cornwell et al., 2006). In terms of the germination niche this would imply having narrow niches that only respond to a restricted set of reliable cues, as this would make species more able to track favourable habitat conditions (Donohue et al., 2010).

Temperate maritime coasts are an adequate system to study whether these hypotheses apply at the community level. Their regional climate is relatively homogeneous, with cool summers and cool winters, infrequent frosts, and the absence of a marked dry season. At the same time, maritime coasts show a well-documented environmental zonation of plant communities. Coastal geomorphology creates a first zonation along the coast, with eroded cliffs alternating with beach deposits. On both cliffs and beaches, plants experience strong water stress due to the combined action of salt spray and the low retention capacity of the substrate (Rodwell, 1999, Ciccarelli et al., 2016). This should favour winter seedling emergence when temperatures are lower and

rainfall higher. Winter is however the season of maximum sand transport in temperate beaches (Davidson-Arnott, 2010). Sand transport threatens seedlings with physical damage and burial, posing a real challenge to plant establishment (Rodwell, 1999, Ciccarelli et al., 2012). This can be expected to favour warm-cued germination in beach plants, thereby preventing winter emergence.

A second zonation can be identified along a sea-inland gradient of environmental filtering, in which the effects of abiotic stresses such as salt spray and wave impacts gradually decrease as distance increases landwards (Rodwell, 1999). In cliffs these stresses affect more severely the rock-crevice vegetation of the cliff face, whereas more sheltered landwards positions allow the formation of some soil and the development of maritime grasslands (Rodwell, 1999). In beaches, the gradient is aggravated by the effect of substrate instability and sand transport, creating a zonation of shifting and fixed dunes (Kim and Yu, 2009). The sea-inland gradient is accompanied by a gradual change in plant community composition (Rodwell, 1999) and functional diversity (Ciccarelli, 2015). The stronger environmental filters that are found seawards should favour narrower germination niches in the cliff crevice and shifting dune communities.

In this article, we propose to test whether these predictions about the shape and breadth of the germination niche hold at the community level in the coastal habitat. To do so we have followed a phylogenetic comparative approach with specialist species of coastal plant communities. In this approach, species are used as samples to test whether the values of certain variables (the community to which they belong) can predict the values of other variables (their seed germination niche breadth and shape). Species are not independent samples as they are related by evolutionary history, so this approach considers their phylogeny. Using seeds of 30 species from the west European Atlantic seaboard, we have conducted a laboratory germination experiment based on field temperatures to characterise their germination niches. Subsequently, we have tested the predictions that (1) the germination niche shape is cold-cued in cliffs to prevent emergence at the time of maximum water stress (summer), and warm-cued in beaches to prevent seedling emergence at the time of maximum sand transport (winter); and (2) the germination niche is narrower in the seawards communities, where environmental filters are stronger.

Material and methods

Study area and species

The coastal plant communities studied here occur in the Atlantic seaboard of northern Spain (43º N, 5º W). Regional climate is temperate oceanic. Temperatures range from 13/6 ºC in January to 22/15 ºC in August. Annual precipitation amounts to 1060 mm, exceeding 100 mm per month from October to January, and being lowest in July with 50 mm (data recorded at the centre of

the study area, Asturias airport station, 850 m away from the sea and 130 m above; 1981-2010 averages provided by the Spanish Meteorology Agency). Along the sea-inland gradient, cliff communities are divided into (1) seawards crevice and bird colony vegetation, and (2) landwards maritime grasslands (Álvarez Arbesú, 2008). Beach communities are divided into (3) seawards strandline and embryonic/shifting dunes, and (4) landwards fixed dunes (Jiménez-Alfaro et al., 2015).

Thirty coastal plant species were included in this study (Table 1). At the local level, these species are specialists of one of the four coastal plant communities, being frequent in one of them and rare or absent in the others. Outside the coastal habitat, some of the species can also be frequent in other non-maritime communities. For the purposes of this study, each species was assigned a plant community (seawards cliffs, landwards cliffs, seawards beaches, landwards beaches) according to its peak occurrence in regional vegetation relevés (Rodwell, 1999, Álvarez Arbesú, 2008, Jiménez-Alfaro et al., 2015). To perform phylogenetically-informed statistical tests, a phylogenetic tree of the 30 species was created using Phylomatic v3 (Webb and Donoghue, 2005). Branch lengths were adjusted with the exponential node ages of Bell et al. (2010), using the ages file provided by Gastauer and Meira-Neto (2016). Undated nodes were positioned evenly in the tree using the 'bladj' function of Phylocom (Webb et al., 2008). The tree is supplied in Appendix S1 in Supporting Information.

Seed germination experiments

Dispersal units (hereafter 'seeds') from the 30 study species were collected from natural populations during the dispersal seasons (June to September) of 2012, 2013 and 2014 (Table 1). In each population, all individuals of the target species that were found bearing ripe seeds were sampled. After collection, seeds spent three weeks in the laboratory (21 ℃, 50 % RH) before seed cleaning and the start of the germination experiments. Because physical seed dormancy was expected in Calystegia soldanella (Ko et al., 2004) and Lavatera arborea (Santo et al., 2015), seeds of these two species were mechanically scarified before further manipulation.

Laboratory germination experiments followed a factorial design with two factors: germination temperature and cold stratification. Germination temperature had three levels: daily alternating regimes (12/12 h) of 14/4 ℃ (autumn - spring), 22/12 ℃ (summer), and 30/20 ℃ (unusual heat). The latter regime is rarely recorded in the air, but it can be reached inside shifting dunes during dry and sunny weather (Willis et al., 1959). Germination temperatures were programmed inside incubators (Grow-S 360, Ing. Climas, Barcelona, Spain) and coupled to a 12 h-light/12 h-darkness photoperiod (c. 20 μmol m-2 s-1 during the light phase, provided by six Philips TLD30W/54-765 cool fluorescent tubes). Cold stratification had two levels: non-stratified seeds, which were incubated at the three germination temperatures described above immediately after being sown in agar;

and cold-stratified seeds, which spent 12 weeks at 3 ℃ in darkness before incubation, to simulate overwintering. A colder-than-natural overwintering temperature was chosen to differentiate its effects from those of incubation at autumn/spring temperatures. The described combination of six experimental treatments was assumed to encompass a good sample of the thermal environments that the seeds may encounter in the field. For each of the six treatments, four replicate Petri dishes holding 1 % distilled water agar were prepared. As a standard, 25 seeds were sown in each Petri dish; but for some species with low seed availability, 15 seeds were sown instead (Appendix S2). Dishes were sealed with Parafilm to prevent desiccation.

Germination (i.e. radicle emergence) was scored weekly during incubation. Germinated seeds were removed from the Petri dishes. After 4 weeks of incubation, non-germinated seeds were cut open and classified as apparently viable, empty and fungi infected. Further statistical analyses were calculated only with potentially germinable seeds (germinated + apparently viable, Appendix S2). Some treatments that lacked variance (i.e. all or none seeds germinated) had to be transformed (by subtracting or adding a germinated seed to one of the dishes) so they could be included in the subsequent statistical analyses. The actual values are shown in the figures. Raw germination data is supplied in Appendix S2.

Germination proportion analyses

Statistical analyses were performed in R (R Core Team, 2016). A script with all the analyses is presented in Appendix S3. Final germination proportions were analysed separately for each species by fitting Generalised Linear Models (GLM) with binomial error distribution and logit link function. Full models were fitted first including as fixed factors germination temperature, cold stratification, and their interaction. A stepwise deletion of non-informative model parameters and factor levels was performed until a minimal adequate model was achieved for each species. Models were then used to estimate the mean final germination proportions in the significantly different treatments. Using these estimated means, a matrix was constructed in which each species was assigned the six germination proportions in each of the germination temperature and cold stratification treatments (Appendix S4). Variation in this matrix was explored using a Principal Component Analysis (PCA) computed with the covariance method.

Germination niche analyses

Germination niche shape was estimated for each species as the temperature that produced the best germination (see estimated values in Appendix S4). For each species, only the results for either non-stratified or cold-stratified seeds were considered, depending on whether cold stratification produced a significant decrease or increase in germination, as per the GLMs. When final germination was significantly higher in one of the treatments, this treatment was considered the best. When

two or more treatments produced similar final germination, the one that produced faster germination (i.e. lower time for 50 % germination, Appendix S2) was considered the best. When the treatments were still equal after considering time, their average was considered the best germination temperature. For these purposes, temperature was calculated as the average of the two steps in the daily alternating regime (14/4 = 9 °C, 22/12 = 17 °C, 30/20 = 25 °C). Germination niche breadth was estimated for each species as Pielou's evenness index (Luna and Moreno, 2010), calculated with the final germination proportions in each of the six experimental treatments. Pielou's index takes values from zero (high sensitivity to one treatment, narrowest germination niche) to one (germination evenly distributed across treatments, broadest germination niche). Because most of the species fell in the upper range of values, germination niche breadth was logit-transformed to achieve normality.

Differences between plant communities in their germination niche shape and breadth were tested by fitting Phylogenetic Generalised Least Squares models (PGLS). PGLS models were calculated using the approach proposed by Revell (2010), in which phylogenetic signal in the residual error and the regression parameters are estimated simultaneously. With this approach, if the phylogenetic signal is zero, the analysis becomes an ordinary linear model. A factorial PGLS model was fitted first for each measure of the germination niche, in which the species niche was explained by its cliff or beach habitat, sea-inland position, and the interaction habitat x sea-inland position. Then, a step-wise deletion of non-informative model parameters was applied to obtain the minimal adequate model.

Results

Germination temperature at the species level

Temperature had a significant effect on the germination of most species (Fig. 1, Table S1), with only six exceptions: Achillea maritima, Calystegia soldanella, Eryngium maritimum, Limbarda crithmoides, Raphanus raphanistrum and Reichardia gaditana. Seven species with the coldest germination preferences had their highest final germination at 14/4 and 22/12 °C: Armeria pubigera, Crucianella maritima, Festuca juncifolia, Festuca rubra, Plantago coronopus, Scilla verna and Spergularia rupicola. The largest group included ten species with the highest germination at the intermediate temperature of 22/12 °C: Angelica pachycarpa, Crithmum maritimum, Glaucium flavum, Jasione maritima, Koeleria glauca, Malcolmia littorea, Pancratium maritimum, Plantago maritima, Schoenus nigricans and Silene uniflora. However, cold stratification changed the optimum germination temperature in Crithmum maritimum (to 14/4 °C) and Schoenus nigricans (to 30/12 °C). A group of four species had warmer germination preferences, with highest germination at 22/12 and 30/20 °C: Carex arenaria,

Helichrysum italicum, H. stoechas and Lavatera arborea. Finally, there were three species that germinated best at the warmest temperature of 30/20 °C: Beta maritima, Cakile maritima and Polygonum maritimum.

Cold stratification effect at the species level

Cold stratification significantly improved germination in half the species, 16 out of 30 (Fig. 1, Table S1). In six of these species it increased overall germination percentages, without affecting the germination response to temperature: Armeria pubigera, Beta maritima, Cakile maritima, Carex arenaria, Festuca juncifolia and Scilla verna. In ten species, cold stratification also increased the germination temperature range: towards 14/4 °C in Crithmum maritimum, Malcolmia littorea, Plantago maritima and Polygonum maritimum; towards 30/20 °C in Crucianella maritima, Festuca rubra and Plantago coronopus; and towards both 14/4 and 30/20 °C in Angelica pachycarpa, Glaucium flavum and Schoenus nigricans. On the other hand, cold stratification decreased germination in nine species: at all temperatures in Calystegia soldanella, Jasione maritima, Koeleria glauca, Pancratium maritimum, Raphanus raphanistrum, Reichardia gaditana and Spergularia rupicola; and only at 14/4 °C in Helichrysum italicum and Lavatera arborea. Cold stratification did not have an effect in five species: Achillea maritima, Eryngium maritimum, Helichrysum stoechas, Limbarda crithmoides and Silene uniflora.

Germination proportions at the community level

The first two PCA axes (Fig. 2) accounted for 83 % of the total variance in germination proportions. Axis 1 explained 65 % of the variance and correlated positively with all germination proportions (Pearson's r > 0.75). This axis was constructed by overall germinability, separating species with poor germination (e.g. Achillea maritima) from those that germinated readily across treatments (e.g. Festuca juncifolia) (Fig. 1). Axis 1 did not produce a clear pattern of separation between plant communities. Axis 2 explained 17 % of the variance, and correlated positively with germination at 30/20 °C (Pearson's r > 0.50) and negatively with germination of non-stratified seeds at 14/4 °C (Pearson's r = -0.49). Thus, axis 2 separated species with warm-cued germination (e.g. Cakile maritima) from those with cold-cued germination (e.g. Armeria pubigera). Axis 2 tended to separate beach species from cliff species, with the former having warmer axis positions than the latter.

To further visualize germination variation between communities, the average germination proportions of each community were calculated (Fig. 3). For this and subsequent analyses, species with < 40 % germination in any treatment were excluded (Achillea maritima, Calystegia soldanella, Eryngium maritimum, Lavatera arborea and Limbarda crithmoides, Fig. 1). In these species, the experimental treatments failed to break dormancy and this could confound the measurement of their germination niche. Within beaches, the landwards group had higher germination especially in the colder

treatments. Cliffs had the opposite pattern, with higher germination seawards. Germination at 30/20 °C was higher in beaches, whereas germination at 14/4 °C was higher in cliffs but only seawards.

Germination niche at the community level

The PGLS model fitted to germination niche shape (i.e. best germination temperature) had a positive phylogenetic signal (Pagel's λ = 0.64). Interaction and sea-inland position were not significant; the minimal adequate model included only a significant effect of habitat (Table 2). Beach species had higher germination temperatures than cliff species, with 6 °C of estimated difference between the two groups (Fig. 4).

The PGLS model fitted to germination niche breadth (i.e. Pielou's index, logit-transformed) had a negative phylogenetic signal (Pagel's λ = -1.71). The full model was the minimal adequate model (Table 2). Interaction and sea-inland position had a significant effect in germination niche breadth, while habitat had a marginally significant effect (Table 2). In beaches, the germination niche was narrower seawards; while in cliffs no differences were found between positions (Fig. 4). On average, beaches and cliffs had the same germination niche breadth, but the cliff average (0.95, reversing the logit transformation of Pielou's index) was narrower than that of landwards beaches (0.99) and broader than that of seawards beaches (0.89) (Fig. 4).

Discussion

Our study shows a clear separation of the germination niche shape between cliffs and beaches. Five out of six species with significantly higher germination at the lowest temperature (14/4 °C) are cliff species. The exception, Glaucium flavum, is an specialist of shingle beaches where water stress might be more pronounced and sand transport less of a concern (Thanos et al., 1989, Rodwell, 1999). Conversely, of the nine species with a preference for the warmest germination temperature (30/20 °C), seven are beach plants. Two of the shifting dune species which we did not include in the niche analyses due to their low germinability (Calystegia soldanella and Eryngium maritimum) have also been reported to germinate with summer temperatures (Walmsley and Davy, 1997, Ko et al., 2004, Di Sacco and Bedini, 2015). The estimated best temperature for germination in cliffs is one degree above winter day temperatures, while in beaches it corresponds with average summer day temperatures. In accordance with our initial prediction, this would prevent emergence during the time of the year when the harshest environmental filters can be expected: summer water stress in cliffs; winter storms and sand transport in beaches. Thus beach communities have a warm-cued germination strategy similar to that of cold-adapted plants (Rosbakh and Poschlod, 2015), whereas cliff communities show a cold-cued strategy analogous to the Mediterranean germination syndrome (Thanos et al., 1989).

Our results partially support a divergence in the germination niche breadth of coastal plant communities depending on their position in the sea-inland gradient. This divergence is only found in beaches, where the germination niche is narrower in the seawards strandline and shifting dune communities. Most of the shifting dune species had low germination in all treatments, and may need some extra signal such as light quality, indicative of an adequately deep burial, as has been described in Glaucium flavum (Thanos et al., 1989) or Pancratium maritimum (Balestri and Cinelli, 2004). Low germination might however be a pattern as it has been reported in the shifting dune specialist Eryngium maritimum (Walmsley and Davy, 1997, Curle et al., 2004, Isermann and Rooney, 2014). The low germination of these species could be due to bad seed quality rather than dormancy, but this should not affect our conclusions since we excluded from the analyses the species with < 40 % germination. In contrast with the seawards beach species, fixed dune plants had a broad germination niche and were able to germinate well in most temperatures, as had been found for Malcolmia littorea (De Vitis et al., 2014). Cliffs were in an intermediate situation, their germination niche being narrower than that of landwards beaches but broader than that of seawards beaches. Taken together, these patterns agree with our original prediction that the germination niche would be narrower seawards, at least in the beach communities. Strandline and shifting dune communities experience a harsh abiotic environment (Ciccarelli et al., 2012), while fixed dune communities are more dependent on predictable macroclimatic seasonality (Jiménez-Alfaro et al., 2015). The lack of difference in cliffs may be due to the fact that, while environmental filters are stronger in the seawards rock-crevice vegetation, cliffs are not subjected to the substrate instability of beaches.

Niche shape and the environment showed a positive phylogenetic signal indicating that closer species are more similar than could be expected by random drift. This implies that closely related species are more likely to occur in one of the communities and to have a given germination niche shape. A possible explanation for this may be niche conservatism, although interpretations of the phylogenetic signal have to be made with care because different evolutionary processes can lead to similar phylogenetic signal values (Revell et al., 2008). Phylogenetically-related species thus seem to share germination strategies, as has been found for germination behaviour (Carta et al., 2016) and dormancy class (Dayrell et al., 2016). Regarding the niche breadth, the negative phylogenetic signal in its relationship with the environment means that related species are more different than could be expected, which might be interpreted as a hint of disruptive selection (Revell et al., 2008). An alternative explanation is that the breadth of the germination niche is being regulated through the degree of physiological seed dormancy, a plastic trait that can show considerable variation in response to the seed maturation environment (Chiang et al., 2011).

Conclusions

The confirmed predictions agree with two hypotheses that have been made about the seed germination niche, and moreover support their generalization to the community level. The first hypothesis is that environmental filters shape the germination niche to avoid germination in the less favourable season for seedling establishment. When communities differ in their unfavourable seasons, differences in their germination cues can be expected (Jurado and Flores, 2005, Rosbakh and Poschlod, 2015). In our coastal habitats, water stress is a major environmental filter driving plant occurrence and adaptation (Ciccarelli et al., 2016). Our results indicate that this filter is overruled in beaches by the physical damage to seedlings that can result from sand transport (Ciccarelli et al., 2012). This suggests that when there are conflicting environmental filters, the one with the highest impact on seedling survival will determine the shape of the germination niche. The hypothesis is that the germination niche breadth responds to the strength of the environmental filtering. Narrow niches allow for a better tracking of favourable regeneration environments (Donohue et al., 2010) and are 'filtered in' by harsher environmental filters. It is worth noting that the hypotheses about the germination niche and the environment seem to be valid even in the small spatial scales that separate our coastal plant communities. This strongly supports the importance of germination traits to understand the environmental filtering that drives plant community assembly (Larson and Funk, 2016). Comparative seed germination studies focused on plant community specialists can be a strong tool to bridge the gap between seed biology and plant community ecology (Jiménez-Alfaro et al., 2016). We argue for the future expansion of similar studies, extending the comparative scale to other plant communities and to broader biogeographical scales.

Acknowledgements

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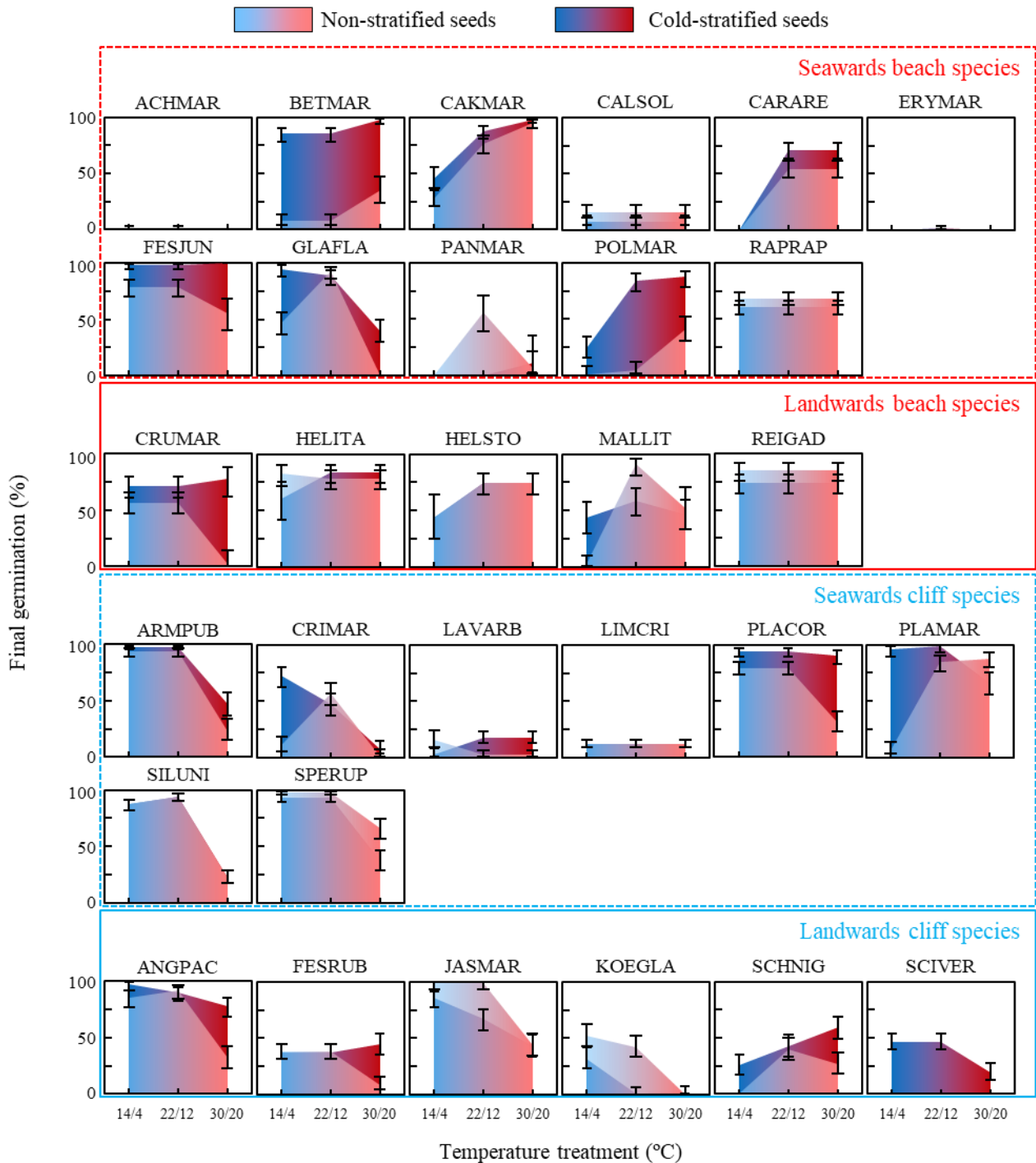
Figure 1 Results of the germination experiments per species. Final germination percentages after 28 days of incubation at one of three temperatures, in non-stratified seeds (lighter areas) and cold-stratified seeds (darker areas). Areas represent mean germination percentages, brackets the 95 % binomial confidence interval, as estimated by the minimal adequate Generalised Linear Models fitted to the results of each species (see Table S1). Species are grouped per their plant community.
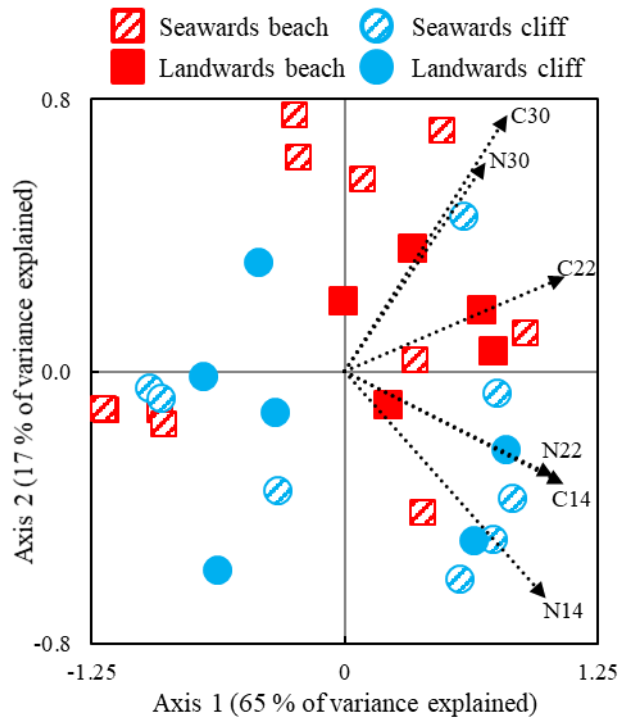
Figure 2 Principal Component Analysis of the final germination per treatment. Arrows represent the contribution of each germination treatment to the axes. Treatments are the combination of two pre-treatments represented by letters (N = non-stratified seeds, C = cold-stratified), and three germination temperatures represented by numbers (14 = 14/4 ℃, 22 = 22/12 ℃, 30 = 30/20 ℃). Arrow lengths have been multiplied by 3.5 for easier visualization. Symbols represent species, coloured per their plant community.
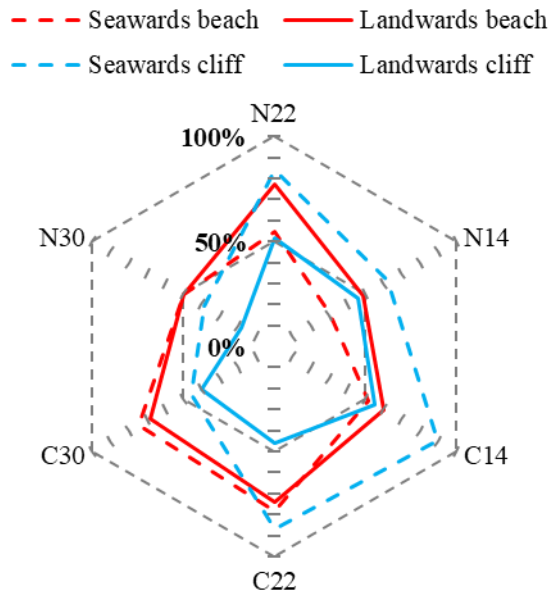
Figure 3 Average final germination per plant community. Germination was tested in six treatments: the combination of two pre-treatments represented by letters (N = non-stratified seeds, C = cold-stratified) and three germination temperatures represented by numbers (14 = 14/4 ℃, 22 = 22/12 ℃, 30 = 30/20 ℃). The figure shows the average final germination of each plant community in the six treatments. Only species with > 40 % germination in at least one treatment were used to calculate the averages.
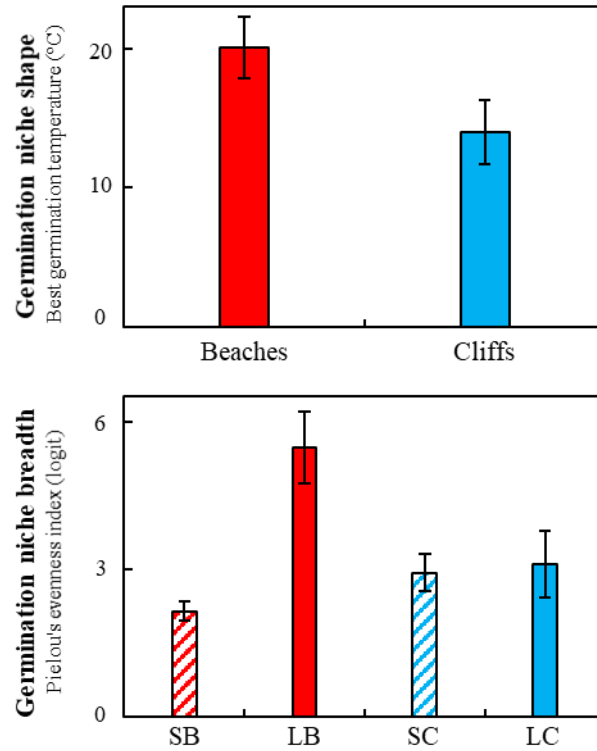
Figure 4 Differences in the seed germination niche among plant communities. Germination niche shape was estimated as the best temperature for seed germination. Germination niche breadth was measured with Pielou's evenness index (logit-transformed), higher values indicate broader niches. Only significant differences are shown, as indicated by Phylogenetic Generalised Least Squares (PGLS) minimal adequate models (Table 2). Bars represent the mean value of each group as estimated by PGLS, and brackets represent the S.E. SB = seawards beaches, LB = landwards beaches, SC = seawards cliffs, LC = landwards cliffs.

Table 1. Species included in this study. Taxonomical sources are indicated with superscript symbols: + Euro+Med PlantBase (http://www.emplantbase.org/home.html, accessed 14 Oct 2015); - Castroviejo, S. (Coord.) (1986–2015) Flora iberica. Real Jardín Botánico – CSIC, Madrid; * Fernández Prieto, J.A. & Loidi, J. (1984) Documents Phytosociologiques, 8, 2; # Fernández Prieto, J.A., Nava, H.S. & Bueno Sánchez, A. (2014) Documentos del Jardín Botánico Atlántico (Gijón), 11, 298-300.

Taxon	Family	Sea-inland position	Habitat	Coordinates of seed collection (N, W)	Date of seed collection
Achillea maritima [=*Otanthus maritimus*][+]	Asteraceae	Seawards	Beach	43º 37' 34", 5º 52' 37"	03/09/2014
Angelica pachycarpa[-]	Apiaceae	Landwards	Cliff	43º 39' 23", 5º 51' 2"	16/09/2013
Armeria pubigera subsp. *depilata**	Plumbaginaceae	Seawards	Cliff	43º 27' 42", 4º 58' 30"	12/06/2014
Beta maritima[-]	Chenopodiaceae	Seawards	Beach	43º 33' 14", 6º 59' 41"	04/09/2014
Cakile maritima subsp. *integrifolia*[-]	Brassicaceae	Seawards	Beach	43º 33' 14", 6º 59' 41"	04/09/2014
Calystegia soldanella[-]	Convolvulaceae	Seawards	Beach	43º 37' 34", 5º 52' 37"	28/07/2014
Carex arenaria[-]	Cyperaceae	Seawards	Beach	43º 28' 48", 5º 8' 10"	24/07/2014
Crithmum maritimum[-]	Apiaceae	Seawards	Cliff	43º 26' 35", 4º 52' 33"	16/09/2014
Crucianella maritima[-]	Rubiaceae	Landwards	Beach	43º 26' 44", 3º 58' 19"	15/08/2014
Eryngium maritimum[-]	Apiaceae	Seawards	Beach	43º 37' 34", 5º 52' 37"	03/09/2014
Festuca juncifolia[+]	Poaceae	Seawards	Beach	43º 33' 14", 6º 59' 41"	04/09/2014
Festuca rubra subsp. *pruinosa*[+]	Poaceae	Landwards	Cliff	43º 39' 23", 5º 51' 2"	30/07/2014
Glaucium flavum[-]	Papaveraceae	Seawards	Beach	43º 37' 34", 5º 52' 37"	03/09/2014
Helichrysum italicum subsp. *picardii*[+]	Asteraceae	Landwards	Beach	42º 26' 55", 8º 52' 26"	01/08/2014
Helichrysum stoechas[+]	Asteraceae	Landwards	Beach	43º 28' 48", 5º 8' 10"	18/07/2012
Jasione maritima subsp. *gallaecica*[#]	Campanulaceae	Landwards	Cliff	43º 35' 35", 6º 14' 37"	19/06/2014
Koeleria glauca[+]	Poaceae	Landwards	Cliff	43º 39' 23", 5º 51' 2"	30/07/2014
Lavatera arborea[-]	Malvaceae	Seawards	Cliff	43º 35' 35", 6º 14' 37"	19/06/2014
Limbarda crithmoides[+]	Asteraceae	Seawards	Cliff	43º 27' 42", 4º 58' 30"	31/07/2014
Malcolmia littorea[-]	Brassicaceae	Landwards	Beach	43º 33' 14", 6º 59' 41"	04/09/2014
Pancratium maritimum[-]	Amaryllidaceae	Seawards	Beach	43º 37' 34", 5º 52' 37"	03/09/2014
Plantago coronopus[-]	Plantaginaceae	Seawards	Cliff	43º 27' 42", 4º 58' 30"	31/07/2014
Plantago maritima[-]	Plantaginaceae	Seawards	Cliff	43º 27' 42", 4º 58' 30"	31/07/2014
Polygonum maritimum[-]	Polygonaceae	Seawards	Beach	43º 32' 0", 5º 22' 50"	18/07/2012
Raphanus raphanistrum subsp. *landra*[-]	Brassicaceae	Seawards	Beach	43º 31' 38", 5º 23' 6"	18/09/2012
Reichardia gaditana[+]	Poaceae	Landwards	Beach	43º 37' 34", 5º 52' 37"	28/07/2014
Schoenus nigricans[-]	Cyperaceae	Landwards	Cliff	43º 26' 53", 4º 53' 9"	18/06/2014
Scilla verna[-]	Liliaceae	Landwards	Cliff	43º 27' 42", 4º 58' 30"	12/06/2014
Silene uniflora[-]	Caryophyllaceae	Seawards	Cliff	43º 26' 53", 4º 53' 9"	18/06/2014
Spergularia rupicola[-]	Caryophyllaceae	Seawards	Cliff	43º 39' 23", 5º 51' 2"	19/06/2014

Table 2. PGLS models fitted to the shape and breadth of the germination niche of 25 coastal plant species. The models are the minimal adequate models obtained by step-wise simplification of a fully factorial PGLS model. The marginal ANOVA table of each model is shown. Germination niche shape was fitted with best germination temperature as response variable. Germination niche breadth was fitted with logit-transformed Pielou's evenness index as response variable.

PGLS model	Parameter	Degrees of freedom	F	p
Germination niche shape	Habitat	1, 23	8.526	0.007
Germination niche breadth	Interaction	1, 21	6.297	0.020
	Habitat	1, 21	3.947	0.060
	Position	1, 21	24.428	< 0.001

Supporting information

Additional supporting information may be found in the online version of this article:

Appendix S1 Phylogenetic tree of the study species, in newick format.

Appendix S2 Raw germination results per Petri dish.

Appendix S3 R script to repeat all analyses in this article.

Appendix S4 Seed germination proportions and best germination temperatures per species.

Table S1 Table with the minimal adequate Generalised Linear Models fitted to the germination data of each species.