

Seed ecology of European mesic meadows

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- **Background and Aims** European mesic meadows are semi-natural open habitats of high biodiversity and an essential part of European landscapes. These species-rich communities can be a source of seed mixes for ecological restoration, urban greening and rewilding. However, limited knowledge of species germination traits is a bottleneck to the development of a competitive native seed industry. Here, we synthesize the seed ecology of mesic meadows.
- **Methods** We combined our own experimental data with data obtained from databases to create a combined dataset containing 2005 germination records of 90 plant species from 31 European countries. We performed a Bayesian meta-analysis of this dataset to test the seed germination response to environmental cues including scarification, stratification, temperature, alternating temperature and light. We also used multivariate ordination to check the relationship between seed traits (germination and morphology) and species ecological preferences, and to compare the seed ecology of mesic meadows with that of other herbaceous plant communities from the same geographic area.
- **Key Results** The seed ecology of mesic meadows is characterized by (1) high seed germinability when compared with other herbaceous plant communities; (2) low correspondence between seed traits and species ecological preferences; and (3) a deep phylogenetic separation between the two major families, Poaceae and Fabaceae. Poaceae produce many light seeds that respond to gap-detecting germination cues (alternating temperatures and light); Fabaceae produce fewer heavy seeds, which need scarification to break their physical dormancy.
- **Conclusions** High germinability of meadow seeds will reduce their capacity to form persistent seed banks, resulting in dispersal limitations to passive regeneration. For centuries, human activities have shaped the regeneration of meadows, leading to a loss of seed dormancy and decoupling seeds from seasonal cycles, as has been found in many domesticated species. The same anthropic processes that have shaped semi-natural mesic meadows have left them dependent on continued human intervention for their regeneration, highlighting the importance of active restoration via seed supply.

Key words: *Arrhenatheretalia*, *Arrhenatherion*, Asteraceae, Europe, Fabaceae, domestication, hay meadows, mesic grasslands, Poaceae, seed germination, seed morphology, semi-natural, species-rich meadows.

INTRODUCTION

The mesic meadows of Europe are semi-natural open habitats that occupy clearings created by human intervention over a temperate wooded landscape (Poschlod *et al.*, 2009; Hejman *et al.*, 2013), in sites with moderately fertile and well-drained soils (Mucina *et al.*, 2016). These novel anthropogenic plant communities, with a composite flora made up of species from different ecological backgrounds, have constantly changed in response to shifts in human society and associated management practices (Chytrý, 2012; Hejman *et al.*, 2013), but nonetheless characterize Europe's natural history (Finck *et al.*, 2002).

Traditional classification of mesic grasslands emphasizes the separation between meadows used for hay making versus

pastures maintained by grazing, but a recent revision at the European level showed that the main driver of variation in species composition is the intensity rather than the type of management (Rodríguez-Rojo *et al.*, 2017). Indeed, changes in the intensity of management, including land abandonment and agricultural intensification, are threatening the maintenance of mesic meadows in large parts of Europe (Carboni *et al.*, 2015). For this reason, the European Habitats Directive (92/43/EEC) lists as habitats of conservation interest those species-rich meadows that are traditionally managed by one or two annual cuts and light grazing.

One of the reasons for the high conservation interest of traditional mesic meadows is their high species richness, and for this same reason they have been highlighted as a valuable source of natural seed material to be used in ecological restoration, rewilding and urban greening (Krautzer *et al.*, 2013;

Haslgrübler *et al.*, 2014; Golińska *et al.*, 2017). However, a lack of knowledge about species germination traits has already been identified as a bottleneck (Ladouceur *et al.*, 2018) hampering the development of a competitive native seed industry (De Vitis *et al.*, 2017) in Europe. Understanding seed germination is part of the practical scientific framework needed to tackle large-scale ecological restoration challenges (Merritt and Dixon, 2011) and to maintain *ex situ* collections of plant genetic resources (Li and Pritchard, 2009). When such knowledge is available, ambitious regional schemes of seed-based landscape restoration can be designed (Jiménez-Alfaro *et al.*, 2020).

Mesic meadows are dominated by mesophilous grasses of the family Poaceae, which make up most of the biomass in the community and define the structure of the vegetation. The dispersal unit in Poaceae is generally the floret (Fig. 1A), a composite structure made up of the caryopsis or grain (a dry, indehiscent and monospermic fruit) and its surrounding bracts (i.e. modified leaves): the lemma and the palea. Removal of the lemma and palea, as well as puncturing the pericarp, can aid in seed germination (Probert *et al.*, 1985). The dispersal unit often carries awns or hairs (Fig. 1B) that are assumed to aid dispersal, although it is not always the case that they do (Schonfeld and Chancellor, 1983). Several genera of Poaceae that grow in European meadows show seed heteromorphism (e.g. *Aegilops*, *Avena*, *Dasypirum*, *Poa*) (Guzzon *et al.*, 2018).

They produce different seed morphs characterized by different seed traits in terms of dormancy, longevity, morphology, phenology, tolerance to abiotic stresses and susceptibility to predation. This is connected to bet-hedging ecological strategies, typical of ruderal/disturbed habitats. Regarding germination, meadow Poaceae seeds have been reported as having physiological seed dormancy in various degrees (Sprague, 1940; Dixon, 1995; Baskin and Baskin, 2014), but germinability is usually high even without treating the seeds with cold stratification (Grime *et al.*, 1981; Schonfeld and Chancellor, 1983; Williams, 1983a; Bean *et al.*, 1984; Froud-Williams *et al.*, 1984, 1986; Froud-Williams and Ferris, 1987; Dixon, 1995; Pérez-Fernández and Rodríguez-Echeverría, 2003; Pérez-Fernández *et al.*, 2006; Stanisavljevic *et al.*, 2011, 2015; Oliveira *et al.*, 2012; Wille *et al.*, 2013). Freshly harvested seeds are comparatively more dormant than stored seeds, but dormancy tends to disappear quickly in dry storage (Sprague, 1940; Dixon, 1995). Germination has been reported to occur at temperatures ranging from 5–30 °C (Grime *et al.*, 1981; Williams, 1983b; Pannangpetch and Bean, 1984; Probert *et al.*, 1986; Froud-Williams *et al.*, 1986; Dixon, 1995). Most Poaceae species have also been reported to germinate better in light than in darkness (Williams, 1983b, c; Froud-Williams *et al.*, 1984; Probert *et al.*, 1985, 1986; Probert and Smith, 1986; Thompson, 1989; Dixon, 1995) and even to be unable to germinate in

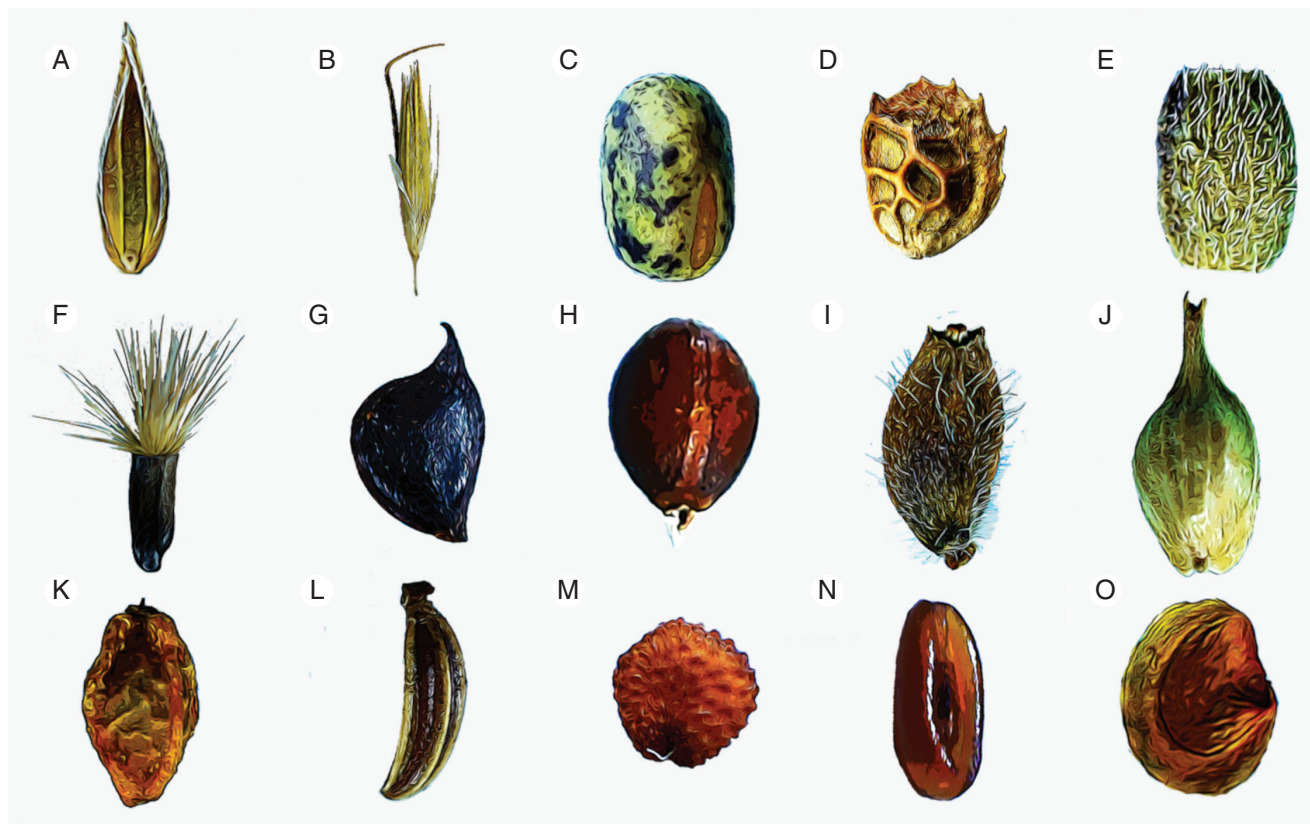


FIG. 1. Diversity of dispersal units in plant species from mesic meadows: (A) Floret of *Cynosurus cristatus* (Poaceae); (B) floret of *Arrhenatherum elatius* (Poaceae); (C) seed of *Lathyrus pratensis* (Fabaceae); (D) legume of *Onobrychis viciifolia* (Fabaceae); (E) loment fragment of *Ornithopus perpusillus* (Fabaceae); (F) achene with pappus of *Centaurea scabiosa* (Asteraceae); (G) achene of *Ranunculus acris* (Ranunculaceae); (H) nutlet of *Prunella grandiflora* (Lamiaceae); (I) achene of *Knautia nevadensis* (Caprifoliaceae); (J) perigynium of *Carex binervis* (Cyperaceae); (K) receptacle of *Sanguisorba minor* (Rosaceae); (L) mericarp of *Carum verticillatum* (Apiaceae); (M) seed of *Cerastium fontanum* (Caryophyllaceae); (N) seed of *Plantago lanceolata* (Plantaginaceae); (O) seed of *Rhinanthus angustifolius* (Orobanchaceae).

darkness (Froud-Williams *et al.*, 1986). Nonetheless, species of *Bromus* have been reported as germinating better in darkness (Thompson, 1989) and *Cynosurus cristatus* as being indifferent to light/darkness (Williams, 1983b, c). In *Poa trivialis*, germination is promoted by light but not by diurnal alternating temperatures (i.e. germination conditions where different temperatures are applied during the day and the night, in diurnal cycles) (Froud-Williams and Ferris, 1987), although alternating temperatures do encourage some germination in darkness (Froud-Williams *et al.*, 1986). Wild Poaceae ecotypes usually have a germination response to alternating temperatures (Schonfeld and Chancellor, 1983; Williams, 1983b, c; Pannangpetch and Bean, 1984; Probert *et al.*, 1985, 1986; Probert and Smith, 1986; Thompson, 1989), although this response is missing in some wild ecotypes and in the domesticated cultivars (Pannangpetch and Bean, 1984), and some species such as *Lolium perenne* have been reported as insensitive to temperature alternation (Thompson *et al.*, 1977; Williams, 1983c).

Next in abundance to Poaceae are the legumes of the family Fabaceae. Fabaceae species contribute to the nutritional value of meadow fodder, as thanks to their N-fixing capabilities they have high N contents (Reiné *et al.*, 2020; Álvarez *et al.*, 2021). The dispersal unit of most Fabaceae is the seed itself (Fig. 1C) but in some species dispersal units are more complex, including indehiscent monospermic fruits (e.g. *Onobrychis*, Fig. 1D) or indehiscent monospermic fruit fragments, i.e. loments (e.g. *Ornithopus*, Fig. 1E). Fabaceae are generally hard-seeded: they have a water-impermeable seed coat that needs to become permeable before germination can happen (i.e. physical seed dormancy) (Grime *et al.*, 1981; Jones and Turkington, 1986; Ehrman and Cocks, 1996; Kupferschmid *et al.*, 2000; Baskin and Baskin, 2014). In *Medicago*, seeds that have not reached full maturity can germinate before they become impermeable, but the completion of maturation imposes coat impermeability, and thereafter the seed must be scarified to allow water imbibition and germination (Gresta *et al.*, 2007). Buried Fabaceae seeds can track the seasons, and in some species germination seems to be promoted by cold stratification and alternating temperatures (Van Assche *et al.*, 2003); some of these species have been described as having combinational dormancy (i.e. physical + physiological) (Van Assche and Vandeloos, 2010). However, Fabaceae seeds have also been reported to germinate without any previous treatment (Marchiol *et al.*, 2000; Nikolic *et al.*, 2007; Kabouw *et al.*, 2010; Oliveira *et al.*, 2012), and to lose dormancy during storage (Van Assche and Vandeloos, 2010). As in Poaceae, seeds of Fabaceae have been reported to germinate in high numbers across a range of temperatures from 5 to 25 °C (Grime *et al.*, 1981; Gresta *et al.*, 2007). Fabaceae seeds have been described as not being responsive to light and as capable of germinating in darkness (Silvertown, 1980; Grime *et al.*, 1981).

Poaceae and Fabaceae are accompanied by a diversity of other families which, even if present in lower abundances, contribute to the high biodiversity of the system and increase the aesthetic value of the meadows as perceived by people (Southon *et al.*, 2017; Chollet *et al.*, 2018). They also add nutritional scope for livestock, being richer than Poaceae and Fabaceae in specific elements (Reiné *et al.*, 2020; Álvarez *et al.*, 2021). In many of these plant families (e.g. Asteraceae, Cyperaceae, Caprifoliaceae, Lamiaceae, Polygonaceae,

Ranunculaceae) the dispersal unit is the achene: dry, indehiscent and monospermic fruits. The morphology of these achenes is varied: cypselae with a hairy pappus in *Centaurea* (Asteraceae, Fig. 1F); beaked in *Ranunculus* (Ranunculaceae, Fig. 1G); hardened nutlets in *Prunella* (Lamiaceae, Fig. 1H); hairy and with an elaiosome in *Knautia* (Caprifoliaceae, Fig. 1I); surrounded by a perigynium which aids in dispersal by water in *Carex* (Cyperaceae, Fig. 1J). In the genus *Sanguisorba* (Rosaceae), the dispersal unit is the urn-shaped receptacle containing one to three achenes (Fig. 1K). In Apiaceae, it is the mericarp (Fig. 1L), an indehiscent monospermic fragment of the fruit. In some other minor families, the dispersal unit is the seed itself, such as in Caryophyllaceae (Fig. 1M), Plantaginaceae (Fig. 1N), Juncaceae or the hemiparasitic species of *Rhinanthus* (Orobanchaceae) (Fig. 1O). In Asteraceae, high germination without previous treatments has been reported in *Taraxacum officinale* (Mezyski and Cole, 1974; Washitani, 1984; Noronha, 1997; Benvenuti and Pardossi, 2016; Masin *et al.*, 2017), *Hypochaeris radicata* (Oomes and Elberse, 1976; Benvenuti and Pardossi, 2016) and *Achillea millefolium* (Oomes and Elberse, 1976). *Taraxacum officinale* germinates between 5 and 30 °C (Mezyski and Cole, 1974; Washitani, 1984; Masin *et al.*, 2017) and has higher germination in light (Thompson, 1989; Letchamo and Gosselin, 1996; Noronha, 1997) and in alternating temperatures (Mezyski and Cole, 1974). In *Stachys officinalis* (Lamiaceae), seeds need either cold stratification, light or alternating temperatures to germinate (Wagner *et al.*, 2011; Kolodziejek *et al.*, 2017). Underdeveloped embryos that need to grow inside the seed before germination (i.e. morphological dormancy) are widespread in Ranunculaceae and Apiaceae (Jauzein and Mansour, 1992; Baskin and Baskin, 2014). *Ranunculus repens* (Ranunculaceae) germinates between 10 and 25 °C, but the germination percentages have been reported to be low (Harris *et al.*, 1998); the same species has been reported to respond to alternating temperatures, which can promote its germination even in darkness (Thompson and Grime, 1983). In Polygonaceae, *Rumex acetosa* can germinate immediately after dispersal and between 7 and 27 °C, while the congeneric *Rumex acetosella* does not: this difference is due to the former being able to germinate at constant temperatures in the darkness (Grime *et al.*, 1981; Van Assche *et al.*, 2002), while the latter has an absolute requirement for light (Van Assche *et al.*, 2002). In *Heracleum sphondylium* (Apiaceae), growth of the embryo only occurs below 10 °C, in moist conditions (Jauzein and Mansour, 1992). *Sanguisorba minor* (Rosaceae) increases its germination after abrasion of the seeds with bleach (Tavşanoğlu *et al.*, 2015; Benvenuti and Pardossi, 2016), although germination without previous treatment has also been reported (Ludewig *et al.*, 2014; Tavşanoğlu *et al.*, 2015). Seeds of the hemiparasitic species *Rhinanthus angustifolius* and *Rhinanthus minor* (Orobanchaceae) require relatively long periods of cold stratification to germinate (Ter Borg, 2005) and can germinate in the dark (Marin *et al.*, 2019).

Although a wealth of studies has accumulated, a synthesis of the seed ecology of European mesic meadows is still missing. In this article, we review for the first time this topic to provide an overview of traits related to plant regeneration by seed, as a knowledge basis to expand our understanding of meadow communities and assist in their management by conservation and restoration practitioners. We combine newly generated data on

seed morphology and germination with records from existing databases (Kleyer *et al.*, 2008; Royal Botanic Gardens, Kew, 2017; Fernández-Pascual, 2021). The resulting dataset contains 2005 germination records of 90 plant species from 31 European countries. We use this dataset to test the seed germination response to environmental cues including scarification, stratification, temperature, alternating temperature and light, applying Bayesian meta-analysis (Pappalardo *et al.*, 2020). Further, using well-preserved meadows of the Iberian Peninsula as a study system, we analyse the covariation between seed traits and species environmental preferences, and compare the germination ecology of mesic meadows with that of other herbaceous plant communities from the same geographic area.

MATERIALS AND METHODS

Data sources

Selection of mesic meadow species. To create a list of representative European mesic meadow species for inclusion in our analysis, we used the list of species provided by Chytrý *et al.* (2020) as dominant, constant and diagnostic species of the EUNIS habitats ‘low and medium altitude hay meadows’ and ‘mountain hay meadows.’ This list included 120 species and was used to test the response to germination cues at the European level (see below).

To conduct further analyses at the Iberian level, we constructed a second species list based on a dataset comprising 118 vegetation relevés (i.e. records of plant species co-occurring in sampling plots) from three Iberian regions with well-maintained mesic meadows: 43 relevés from Northern Portugal, 25 from the Cantabrian Mountains of Spain and 50 from the Pyrenees. These relevés were used to assess variation in seed responses along environmental gradients (i.e. the relationship between species seed traits and species ecological requirements, see below), as they were recorded along a major stress gradient related to summer drought (Rodríguez-Rojo *et al.*, 2014): the Pyrenees and Cantabrian Mountains have a temperate macroclimate, whereas Northern Portugal is transitional between the temperate and Mediterranean macroclimates. Furthermore, meadows from the Pyrenees are closest to the European optimum of mesic meadow vegetation while the Portuguese ones are in suboptimal areas at the limit of the European distribution of temperate meadows (Rodríguez-Rojo *et al.*, 2017). Finally, the traditional management of meadows (i.e. mowing for haymaking once or twice per year plus light grazing) is relatively well preserved in these three regions compared with their European context (Prince *et al.*, 2012; Guadilla-Sáez *et al.*, 2019).

The Iberian meadows were maintained by traditional agricultural practices: mowing for haymaking once or twice per year plus light grazing. Each vegetation sampling plot was placed in a square area (25–100 m² area) situated in the central part of the meadow, avoiding the margins. Vegetation sampling took place in 2016–17, at the peak of plant development, just before mowing. All vascular plant species in the plots were recorded and given a cover value using the transformation of the Braun-Blanquet scale to coverage (+ = 0.1 %, 1 = 5 %, 2 = 17.5 %, 3 = 37.5 %, 4 = 62.5 % and 5 = 87.5 %). All plant names were

assigned following the nomenclature of Euro+Med (2006), which is used throughout this article. As expected, the vegetation of the sampled meadows was dominated by Poaceae and Fabaceae: these two families represented 47 and 17 %, respectively, of the total plant cover recorded in all the plots. Other 20 families were recorded, the largest of which was Asteraceae; each of these other families represented <10 % of the total cover.

Using all the relevés, we calculated the cumulative cover of each species in the entire area. To perform the calculation, first we standardized the cover values of the plots by dividing the cover of each species in each plot by the total plant cover in that plot. Then, for each species, we calculated its total cover in the dataset, by summing its standardized cover values from all the plots. Finally, we rescaled the values of all species to a 1–100 scale to obtain the cumulative cover values. From the resulting list of species, we removed 208 species with cumulative cover values <2 %, considering them to be transient species (Mariotte, 2014) that might have been recorded by chance and may not represent the core mesic meadows flora. We used the remaining 116 species as the core list of Iberian meadow species. Most of these species were hemicryptophytes (78 %), with some therophytes (16 %) and a few chamaephytes and geophytes (3 % of each). The family with most species in the list was Poaceae (22 %), with another 17 % belonging to Fabaceae, 15 % to Asteraceae, 8 % to Apiaceae, and the rest of the families representing <5 % each.

Species ecological requirements. We also used the Iberian relevés as a basis to characterize the preferences of the selected species for three environmental factors (cold, summer drought and soil reaction) that have been found to be major ecological drivers of mesic meadow plant diversity (Rodríguez-Rojo *et al.*, 2014). For cold and drought, we used the coordinates of the plots to retrieve from CHELSA (Karger *et al.*, 2017) the bioclimatic variables bio06 (minimum temperature in the coldest month) and bio14 (precipitation in the driest month). For soil reaction (pH), we took from each plot five soil samples from between 0 and 20 cm depth with a Dutch auger and combined them to make a bulk soil horizon, which we subsequently air-dried, crumbled, finely crushed and sieved with a 2-mm screen, to finally measure the pH in H₂O with a glass electrode in a suspension of soil:water (1:2.5). With each of these three environmental variables (bio06, bio14, pH) measured at the plot level we calculated the species niche centroids (SNCs). The SNC for any given species and variable is the mean of the environmental variable in all the plots where the species occurs, weighted by species cover in each plot (Zelený, 2018).

Seed morphology. From the vegetation plots described above, we collected dispersal units (hereafter called seeds) during the dispersal seasons of 2016, 2017 and 2018. Seed collection followed the methodology of ENSCONET (2009). To describe seed morphology, we acquired images of 100-seed samples of each species using a flatbed scanner (Brother LC985) with a resolution of 200 dpi and a scanning area of 1024 × 1024 pixels (Bacchetta *et al.*, 2008). We distributed the seeds on the transparent glass of the scanner, in a 10 × 10 grid. For each sample, and without moving the seeds, we repeated the scan with black and white backgrounds. In the case of the black background, we covered samples with a black box to avoid interference from environmental light. For the white background, we used

the scanner cover. We digitized the obtained images and stored them in JPEG format (Joint Photographic Experts Group). We processed the scanned images using ImageJ, an open-source image processing program designed for scientific multidimensional images (Schneider *et al.*, 2012). The program calculates several biometric parameters for each seed on the sample, and among those we chose seed length and width. Additionally, we retrieved species values of seed mass from the Seed Information Database (Royal Botanic Gardens, Kew, 2017) and of seed number at the individual/ramet level from the LEDA database (Kleyer *et al.*, 2008). The dataset with the length and width measures is available at GitHub (see Data Availability Statement).

Seed germination. We constructed a germination dataset by combining the results of our own germination experiments with Iberian seeds, and records of seed germination of meadow seeds from across Europe.

We germinated the collected Iberian seeds using three germination treatments to determine the germination response to temperatures that are representative of the study area: 14/4 °C representing the capacity of freshly-dispersed seeds to germinate at cool temperatures of spring and autumn, 22/12 °C as summer temperature, and 30/20 °C as sun-heated soil, e.g. soil exposed to sun after hay cutting. Additionally, we compared, for each of these temperature regimes, the germination of fresh seeds versus seeds subjected to a dormancy-breaking treatment. This treatment was adjusted to the most probable dormancy type per taxonomic group (Baskin and Baskin, 2014). In the case of Fabaceae and other families that might present physical dormancy (Baskin and Baskin, 2014), the treatment consisted in scarification by chipping the seed coat with a scalpel. For the rest of the families, we used gibberellic acid (GA₃; 0.0645 mM) in darkness for 24 h, as a treatment to remove potential physiological seed dormancy (Blandino *et al.*, 2019). For each species and treatment, we sowed four Petri dishes with 25 seeds each. The germination substrate was 1 % distilled water agar. We sealed dishes with Parafilm to prevent desiccation. Trials took place in a germination chamber (KBW 400, Binder, Tuttlingen, Germany) with a 12/12-h photoperiod (the light period corresponding to the higher temperature). Experiments lasted for 4 weeks, with germination scoring once per week. The germination criterion was 2-mm radicle emergence. After 4 weeks, we cut the seeds that failed to germinate and examined them under a magnifying glass. We classified them as normal when the embryo was visible and firm, empty when they lacked an embryo, and contaminated when they were mouldy. We only considered normal seeds when calculating germination proportions and conducting subsequent analyses.

In addition to these experimental germination data, we retrieved European seed germination records for the European list of study species from ENSCOBASE, the seed germination database of the European Native Seed Conservation Network (<http://enscibase.maich.gr/index.tml>); and the *SylvanSeeds* database of seed germination records for the nemoral biome (Fernández-Pascual, 2021). These new records included additional records of species in our experimental dataset, plus records of new species absent from our experimental dataset, and in all cases corresponded to seed lots originally collected within Europe. The combined dataset, including our own experimental

data and the records from ENSCOBASE and *SylvanSeeds*, contained 90 species (i.e. 90 % of the core list of meadow species was covered) and 2005 germination records (i.e. germination proportions for a given seed lot of a species, recorded in a set of laboratory experimental conditions) from 31 European countries. Overall, 156 252 seeds had been used in the experiments. The range of experimental germination temperatures (weighted average of the daily thermoperiod) that had been used in the experiments spanned from 2 to 31 °C, with 1226 records of constant temperatures (i.e. experiments that used the same temperature during all their duration) and 779 of alternating temperatures (i.e. experiments where different temperatures were applied during the day and the night, in diurnal cycles). Seeds had been exposed to light during some part of the diurnal cycle in 1907 records or kept in total darkness in 98 records. Experiments had been performed with unstratified seeds (i.e. not subjected to a previous dormancy-breaking incubation) in 1726 records and with stratified seeds (i.e. subjected to previous incubation in dormancy-breaking conditions, including treatments of wet incubation under cold, warm and combinations of cold and warm conditions) in 279 records. There were 262 records where GA₃ had been applied, and 369 records where seeds had been scarified.

Finally, to compare the germination of mesic meadow species with that of other herbaceous communities, we retrieved data from previous works on the seed germination ecology of bogs and fens (Fernández-Pascual *et al.*, 2013; Fernández-Pascual, 2016), alpine and subalpine grasslands (Fernández-Pascual *et al.*, 2017a), and coastal plant communities of rocky cliffs and sand dunes (Fernández-Pascual *et al.*, 2017b). These additional germination records had been obtained using the same experimental methodology as the one employed for some of the germination experiments of this study: recently collected seeds, untreated for physiological dormancy (but scarified in the cases of families known for having physical dormancy), had been subjected to three germination thermoperiods (14/4, 22/12, 30/20 °C). All seeds had been collected in the Cantabrian Mountains of Spain or the neighbouring coast. We combined these records with the records with matching experimental conditions from the meadows germination dataset to create a Cantabrian dataset.

Statistical analysis

We conducted all analyses in R (R Core Team, 2020), and the code for analysis and creation of the figures and manuscript is available at GitHub (see Data Availability Statement).

Seed responses to germination cues. We used the European germination dataset (available at GitHub, see Data Availability Statement) to test the effect of germination treatments on seed germination proportions. The dataset used included 2005 germination records of 90 species; of these, 222 records came from our own experimental data and the rest from the databases. We performed a meta-analysis (Pappalardo *et al.*, 2020) of the germination dataset by fitting binomial generalized mixed models with Bayesian estimation [Markov chain Monte Carlo generalized linear mixed models (MCMCglmm)] (Fernández-Pascual *et al.*, 2021) using the R package *MCMCglmm* (Hadfield, 2010).

We fitted four models: (1) to the entire dataset; and separately for each of the three botanical groups of mesic meadows: (2) Poaceae, (3) Fabaceae, and (4) a third group including the rest of the families. To account for the effect of a shared phylogenetic history in species traits, models included as a random effect a reconstructed phylogenetic tree of the study species. We created the phylogeny using the R package *V.PhyloMaker* (Jin and Qian, 2019), which contains an updated mega-tree of the seed plants based on Smith and Brown (2018). We placed taxa absent from the mega-tree at the genus-level basal node. The phylogenetic tree is available at GitHub (see Data Availability Statement). The response variable of the models was the proportion of germinated seeds. The fixed effects were the germination treatments (scarification, stratification, GA₃, temperature, alternating temperature and light). Random effects included the phylogenetic tree, species identity, seed lot and source of the data. In all models, fixed effect variables were scaled so their contribution to the effect sizes could be compared. We used weakly informative priors in all models, with parameter-expanded priors for the random effects. Each model was run for 500 000 MCMC steps, with an initial burn-in phase of 50 000 and a thinning interval of 50 (De Villemereuil and Nakagawa, 2014), resulting, on average, in 9000 posterior distributions. From the resulting posterior distributions, we calculated mean parameter estimates and 95 % highest posterior density (HPD) and credible intervals (CIs). We estimated the significance of model parameters by examining CIs, considering parameters with CIs overlapping with zero as non-significant. To estimate the phylogenetic signal in the models we used Pagel's λ (Pagel, 1999), estimated simultaneously with the models by calculating the mean of the posterior distribution and the 95 % CI of λ as indicated by De Villemereuil and Nakagawa (2014). When $\lambda = 0$, related taxa are no more similar than expected by chance, while when $\lambda = 1$, the trait is evolving following a constant variance random walk or Brownian motion model; intermediate values of λ indicate a phylogenetic correlation in trait evolution that does not fully follow a Brownian motion model (Pagel, 1999). The detailed output of the models is available at GitHub (see Data Availability Statement).

Seed traits versus species environmental preferences. We used the Iberian dataset (available at GitHub, see Data Availability Statement) to check whether seed traits and plant ecological preferences were related. The used dataset contained 76 species with complete trait data for seed germination, seed morphology and ecological requirements. We did a principal component analysis (PCA) of seed traits and species SNCs for cold, drought and pH. We performed the PCA ordination at the species level, i.e. calculating a series of continuous seed traits for each species. We transformed the final germination proportions to create a continuous variable for the germination cues (i.e. stratification, scarification, temperature, alternating temperatures and light). To do so, for each cue and species, we calculated a weighted average of the cue levels (in the case of temperature, cue levels were the temperature treatments; for the other cues, the levels were 0 = absence and 1 = presence), weighting by the germination proportion at each level. This approach underrepresents the importance of the levels that had not been tested for a given species, but can serve as a proxy of the response to the

germination cues when visualized across the whole dataset. We also included seed mass and seed number in the ordination (species average values). We left GA₃ out of the PCA because its ecological interpretation is subordinated to stratification (as both cues break physiological seed dormancy). We also left seed length and width out because these values were not available for enough species. We calculated the PCA with the package *FactoMineR* (Lê et al., 2008).

Germination in meadows versus other habitats. We used the Cantabrian dataset (available at GitHub, see Data Availability Statement) to compare the germination of mesic meadows with that of other herbaceous communities from the same region. The Cantabrian dataset included 131 plant species. We analysed this dataset by PCA.

RESULTS

Seed morphology

Poaceae had lower values of seed mass and higher values of seed number, while Fabaceae had heavier but fewer seeds (Fig. 2). The other families covered the range of values showed by Poaceae and Fabaceae, but their median values were high for both traits: their median seed mass was close to that of the Fabaceae, while their median seed number was higher than that of the Poaceae (Fig. 2). Seed shape also showed a divergence between Poaceae and Fabaceae, with seeds being elongated in the former and round in the latter (Fig. 2). The other families covered the full range of variation, with both elongated and round seeds (Fig. 2).

Seed germination

When considering the full set of mesic meadow species, all the studied germination cues except for stratification had a significant effect on final germination proportions (Fig. 3). The germination of meadow seeds was positively associated with scarification, GA₃, alternating temperatures, and light. Average temperature had a negative effect, indicating a trend towards higher germination at lower temperatures. Averaging the whole dataset, the highest germination proportions were achieved at 20 °C. Between 0 and 20 °C, germination proportions increased steadily with increasing temperatures. Above 20 °C, germination declined more sharply, and meadow seeds rarely germinated at 30 °C.

Some differences became apparent when dividing the dataset in the three floristic groups that compose mesic meadow vegetation. In Poaceae (Fig. 3), no effect was found for scarification, GA₃ or temperature. Stratification had a negative effect on germination. The major drivers of Poaceae germination appeared to be alternating temperatures and light, with both having a positive effect. In Fabaceae (Fig. 3), the largest positive effect on germination was produced by scarification, with no effect of stratification, GA₃, alternating temperatures or light. Temperature had a negative effect, and in fact Fabaceae species had higher germination proportions at temperatures under 20 °C. Finally, in the remaining families (Fig. 3), the main cues

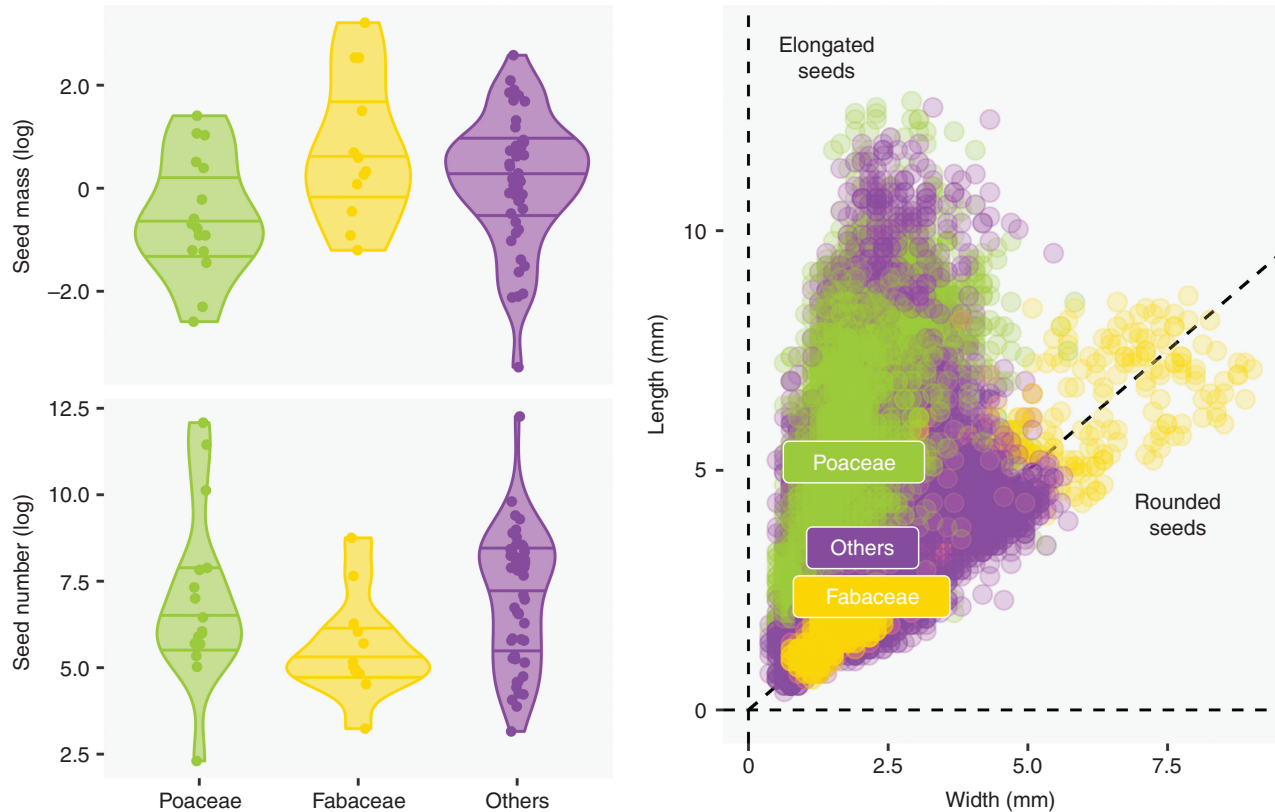


FIG. 2. Morphology of mesic meadow seeds. The two panels on the left show the probability densities of species values for seed mass and seed number, log-transformed for ease of visualization. The three horizontal lines within the probability densities represent the first quartile, the median and the third quartile of the values. The panel on the right shows values of seed length and width obtained by image analysis, each point being a seed. In all cases, data are divided between the grasses (Poaceae), legumes (Fabaceae) and the other plant families.

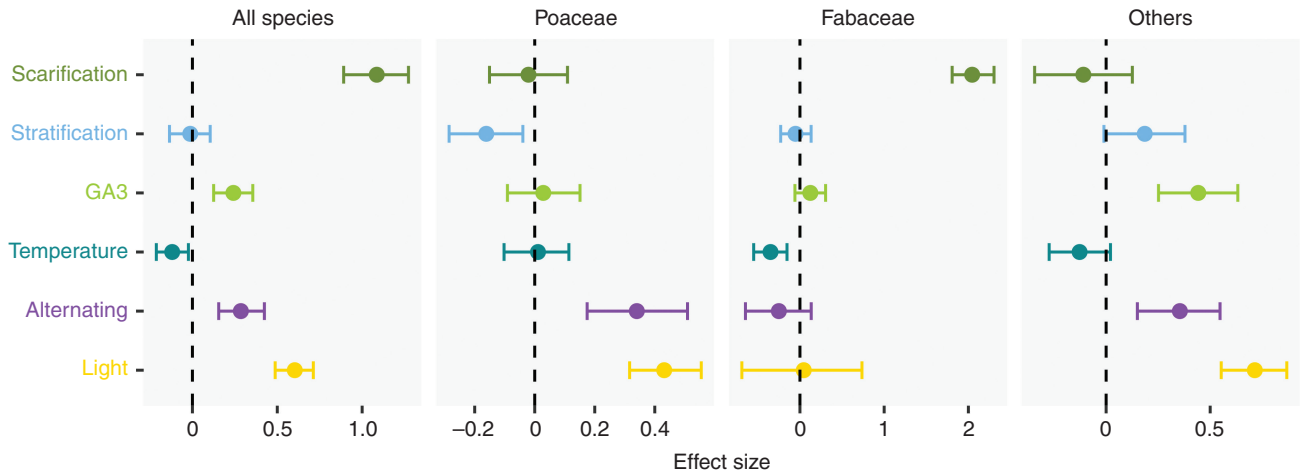


FIG. 3. Germination cues in mesic meadows. Effect of germination cues simulated in the laboratory over the final germination proportions of mesic meadow seeds. Dots indicate the posterior mean of the effect size for each cue, and whiskers the 95 % CI of the effect size. The line of zero effect is shown: when a credible interval overlaps with the zero-effect line, the effect can be regarded as non-significant. In separate panels, the figure shows the results of a general model including data for all species, plus specific models for the three main botanical groups of mesic meadows: grasses (Poaceae), legumes (Fabaceae) and the other families.

having a positive effect on germination were GA₃, alternating temperatures and light. These species did not respond to scarification, stratification or average temperatures.

To describe the effects of the random factors, we will refer only to the full model that included all species in the dataset.

The strongest effect was that of the phylogeny (mean = 5.42, CI = 2.91–8.2), followed by the source of the data (mean = 3.06, CI = 1.43–5.13) and the seed lot (mean = 1.28, CI = 0.94–1.64). The phylogenetic signal in the germination responses was relatively high ($\lambda = 0.68$, CI = 0.57–0.79).

Seed traits and species ecological preferences

Principal component analysis indicated a clear separation between environmental preferences and seed traits, with each set of variables contributing to different axes (Fig. 4). The first PCA axis explained 29 % of the variation and was related to environmental preferences. The variables with the largest contribution to this first axis were soil reaction (pH), winter cold (bio06) and summer rainfall (bio14). This horizontal axis separated (i, left) species with preferences for sites with warm winter temperatures from (ii, right) species with preferences for sites with high summer rainfall and less acidic soils. Axis 2 explained 17 % of the variability, mostly related to seed traits. The main contributing variables were seed mass, seed number and the germination response to scarification. This axis separated (iii, bottom) Poaceae species that produce many seeds with a positive germination response to alternating temperatures from

(iv, top) Fabaceae species that produce heavy seeds with a positive response to scarification.

Comparison with other habitats

Ordination resulted in a separation of mesic meadow species from plant species belonging to natural herbaceous communities of the same region (Fig. 5). Axis 1 explained 78 % of the variance and was positively associated with high germination at all three temperature treatments. This horizontal axis separated (i, left) species with low germination across treatments from (ii, right) species with high germinability across treatments. The second axis explained 17 % of the variation and separated (iii, bottom) species that responded more to the cool germination temperature from (iv, top) species that responded more to the warm germination treatment. Meadow species tended to

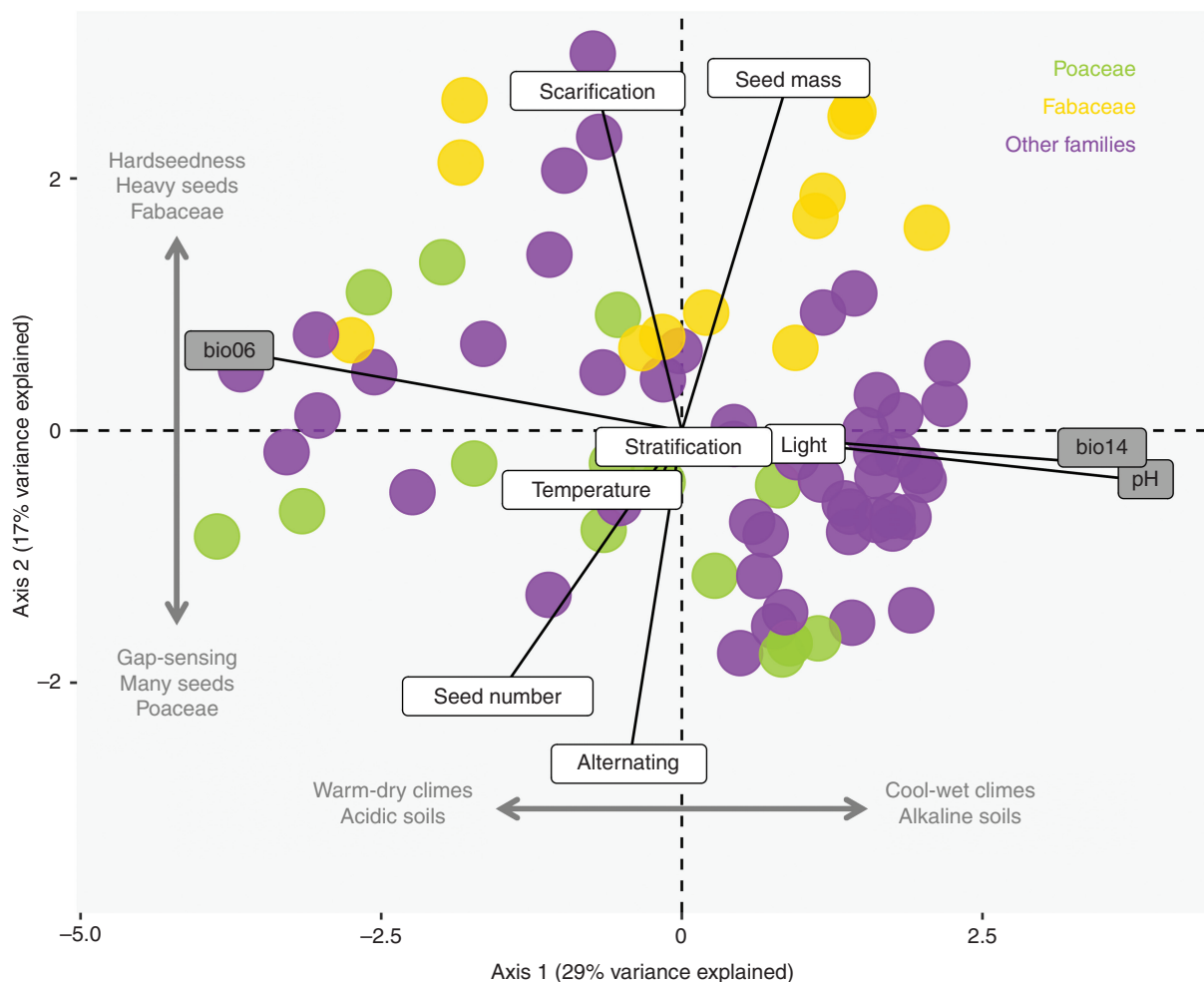


FIG. 4. Environment and seed traits are separate axes of variation in mesic meadows. Principal component analysis ordination of mesic meadow species considering their environmental preferences and their seed traits. Each point is a species, coloured by the three main botanical groups of mesic meadows: grasses (Poaceae), legumes (Fabaceae) and the other families. Labels indicate the contribution of the variables to the axes: grey-background labels for environmental preferences and white-background labels for seed traits. Environmental preferences were calculated as species niche centroids (SNCs) for the minimal temperature of the coldest month (bio06), precipitation of the driest month (bio14) and soil pH. To calculate the SNCs, a vegetation dataset of mesic meadows of the Iberian Peninsula was used. The seed traits are seed mass and the germination relative indices for the response to scarification, stratification, average germination temperature (temperature), alternating temperature (alternating) and light. All environmental preferences aligned to the first axis, while seed traits aligned to the second axis, showing that environment and seed traits are separate axes of variation in mesic meadows. Stratification and average germination temperature showed very low variation, in accordance with their small effect in Fig. 2.

be situated in the right sector of the ordination, indicating high germinability, and at the centre of the vertical axis, indicating a neutral response to temperature. On the other hand, species from the other communities were positioned at the left of the horizontal axis, indicating a lower germinability; and were more separated along the vertical axis, indicating a preference for either warmer (bogs and fens) or cooler (alpine grasslands, coastal communities) germination treatments.

DISCUSSION

Our meta-analysis of germination records showed that the seed ecology of mesic meadows is characterized by (1) high germinability when compared with other herbaceous plant communities; (2) low correspondence between seed trait variability and the natural environmental drivers of mesic meadow diversity; and (3) a deep phylogenetic separation between the two major families, Poaceae and Fabaceae.

We found that alternating temperatures and light are the two most influential factors triggering germination in all taxonomic groups except for Fabaceae. Alternating temperatures and

light are usually considered to be micro-environmental cues that indicate, at a fine scale, the existence of safe sites for regeneration (Jumpponen *et al.*, 1999). The diurnal alternation of temperature decreases with burial depth in the soil, and by the presence of vegetation cover (Thompson *et al.*, 1977; Van Assche and Vanlerberghe, 1989; Saatkamp *et al.*, 2011). Thus, a positive germination response to alternating temperatures could detect the depth of seed burial and prevent the germination of seeds that are buried too deep for seedlings to survive before emerging from the ground (Bond *et al.*, 1999). Perhaps more importantly in mesic meadows, alternating temperatures could also indicate that the vegetation cover has been diminished by either mowing or grazing, marking an appropriate time for seedling regeneration, when the competition by the established plants is lessened. Similar functions can be ascribed to the germination response to light, as physiologically active light in significant amounts only travels the uppermost millimetres of soil (Tester and Morris, 1987) and the quality of light will be affected by vegetation cover (Jankowska-Błaszczuk and Daws, 2007). Therefore, the germination response to alternating temperatures and light indicates conditions that are

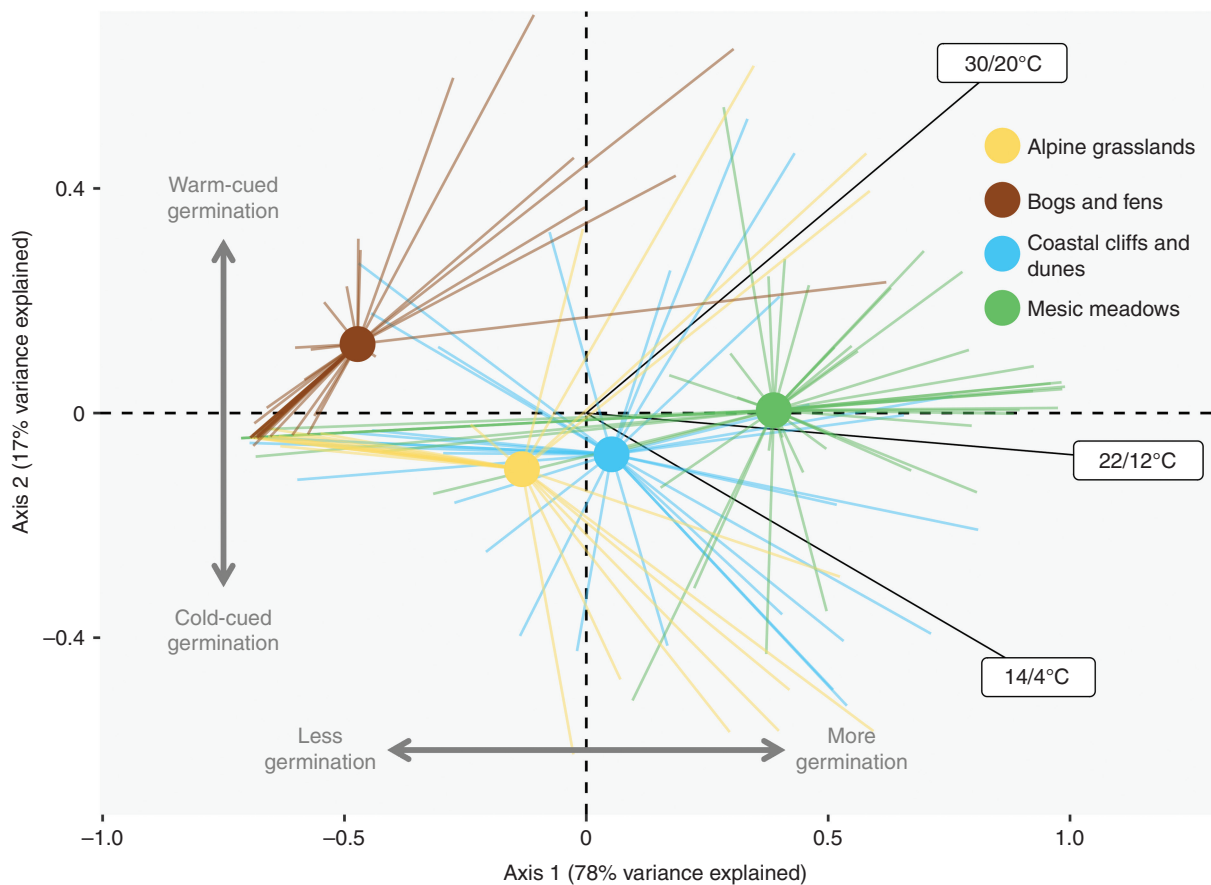


FIG. 5. Higher germinability in mesic meadows compared with other herbaceous plant communities. Principal component analysis ordination of species responses to three seed germination treatments (fresh seeds germinated at 30/20, 22/12 and 14/4 °C in a 12/12-h light/darkness photoperiod). Species are grouped by their habitat, with points showing the centroid for each habitat group, and rays linking the centroid with the position of each species in the group. Labels indicate the contribution of the germination treatments to the axes. All species had been collected in herbaceous vegetation types of the Cantabrian Mountains of Spain and the neighbouring coast. All seeds were untreated, except for scarification, which was applied routinely to all botanical families presenting physical dormancy. Germination treatments consisted in 12/12-h periods with a warmer phase in light and a cooler phase in darkness. The position of mesic meadows compared with the other habitats indicates that their species tended to have higher germinability even if untreated (i.e. less seed dormancy) and were more neutral in their thermal requirements.

found immediately after mowing for haymaking, a predictable perturbation that occurs approximately at the same time every year, and which forces meadow plants to establish, grow and reproduce in a regular time frame (Grime, 2006; Klimešová *et al.*, 2010). Theoretically, these germination traits would also promote the formation of a transient soil seed bank (Williams, 1983b; Venn and Morgan, 2010). But it is worth mentioning that Poaceae, which show the germination response to alternating temperatures and light, also have the more elongated seed shape, a trait that is thought to reduce the capacity of seeds to enter the soil seed bank in temperate meadows (Thompson *et al.*, 1993; Funes *et al.*, 1999).

The strong effect of scarification was specifically related to the hard-seeded Fabaceae. In this family, physical dormancy as a result of an impermeable seed coat works as a mechanism to detect seasonal cycles of temperature and humidity (Van Assche *et al.*, 2003). It has been also proposed that physical dormancy can prevent seed predation, by preventing the emission of olfactory cues that are elicited by seed imbibition and that can be perceived by seed predators (Paulsen *et al.*, 2013). In the case of this study, Fabaceae seeds are amongst the heaviest, and they clearly follow a separate regeneration strategy from that of the other families. While other groups rely on alternating temperatures and light as gap-detecting mechanisms, Fabaceae seeds are regulated instead by scarification, while they also respond to cooler temperatures than the rest of the families. This suggests that Fabaceae seeds would tend to germinate when temperatures are cooler, and thus before or after the summer haymaking season. Their larger size, and the related larger reserves, could allow Fabaceae seedlings to emerge from greater depths (Bond *et al.*, 1999), e.g. when the meadow grass is still high before mowing. Furthermore, larger seed size also increases seed survival in cattle dung (Peco *et al.*, 2006) and thus improves the capacity to be dispersed by animal depositions (Traba *et al.*, 2003).

Seed germination responses to average temperatures and stratification are understood to be a mechanism to track seasonal climatic cycles (Finch-Savage and Leubner-Metzger, 2006). Apart from the response to cool temperatures in Fabaceae, the rest of the families did not show a response to average temperatures, further highlighting their reliance on micro-habitat and short-term cueing. Moreover, the dominant family, Poaceae, showed a negative response to cold stratification. The less frequent families, however, showed a divergence from the Poaceae strategy: their positive response to GA₃. Gibberellic acid is a phytohormone that can work as a substitute for cold stratification to overcome physiological dormancy (Bewley *et al.*, 2013). The response to GA₃ indicates which, the non-dominant species have a degree of physiological dormancy, which could postpone their germination until overwintering has occurred (Baskin and Baskin, 2014).

The strong phylogenetic signal in germination responses highlights the phylogenetic clustering of regeneration strategies that we have described so far. (1) The dominant family Poaceae shows a lack of response to seasonal cues (average temperature and cold stratification). Instead, Poaceae rely on large amounts of propagules and on detecting micro-niche cues (alternating temperatures, light) that can be associated with the yearly perturbation of mowing. (2) The second most-dominant family, Fabaceae, does not respond to micro-niche cues, and regulates

germination timing through scarification and cooler germination temperatures, possibly because their larger size and reserves allow their seeds to decouple their emergence timing from the mowing disturbance. (3) The minor families respond to micro-cues in a similar way to Poaceae, but are differentiated from them by showing a positive response to GA₃, indicating that they rely on physiological seed dormancy to track seasonal cycles. This third strategy would make it possible to fine-tune their germination to the micro-environmental conditions plus the inter-annual climatic variation. Phylogenetic clustering is also apparent regarding seed shape, mass and number, with Poaceae producing many elongated and light seeds, and Fabaceae producing fewer, rounder and heavier seeds. The shape of Poaceae seeds could make them particularly successful in attaching to hay and dispersing with it, and Poaceae seeds tend to be overrepresented in seed mixes obtained via haymaking (Scotton *et al.*, 2009; Haslgrübler *et al.*, 2014). Asteraceae and Apiaceae, some of the most abundant among the minor families, also tend to have shapes resembling those of Poaceae.

As we have seen, the regeneration strategy of high germinability appears to be general in meadow species, across regional environmental gradients. Seed traits usually show variation along environmental gradients; some examples include warm-cued germination in species from colder sites (Rosbakh and Poschlod, 2015) or smaller seeds in species from higher elevations (Wang *et al.*, 2014). Such relationships are missing in the hay meadows of the Iberian Peninsula, highlighting the semi-natural character of this vegetation, conditioned by human management as much as by environmental constraints. To some extent, the lower germinability that we found in coastal habitats, mires and alpine grasslands could be a result of the stressors imposed by salinity, waterlogging and temperature extremes. At the same time, the seed ecology of meadow species could be a response to the potential selective force that is the predictable perturbation by yearly mowing. Past research supports the relationship between mowing and germination timing. In an abandoned meadow of the Swiss Prealps, resuming mowing promoted germination and emergence, although natural regeneration was limited due to a lack of seed dispersal to the site (Kupferschmid *et al.*, 2000). Mowing also promoted autumn germination in a dry grassland of northern Germany (Kahmen and Poschlod, 2008). Past studies also agree with the lack of dormancy and the high germinability of meadow seeds that we have found. For example, meadow seeds of *Poa trivialis* were less dormant than seeds of the same species collected in an arable field (Froud-Williams *et al.*, 1986). Ten Brink *et al.* (2013), when comparing congeneric herbaceous species from open grasslands or forest habitats, found that species from the open grasslands needed less cold stratification. Similarly, seeds collected from a hay meadow germinated better when left untreated, rather than when being exposed to dormancy-breaking treatments (Haslgrübler *et al.*, 2014). The high-germinability syndrome as a response to artificial selection by mowing is indeed an intriguing hypothesis, and future work should be designed to test it.

The high-germinability strategy, however, has the practical consequence of greatly limiting the long-term seed bank persistence of mesic meadow species: the soil seed bank has been reported as being transient in hay meadows and related grasslands

(Milberg, 1992; McDonald, 1993; Hutchings and Booth, 1996). In Poland, the soil bank of a hay meadow was dominated by arable and weedy forbs, with low representation of Poaceae and Fabaceae (Janicka, 2017). The inability to form persistent seed banks leads to dispersal limitations to passive natural regeneration (Kupferschmid *et al.*, 2000), making mesic meadows quite sensitive to land use change, and stressing the importance of active actions of meadow restoration via seed supply.

Understanding the germination requirements of the different plant groups that coexist in mesic meadows can help to manage, conserve and restore their biological diversity. For centuries, human activities have shaped the regeneration of hay meadows, and apparently this has led to a loss of seed dormancy, decoupling seeds from seasonal cycles, as has been found in many domesticated species (Dürr *et al.*, 2015). The same anthropic processes that have shaped semi-natural mesic meadows may have left them dependent on continued human intervention for their regeneration. The high germinability of meadow seeds makes them relatively easy to use in restoration projects. But it also creates a fascinating dilemma to the restoration practitioner: instead of sourcing seed from existing – and seemingly domesticated – meadows, it could be advisable to also source seeds from related wild populations of meadow species, which may hold the genetic variability and phenotypic plasticity to cope with the threats posed by new environmental challenges (Parmesan and Hanley, 2015).

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AUTHOR CONTRIBUTIONS

All authors contributed data. E.F.P. conceived the study and performed the analyses. E.F.P. wrote the manuscript with help from A.C. All authors revised the manuscript and approved the final version.

OPEN DATA

The original data, R code for the analysis and creation of the manuscript can be accessed at the GitHub repository <https://github.com/efernandezpascual/meadows>. Upon publication, a version of record of the repository will be deposited in Zenodo.

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