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External conditions, seed characteristics and other factors affecting seed germination of alpine species

Vnější podmínky, vlastnosti semen a další faktory ovlivňující klíčení semen alpínských druhů

Doctoral thesis/Disertační práce

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tuto práci ani její podstatnou část jsem nepředložila k získání jiného nebo stejného akademického titulu.

I hereby declare that I made this thesis independently, using only the mentioned references. I did not submit this thesis nor its part for any other degree or diploma.

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Content

ABSTRAKT

Klíčení je nejkritičtější a nejzranitelnější fází v životním cyklu mnoha rostlin a ovlivňuje regenerační potenciál populací, a tím i dlouhodobé přežití druhů. Klíčivost je ovlivněna vnějšími podmínkami, jako je světlo, teplo a vlhkost, působících jak během klíčení (cílové podmínky), tak na mateřské rostliny během jejich života (původní podmínky). Cílové a původní podmínky mohou interagovat a ovlivňovat klíčení semen. Silný vliv teploty a vlhkosti na klíčení semen naznačuje, že úspěšné rozmnožení může být silně ovlivněno probíhajícími klimatickými změnami. Současně je klíčivost druhů ovlivňována vlastnostmi samotných semen jako jejich hmotnost nebo obsah živin v semeni. Tyto vlastnosti mohou být také ovlivněny vnějšími klimatickými podmínkami.

Cílem této disertační práce je i) prozkoumat vliv teploty a vlhkosti působící jednotlivě i v interakci během aktuálních podmínek klíčení (vliv cílových podmínek), během života mateřských rostlin (vliv původu) a v interakci cílových a původních podmínek na klíčivost alpínských druhů; ii) studovat vliv původu na hmotnost semen, obsah živin v semenech a jejich vliv na klíčivost; iii) prozkoumat, jak je hojnost druhů ve středoevropském pohoří ovlivněna jejich klíčivostí.

Zjistila jsem, že i) cílové podmínky, původní podmínky i interakce mezi cílovými a původními podmínkami ovlivnily klíčení. Nejnižší procento vyklíčených semen a nejvyšší rychlost klíčení byla v extrémně teplých a suchých cílových podmínkách. V těchto cílových podmínkách se podíl dormantních semen liší mezi studovanými druhy od velmi nízkého po vysoký počet semen. Nejvyšší procento klíčených semen bylo v teplých a vlhkých cílových podmínkách. Výjimkou byl studovaný rod *Impatiens* (pocházející z Himalájí), jejichž procento klíčených semen se zvýšilo v chladných cílových podmínkách. Rostliny pocházející z teplých původních podmínek vykazovaly nejvyšší procento vyklíčených semen. Interakce mezi cílovými a původními podmínkami dále ukázaly, že podmínky, ve kterých druhy běžně rostou, nejsou vždy ideální pro klíčivost semen, jelikož změna podmínek zvýšila klíčivost. ii) hmotnost semen a obsah dusíku v semenech byly výrazně ovlivněny podmínkami původu, zatímco obsah sacharidů a fosforu ne. Vyšší hmotnost semen způsobila vyšší a rychlejší klíčení, zejména při vyšší teplotě klíčení; iii) vzácné druhy měly vyšší podíl vyklíčených semen než jejich blízce příbuzné druhy hojné. Načasování klíčení bylo však odlišné. Vzácné druhy klíčily zejména po projití chladovou stratifikací, zatímco běžné druhy klíčily především během chladové stratifikace.

Tato práce ukazuje, že je důležité zahrnout cílové i původní podmínky a zejména jejich interakci do studií klíčení. Pro predikci budoucího vývoje druhů v odezvě na změny klimatu je nutné se zaměřit na reakce druhů na změny podmínek z původních na nové. Dále práce ilustrovala, že klíčení je modifikováno hmotností semen, obsahem živin v semenech a současně jsou tyto vlastnosti ovlivněny klimatem původu. Nakonec jsem ukázala, že vzácné druhy jsou dobře přizpůsobeny místním podmínkám v horách a neschopnost klíčit není příčinou jejich vzácnosti.

Klíčová slova: reprodukční vlastnosti; hmotnost semen; alpínské druhy; podmínky původu; podmínky v průběhu klíčení; efekt matky; fylogenetika; druhová vzácnost

ABSTRACT

Germination is the most critical and vulnerable stage in the life cycle of many plants and it directly influences the regeneration potential of populations and thus long-term survival of species. Species germination is affected by external conditions such as light, temperature and moisture acting both during germination (target conditions) and on maternal plants during their life (original conditions). Target and original conditions can interact and affect seed germination as well. The strong temperature and moisture control of seed germination suggests that species recruitment rates and success may be strongly impacted by ongoing climatic changes. Simultaneously, species germination is influenced by characteristics of the seeds themselves, such as, seed mass or seed nutrient content, which can be affected by external climatic conditions as well.

This thesis aimed to i) investigate the effect of temperature and moisture acting separately and in interaction as target conditions, original conditions and as interaction between target and original conditions on germination behaviour of alpine species; ii) study effect of seed origin on seed mass and seed nutrient content and their effect on germination; iii) explore how abundance of species is affected by germination behaviour in central European mountains.

I found that i) both target and original conditions and their interaction affected germination behaviour. The lowest germination percentage and the highest speed was observed in extreme warm, dry target conditions. In these conditions, proportion of dormant seeds varied between study species from very low to high. The highest germination percentage was found in warm, wet target conditions. The exception was genus *Impatiens* (coming from the Himalayas), in which germination percentage increased in cold target conditions. Plants coming from warm original conditions showed the highest germination percentage. Further, interactions of target and original conditions demonstrated that the conditions in which species commonly grow are not necessarily ideal for seed germination, as a change in conditions increased the germination. ii) seed mass and nitrogen content in seeds were significantly affected by original climate, while carbohydrates and phosphorus were not. Higher seed mass caused higher and faster germination especially in warmer target temperature; iii) rare species had higher germination than those of their common congeners. Timing of germination was different. Rare species had higher germination after cold stratification and common species had higher germination during cold stratification.

Overall, I showed the importance of including both target and original conditions and especially their interaction into germination studies. For predicting future fates of species under climate change it is necessary to focus on the species responses to changes in conditions from original to novel. Further, the thesis illustrated that germination behavior is modified by seed mass, seed nutrient content and simultaneously, these traits are affected by climate of origin. Finally, I showed that rare species are apparently well adapted to local conditions in the mountains and the inability to germinate is not a cause of their rarity.

Key words: reproduction characteristics; seed mass; alpine species; original environment; target environment; maternal effects; phylogeny; species rarity

INTRODUCTION

Germination is one of the most critical stages in the life cycle of many plants (Donohue 2005). It is the primary source of variation in the regeneration niche (Pearson et al., 2002) and has an impact on plant fitness (Moles et al. 2005; Moles et al. 2007; Bolmgren and Cowan 2008; Hallett, Standish and Hobbs 2011; Turnbull et al. 2012; Baskin and Baskin 2014). During germination, respiratory activity rapidly increase, nutrients are mobilized and it is initiated of embryo growth (Fenner and Thompson 2005; Baskin and Baskin 2014). Germination at the right time and place largely determines the ability of the seeds to grow up to adult plants. Not surprisingly, the dormancy characteristics and the ability to germinate are under strong selection pressure and are considered as a key elements in plant life strategies (Schütz and Rave 1999).

Role of germination in life cycle of plants

The plants follow cycling process, which can be divided into several developmental phases: seed, germination, growth, reproduction and seed spreading. Monocarpic plants die at the end of this cycle, while polycarpic not and the length of the cycle can range from one year to decades, which depends on plant life form and environmental conditions (Martinez-Zapater et al. 1994).

The plant life cycle starts with seeds, which fundamentally are means of reproduction, and most seeds are the product of sexual reproduction which produces novel genetic combinations leading to novel phenotype variability on which natural selection can act. Seed protects the embryo and seed reserves nourish the embryo or seedling. When a seed falls on the soil, they commonly enter primary dormancy (Baskin and Baskin 2014), which develops during seed development on the mother plants (Hilhorst 1995; Bewley 1997; Finch-Savage and Leubner-Metzger 2006). This mechanism is used for protection of sensitive parts of plants and affects the timing of germination and thus increases the likelihood of seedling survival as the probability of seeds germinating under very suitable conditions increases (Fenner and Thompson 2005). There are species, which do not enter the dormancy and germinate immediately after ripening, however, most of them grow in tropical zone (Baskin and Baskin 1988). Different classes of seed dormancy have been described that include physiological dormancy, morphological dormancy, morphophysiological dormancy, physical dormancy, and combinational dormancy (Finch-Savage and Leubner-Metzger 2006; Baskin and Baskin 2014). The intensity of dormancy (deep, intermediate and non-deep) depends on genotype and environmental conditions during seed development (Haile and Shirdiffe 2014; Huang et al. 2016). Similarly, seed ability to stay viable in the soil is different and is affected by genotype and environmental conditions. Alpine seeds remain viable over several years (Weilenmann 1981; Schwienbacher, Marcante and Erschbamer 2010), in some cases germination even increases with time (Billings and Mooney 1968).

Seeds, which do not enter primary dormancy or have entered primary dormancy and are still in the soil, can enter secondary dormancy as a reaction to unfavourable conditions for gemination (Fenner and Thompson 2005; Baskin and Baskin 2014; Soltani, Baskin and Baskin 2019). This mechanism can be used, for instance, in summer to prevent germination in response to high temperatures or drought (Schütz and Rave 1999).

Before germination itself, dormancy must be broken, which is affected by many environmental factors (Fenner and Thompson 2005). Temperature, moisture and light are consider to be the most important in the timing of germination (Baskin and Baskin 1988) and thus are described in more details bellow. Process of germination itself is following. During the beginning stage of the germination, the seeds sucking water rapidly and this results in swelling and softening of the seed coat at an optimum temperature. Rupturing of the seed coats helps to emerge the radicle and the plumule to form a primary root. After the emergence of the radicle and the plumule, the seed activates its internal physiology and starts to respire and produce proteins and metabolize the stored nutrients (Baskin and Baskin 2014). Because characteristics of dormancy and subsequent germination varies within the genus and species, their knowledge may provide insights into how each species adapts to different natural habitats (Schütz and Rave 1999).

The seedling continues to grow to maturity. When plants become mature, they start to make flowers and pollen. When the pollen reaches the female part (ovary) of the flower, it fertilizes the cells inside it and produces new seeds (Raven, Evert and Eichhorn 2005). New seeds are dispersed by wind, animal, water or human activities (Fenner and Thompson 2005) and plant life cycle can start over again.

Many constrains, which not allow to successfully complete or start new plant life cycle, can occur. It was demonstrated that problems in the phase of seed production and germination of plant life cycle can contribute to lower population growth rate and thus can lead to species rarity (e.g., Baskin et al. 1997; Münzbergová 2005; Mattana, Daws and Bacchetta 2010; Combs, Lambert and Reichard 2013; Stachurska-Swakon et al. 2018). Phases of seed and germination is rightly considered to be the most vulnerable to injury, disease, and water stress (Raven, Evert and Eichhorn 2005). Particularly under harsh environmental conditions, such as, alpine conditions, where time windows favourable for seedling establishment and survival are short and unpredictable, germination might play a highly adaptive role. The mortality between dispersal of seeds and completion of establishment can be so high that this phase in life cycle plays a key role in population dynamics, genetic composition of population and thus evolution changes.

Genetic composition of population is affected not only by the fact of which seeds germinate, but also by seed and/or pollen dispersion between and within population (Wade and McCauley 1988; Whitlock and McCauley 1990; Ellstrand 1992). In addition, broader range of genotypes can persist in soil seed bank, which can enhance adult population (Cruzan 2018). Evolutionary changes have been demonstrated, for instance, for seed size (Rees 1996; Alcantara and Rey 2003), seed dispersion (Vander Wall 2001) or germination speed (Donohue et al. 2005; Kadereit, Newton and Vandelook 2017). As follows from the text above and as some studies indicate, seed dormancy and germination may account for the success of the dominant species and relative abundance of species (Amen 1966; (Brändle et al. 2003; Brown, Enright and Miller 2003; Simon and Hay 2003; Ramirez-Padilla and Valverde 2005; Mattana et al. 2010; Hewitt et al. 2015).

Environmental conditions in alpine areas

Environmental conditions, with which plants and their seeds must cope in temperate alpine areas are considerably different from conditions in lowlands. Temperature conditions are considered to be the most limiting factor with regard to plant growth and reproduction there (Bliss 1962). Alpine areas are characterized by short and cold vegetation period (Körner 2003) with high differences in temperature between day and night, thus plants have to adapt their life cycle (Billings 1987). Precipitation increase with altitude and most of them is in the form of snow. Snow cover often lasts for several months (Körner 2003). In spring, late frosts commonly occur, which may damage the seedlings (Baskin and Baskin 2014). Seeds for successfully germination commonly did not require special light/dark regime (Körner 2003). Soils in alpine areas tend to be nutrient poor, which requires high maternal investments in sexual reproduction (Urbanska and Schutz 1986). All these reasons indicate that time windows favourable for germination are shorter and harder predictable than in lowlands, which lead to that alpine species are more limited by seedling establishment than species with lowland distribution (Mendoza et al. 2009).

In the context of climate change, alpine environments require special attention as they are considered of the most vulnerable system (Körner 2007). Species have limited possibilities to migrate to colder conditions (they are already at the peak of mountains, (Bliss 1962)) and must therefore respond via adaptation or phenotypic plasticity. Simultaneously, alpine areas host considerable part of plant biodiversity (in Europe it is about 20% of total plants) with many endemic and rare species (Väre 2003). Climatic change has already increased the altitudinal and latitudinal distribution of many species from a wide range of taxa (Parmesan and Yohe 2003; Lenoir et al. 2008; Ruiz-Labourdette et al. 2012), which can lead to increasing competition in alpine areas between adult plant as well as between seedlings. However, climate change can be advantageous to some species, since it can induce longer vegetation periods due to reduced snowfall and earlier snowmelt (Beniston 2012; Pederson, Betancourt and McCabe 2013; Sanchez-Bayo and Green 2013). However, the effect of climate change on seeds and seedlings of alpine species has been largely neglected (reviewed in Briceno, Hoyle and Nicotra 2015). Thus, I focused on germination of alpine species in all my studies.

Factors and processes determining plant germination

A large part of the seeds fails to become a seedling. Their fate after dispersion depends on both biotic and abiotic factors (Koike et al. 2012). Seeds can get too deep into the soil and the sprout is not able to get the surface (Koike et al. 2012), can be attacked by pathogens (Pringle, Alvarez-Loayza and Terborgh 2007; Beckman and Muller-Landau 2011) or can become part of the animal food (Fenner and Thompson 2005; Koike et al. 2012). Risk of pathogens and herbivory especially by vertebrates decreases with elevation (Urbanska and Schutz 1986; Galen 1990; Gworek, Wall and Brussard 2007; Speed et al. 2012).

The breaking of dormancy and subsequent germination is strongly influenced by light, temperature and moisture. Effect of light is driven by phytochrome system (Flint, 1934). Phytochrome system provides information to seeds about sunlight quality, quantity and ratio between its wavelengths, which indicates the length of day a thus part of year and presence of vegetation in the surrounding (Borthwick et al. 1952). There are species that germinate significantly more in the dark and species that have no preference in this regard. However, most of species prefer light to dark for germination, therefore variability between species is not too high (Grime et al. 1981; Baskin and Baskin 1988) and in comparison with temperature, light plays minor role in determining the season suitable for germination (Baskin and Baskin 1988).

The most important factor in breaking dormancy and germination is temperature during these processes (Baskin and Baskin 1988) (further referred as a target temperature). Temperature is a good indicator of what part of the year it is (Fenner and Thompson 2005). From these reasons the response of seed germination to temperature has been extensively studied (e.g., Baskin and Baskin 1994; Schütz and Rave 1999; Gardarin, Daurr and Colbach 2011; Rosbakh and Poschlod 2015). Alpine species are exposed high temperature differences between day and night, thus higher germination was observed in fluctuating temperatures than in constant temperatures (Billings and Mooney 1968; Bliss 1971). The strong temperature control of seed germination implies that species' recruitment rates and success may be strongly impacted by the ongoing climatic changes, which is studied less frequently, but number of studies slowly increase (reviewed in Walck et al. 2011).

As germination is a catabolic process that supplies important nutrients to the future plant, chemical changes occur within the seed before germination itself. This process is mainly controlled by the phytohormone abscisic acid (ABA), which prevents untimely seed germination. If the seeds are exposed to a temperature that breaks the dormancy of the species, whether higher or lower, ABA is inactivated (Kushiro et al. 2004). At this point, the concentration of lipids and phytic acid decreases and the amount of starch and vitamins increases. After these changes in the chemical composition, germination itself takes place (Deruiz and Bressani 1990).

The optimal temperature for breaking dormancy and subsequent germination vary among species (Grime et al. 1981), but species that require cold stratification for breaking dormancy are the most common, especially in temperate zone (Baskin and Baskin 1988) or alpine areas (Söyrinki 1938; Billings and Mooney 1968; Meyer 1992). These species break dormancy during the cold period and germination occurs with increased temperatures (Baskin and Baskin 1988). Variation in duration of cold period along altitudinal gradients has promoted different adaptations in many alpine plants, such as the generation of frost resistance (Venn, Morgan and Lord 2013) or postponing germination until accumulation of warm temperatures will be enough to avoid damages of seedlings by late frosts (Billings and Mooney 1968; Schütz 2002; Fernandez-Pascual, Jimenez-Alfaro and Bueno 2017). Therefore, necessary stratification duration is usually prolonged along elevation gradient (Cavieres and Arroyo 2000). The ongoing climate change may shorten the stratification period (IPCC 2014) possibly leading to decreasing germination in some species (Garcia-Fernandez et al. 2015; Carta et al. 2016b). However, the effects of reduced stratification on species germination in warmer climates as a possible consequence of climate change, has not yet been studied, thus I included effect of this temperature changes on germination behaviour into my research (**Study 3**).

Beside light and temperature, germination is strongly dependent on moisture (Baskin and Baskin 2014), since germination is initiated by sucking of water (Fenner and Thompson 2005). Similar to temperature, strong moisture control of seed germination implies that species' recruitment rates and success may be strongly impacted by the ongoing climatic changes. Climate change could bring changes in precipitation. The general pattern suggests that the frequency of extreme meteorological events (drought and floods) will increase (IPCC 2014). The species response to different moisture conditions is studied less frequently than response to temperature since it is more difficult to control it. Several recent studies have shown that the number of germinated seeds and the germination speed decreases with decreasing moisture (e.g., Wen et al. 2015; Ruhl et al. 2015).

The effects of target temperature and moisture may also interact in their effects on seed germination and the interaction may be more important than either variable alone (e.g., Rivas-Arancibia et al. 2006; Walck et al. 2011; Gurvich et al. 2017; (Flores, Perez-Sanchez and Jurado 2017). The interaction of temperature and moisture has also been shown by several recent studies at intraspecific level (e.g., Miranda et al. 2014; Simao, Takaki and Cardoso 2010; Bauk et al. 2017). These previous studies on temperature and moisture interaction are for arid or semiarid species. To our best knowledge, only one such study exists for alpine environments (Orsenigo et al. 2015) indicating decrease of germination in warm, dry conditions. To extend our knowledge of temperature and moisture interactions for alpine species, I studied this in my research (**Study 1, 2**).

Germination response may depend not only on current conditions, but can also differ intra-specifically due to species adaptation to the climate of origin (Meyer, Allen and Beckstead 1997; Qaderi and Cavers 2002; Degreef et al. 2002). Recent studies, for instance, showed that germination percentage is significantly different between populations of a single species and that the highest germination is in populations coming from the warmest (i.e. lowlands) conditions (Cruz et al. 2003; Michael, Steadman and Plummer 2006; Ndihokubwayo, Nguyen and Cheng 2016; Santo, Mattana and Baechetta 2015; Bauk et al. 2017). However, Ooi, Auld and Denham (2012) illustrated the opposite trend. To broader our knowledge on this topic, I study effect of original conditions on species germination behavior in alpine areas (**Study 1, 2, 3**).

The studies cited above show that germination is highly responsive to the immediate conditions of the seeds and simultaneously is affected by the environment experienced by the mother plant i.e. target and original conditions may interact (Bauk et al. 2017). Study Bauk et al. (2017), for instance, demonstrated that populations from colder and wetter climatic conditions were more strongly affected by the combination of high temperatures and low moisture than populations from warmer and dried conditions, which indicate species adaptation to specific climatic conditions. Including different target and original conditions into one study system, allow us to simulate different climate change scenario and thus help us to understand species future fates. This knowledge is important especially in alpine regions, since there are considered as the most vulnerable system to climate changes (Körner 2007). From these reasons, I included both target and original conditions into three of my studies (**Study 1, 2, 3**).

Maternal effects

Besides the effects of genetic properties of the mother plants, germination may be affected by original environment (Roach and Wulff 1987) and by environment induced changes in epigenetic set up (Rossiter 1996; Boyko et al. 2010; Münzbergová et al. 2018) resulting in so called ‘maternal effects’. Despite the general understanding that maternal effects may be important in regulating species germination, their effects on germination have rarely been explicitly tested. To control for the effects caused by the maternal environment, many experiments do not use field-collected seeds, but instead use seeds propagated from maternal plants cultivated in common garden conditions (reviewed in Latzel 2015). However, in doing so we may miss important patterns and responses operating in the field (Münzbergová and Hadincová 2017). Further, not all epigenetic changes may be removed, due to the potential stability of some epimutations across several generations (Latzel 2015). This approach may also lead to new adaptations of the plants to the common garden environment (Münzbergová et al. 2018). To disentangle direct environmental responses and maternal effects, studies that explicitly compare patterns and responses between field-collected and garden-cultivated seeds are needed (De Vitis et al. 2014). To broader our knowledge how maternal effect influence germination response, I used field-collected and garden-cultivated in my research and compared the results **(Study 1**).

Effect of seed mass and seed nutrients on germination

One possible explanation of maternal effects could be differences in seed mass and nutrient content in the seeds. Favorable conditions can contribute to higher seed reserve and thus to higher seed mass. Relationship between seed mass and germination is commonly studied. Positive effect of seed mass on germination was demonstrate in studies of (Navarro and Guitian 2003; Wu and Du 2007; (Münzbergová and Plačková 2010; Paulů, Harčariková and Münzbergová 2017), but see e.g. (Wang et al. 2009; Wu, Li and Du 2011) for an opposite trend. The effects of seed mass on species response to changing climatic conditions, however, remain to be explored. Thus, I studied effects of seed mass, original conditions and conditions during germination and their interaction on germination behavior (**Study 2**).

Seed mass is not change only within original conditions, but it is strongly associated with phylogeny (Zhang, Du and Chen 2004; Moles et al. 2005; Norden et al. 2009; Barak et al. 2018), thus is different between group of species. Comparisons of behavior of species with different seed mass thus have to control for species relationships. The comparison of congeneric species is an easy way to achieve this. The comparison of rare and common *Cirsium* species in northern Bohemia, Czech Republic it was shown that the common species had larger seeds than rare one (Münzbergová 2005). On the other hand, Lavergne et al. (2004) and Gabrielová et al. (2013) found that seed mass was not significantly different between rare and common species. On large data set, I studied the differences in seed mass between a pair of rare and common species, because this knowledge could contribute to explanation of differences in their germination (**Study 4**).

Studies focusing on seed nutrients are less frequent than studies focusing on seed mass. Most of the previous studies focused on the nutrient allocation to plant leaves, stems, and roots, and only few studies investigated the nutrient contents of seeds (Bu et al. 2018). However, from the seed imbibition to the early growth of the radicle, a series of physiological and morphological process highly depends on the reserves in the seeds which provide the initial energy and nutrients for growth of the embryo (Muthukumar and Udaiyan 2000). Higher C content might provide enough energy for seed germination and seedling development (McGinley and Charnov 1988; Caliskan and Makineci 2014); higher contents of N and P could provide sufficient energy and material for ribosomes for their rapid germination and seedling growth (Elser et al. 2000; Makino et al. 2003; Slot, Palow and Kitajima 2013). Rees et al. (2001) suggested that heavier seeds have higher content of nutrients, which should contribute to their higher germination, but did not quantitatively demonstrate the relationship between nutrients and seed mass. I thus decided to explore this relationship in my study (**Study 2**). Effect of climate on seed nutrients is demonstrated in the study of Bu et al. (2018), where was showed interspecific differences between populations in the content of nitrogen and phosphorus, with higher content of both nutrients in alpine conditions. Intra-specific variability of nutrient content in seeds is not sufficiently explored (but see Vaughton and Ramsey 2001; Obeso 2012; Kolodziejek 2017) and to our best knowledge, the effects of nutrient content on germination behavior have not been studied yet. To broader our knowledge, I studied effect of origin climate on seed nutrients and effect of seed nutrients on germination behavior (**Study 2**).

Role of phylogenetic relatedness between species in germination

As mentioned above, seed traits including seed mass are strongly associated with phylogeny (Zhang et al. 2004; Moles et al. 2005; Norden et al. 2009; Barak et al. 2018). Phylogeny explains considerable part of variance in germination among species (Bu et al. 2008; Wang et al. 2009; Xu et al. 2014; Seglias et al. 2018) and strong phylogenetic signal was found also in seed dormancy traits (Dayrell et al. 2017). The likely cause is that phylogeny imposes limits to variability in reproductive attributes within a clade because of similar developmental and design constraints in related species (Ackerly and Donoghue 1995; Figueroa and Armesto 2001). To our knowledge, there is, however, only one study focusing on the effects of within-genus phylogeny on species' germination. It is based on seven species and showed that more closely-related species had more similar germination behaviour and the authors (Carta, Hanson and Muller, 2016a) point out that this subject requires further attention. To increase our understanding of evolution of species germination requirements, I focus on the effects of phylogeny on species germination (**Study 3**).

Possible ecological consequences of germination

As described above, germination has irreplaceable role in life cycle of many plants (Donohue et al. 2005) and therefore can have ecological and ecogeographic consequences. Thompson, Gaston and Band (1999) found that species' geographic ranges are significantly associated with the range of temperatures in which the species can germinate. Similarly, Grime et al. (1981) and Brändle et al. (2003) showed that species with a wider range of germination temperature had larger geographic ranges. Similar associations were found between the width of the germination conditions and the species ecological or geographic range in invasive and non-invasive species (e.g., Cervera and Parra-Tabla 2009; Hierro et al. 2009; Moravcová et al. 2010). However, Baskin and Baskin (1988) demonstrated that widely distributed and narrowly endemic temperate species do not differ consistently in germination characteristics. From these studies, it follows that in some cases, narrower germination conditions are associated with narrower ecological range and vice versa, which could contribute to species rarity or commonness (reviewed in Donohue et al. 2010), but this pattern is not applicable in all cases.

Studies, which compare germination characteristics in closely related species to avoid distortion by phylogeny, demonstrated that common species germinate more and on a wider range of conditions than rare species (e.g., Brown et al. 2003; Simon and Hay 2003; Ramirez-Padilla and Valverde 2005; Mattana et al. 2010; Hewitt et al. 2015). But these studies show differences in only a few closely related species (maximum 4 species) of lowland habitats. Harsh alpine environment, where suitable germination conditions are short and hard predictable, can lead to deepen differences in germination of rare and common species. To broaden our knowledge on this issue, I tested whether germination characteristics have an impact on the rarity of species, which I studied on a large data set of alpine species (**Study 4**).

The aim of this thesis is to assess the effect of temperature and moisture as target and original conditions and their interactions on germination behaviour of alpine species both intraspecifically and interspecifically. As alpine species regularly experience cold stratification and it plays important role in breaking dormancy and germination, the thesis explores effects of different length of cold stratification. This knowledge could help us predict species fate under novel climatic conditions. Simultaneously, the thesis aimed to include seed mass and seed nutrient content into germination studies, as this information could bring new insights into germination behaviour. Since knowledge of the effect of phylogeny on germination behaviour likely increase our understanding of evolution of species germination requirements, the thesis investigates the issue as well. To find out, how abundance of species is affected by germination behaviour and to account for possible differences due to phylogeny, the thesis also compares species germination behaviour between rare and common congeners in alpine area.

Overview of my research

The thesis comprises four studies that are briefly described in this chapter:

Study 1: Veselá A., Hadincová V., Vigdis V, Münzbergová Z. (submitted): Maternal effects strengthen the interaction between changes in temperature and precipitation in determining seed germination of dominant grass species

Study 2: Veselá A., Lien D., Münzbergová Z. (submitted): Plant origin determines seed mass, seed nutrients and germination behaviour of a dominant grass species

Study 3: Veselá A., Dostálek T., Rokaya M., Münzbergová Z. (submitted): Seed mass and plant origin interact to determine alpine species germination patterns along an gradient

Study 4: Paulů A., Harčariková L., Münzbergová Z., 2017: Are there systematic differences in germination between rare and common species? A case study from central European mountains. *Flora* 236–237: 15–24.

Study 1

In Study 1, we were studying species germination responses to changing climate (temperature and moisture) and how these responses depend on plant origin and are affected by maternal environment. We studied the species at twelve localities distributed along a unique natural ‘climate grid’ in western Norway (see Meineri et al. 2013; Meineri et al. 2014; Klanderud, Vandvik and Goldberg 2015). Each locality represents different type of climate while other factors (e.g. slope, bedrock, grazing history) are as similar as possible (Meineri et al. 2014). We used seeds collected from mother plants in the field and from plants collected in the field and cultivated in the experimental garden (from all the 12 localities, cultivated for 2 years) to test how possible maternal effects modify the patterns. By using two species (*Anthoxanthum alpinum*, *A. odoratum*) both collected from multiple localities, this system allows us to study interspecific and intraspecific variability in germination responses to climate change. We aimed to answer following questions: i) What is the importance of changes in temperature and moisture between site of plant origin and conditions of germination for species germination behavior and do changes in temperature and moisture interact with each other?, ii) Do the germination responses to changing climate vary between the two closely-related species possibly favoring one of the species at the expense of the other?, iii) Are these climate responses affected by plant cultivation in a common garden indicating that maternal environment is crucial in determining species germination patterns?

Results of this study indicate that germination percentage, speed as well as seed dormancy depend on the range of change in temperature and moisture between the current and the original conditions and these two factors interact. The study also demonstrated that conditions in which species commonly grow are not necessarily ideal for seed germination, as a change of conditions increased germination of *A. alpinum*. However, a change in conditions did not bring a big benefit to *A. odoratum*. Germination response to drought was strongly affected by previous cold stratification of the seeds, indicating that stratification is crucial for species ability to respond to novel climates. Finally, we demonstrated that the maternal effects can be crucial for driving seed responses to changing climate. Cultivation of maternal plants in common garden conditions strongly affected the conclusions on ability of species to adapt to novel conditions.

Study 2

In Study 2, we were focusing on the importance of original and actual climatic conditions, and thus the importance of genetic differentiation and phenotypic plasticity, for species germination using natural factorially crossed temperature and moisture gradients and widespread clonal grass species, *Festuca rubra*, as the model system. We used the same study system as in Study 1. Further, we studying the effect of plant origin on seed mass and nutrient concentration and the effect of seed mass and nutrient concentration on seed germination in different climatic conditions, because seed mass and nutrient content in the seeds may be an important mechanism affecting species germination patterns and at the same time, they may be affected by species origin further referred to as a target condition. To assess to what extent the effects detected at the level of germination correspond to the effects detected using grown-up plants, we used vegetative data from the same study system published in (Münzbergová et al. 2017) and compared the patterns to the results obtained here. The aim of this study was to answer following questions: i) Does the original climate affect seed nutrient content and seed mass and do these differences, if any, relate to seed germination patterns?, ii) What is the effect of original and target conditions on species germination patterns and do these factors interact?, iii) What is the relationship between traits related to generative and vegetative reproduction and their responses to changing climatic conditions?

Our results showed that seeds mass was strongly affected by climate with increasing seed mass in warm, wet conditions. Further, that higher seed mass is caused by higher concentration of carbohydrates. It seems that higher seed mass, and probably higher concentration of carbohydrates, may play role in higher and faster germination in seeds coming from warmer localities and simultaneously also in higher and faster germination in warm target conditions. Germination response of *F. rubra*, as a dominant species of meadows, shows high proportion of germinated or dormant seeds, which suggest phenotypic plasticity of study species. Phenotypic plasticity allows to species cope with changing climate. When the conditions during germination were unsuitable and germination was low, seeds stay dormant and probably they would be able to germinate in more suitable condition. Simultaneously, *F. rubra* show trade-off between generative and vegetative reproduction indicating that the species is able to modify investments to generative or vegetative reproduction depending on the actual conditions.

Study 3

In Study 3, we were studying germination response and necessary stratification duration of 40 populations belonging to 14 species of genus *Impatiens* coming along elevation gradient of the Himalaya mountains in Nepal. Because germination characteristics do not depend only on environmental conditions but also on seed traits such as seed mass (Wang et al. 2009; Wang et al. 2012; Liu et al. 2013; Hradilová et al. 2019), we were studying effect of seed mass and their interaction with original and target conditions on germination behaviour. Further, we were focusing on the effects of phylogeny within genus on species germination, since such knowledge is likely to increase our understanding of evolution of species germination requirements. Specifically, we were aiming to answer following questions: i) What is the effect of seed mass and original and target conditions on species germination and the necessary stratification duration?, ii) Does shorter stratification influence germination response to warmer conditions?, iii) Are the patterns affected by phylogenetic relationships among the species?

Our results demonstrate strong dependency of necessary stratification duration on original climate. Predicted climate change represented by shorter stratification period followed by warmer temperature negatively affected species germination. Germination of *Impatiens* species will thus be probably negatively affected by climate change forcing the species to migrate to higher altitudes. Heavy seeds germinate the best and the fastest when they come from warm and dry localities, while light seeds germinate the best when they come from cold and wet localities. Germination response of *Impatiens*, with exception of germination speed (T30), is driven by environmental conditions rather than by phylogeny. The only variable affected by phylogeny was seed mass indicating, that unlike germination behaviour, seed mass is unlikely to change with changing conditions.

Study 4

In Study 4, we conducted a comparative analysis of germination requirements and proportions of germinated seeds in 18 congeneric pairs of rare and common plant species occurring in the Krkonoše Mts. in the Czech Republic. Because germination depends on seed mass (e.g., Wu and Du 2007; Liu et al. 2013), we also explored differences in seed mass between the rare and common species. i) Do rare and common species differ in terms of seed mass? ii) Do rare and common species differ in germination response to temperature and light? iii) Do rare and common species have differences in proportions of germinated seeds? iv) Do differences in seed mass explain the possible effects of species rarity on germination? v) Do rare and common species have differences in the range of germination conditions?

The study showed that seed mass of rare and common congeners was not significantly different. The results indicated that after cold stratification, the proportions of germinated seeds were higher in rare than in common species. In common species, the proportions of germinated seeds were significantly higher during cold stratification than those of rare species. Rare species had higher proportions of germinated seeds than those of their common congeners, and total proportions of germinated seeds were influenced by seed mass and species rarity. The results indicated that an inability to germinate clearly did not cause species rarity in the Krkonoše Mts., and that rare species were apparently well adapted to local conditions. The range of germination conditions did not differ significantly between rare and common species.

Summary

In the presented studies, I showed strong impact of temperature and moisture acting as target conditions, original conditions and their interactions on germination of alpine species and possible ecological consequences of germination. The strong temperature and moisture control of seed germination suggests that species recruitment rates and success may be strongly impacted by ongoing climatic changes. This issue I studied in three my studies (Study 1, 2, 3) and in two studies the expectation was confirmed (Study 1, 3). Non-confirmation of this expectation in Study 2 indicates that these patterns are not universal and widely applicable without further research. These studies fill the knowledge gap of germination of alpine species, their reactions with ongoing changes and indicate methods how to study effects of climate changes. The Study 1 highlighted that for predicting the future fates of species, it is necessary to focus on the species responses to changes in conditions from original to novel. Studying only novel conditions does not reveal the response to ongoing climate change. Further, results of my studies indicate that the conditions in which species commonly grow are not necessarily ideal for seed germination, as a change in conditions increased the germination of *Anthoxanthum alpinum* (Study 1) and of some species of genus *Impatiens* (Study 3). In addition, I demonstrated that the cultivation of maternal plants in common garden conditions strongly affected the ability of species to adapt to novel conditions (Study 1).

My research highlighted the importance of cold stratification in germination of alpine species. Cold stratification affects timing of germination, which was demonstrated also in previous studies. However, my study showed contribution of this timing to explaining species rarity as an ecology consequence of germination (Study 4). Within lack of studies about effect of climate change on alpine species I showed that the response to drought was strongly affected by previous cold stratification of the seeds (Study 1). And combination of shorter stratification followed by warmer growing season, as a potential result of climate change, will lead to faster but reduced germination (Study 3).

Original conditions significantly affect not only germination, but also seed mass (Study 2, 3) and nitrogen content in the seeds (Study 2). As seed mass is strongly affected by climate, it is possible that ongoing climate change will have impact on it, which could further affect for instance dispersal, predation, seed-bank persistence or seedling establishment. However, seed mass is strongly phylogenetically constrained (Study 3), which indicates that changes will proceed rather slowly. Further, my results indicated that higher seed mass leads to higher germination (Study 2, 3, 4), especially in warm target conditions (Study 2), which could be caused by higher content of carbohydrates (Study 2). However, more extensive studies are required to confirm this since to our best knowledge this is the first study dealing with the effect of the nutrient content on germination behavior. Simultaneously, to our best knowledge our two studies (Study 2, 3) are the first studies, which show that the effects of seed mass interact with original climate in determining species germination patterns under changing climate. The differences in seed mass are thus likely crucial for species ability to adapt to novel conditions. However, further studies exploring how seed mass modifies species germination under changing climate are needed to confirm generality of these findings.

Knowledge about effects of phylogeny on germination behaviour is likely to increase our understanding of evolution of species germination requirements. I showed that germination response of *Impatiens*, with exception of germination speed, is driven by environmental conditions rather than by phylogeny, which indicates that germination behaviour will probably change with changing conditions (Study 3). However, more studies focusing on the effects of within genus phylogeny on species germination are needed to confirm this.

In Study 4, I focused on relationship between germination behaviour and species rarity using pairs of congeneric species. I pointed out that rare species have higher germination percentage than their common congeners. Simultaneously, seed mass was not different between congeners. Timing of germination was different since rare species had higher germination after cold stratification and common species had higher germination during cold stratification. This result indicates that rare species postponed germination to avoid frost. The common species risk more and in cases of successful germination gain competition advantage. This result suggests that an inability to germinate clearly did not cause species rarity in the Krkonoše Mts., and that rare species were apparently well adapted to local conditions.

To conclude, my research contributed to growing knowledge of conditions affecting germination especially in alpine species. Complexity of external conditions and characteristics of seeds make process of germination hard to understand in all details and variability. If the factors such as climate change or species rarity come into this process so far observed patterns may not have to be still valid. Including all these factors in one study system is a challenge for researchers, however, only these studies can bring satisfying answers for our questions.

Contribution statement

This thesis consists of general introduction, 1 published paper and 3 manuscripts. All papers are co-authored. My contributions to particular papers are as follows:

Study 1: Veselá A., Hadincová V., Vigdis V, Münzbergová Z. (submitted): Maternal effects strengthen the interaction between changes in temperature and precipitation in determining seed germination of dominant grass species

AV, VH, ZM conceived and designed the experiments. VV provided the experimental infrastructure. VH and ZM conducted the field work and AV performed the germination experiments. AV with help of ZM analysed the data. AV wrote the manuscript.

Study 2: Veselá A., Lien D., Münzbergová Z. (submitted): Plant origin determines seed mass, seed nutrients and germination behaviour of a dominant grass species

LD and ZM conceived and designed the experiments. ZM provided the plant material and LD performed the germination experiments. AV with help of ZM analysed the data. AV wrote the manuscript.

Study 3: Veselá A., Dostálek T., Rokaya M., Münzbergová Z. (submitted): Seed mass and plant origin interact to determine alpine species germination patterns along an gradient

AV, TD, ZM conceived and designed the experiments. MR conducted the field work and AV performed the germination experiments. AV with help of ZM analysed the data. AV wrote the manuscript.

Study 4: Paulů A., Harčariková L., Münzbergová Z., 2017: Are there systematic differences in germination between rare and common species? A case study from central European mountains. *Flora* 236–237: 15–24.

AV and ZM conceived and designed the experiment, LH designed the germination tests, AV and LH collected seeds and performed the germination experiments. AV with help of ZM analysed the data. AV wrote the manuscript.

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Study 1: MATERNAL EFFECTS STRENGTHEN THE INTERACTION BETWEEN CHANGES IN TEMPERATURE AND PRECIPITATION IN DETERMINING SEED GERMINATION OF DOMINANT GRASS SPECIES

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Manuscript

Abstract

Despite the many existing studies on plant species responses to changing climate, there is a knowledge gap on how specific climatic factors and their interactions regulate seed germination. This understanding is complicated by the interplay between responses of seeds to the environment experienced during germination, the environment experienced by the maternal plant during seed development and the genetic adaptation of the maternal plant to its environment of origin. We studied two grasses (*Anthoxanthum odoratum, A. alpinum*) with contrasting ecology. The plants originated from twelve localities with factorial combinations of temperature and precipitation. The seed germination was tested in conditions simulating the extreme ends of the current field conditions and a climate change scenario. We compared the performance of field-collected seeds with that of garden-collected seeds. The responses to changes in climate differed between the species. A change to warmer and wetter conditions resulted in the highest germination of *A. alpinum*, while *A. odoratum* germinated the most in colder temperature and home moisture. Maternal effects modified the plant performance. The field-collected seeds of *A. alpinum* tolerated warmer conditions better than the seeds from the experimental garden. The results demonstrate how knowledge of responses to climate change can increase our ability to understand and predict the fates of species. Studies that aim to understand the realized behaviors of seeds under future climates should use experimental designs allowing the separation of genetic differentiation, plasticity and maternal effects and their interactions, as all these mechanisms play an important role in driving species germination patterns.

Key words: climate change, graminoid, maternal effects, reproductive characteristics, seed dormancy

Introduction

Germination is one of the most critical stages in the life cycle of many plants (Donohue 2005) and is controlled by a suite of environmental factors, among which water availability and temperature are particularly important (Baskin and Baskin 2014; Fenner and Thompson 2005). The strong temperature and moisture control of seed germination suggests that species recruitment rates and success may be strongly impacted by ongoing climatic changes. Climate projections for Europe predict temperature increases in the range of 1.5-4.0°C by 2080 compared to 1980 (IPCC 2014). Projections for precipitation change are more complex and regionally variable, but the general pattern suggests that the frequency of extreme meteorological events (drought and floods) will increase. In combination with increased temperatures, these changes will lead to increased drought risk during the growing season (IPCC, 2014). Plants will thus be more frequently exposed to episodes of extremely high temperature and/or low soil moisture, which may have an especially strong impact during vulnerable life-history stages, such as germination, seedling emergence and reproduction (Knapp et al. 2008; Walck et al. 2011; Dreesen et al. 2014).

Understanding the effect of these climatic extremes on plant recruitment success is important for predicting future changes in plant population dynamics (e.g., Morin and Thuiller 2009; De Frenne et al. 2010). Seed germination is a key transitional life-history state in this respect, as the early developmental stages of plants are expected to be more sensitive to climate change than the adult stages (Lloret, Penuelas and Estiarte 2004; Fay and Schultz 2009; Dalgleish, Koons and Adler 2010). The response of seed germination to temperature has been extensively studied (e.g., Baskin and Baskin 1994; Schütz and Rave 1999; Gardarin, Daurr and Colbach 2011; Rosbakh and Poschlod 2015); studies exploring the germination response to variation in moisture are less common (but see, e.g., Wen et al. 2015; Ruhl et al. 2015). The effects of temperature and moisture on seed germination may also interact, and the interaction may be more important than either variable alone (e.g., Rivas-Arancibia et al. 2006; Walck et al. 2011; Gurvich et al. 2017). Most importantly, it has been shown that temperature increase is likely more important for seed germination when coupled with a simultaneous decrease in moisture (Gurvich et al. 2017). There are studies of these interactive effects on germination using species from arid regions (e.g., Rivas-Arancibia et al. 2006; Gurvich et al. 2017; Flores, Perez-Sanchez and Jurado 2017). To our knowledge, only one such study exists for alpine environments (Orsenigo et al. 2015), in which a decrease in germination in warm, dry conditions was found.

Previous studies on species responses to environmental variability have been done at the interspecific level (e.g., Pearson et al. 2002; Ooi, Auld and Denham 2012). More recently, emphasis has been placed on the importance of intraspecific trait variation (reviewed in Cochrane et al. 2015). These studies are important, for example, to inform practitioners on which genotypes are best adapted to given conditions. Generally, germination is highly responsive to the immediate environment of the seeds (e.g., Grime et al. 1981; Khan and Gulzar 2003; Shahriari et al. 2014) as well as to the environment of their origin (e.g., Roach and Wulff 1987; Cendan, Sampedro and Zas 2013; Walter et al. 2016). Recent studies found considerable intraspecific variation in germination percentage, with populations from the warmest conditions showing the highest germination (e.g., Santo, Mattana and Baechetta 2015; Ndihokubwayo, Nguyen and Cheng 2016; Bauk et al. 2017; Mira, Arnal and Perez-Garcia 2017). Cavieres and Arroyo (2000) and Spindelbock et al. (2013) demonstrated higher temperature requirements for germination in populations coming from colder conditions, which was explained as cold avoidance. Furthermore, Bauk et al. (2017) reported that the germination percentage of seeds coming from colder conditions exposed to warmer and drier conditions decreased more rapidly than that of seeds coming from warmer conditions.

In addition to the effects of genetic properties of mother plants, the original environment of a plant (Roach and Wulff 1987) and environmentally induced epigenetic changes (Rossiter 1996; Boyko et al. 2010; Münzbergová et al. 2018) may have effects on plants, resulting in so-called ‘maternal effects’. Despite the general understanding that maternal effects may be important in regulating species germination, their effects on germination have rarely been explicitly tested. To control for the effects caused by the maternal environment, many experiments do not use field-collected seeds, but instead use seeds propagated from maternal plants cultivated in common garden conditions (reviewed in Latzel 2015). However, in doing so, we may miss important patterns and responses operating in the field (Münzbergová and Hadincová 2017). Furthermore, not all epigenetic changes may be removed due to the potential stability of some epimutations across several generations (Latzel 2015). The cultivation of seeds from common gardens may also lead to new adaptations of the plants to the common garden environment (Münzbergová et al. 2018). To disentangle direct environmental responses and maternal effects, studies that explicitly compare patterns and responses between field-collected and garden-cultivated seeds are needed (De Vitis et al. 2014).

In this study, we aimed to understand species germination responses to changing climatic conditions and how these responses depend on plant origin and are affected by maternal environment. We used two closely related grass species as the model species: the diploid *Anthoxanthum alpinum*, occurring primarily in alpine or northern areas of Europe, the and tetraploid *A. odoratum*, with a distribution centered on areas with temperate lowland conditions. These two species may grow together at intermediate locations (Filipová and Krahulec 2006). *Anthoxanthum* species are typical grassland-dominant species. The changes in their distribution may thus affect the composition and functioning of whole ecosystems (Bruelheide 2003). Understanding the responses of these two species to a novel climate may also help elucidate the fate of alpine species exposed to future climate change. It can be expected that the alpine diploid will show lower plasticity than the lowland tetraploid and, thus, will have a lower ability to cope with climate change (Eliášová et al. 2014; Eliášová and Münzbergová 2014).

We studied the species in twelve localities distributed along a unique natural 'climate grid' in western Norway (see Meineri, Spindelbock and Vandvik 2013; Meineri et al. 2014; Klanderud, Vandvik and Goldberg 2015). We tested the germination of both species under conditions simulating those in the home localities of the plants and under projected climate change. We used seeds collected from mother plants in the field and from plants collected in the field and cultivated in an experimental garden (collected from all 12 localities and cultivated for 2 years) to test how possible maternal effects modify germination patterns. The use of two species collected from multiple localities allows us to study the interspecific and intraspecific variability in germination responses to climate change. Furthermore, this approach provides insights into the importance of maternal effects for species responses to climate change.

Specifically, we aimed to answer the following questions: i) What is the importance of changes in temperature and moisture between the original site of a plant and its germination site for species germination behavior during germination, and do changes in temperature and moisture interact with each other? ii) Do the germination responses to changing climate vary between the two closely related species, possibly favoring one of the species at the expense of the other? iii) Are these climate responses affected by plant cultivation in a common garden, thus indicating that maternal environment is crucial for determining species germination patterns? As a prerequisite of this study, we investigated the distributions of the two studied species in the study system, as these species cannot be easily distinguished morphologically, and their distribution patterns have been unknown.

We expected that (i) a shift to higher temperature will have positive effects on germination percentages and speed, but only under non-limiting, relatively high moisture conditions, while decreasing moisture will have negative effects on germination, (ii) *A. alpinum* will have a less plastic response and be more sensitive to moisture reduction than *A. odoratum*, (iii) the seeds from maternal plants reared in a common garden will be more sensitive than seeds collected directly from the field to changes in climatic conditions relative to the conditions at the population origin because the former group may lack maternal effects pre-adapting them to live under the climatic conditions likely to be encountered in the field.

Materials and methods

Study species

We studied two species of perennial grasses representing two cytotypes of the genus *Anthoxanthum*. The diploid species, *A. alpinum*, occurs in the northern part of Eurasia and at high elevations in European mountains. The allotetraploid species, *A. odoratum,* of which *A. alpinum* is one of the supposed parental species (Chumová et al. 2015), is widespread in low elevations of the Eurasian temperate zone. The two species can sometimes co-occur in intermediate climates (Filipová and Krahulec 2006; Flégrová and Krahulec 1999). The two species are difficult to distinguish morphologically, especially when sterile. The only widely accepted morphological characteristic with which to distinguish them is the short-bristled lemma of the fertile flowers of *A. alpinum* (Conert 2000). The bristles are, however, not always visible, and this character cannot be reliably applied to distinguish the two species, especially under field conditions (pers. obs.). As the species represent distinct cytotypes, we determined the species using flow cytometry (Otto et al. 1981). This is a faster and more reliable method than distinguishing them morphologically. Flow cytometry has been previously successfully used to distinguish these species by (Chumová et al. 2015). Another cytotype of *Anthoxanthum* has been described from the northwestern Alps and Massif Central. Based on karyological and flow cytometry studies, this cytotype was suggested to be an autotetraploid of *A. alpinum* (Hedberg 1990; Chumová et al. 2015). The genome size of this cytotype differs significantly from that of *A. odoratum* (Chumová et al. 2015) and thus could also be identified using flow cytometry.

Study localities

The experimental plants and seeds were collected in the SeedClim grid, a set of localities occurring along a natural climatic gradient in western Norway (Meineri et al. 2014; Klanderud et al. 2015). This gave access to a set of sites that differ systematically in climate and at which other biotic and abiotic conditions were as similar as possible. The grid consists of 12 grassland localities (Fig. 1), combining four levels of mean annual precipitation [ca. 600 (1), 1300 (2), 2000 (3) and 2700 (4) mm/year] and three levels of mean summer temperature [defined as the mean of the four warmest months; ca. 6.5°C (alpine, ALP), 8.5°C (subalpine, SUB) and 10.5°C (boreal, BOR)]. The communities are grazed, intermediate-rich meadows (Potentillo-Festucetum ovinae; G8 *sensu* Fremstad 1997) occurring on southwestern facing (with the exception of BOR3, which is exposed to the east), shallow slopes (5-20°) and relatively calcareous bedrock. The geographical distance between the sites ranges from 0.7 km to 175 km (BOR1 and BOR4). The two geographically closest localities, BOR2 and SUB2, differ by 400 m in elevation and hence differ substantially in climate (Meineri et al. 2014).

Plant material and seed collection

At each locality, we collected at least 40 *Anthoxanthum* plants, with a minimum distance between individuals of 1 m, in July 2014. The live plants were transported to the experimental garden of the Institute of Botany, Czech Academy of Sciences in Průhonice, Czech Republic (49°59'38.972"N, 14°33'57.637"E; mean temperature of the four warmest months: 16.5°C; plants were regularly watered during the growing season), and planted in pots (10 × 10 × 10 cm, filled with a mixture of common garden soil and sand in a 1:2 ratio) just after transport at the end of July 2014. The common garden soil comprised compost from the experimental garden containing approximately 0.135% nitrogen, 1.35% carbon and 46.5 mg phosphorus in 1000 g of soil. After the plants recovered from transplanting, they were extracted from the pots and reduced to a single ramet. This was done to ensure that we only had one genotype per pot, thereby avoiding the possibility that we originally collected multiple intermingled genotypes.

The plants from all 12 localities were cultivated for 2 years before collecting the seeds. In early spring 2016, the plants cultivated in the common garden that were about to flower (13 to 31 individuals per population and species) were transferred into 120 × 80 × 60 cm cages covered with a double layer of fine mesh fabric. The mesh size enabled the light and wind to pass through the cage but prevented pollen dispersal into the cages. Only plants from one locality and species were placed in each cage. In this way, the plants could be cross-pollinated within species and locality but not between species and localities. From June to August 2016, we collected ripe seeds (hereafter referred to as garden seeds).

In August 2016, we also collected seeds in the field (hereafter referred to as field seeds) at the SeedClim grid localities in western Norway. Seeds of at least 40 maternal plants from localities that were known to host only one species based on our previous sampling in 2014 were mixed together. From the localities with both species, we collected seeds from each maternal plant separately (46 maternal plants). We used three seeds from each of the maternal plants from the mixed populations to identify the species using flow cytometry. Based on this information, we created two seed mixtures, each containing seeds of one species from each mixed locality. Both the garden and field seeds were stored at room temperature (approximately 20°C and an ambient moisture of approximately 55%) for 2 months before the experiments.

Germination tests

Undamaged, fully developed seeds were used for germination tests in growth chambers. We used 12 combinations of germination conditions, hereafter referred to as target conditions. Specifically, we combined 2 levels of stratification, 3 levels of temperature, and 2 levels of moisture (see below for details). For each locality, species, target condition, and maternal environment (i.e., seeds from the garden/field), we established 10 replicates with 10 seeds each, which were incubated on moist filter paper in 5 cm diameter Petri dishes.

Half of the Petri dishes were placed in the growth chambers without stratification; the remaining Petri dishes were stratified for 1 month in the dark at 1°C (on moist filter paper) before the seeds were placed in the growth chambers. All the seeds were placed in the growth chambers or stratified at the same time. The stratification simulated conditions under snow cover in winter. The seeds were placed in growth chambers with three temperature regimes, two moisture regimes, and a summer day/night length of 20/4 hours (mimicking the light conditions in the field sites). Two of the temperature regimes were derived from long-term measurements in the SeedClim grid in Norway (for details see Tingstad et al. 2015). The selected temperature regimes were based on data from the four warmest months of the year (i.e., the growing season) at the coldest and the warmest plant origin localities. The daytime temperature was set to correspond to the mean highest daytime temperature, and the night temperature corresponded to the mean of the lowest nighttime temperatures at these localities. The resulting day/night temperatures were 12/5°C (simulating alpine conditions) and 25/5°C (simulating boreal conditions). As it is expected that the mean temperature will increase and the differences between daytime and nighttime temperatures will decrease with climate change (IPCC 2014), we introduced a third temperature regime, with day/night temperatures of 25°C/12°C.

A wet regime (an approximation for the wettest localities, i.e., 2700 mm) was simulated by watering the Petri dishes with demineralized water (water potential 0.0 MPa). A dry regime was simulated by decreasing the water potential by adding polyethylene glycol (PEG, molecular weight 6000) into the water. To simulate the driest localities, i.e., precipitation of 600 mm per year, we reduced the water potential to -0.7 MPa. According to (Young and Nobel 1986), this water potential approximately corresponds to 600 mm of rainfall. According to (Evans and Etherington 1990), such a water potential represents an intermediately dry environment before rain, i.e., continuous water stress. The solutions of PEG were prepared and adapted to each germination temperature according to Michel (1983). PEG adjusts the water potential by creating water stress without affecting seed germination (Hardegree and Emmerich 1994).

The number of germinated seeds was recorded every week, and a seed was considered to be germinated if the radicle was visible to the naked eye. When at least 60% of the seeds had germinated and no further seeds germinated for two subsequent weeks in dishes belonging to a specific germination regime, we checked the rest of the seeds by gently pushing on the seed coat. Moldy, soft seeds were considered dead and were counted and removed. The remaining seeds were left in the Petri dishes. As testing the viability of all the remaining seeds with a cut test or tetrazolium chloride would be very time consuming, we stimulated their germination with gibberellic acid at a concentration of 0.05 g/100 ml of demineralized water (Kahn 1960). After the gibberellic acid application, we continued recording seed germination every week. The additional germination after the application of gibberellic acid was used to assess seed viability, but these seeds were not scored as 'germinated' within the main experiment. The experiment was terminated 4 weeks after the application of gibberellic acid. At this time, we again checked the remaining seeds by gently pushing on the seed coat as described above. To be sure that healthy-looking non-germinated seeds were truly alive, we tested them for viability using tetrazolium chloride according to methods described by (Cottrell 1947). Because there were many healthy-looking non-germinated seeds (approximately 10%) and the viability test is very time consuming, we performed this test on subsets of the seeds from each locality, species, and treatment. In this way, we proved that all the healthy-looking ungerminated seeds of *Anthoxanthum* could generally be considered viable.

Data analysis

We analyzed the effects of the treatments on the proportion of germinated seeds, germination speed (expressed as the time to 30% germination (T30)), and germination index (GI), which describes the ratio of the germination percentage and speed, the proportion of dormant seeds and seed viability. We used T30 instead of the more commonly used T50 because of the low germination rates in some treatment combinations (Benech-Arnold and Sánchez 2004). The germination percentage was defined as the percentage of germinated seeds from all the seeds in one Petri dish over the period before the application of gibberellic acid. T30 was calculated according to methods described by (Coolbear, Francis and Grierson 1984) and modified by (Farooq et al. 2005) (see Equation 1).

$$T_{30} = t_i + \frac{(\frac{N}{2} - n_i)(t_j - t_i)}{(n_j - n_i)}$$

where N is the final number of germinating seeds and

n_j and n_i are the cumulative number of seeds

germinated by adjacent counts at times t_j and t_i, (1)

The germination index was calculated as described by the Association of Official Seed Analysts (AOSA 1983) (see Equation 2).

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \cdots\cdots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}} \quad (2)$$

The proportion of dormant seeds was defined as the proportion of all the seeds that germinated only after gibberellic acid application or were found to be viable after tetrazolium chloride application. Seed viability was defined as the percentage of germinated seeds that were found to be viable after gibberellic acid or tetrazolium chloride application out of all the seeds in one Petri dish. The pairwise correlation matrix of the variables, based on Pearson's correlation coefficient, is presented in Supporting information 1A. Because of the strong positive correlation of seed viability with germination percentage and proportion of dormant seeds, we did not use seed viability in further analyses.

To assess the effect of the conditions of the original localities and the target conditions in the experiments on germination, we coded the climatic conditions of each original locality. To do this, the original temperature was coded with respect to the mean temperature of the four warmest months for each locality type, i.e., as 6.5, 8.5 and 10.5°C. Similarly, the original moisture was coded by the mean annual precipitation at the localities, i.e., 600, 1300, 2000 and 2700 mm. The target moisture and temperature conditions in the growth chambers were coded using the same approach, i.e., the mean temperature of the growth chambers (6.5°C, 10.5°C and 12.5°C) and dry and wet moisture regime (600 and 2700 mm). To see how the seeds of different populations responded to changes in temperature and moisture, we expressed the difference between the original and target conditions. In this way, we obtained the codes -2°C, 0°C, 2°C, 4°C and 6°C for temperature and -2100 mm, -1400 mm, -700 mm, 0 mm, 700 mm, 1400 mm and 2100 mm for moisture. Zero indicates plants growing in their original (home) conditions, negative values indicate germination in colder or drier conditions, and positive values indicate germination in warmer or wetter conditions than those in the original environments of plants (see Münzbergová et al. 2017, in which the same approach was used). These codes have been used in the tests described below.

Due to an unbalanced study design caused by the patterns of distribution of the two species (for details see Fig. 1), it was not possible to reliably analyze the whole dataset. Thus, we split the dataset and performed the analyses on two independent sets of tests.

First, we used 3 alpine populations of *A. alpinum* and 3 boreal populations of *A. odoratum* from the localities with original moisture ranging from 1300 mm to 2700 mm (ALP 2-4 and BOR 2-4 in Fig. 1) to explicitly test for differences in germination responses between the two species. For these populations, we used seeds placed in temperature conditions corresponding to both original conditions, i.e., 12/5°C and 25/5°C. We coded the temperature as corresponding to home or shifted conditions (to warmer conditions for *A. alpinum* and to colder conditions for *A. odoratum*). Then, we tested the effect of maternal environment (seeds from the garden/field), species identity, change in moisture (the codes for these changes are -2100 mm, -1400 mm, -700 mm, 0 mm, 700 mm, and 1400 mm), stratification (yes/no), and home temperature (home/warmer or colder) and their interactions on the germination percentage, germination speed (T30, germination index) and proportion of dormant seeds.

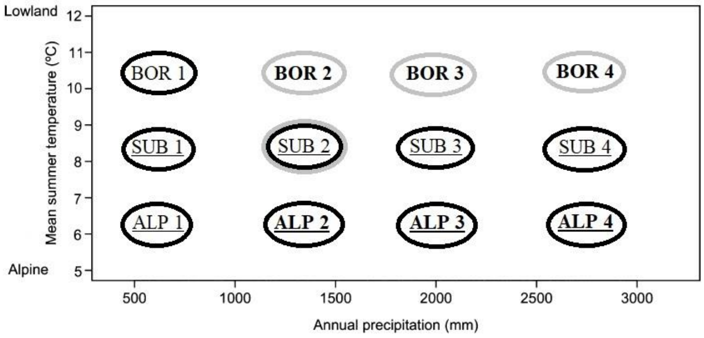


Fig. 1. Position of the studied localities along the temperature and precipitation gradient. The populations of *A. alpinum* are circled in black, the populations of *A. odoratum* are circled in grey. The populations included into model of the comparison between species are highlighted in bold (the first analysis), the populations included into model of determinants of *A. alpinum* germination are underlined (the second analysis).

The second test was carried out only for *A. alpinum* using populations from all 4 original moisture levels and 2 original temperature levels (ALP and SUB, underlined in Fig. 1). Population BOR1 of *A. alpinum* was excluded to maintain the factorial combination of temperature and moisture. We tested the effect of maternal environment (seeds from the garden/field), change in moisture (the codes for the changes range from -2100 mm to 2100 mm), change in temperature (the codes for the changes range from -2°C to 6°C), stratification (yes/no), and their interactions on the germination percentage, germination speed (T30, germination index) and proportion of dormant seeds.

The third test was carried out using the same dataset as that in the second test. The effect of changes in temperature and moisture was substituted by the effect of origin (temperature and moisture) and target conditions (temperature, moisture and stratification (yes/no)). The other parameters of the third test were the same as those used in the second test. We present the results of the third test in Supporting information 1B – D. The effect of the change in conditions is presented in the main text, since the interaction between moisture change and temperature change is easier to understand than three- and four-way interactions between the target and original moisture and temperature.

All the tests were performed using linear mixed effect models as implemented in the lme4 package in R (Bates et al. 2015) with population as a random factor. All the two- and three-way interactions were also tested. We did not include the higher-level interactions into the models, as this would make the models too complex and hard to interpret. As there was always some proportion of non-germinated seeds, all the dependent variables, including germination percentage, followed a normal distribution.

Results

Differences between species and their germination responses to climate change

A. alpinum had significantly lower germination speed (T30; 24 days ± 0.70, mean ± SE) and germination percentage (11% ± 1.88) than *A. odoratum* (13 days ± 0.42; 56% ± 4.61, respectively) across germination regimes. There were significant interactions between species and changes in moisture and temperature, maternal environment and stratification for at least one of the seed germination characteristics (Table 1). Specifically, the seeds originating from plants cultivated in the experimental garden were more sensitive to drought (lower germination) than those from the field, and *A. alpinum* was more strongly affected than *A. odoratum*.

Table 1. *Anthoxanthum alpinum* versus *A. odoratum*. Effect of the species, maternal environment (garden/field), stratification, change of original moisture (difference between target and original conditions) and home temperature (home/ warmer resp. colder) on germination percentage, time to 30% germination (T30, referred to as germination speed in the text), germination index (GI) and proportion of dormant seeds in *A. alpinum* and *A. odoratum*. Significant values (≤ 0.05) are in bold. N = 720.

	Germination percentage		**T30**		**GI**		**Proportion of dormant seeds**	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Species	**15.92**	**0.014**	**33.75**	**<0.001**	0.41	0.548	1.04	0.356
Change moisture	**99.21**	**<0.001**	**6.45**	**0.011**	0.06	0.896	**7.53**	**0.006**
Change temp	**40.18**	**<0.001**	**12.84**	**<0.001**	**7.95**	**0.005**	**14.62**	**<0.001**
Species: Change moisture	**6.46**	**0.011**	**25.39**	**<0.001**	0.07	0.786	1.43	0.232
Species: Change temp	**4.54**	**0.035**	0.69	0.407	**5.13**	**0.024**	0.35	0.549
Species: Stratification	0.61	0.432	**17.80**	**<0.001**	0.01	0.981	0.02	0.888

	Germination percentage		**T30**		**GI**		**Proportion of dormant seeds**	
Species: Mat. envi	0.85	0.355	1.02	0.313	0.01	0.923	**5.23**	**0.022**
Change moist: Change temp	0.18	0.671	0.69	0.467	**9.39**	**<0.001**	0.02	0.909
Species: Change moist: Change temp	**15.75**	**<0.001**	2.27	0.133	**9.15**	**0.002**	2.68	0.101
Species: Stratification: Mat. envi	0.13	0.715	**5.72**	**0.017**	0.01	0.946	**4.15**	**0.042**
Species: Change temp: Stratification	1.93	0.165	0.23	0.635	**9.49**	**0.002**	0.01	0.982
Species: Change temp: Mat. envi	0.43	0.514	0.27	0.60	0.81	0.368	**12.41**	**<0.001**
Species: Change moist: Stratification	0.33	0.567	0.63	0.427	0.01	0.986	0.63	0.428
Species: Change moist: Mat. envi	**18.34**	**<0.001**	**7.83**	**0.005**	0.15	0.700	0.06	0.796

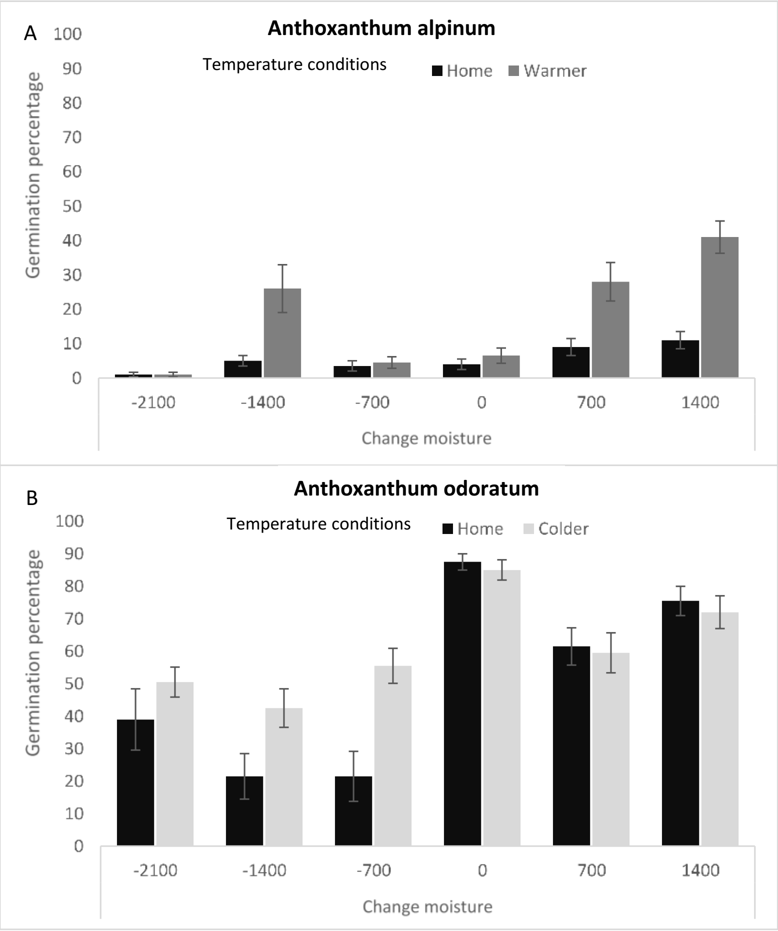


Fig. 2. Effect of moisture change and home temperature (home/warmer resp. colder) on germination of A) alpine populations of *A. alpinum* and B) boreal populations of *A. odoratum*. Zero indicates plants growing under their home conditions, the negative values indicate germination in drier conditions and positive values indicate germination in wetter conditions in comparison with the environment of their origin. The graphs show mean ±SE.

Both species germinated equally well or more and faster under climate regimes different from their home climate, but the specific responses varied between species. There were strong interactions between the effects of temperature and moisture change (Table 1). *A. alpinum* generally had significantly higher germination speed (from a mean of 24 to 19 days ± 2.25) and germination (from a mean of 11% to 41% ± 2.50) under warmer than home conditions, except in the extreme moisture reduction treatment, under which this species germinated less irrespective of temperature (Fig. 2A). When *A. odoratum* seeds were germinated under home or wetter conditions, the germination percentage remained high irrespective of temperature. In contrast, germination decreased under colder and drier conditions, from a mean of 55% to 43% (± 6.97) (Fig. 2B). Even higher decreases (up to 21% (± 4.61)) were observed under home and drier conditions. Stratification caused slower germination of both species, with a stronger effect observed in *A. odoratum* (from a mean of 13 to 16 days ± 0.44).

A. alpinum germination response to climate change

Changes in temperature, moisture and their interaction affected various aspects of *A. alpinum* germination (Table 2). The general observed trend was that in warmer conditions, the germination percentage was higher in the seeds in wetter conditions than those in drier conditions. The lowest germination (3% ± 0.68) was found in the seeds subjected to the drier and warmer conditions, while the seeds subjected to wetter and warmer conditions had the best germination (65% ± 2.65). The germination percentages were generally very low in the treatments with home temperatures (Fig. 3A) due to a high proportion of dormant seeds (more than 30% ± 5.96, with the exception of seeds from the driest sites, Table 2; Fig. 3B). The proportion of dormant seeds increased in the seeds shifted to both drier and warmer conditions, from a mean of 17% up to 27% (± 7.30). Again, the exception was the treatment with the most extreme drought conditions, in which the proportion of dormant seeds increased only with transfer to colder conditions, from the minimum germination (1%) up to 13% (± 5.94). In populations exposed to wetter conditions, the proportions of dormant seeds decreased with increasing temperature, from a mean of 16% to 5% (± 2.66) (Fig. 3B).

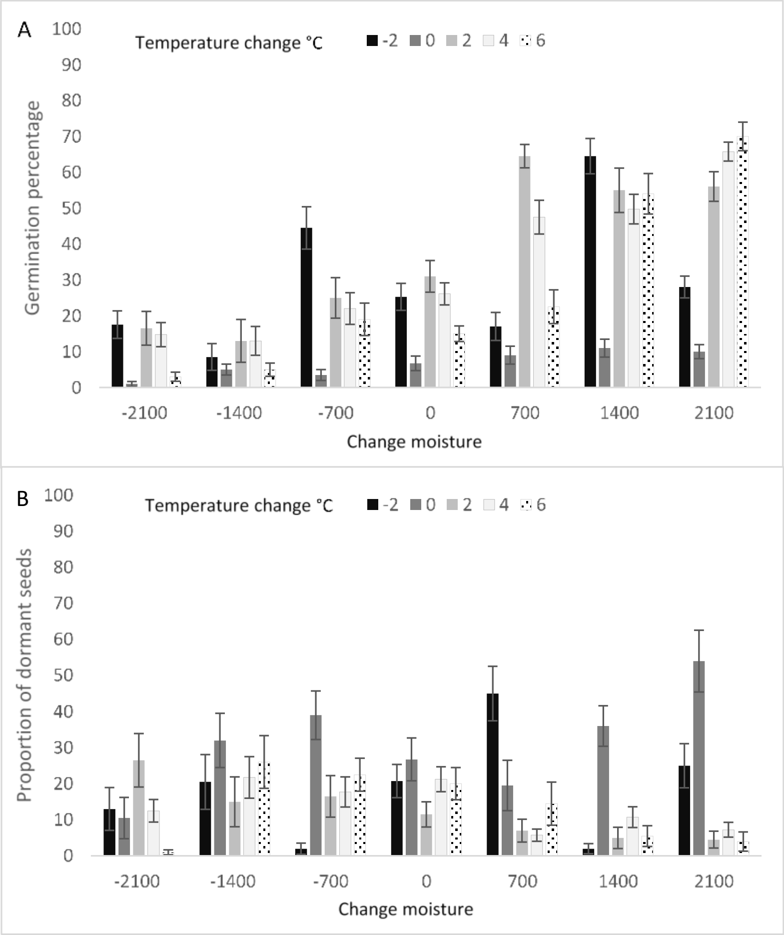


Fig. 3. Effect of moisture and temperature change on A) germination proportion and B) on proportion of dormant seeds of alpine and subboreal populations of *A. alpinum*. Zero values indicate plants growing under their home conditions, the negative values indicate germination in colder or drier conditions and positive values indicate germination in warmer or wetter conditions in comparison with the environment of their origin. The graph shows mean ±SE.

Table 2. *Anthoxanthum alpinum*. Effect of the change of original conditions (moisture and temperature), stratification and maternal environment (garden/field) on germination percentage, time to 30% germination (T30, referred to as germination speed in the text), germination index (GI) and proportion of dormant seeds in *A. alpinum*. Significant values (≤ 0.05) are in bold. N = 960.

	Germination percentage		**T30**		**GI**		**Proportion of dormant seeds**	
	F-value	p-value	F-value	p-value	F-value	p-value	F-vale	p-value
Change moisture	**131.34**	**0.001**	0.01	0.917	**5.92**	**0.015**	1.95	0.162
Change temperature	**105.98**	**0.001**	0.04	0.848	1.07	0.299	**46.74**	**0.001**

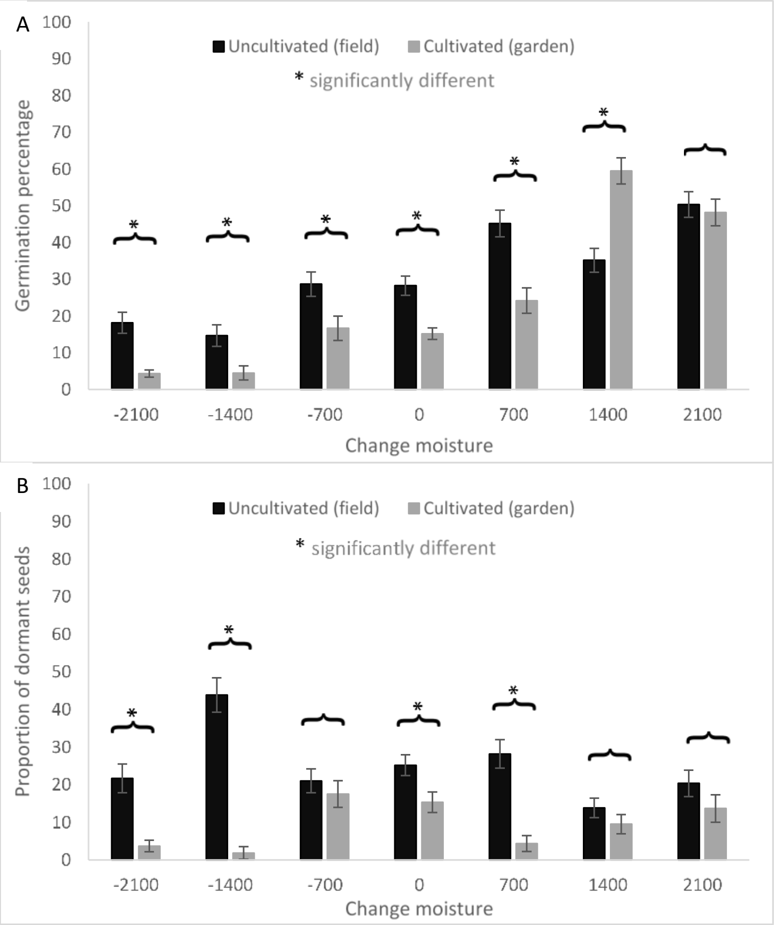
	Germination percentage		T30		GI		Proportion of dormant seeds	
Change moist: Change temp	**44.34**	**0.001**	2.03	0.154	0.39	0.529	**30.90**	**0.001**
Change mois: Mat. envi	**15.15**	**0.001**	**4.80**	**0.029**	1.55	0.212	**7.50**	**0.006**
Change mois: Strati	**5.01**	**0.025**	1.04	0.307	2.95	0.086	1.46	0.227
Change temp× Mat. envi	1.01	0.316	3.18	0.075	0.71	0.401	0.01	0.998
Change temp: Strat	**5.65**	**0.017**	**13.25**	**0.001**	0.86	0.354	0.93	0.335
Change moist: Change temp: Mat. envi	0.143	0.706	1.55	0.213	0.83	0.362	0.09	0.761
Change moist: Change temp: Strat	0.44	0.507	0.06	0.801	1.27	0.260	1.18	0.673

Germination was also affected by interactions between climate change (i.e., changes in temperature and moisture) and stratification. The stratified seeds had a significantly higher germination percentage in the treatments with drier conditions than the non-stratified seeds (Table 2). The non-stratified seeds were more sensitive to warming and had lower germination percentages but faster germination times than the stratified seeds.

Effects of maternal environment

The maternal environment had a significant effect on germination (garden plants vs. field collections; Table 2). The germination speed was significantly impacted by the interactions between the maternal environment and temperature change (Table 2). Germination was slower with a shift to warmer conditions for both the garden (from a mean of 20 to 25 days ± 1.01) and field seeds (from a mean of 19 to 21 days ± 0.83). Furthermore, the germination percentage and proportion of dormant seeds were impacted by the significant interaction between the maternal environment and moisture change (Table 2). The germination percentage of the garden seeds decreased with decreasing moisture more strongly than that of the field-collected seeds (Fig. 4A). In all of the treatments with drier conditions, home conditions and slightly wetter conditions, germination was significantly higher (on average by 12% ± 3.63) for the field-collected seeds than the garden seeds (F ≥ 8.34, P ≤ 0.003 in all cases; Fig. 4A). Only in moderately wetter conditions did the garden seeds have significantly

Fig. 4. Effect of moisture change and maternal environment on A) germination proportion and B) on proportion of dormant seeds of alpine and subboreal populations of *A. alpinum*. Zero indicates plants growing under their home conditions, the negative values indicate germination in drier conditions and positive values indicate germination in wetter conditions in comparison with the environment of their origin. The graph shows mean ±SE.



higher germination (60% ± 3.55) than the field seeds (35% ± 3.23; F = 12.85, P < 0.001; Fig. 4A). In the wettest condition, the germination percentage was not significantly affected by the maternal environment (F = 1.84 P = 0.184; Fig. 4A).

The proportion of dormant seeds increased with changes to wetter conditions in the garden-collected seeds, while the field-collected seeds showed the opposite trend (Table 2; Fig. 4B). In the treatments with the conditions, home conditions and in slightly wetter conditions, the proportion of dormant seeds was significantly higher for the field-collected than the garden seeds (F ≥ 8.29, P ≤ 0.003 in all cases; Fig. 4B). The mean difference was 23% (± 3.74). There was no significant difference between the field and garden seeds in the other treatments with changes in moisture ($F \leq 1.02$, $P \geq 0.202$ in all cases; Fig. 4B). We did not find any three-way interactions with the maternal environment (Table 2).

Discussion

Germination was affected by the original and target climate and differed between the two species. *A. alpinum* seeds germinated faster and to a higher percentage under warmer and wetter conditions than under their home conditions. In contrast, *A. odoratum* seeds generally germinated to a higher percentage under wetter conditions, independent of temperature. Under dry conditions, germination of *A. odoratum* increased when the seeds were exposed to colder temperatures. Cold stratification seems to be an important factor enabling both species to germinate under drought conditions. The germination response to changing conditions was also dependent on whether the seeds were collected in the field or in the garden, suggesting that the maternal environment strongly affects the germination response. Climate change thus could potentially affect both the timing of germination and the overall ability of recruitment from seeds.

Germination differences between A. odoratum and A. alpinum

The two closely related species, *A. odoratum* and *A. alpinum*, have previously been reported to have different distributions, with infrequent co-occurrence in mid-elevations (Rozmus 1958; Hedberg 1990; Filipová and Krahulec 2006). Our flow cytometry results indicated that only one locality out of the 12 studied included both species. We also found one population of *A. alpinum* in a boreal site, i.e., relatively warm lowland conditions. A similar lowland occurrence was also reported from extreme serpentine areas in Austria (400-600 m asl.) (Melzer, 1986).

The comparison of the germination of the two species showed that *A. alpinum* may germinate faster and to higher final percentages under future warmer conditions than in its home climates. However, fast germination is not necessarily advantageous, as seed dormancy may in fact be more important for the long-term fitness of populations (Baskin and Baskin 2014). Alternatively, climate *per se* may not be limiting for *A. alpinum* in lowlands, and the species may instead be excluded from the stands by competitive interactions, as hypothesized by (Flégrová and Krahulec 1999). This could be the reason why *A. alpinum* survives in the dry lowland locality (BOR1), at which productivity is relatively low and strong competitors, including *A. odoratum*, are absent.

Our results also suggest that *A. odoratum* could germinate in colder conditions than those occurring where it naturally grows. In line with (Flégrová and Krahulec 1999), this suggests that *A. odoratum* could occur in alpine and sub-alpine localities. However, (Flégrová and Krahulec 1999) also demonstrated that *A. odoratum* is not able to flower at alpine elevations, which can lead to the absence of *A. odoratum* at these elevations despite high germination and good growth. However, it is also possible that there is a time lag in the current distribution of this species in comparison to climate change, as previously demonstrated for another species (Peterson, Doak and Morris 2019). Due to climate change, *A. odoratum* is able to grow in higher elevation sites, but it still has not dispersed and become established there. Such delayed responses of species to environmental changes have been shown in a range of previous systems (e.g., Herben et al. 2006; Hemrová and Münzbergová 2015).

Germination response to climate change

We are paying special attention to *A. alpinum,* as alpine species have limited possibilities to migrate to colder conditions and must therefore respond to changing climate via adaptation or phenotypic plasticity. However, the effect of climate change on the seeds and seedlings of alpine species has been largely neglected (reviewed in Briceno, Hoyle and Nicotra 2015). Climate change can be advantageous to species, as it can induce longer vegetation periods due to reduced snowfall and earlier snowmelt (Beniston 2012; Pederson, Betancourt and McCabe 2013; Sanchez-Bayo and Green 2013). This phenomenon may provide an advantage to species such as *A. alpinum*, which are able to germinate early in the spring under cold conditions. On the other hand, soil warming in alpine areas may lead to reduced germination from the soil seed bank because many species from these areas undergo physiological dormancy (Hoyle et al. 2013). Our results suggest a similar trend, as the proportion of dormant seeds in *A. alpinum* was very low in warmer and especially warmer and drier conditions.

Generally, it is known that original (home) (e.g., Meyer, Allen and Beckstead 1997; Qaderi and Cavers 2002; Santo et al. 2015) and target (e.g., Grime et al. 1981; Schütz and

Rave 1999; Gardarin et al. 2011) environments affect germination. Similarly, the germination response of *A. alpinum* was significantly influenced both by its original conditions and by the target temperature. The germination percentage and rate decreased with decreasing target moisture in all the target temperatures (e.g., Shahriari et al. 2014; Wen et al. 2015; Ruhl et al. 2015), and the proportion of dormant seeds increased with decreasing target moisture. Generally, the ability of grass seeds to form persistent seed banks is limited (Grime et al. 1981; Eriksson 1989; Baskin and Baskin 2014), but Vandvik et al. (2016) found a substantial number of *Anthoxanthum* seeds in the transient seed banks of our study localities. The functions of seed dormancy include risk-spreading under harsh conditions, such as drought, and the ability to germinate under favorable conditions (Baskin and Baskin 2014). It is expected that in the future, there will be long periods of drought disrupted by heavy rains (IPCC 2014); therefore, it is important that the seeds of *A. alpinum* will be able to stay alive and have a chance to germinate later in the wet season and thus contribute to population growth (Baskin and Baskin 1988). However, the seeds will probably not be able to stay alive if the conditions are dry and warm, as the proportion of dormant seeds was very low and seed mortality was high in the most extreme shift to the warmest and driest treatments. Similarly, Swain et al. (2006) found the lowest proportion of dormant seeds in dry conditions. It seems that moist soil may improve seed survival.

The effects of the original and target conditions were weak, but changes in temperature and moisture strongly affected seed germination. At the stage of germination, *A. alpinum* is likely to profit from the expected climate change when the timing of germination and rain coincide. It is, however, not clear if it would be able to compete with other species, which may migrate to the localities of *A. alpinum* due to changing climate. In the long-term view, increasing germination does not have to be only beneficial for overall fitness, as increasing germination can also increase intraspecific competition (Cheplick 1992; Milla et al. 2009), which can lead to higher mortality. If the timing of germination and rain do not coincide, the benefit from the expected climate change will be lost. The seeds shifted to warmer and drier conditions germinated faster but in very low proportions, and the proportion of dormant seeds was low, as the rest of the seeds died. However, the seeds better tolerated a shift to drier conditions if they were cold stratified. This suggests that cold stratification may be crucial for species' ability to respond to future climates and needs to receive more attention in future experiments. Its importance is in line with the fact that cold stratification has an important role in breaking dormancy, especially for species of temperate and alpine zones (Baskin and Baskin 1988; Meyer 1992). The optimal temperature of cold stratification for most species is in the range 0°C – 10°C (Baskin and Baskin 2014). However, it is likely that seeds in Norwegian alpine and boreal areas will experience these low temperatures during winter for at least one month even under climate change.

Effects of the maternal environment

Germination is influenced by the actual environmental conditions during germination and by the environment experienced by the maternal plant during seed development (Donohue et al. 2008; Chiang et al. 2013). Consistent with this, we demonstrated that the germination response to changes in temperature and moisture depends on whether the seeds originated from the field or from plants cultivated in the experimental garden. The garden-cultivated seeds of both species were more sensitive to reduced moisture than the field-collected seeds. This may be explained by acclimatization to the garden conditions (mainly acclimatization to regular irrigation) and the suppression of original adaptations. In the context of climate change, our results showed that seeds of plants grown in the field, where they were exposed to irregular precipitation, were able to germinate even in the driest conditions, with many non-germinated viable seeds remaining. These results suggest that a soil seed bank would be found.

In the same system but using another grass species, Münzbergová and Hadincová (2017) demonstrated that the maternal environment may be more important for plant performance than the current and original conditions. Postma and Agren (2015) found that differences in seed dormancy could also be affected by the maternal environment. This is in line with our results, as most seeds from the garden-cultivated plants were not dormant. To understand the phenotype and germination behavior in the field, we need to incorporate genetic differentiation, plasticity and maternal effects into one experiment to disentangle their relative contributions.

The differences between the field-collected and garden-cultivated seeds could potentially be affected by the lower genetic diversity of the seeds from the garden due to the limited pool of paternal plants available for pollination. Genetically impoverished populations may react sensitively to unexpected or rapid changes in their environment (Leimu and Fischer 2008), exhibiting lower adaptability (Stöcklin, Kuss and Pluess 2009) and lower germination under certain conditions. However, we had at least 13 paternal plants in each population, selected from natural large populations. We thus expect that this effect is much less important than acclimatization to the garden conditions, as discussed above.

Conclusions

This study highlighted that for predicting the future fates of species, it is necessary to focus on the species responses to changes in conditions from original to novel. Studying only novel conditions does not reveal the response to ongoing climate change. Our results indicate that germination percentage, germination speed and seed dormancy depend on the range of the change in temperature and precipitation between the current and the original conditions, and these two factors interact. This study also demonstrated that the conditions in which species commonly grow are not necessarily ideal for seed germination, as a change in conditions increased the germination of *A. alpinum*. However, a change in conditions was not very beneficial to *A. odoratum*. The germination response to drought was strongly affected by previous cold stratification of the seeds, indicating that stratification is crucial for the ability of species to respond to novel climates. Finally, we demonstrated that maternal effects can be crucial for driving seed responses to changing climates. The cultivation of maternal plants in common garden conditions strongly affected the ability of species to adapt to novel conditions. Future studies should investigate the interactions between temperature and moisture and cold stratification with respect to patterns operating directly in the field and in common garden conditions to develop a possible generalization of the expected changes in and the fate of species and ecosystems.

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Study 2: PLANT ORIGIN DETERMINES SEED MASS, SEED NUTRIENTS AND GERMINATION BEHAVIOUR OF A DOMINANT GRASS SPECIES

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Manuscript

Abstract

Although number of studies exploring effects of climate change on plants is increasing, only few studies pay attention to germination. Understanding of germination behaviour is complicated by impact of climate on seed mass and possibly also seed nutrients, which play irreplaceable role in nourishing the sprout. The germination behaviour of species may also depend on trade-off between investment to generative and vegetative reproduction. We studied a dominant grass, *Festuca rubra*, originating from localities situated along a natural climatic grid. Seeds of different origin were germinated in two temperature and two moisture regimes, simulating the extremes of the grid. To study the relationship between generative and vegetative reproduction, we used data on vegetative traits of the species originating from the same study system. Seed mass and nitrogen content in seeds were significantly affected by original climate, while carbohydrates and phosphorus were not. Higher seed mass and warmer temperature of origin caused higher and faster germination. Warm and dry condition during germination caused the lowest germination but the highest dormancy. Total germination and proportion of viable seeds negatively correlated with plant performance variables contributing to vegetative reproduction. Despite this, the patterns detected using datasets of germination and plant performance, did not provide fully comparable results. Changes in climatic condition can modify seed mass and seed nutrients and these changes translate into changes in species germination behavior. After accounting for seed mass, both original and target conditions determine species germination indicating that both genetic differentiation as well as actual conditions drive the germination patterns. As the patterns detected at the level of seed germination do not fully match those detected for the vegetative traits, we urge that future studies should focus on multiple stages of plant life to understand species responses to future climates.

Key words: reproduction characteristics, climate change, graminoid, seed mass, seed nutrient content, effect of original condition, dormancy

Introduction

One of the most commonly observed responses to climate change is migration, often to higher altitudes (Jump and Penuelas 2005). However, species are not always able to migrate due to slow migration rates (Huntley 1991; Neilson et al. 2005) or lack of habitats suitable for migration (e.g., in alpine species already at the peak of mountains (Bliss 1962)). Species thus need to use other strategies to respond to the changes.

One possibility is the response through phenotypic plasticity, which involves expression of different morphology or physiology by plants of the same genotype under different environment. It can be observed e.g. in seed traits (including germination), plant height or flowering time (Nicotra et al. 2010; Anderson et al. 2012; Wainwright and Cleland 2013; Anderson and Gezon 2015). This mechanism allows species to cope mainly with short-term undirected changes. Alternatively, species may respond by genetic adaptation resulting from climate selecting individuals with traits advantageous for survival in the specific conditions. Although genetic adaptation is usually slower, it allows the species to respond to stronger directional changes occurring over longer time periods. Despite the increasing number of studies assessing the importance of these two mechanisms to changing climatic conditions (e.g., Jump and Penuelas 2005; Wilczek et al. 2010), our knowledge on their importance in species germination behaviour is still rather limited (Nicotra et al. 2010).

Paying special attention to germination is important as early developmental stages of plants are expected to be more sensitive to climate change than adult stages (Lloret, Penuelas and Estiarte 2004; Fay and Schultz 2009; Dalgleish, Koons and Adler 2010). Germination is mainly affected by light, temperature and water availability (Baskin and Baskin 2014; Fenner and Thompson 2005) and may thus be strongly affected by ongoing climate change. The response of seed germination to temperature has been extensively studied (e.g., Grime et al. 1981; Schütz and Rave 1999; Gardarin, Daurr and Colbach 2011), studies exploring germination response to variation in moisture are less common (but see e.g., Wen et al. 2015; Ruhl et al. 2015). In addition, some studies demonstrated interaction of temperature and moisture on plant performance (e.g.; Suseela et al. 2012; Meineri, Spindelbock and Vandvik 2013; Münzbergová et al. 2017). Studies of these interactive effects on germination use species from arid regions (e.g.; Rivas-Arancibia et al. 2006; Gurvich et al. 2017; Flores, Perez-Sanchez and Jurado 2017). Species of alpine conditions, likely being most threatened by the ongoing change in climate, are usually neglected (but see Orsenigo et al. 2015; Veselá et al. submitted). Both these studies indicate decreasing germination in warm, dry conditions.

Veselá et al. (submitted) also found relatively high dormancy in these conditions. This suggest that studying seed dormancy is an important part of studies of seed germination.

Germination response may depend not only on current conditions, but can also differ intra-specifically due to species adaptation to the climate of origin (Meyer, Allen and Beckstead 1997; Qaderi and Cavers 2002; Degreef et al. 2002). Many studies, for example, demonstrated that populations of a single species differ in germination percentage with populations coming from the warmest conditions having the highest germination (Cruz et al. 2003; Ndihokubwayo, Nguyen and Cheng 2016; Santo, Mattana and Baechetta 2015; Bauk et al. 2017; Mira, Arnal and Perez-Garcia 2017). In addition, the differences between seeds of different origin may depend on the actual conditions in which the seeds are produced (Veselá at al. submitted).

Effect of seed origin on germination response could be also caused by different seed mass and nutrient content in the seeds because favorable conditions can contribute to higher accumulation of reserves in the seed. Relationship between seed mass and germination is commonly studied. Positive effect of seed mass on germination was demonstrate in studies of Münzbergová and Plačková (2010); Wu and Du (2007) Paulů, Harčariková and Münzbergová (2017), but see e.g., Wang et al. (2009); Wu, Li and Du (2011) for an opposite trend. Studies focusing on seed nutrients are less frequent, but Bu et al. (2018), for instance, demonstrated differences between populations in the content of nitrogen and phosphorus, with higher content of both nutrients in alpine conditions. Carbohydrates, nitrogen and phosphorus provide the initial energy and nutrients for growth of the sprout (Muthukumar and Udaiyan 2000; McGinley and Charnov 1988) and the differences in their content may thus drive the differences in seed germination behavior. Rees et al. (2001) suggested that heavier seeds have higher content of nutrients, which should contribute to their higher germination, but did not quantitatively demonstrate the relationship between nutrients and seed mass. In addition, intra-specific variability of nutrient content in seeds is not sufficiently explored (but see Vaughton and Ramsey 2001; Obeso 2012; Kolodziejek 2017) and to our best knowledge, the effects of nutrients on germination behavior have not been studied yet.

Despite the increasing bulk of knowledge on the determinants of species germination (Bauk et al. 2017; Veselá et al. submitted) and species performance (Meineri et al. 2013; Münzbergová et al. 2017) in response to climate, our knowledge on the correspondence of the patterns of responses between the seeds and grown up plants in long-lived species under climate change is largely missing. Understanding the effects of changes in climatic conditions on multiple parts of life cycle is, however, important as the patterns detected in single life cycle stages may contradict the conclusions based on other stages (Münzbergová 2005; Kolb, Dahlgren and Ehrlen 2010; Laughlin et al. 2018). In natural conditions, several studies have detected negative correlation between generative and vegetative reproduction (e.g., Cheplick 1995; Worley and Harder 1996; Ronsheim and Bever 2000; van Kleunen, Fischer and Schmid 2002; Herben et al. 2012). This indicates that species have limited resources and there is thus a trade-off between investment into the two modes of reproduction. However, no such trade-off has been detected in other studies (e.g., Reekie 1991; Cain and Damman 1997). Existence of the trade-off may suggest that the patterns detected at the stage of generative reproduction may go in the opposite direction than the patterns detected for vegetative reproduction. To what extent the mechanisms driving these two types of responses to climate change correspond to each other and to what extent the values of the different traits correlate, however, remains largely unexplored.

The aim of this study is to understand the importance of original and actual climatic conditions, and thus the importance of genetic differentiation and phenotypic plasticity, for species germination using natural factorially crossed temperature and moisture gradients and widespread clonal grass species, *Festuca rubra*, as the model system. Each locality represents different type of climate while other factors (e.g., slope, bedrock, grazing history) are as similar as possible (Meineri et al. 2014). Seed mass and nutrient content in the seeds may be an important mechanism affecting species germination patterns and at the same time, they may be affected by species origin. We thus studied the effect of plant origin on seed mass and nutrient concentration and the effect of seed mass and nutrient concentration on seed germination in different climatic conditions further referred to as a target condition. To assess to what extent the effects detected at the level of germination correspond to the effects detected using grown-up plants, we used vegetative data from the same study system published in (Münzbergová et al. 2017) and compared the patterns to the results obtained here.

Specifically, we aimed to answer the following questions: i) Does the original climate affect seed nutrient content and seed mass and do these differences, if any, relate to seed germination patterns?, ii) What is the effect of original and target conditions on species germination patterns and do these factors interact?, iii) What is the relationship between traits related to generative and vegetative reproduction and their responses to changing climatic conditions?

We tested the following hypotheses: i) Seed mass and seed nutrient concentration, especially the concentration of nitrogen and phosphorus, will be affected by original climate, with the highest nitrogen and phosphorus content in the alpine populations. Higher seed mass will be caused mainly by higher content of carbohydrates, because these compounds have higher molecular weight than nitrogen and phosphorus. Simultaneously, higher seed mass and higher content of nutrients, especially carbohydrates, will contribute to higher germination., ii) Target temperature and moisture will interact in their effect on seed germination, and germination percentage will decrease with increasing temperature and decreasing moisture. We also expected that germination response will be strongly affected by origin of the population and the highest germination percentage will be observed in populations coming from the warmest conditions. Also, origin and target conditions will interact. iii) Total germination and/or proportion of viable seeds will negatively correlate with plant performance variables contributing to vegetative reproduction. Factors determining species germination patterns will be the same as the factors determining vegetative reproduction, just acting in the opposite direction.

Materials and methods

Study species and localities

We chose *Festuca rubra* L., a widespread perennial grass species of temperate grasslands in Europe, as a model plant. In the experiment, we used a widespread hexaploid type from the *F. rubra* complex (Šurinová et al. 2019). It reproduces by seeds as well as vegetatively, producing both intravaginal and extravaginal tillers on rhizomes. It grows at different densities in grasslands, both as a dominant with only a few other species and also as a subordinate of species-rich stands. *F. rubra* possesses considerable genetic variability and plasticity and can adapt to a wide range of climatic conditions (Skalova et al. 1997; Herben et al. 2001; Münzbergová et al. 2017; Münzbergová and Hadincová 2017).

The experimental plants originated from localities occurring in a unique natural climatic grid in western Norway previously used e.g. by (Meineri et al. 2014) and (Klanderud, Vandvik and Goldberg 2015) as we wanted to have plants from defined environmental conditions. The grid is represented by twelve grassland localities (Fig. 1) combining four levels of mean annual precipitation [ca. 600 (1), 1300 (2), 2000 (3) and 2700 (4) mm/year] and three levels of mean summer temperature [defined as the mean of the four warmest months; ca 6.5°C (alpine, ALP), 8.5°C (sub-alpine, SUB) and 10.5°C (boreal, BOR). *F. rubra* occurs on 11 localities in climatic grid and does not occur on the twelfth locality (ALP2). Localities were selected specifically to ensure that grazing regime and grazing history, bedrock, slope, aspect and vegetation types are as similar as possible (Meineri et al. 2014). The communities are grazed intermediate-rich meadows (Potentillo-Festucetum ovinae; G8 *sensu* Fremstad 1997) occurring on south-west facing (with the exception LOW3, which was exposed to the east), shallow slope (5-20°) with relatively rich bedrock in terms of nutrient availability. Geographical distance between the sites ranges from 0.65 km to 175 km (BOR1 and BOR4). The two geographically closest localities, BOR2 and SUB2, which are only 0.65 km apart are 400 m in altitude, and hence differ substantially in climate (Meineri et al. 2014).

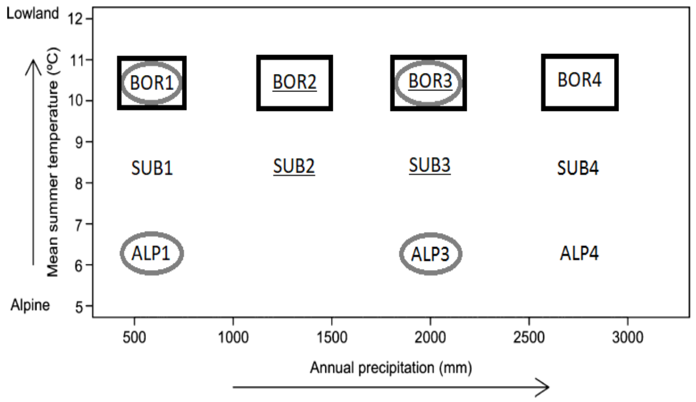

Fig 1. Position of the localities in the climate grid. All shown populations were included into the first analysis. The populations included into the second analysis are highlighted by square, the populations included into the third analysis are underlined and populations included in nutrient analysis are circled.

Plant material and seed collection

For this study, we used plants collected for the purpose of our previous studies (Münzbergová and Hadincová 2017; Münzbergová et al. 2017; Knappová et al. 2018;

Münzbergová et al. 2018). Specifically, the material consisted of 40 *F. rubra* plants collected in July 2014, being at least 1 m apart from each other at each locality. Living plants were transported to the experimental garden of the Institute of Botany, Czech Academy of Sciences in Průhonice, Czech Republic (49°59'38.972"N, 14°33'57.637"E; mean temperature of the four warmest months 16.5°C and regular watering during the vegetation season) and planted into pots (16 × 16 × 16 cm, filled with a mixture of common garden soil and sand in 1:2 ratio) just after the transport at the end of July 2014. The common garden soil comprised compost from the experimental garden containing approximately 0.135 % of nitrogen, 1.35% of carbon and 46.5 mg of phosphorus in 1000 g of soil. After the plants recovered from the transport, they were extracted from the pots and reduced to a single ramet. This was done to make sure that we only have one genotype per pot avoiding the possibility that we originally collected multiple intermingled genotypes.

Plants from all 12 populations were cultivated for 2 years before collecting the seeds. In early spring 2016, the plants, that were about to flower were transferred into 120 × 80 × 60 cm cages covered with a double layer of fine mesh fabric. The mesh size was enabling the light and wind to go through the cage, but preventing the pollen from outside to penetrate in. In each cage, only plants from one locality were placed. In this way, the plants could be cross-pollinated within population but not between populations. From July to August 2016 we collected the ripe seeds and kept the seeds of each mother plant separately. The seeds were stored at room temperature (about 20°C and ambient moisture of about 55%) for 10 weeks before the experiments. All plants performed well in the experimental garden, and it is thus unlikely that quality of the seeds would be affected by low performance of some of the plants in the garden. We thus expect that differences in seed germination behavior are not due to different distance between the natural sites and the experimental garden, but rather due to effect of the original conditions (see discussion for more details on the issue).

Nutrient analyses

To assess whether concentrations and/or content of nutrients depend on original climatic conditions, we determined the concentrations of nitrogen, phosphorus and carbohydrates in the form of starch and fructans in seeds from 4 populations (circled in Fig 1.). All nutrients were expressed as a percentage of dry biomass i.e. concentration. To observe if the negative relationship between concentrations of nutrients and seed mass is caused by dilution of the content in case of larger seeds, we multiplied concentrations of all study nutrients by seed mass. We refer to these values as contents of nutrients.

While we initially planned to used seeds from the 4 most extreme populations, we had to replace seeds from ALP4 and BOR4 with seeds from ALP3 and BOR3 because of insufficiency of seeds from ALP4. To assess whether seed nutrients differ between mother plants, we determined the concentrations of nutrients in 10 randomly chosen mother plant from each population (subset of plants used for the germination test) i.e. we analyzed 40 mothers from 4 populations. While we initially intended to analyze the nutrients in the remaining populations in the next step, we did not do this as this initial testing showed strong correlation of all the nutrients with seed mass (Supporting information 2A).

Concentrations of nitrogen was determined according the Kjeldahl methodology (Kjeldahl 1883), concentrations of phosphorus was determined by mineralization by perchloric acid ($HClO_4$) (Talvitie, Illustre and Perez 1962). Concentrations of starch and fructans was measured by enzymatic procedure Megazyme (McCleary et al. 1994). All analyses of all mothers included 3 replicates. The nutrient analyses were performed in the Analytic Laboratory of the Institute of Botany of the Czech Academy of Sciences.

Seed mass

For each population (11), we randomly selected at least twenty mother plants, for which we determined weight of all collected seeds of one mother plant and number of seeds in the sample. These values were used to express weight of thousand seeds.

Germination tests

From each population (11), we chose 14 to 20 mother plants which produced at least 20 undamaged, fully developed, seeds. We put a mix of 20 seeds from one population from different mother plants into one Petri dish (5 cm in diameter) with three layers of filter paper. In each case, we created 20 Petri dishes composed of seeds from exactly the same maternal plants in the same proportions. These identical 20 dishes were divided between 4 treatments – 5 for each treatment (warm/cold × wet/dry). Another mixture of mother plants was created for other 20 Petri dishes of seeds from one population. This means that we had 2 sets of dishes (40 dishes in total) from each population. For ALP3, ALP4, SUB1 and SUB4 we were able to create only 10 identical Petri dishes and 2 different sets of genotype mixtures (20 dishes in total from one population), due to shortage of seeds. These populations were exposed to warm-wet and cold-wet regimes only, the dry regimes were not established for these.

We used 4 germination conditions in growth chambers, further referred to as target conditions. Specifically, we combined 2 levels of temperature × 2 levels of moisture. Two temperature conditions, warm and cold, were derived from long term measurements at the localities in Norway (for details see Tingstad et al. 2015) and were based on temperatures of the four warmest months of the year. The day temperature corresponded to average highest day temperatures at the coldest and the warmest localities of origin of the plants, the night temperature corresponded to the mean of the lowest night temperatures at the same localities. The resulting night/day temperature was 3°C/12.5°C (as a proxy for alpine conditions). The warm condition was set to 3°C/24.5°C (as a proxy for boreal conditions). The daylight period lasted from 6-22 o'clock. Dawn and twilight lasted for 2 hours before and after the night. The night period lasted from 24 till 4 o'clock.

The wet conditions were simulated by watering with demineralized water (water potential 0.0 MPa) and dry conditions by decreased water potential by addition of polyethylene glycol (PEG, molecular weight 6000) into the water. The water potential was kept approximately -0.7 MPa (intermediate dry) throughout the experiment. According to (Young and Nobel 1986), this water potential approximately corresponds to the rainfall of 600 mm. According to (Evans and Etherington 1990), such water potential represents intermediately dry conditions. The solutions of PEG were prepared and adapted to each germination temperature according to (Michel 1983). PEG adjust water potential without affecting seed germination in other way than due to the moisture itself (Hardegree and Emmerich 1994). From the 12th week of the experiment, we were using more water in the PEG solution (40% more water i.e. approximately -0.4MPa), because a lot of dishes did not germinate at all.

The germinated seeds were recorded every week. The seed was considered germinated if the radicle was visible to the naked eye. Positions of Petri dishes were shifted randomly after a week. Mouldy seeds with decomposed embryo were removed from the dish. When at least 60% of the seeds had germinated and no further seeds germinated for two subsequent weeks in a specific germination conditions, we applied Gibberellic acid in concentration 0.05 g/100 ml of demineralized water to the seeds to stimulate germination (Kahn 1960). After Gibberellic acid application, we continued seed germination recording every week. The germination after application of the Gibberellic acid was used to assess seed viability, but these seeds were not scored as ‘germinated’ within the main experiment. The experiment was terminated four weeks after application of the Gibberellic acid. At this time, we removed all rotten seeds from the dishes. Healthy looking ungerminated seeds were tested for viability by tetrazolium chloride method according to (Cottrell 1947). Because there were many healthy ungerminated seeds and the viability test is very time consuming, we only selected subsets from each population and treatment. In this way, we proved that the healthy-looking ungerminated seeds can really be considered as viable.

Data analysis

Effects of seed origin on nutrients and seed mass

Pair-wise correlation matrix, based on Pearson’s correlation coefficient, of concentrations and content of all studied nutrients (nitrogen, phosphorus, starch and fructans) and seed mass is presented in Supporting information 2A. Because of strong correlation of concentrations (all $r \geq |0.427|$) and most of contents of all nutrients ($r \geq |0.451|$) with seed mass, we used only seed mass for testing the effect on germination behavior.

We used ANOVA to test effect of original temperature and moisture and of mother plant nested within population on seed mass and all nutrients (nitrogen, phosphorus, starch and fructans, both concentration and content). As results on nutrient concentrations were similar to results of nutrient content, we present the results for nutrient concentrations only in Supporting information 2B.

Germination patterns

Germination behavior was defined as total germination, the time to 50% germination (T50), germination index (GI – describes ratio of the germination percentage and speed), proportion of dormant seeds and proportion of viable seeds. Total germination was defined as the sum of germinated seeds in one Petri dish over the period before application of the Gibberellic acid. Proportion of dormant seeds was defined as the proportion of all seeds that germinated only after Gibberellic acid application or were found to be viable after tetrazolium chloride application. Proportion of viable seeds was defined as sum of germinated seeds during whole experiment (before and after Gibberellic acid application) and seeds found to be viable after tetrazolium chloride application. GI was calculated with formula from (Liu et al. 2014):

$$GI = \sum \left(\frac{n_i}{t_i} \right)$$

where n_i is the cumulative number of germinated seeds in time t_i (in our case one-week intervals)

T_{50} was calculated by following formula:

$$\mathbf{T50 = \quad ti + \frac{\{(N/2) - ni\}\ (ti - tj\ )}{ni - nj}}$$

N is the final number of germinated seeds and n_i and n_j are cumulative number of seeds germinated by adjacent counts at times (weeks) t_i and t_j when $n_i < N/2 < n_j$ (Sadeghi et al. 2011). GI express the initial slope of the germination curve - greater GI indicates faster initial germination. T50 covers the complete germination curve. Lower T50 means overall faster germination. In cases, when no seeds germinated the value for total germination was zero and the value for GI and T50 was classified as a missing value. Pair-wise correlation matrix of the variables, based on Pearson's correlation coefficient, is presented in Supporting information 2C. Because of strong correlation of GI with total germination ($r = -0.861$), we did not use GI in the subsequent tests. Proportion of dormant seeds correlated with all variables (all $r \geq 0.419$), included high proportion of zero values and did not follow Gaussian distribution, thus we did not use proportion of dormant seeds in the subsequent tests. Comparison of the results using total germination and proportion of viable seeds indicate the proportion of dormant seeds. Specifically, high proportion of viable seeds and low germination suggests high proportion of dormant seeds.

We tested the effect of the temperature and moisture of the origin, the target temperature and moisture, seed mass and all their interactions on germination. A significant effect of target conditions will indicate phenotypic plasticity, a significant effect of origin will indicate genetic differentiation, and the interaction between target and origin will indicate genetic differentiation in plasticity.

Due to unbalanced design caused by not including four populations into dry target conditions (for details see section Germination tests) it was not possible to reliably analyze the whole dataset. Thus, we split the dataset and did three independent sets of tests.

First set of tests was carried out for all populations (11), but only wet target conditions were included into the test. To study effect of dry target condition, the second set of tests used both wet and dry target conditions, but only for populations BOR1 to BOR4 (4 populations, highlighted by square in Fig. 1), which did not allow to study effect of original temperature. The third set of tests was done using populations BOR2, BOR3, SUB2 and SUB3 (4 populations, underlined in Fig. 1), which allows us to test effects of original temperature, moisture and target temperature and moisture. All tests were done with and without including seed mass into the statistical model. As the first set of tests (including all populations without dry target conditions) is the most suitable for study effect of original conditions, we present them (both with and without seed mass) in the main text. The second and the third set of tests are presented in Supporting information 2D, only the effect of target moisture is described in the main text. Because germination tests were done as a mixture of mother plants in one Petri dishes (see section Germination tests), we calculated mean of seed mass for each population for these analyses.

All the tests of germination characteristics were done using general mixed effect models as implemented in the lme4 package in R (Bates et al. 2015) with population as a random factor. We assumed binomial distribution of total germination and proportion of viability seeds (information on number of germinating seeds/non-germinating and germinating + dormant/died seeds linked using c-bind function in R). T50 followed Gaussian distribution after log-transformation. All statistical analyses were done in the R programme (version 3.6.0.).

Correlation of plant performance and germination

To assess how the germination behavior correlates with plant performance, we created pair-wise correlation matrix based on Pearson's correlation coefficient (Table 1) with using data describe above and the data previously published in (Münzbergová et al. 2017). For this purpose, was used whole dataset of germination data in wet regime. Single values represented means for each population in each target conditions. Both these data sets used plants originated from the same localities and the temperature and moisture settings in the growth chambers were the same as well. As vegetative plant performance is the best characterized by number of ramets, weight of aboveground biomass and weight of belowground biomass, we used these variables.

Table 1. Pair-wise correlation matrix of germination response and plant performance (the means across the target conditions). Significant values (≤ 0.05) of Pearson correlation coefficients are in bold.

	Total germination	T50	Prop. viable seeds
Above.biom	**0.401**	-0.216	-0.231
Below.ground	**-0.743**	**0.655**	0.283
No.ramets	**-0.435**	0.224	**0.521**

Because the previous study (Münzbergová et al. 2017) providing data on plant performance was based on both wet and dry regimes, we had to re-analyse the data using only the wet regimes (identical as for germination). To do this, we used the same model as is describe in Test 1 for seed germination. As a dependent variable we used number of ramets, weight of aboveground biomass and weight of belowground biomass. All variables follow Gaussian distribution without any transformation.

Results

Effects of seed origin on nutrients and seed mass

We did not find any differences between mother plants in the content or concentration of nitrogen, phosphorus, starch and fructans (all p-values ≥ 0.259). Populations significantly differed in the content of nitrogen (F = 7.21, p = 0.011; Supporting information 2B), with the highest concentration in seed originated from cold, wet conditions (ALP3) and the lowest concentration in seed originated from warm, wet conditions (BOR3). We did not find significant differences in content of phosphorus (F = 0.12, p = 0.734; Supporting information 2B), but we found marginally significant difference in concentration of phosphorus (F = 2.76, P = 0.061, Supporting information 2B), with the highest concentration in cold, wet conditions (ALP3) and the lowest concentration in cold, dry conditions (ALP1). Content of starch and fructans did not differ between population (F = 0.22, p = 0.640 resp. F = 1.22, p = 0.277; Supporting information 2B).

Seed mass negatively correlated with concentration of nitrogen and phosphorus and positively with starch and fructans (Supporting information 2A). The relationship between nitrogen resp. phosphorus and seed mass disappeared after multiplying their concentration by seed mass, i.e. calculating their total content (Supporting information 2A). The highest seed mass was observed in the seeds coming from the warm and wet conditions and the lowest in the seeds from cold and wet conditions.

Effect of seed mass, original and target conditions on germination

The test of the effects of seed mass, target and original temperature and their interactions showed that target temperature significantly affected all germination-related variables (Table 2). Total germination and proportion of viable seeds increased and T50 decreased in

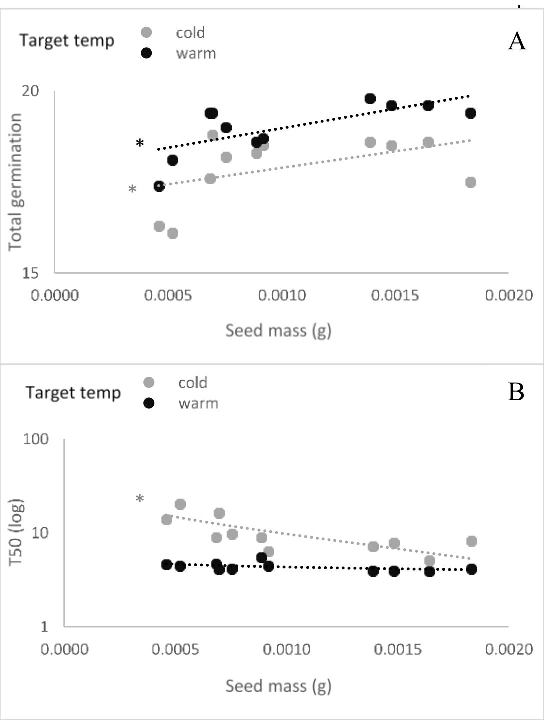

Fig 2. Effect of seed mass and target temperature on A) total germination (number of seeds out of 20) and B) T50. In both cases, there is a significant interaction between target temperature and seed mass. The graphs represent the first set of tests (including all populations without dry target conditions). One point in the graph represent mean total germination resp. germination speed of one study population in one target conditions. * indicates significant ($p \leq 0.05$) relationship between seed mass and the dependent variable in the given target temperature.

warmer target temperature. Original temperature significantly influenced total germination and proportion of viable seeds (Table 2) with higher total germination and proportion of viable seeds in seeds coming from warm conditions. Original moisture significantly affected only T50 (Table 2) with the fastest germination in seeds coming from the wettest condition and the slowest germination in seeds coming from the driest conditions. Seed mass significantly affected all germination-related variables (Table 2). Total germination and proportion of viable seeds increased and T50 decreased with increasing seed mass.

We found only two significant double interaction, specifically interaction of target temperature with seed mass and original temperature with seed mass (Table 2). Interaction of target temperature and seed mass significantly influenced all germination-related variables (Table 2). Total germination and proportion of viable seeds were the highest in heavy seeds exposed to warm target temperature and the lowest in light seeds exposed to cold target temperature (Fig. 2). T50 was the highest (i.e. germination speed was low) in light seeds exposed to cold target temperature and the lowest (i.e. germination speed was

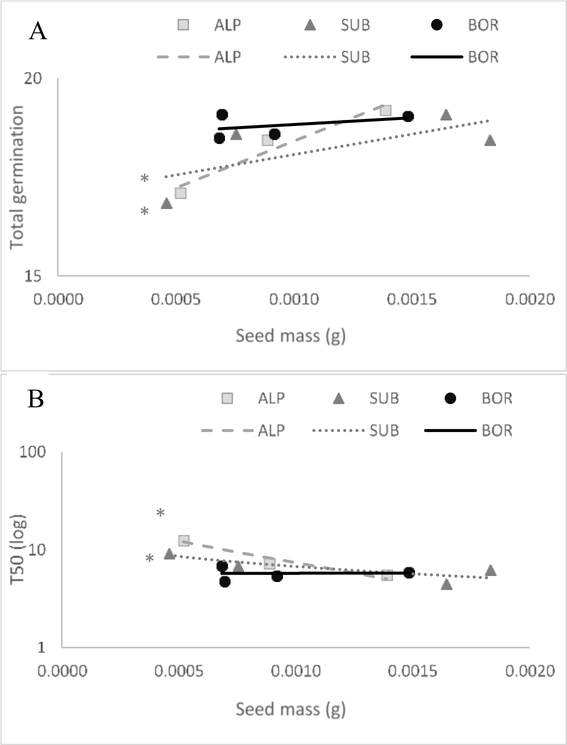

Fig 3. Effect of seed mass and original temperature (ALP, alpine is the coldest, BOR, boreal is the warmest) on A) total germination (number of seeds out of 20) and B) T50. In both cases, there is a significant interaction between target temperature and seed mass. The graphs represent the first set of tests (including all populations without dry target conditions). One point in the graph represent mean total germination resp. germination speed of one study population. * significant relationship between seed mass and the dependent variable in the given original temperature.

high) in heavy seeds exposed to warm target temperature. In warm target temperature the differences between different seed mass was lower than in cold target temperature (Fig. 2). Interaction of original temperature and seed mass significantly influenced all germination-related variables (Table 2). Total germination and proportion of viable seed were the highest in heavy seeds coming from the warmest conditions and the lowest in light seeds coming from colder conditions (Fig. 3). T50 was the highest (i.e. germination speed was low) in light seeds coming from the warmest conditions and the lowest (i.e. germination speed was high) in heavy seeds coming from the warm conditions (Fig. 3).

The results of the model not including seed mass were largely the same as above with few exceptions. Effect of original temperature and moisture became non-significant when seed mass was not included in the model (Table 2). In contrast, we found significant effect of interaction of original moisture and temperature on total germination in this case (Table 2). The highest total germination was in seeds coming from the warmest and wettest conditions and the lowest in seeds coming from the coldest and intermediate wet conditions.

Table 2. Effect of seed mass, original (O) and target (T) conditions on total germination, proportion of viable seeds and time to 50% germination (T50) assessed using mixed effect models with population used as a random factor. Table represents results all populations without wet regime (the first analysis). Significant values (≤ 0.05) are in bold. * indicate significant result in the model not including seed mass. ● indicate non-significant results in the model not including seed mass.

	Total germination		**Prop. viable seeds**		**T50**	
	F-values	p-value	F-values	p-value	F-values	p-value
TTemp	**32.27**	**<0.001**	**23.53**	**<0.001**	**6.85**	**0.010**
OTemp	**6.60**	**<0.001●**	**3.66**	**0.038●**	1.29	0.265
OMoist	1.70	0.152	1.07	0.304	**4.25**	**0.048●**
Seed mass	**19.39**	**<0.001**	**13.49**	**<0.001**	**9.44**	**0.004**
TTemp: OTemp	0.41	0.351	1.00	0.356	0.88	0.350
TTemp: OMoist	0.13	0.700	0.14	0.693	2.92	0.089
OTemp: OMoist	0.13	0.701*	0.05	0.738	0.28	0.600
TTemp: Seed mass	**4.05**	**0.017**	**5.03**	**0.009**	**5.48**	**0.020**
OTemp: Seed mass	**4.59**	**0.019**	**2.67**	**0.036**	**4.98**	**0.033**
OMosit: Seed mass	1.38	0.194	0.84	0.396	3.90	0.057
TTemp: OTemp: OMoist	0.24	0.656	0.50	0.637	0.30	0.586
TTemp: OTemp: Seed mass	0.21	0.598	0.00	0.859	2.93	0.089
TTemp: OMoist: Seed mass	0.06	0.741	0.11	0.751	2.32	0.130
OTemp: OMoist: Seed mass	0.80	0.438	1.39	0.215	1.91	0.177

The other two models, which included target moisture, showed significant effect of target moisture on all germination-related variables (Supporting information 2C), with higher total germination in wet condition and higher T50 and proportion of viable seeds (especially high proportion of dormant seeds) in dry conditions. The most interesting interactions were the following. Target moisture significantly interacted with target temperature and affected total germination and proportion of viable seeds (Supporting information 2D). The highest values of total germination were in wet and warm conditions and the lowest in dry and cold conditions. Proportion of viable seeds had the lowest numbers in wet and cold target conditions and the highest numbers (especially high proportion of dormant seeds) in dry and cold target conditions. Target moisture interacted with original temperature and affected proportion of viable seeds (Supporting information 2D), with the highest numbers (especially high proportion of dormant seeds) in seeds coming from warmer condition and exposed to dry target condition. The lowest numbers were observed in seeds coming from colder conditions and exposed to wet target conditions. In these two models were detected interactions of target and original conditions (Supporting information 2D).

Correlation of plant performance and germination response

Total germination positively correlated with aboveground biomass and negatively with belowground biomass and number of ramets (Table 1). T50 positively correlated only with belowground biomass and number of viable seeds positively correlated with number of ramets (Table 1). No other correlations between plant performances and germination responses were significant.

Target temperature significantly affected all germination characteristics, as described above, as well as belowground biomass, aboveground biomass and marginally number of ramets ($F = 5.25$, $p = 0.022$, $F = 7.59$, $p = 0.006$, $F = 2.98$, $p = 0.085$, respectively). Original temperature and moisture had significant effect on germination (Table 2), but not on any of the vegetative characteristics ($p \geq 0.248$). In contrast, belowground biomass ($F = 5.05$, $p = 0.025$) and number of ramets ($F = 3.19$, $p = 0.075$, marg. sig.) were affected by interaction of target temperature and original moisture, while germination characteristics were not (Table 2). We did not find any other significant effects of interactions on germination (Table 2, except interactions with seed mass) or on plant performance ($p \geq 0.096$).

Discussion

Seed mass, content of nitrogen, but not of carbohydrates were affected by original conditions. Seed mass correlated with all the studied nutrients with heavier seeds having higher content of carbohydrates. In all analyses, increasing seed mass had positive impact on germination (higher and faster germination), while effect of original conditions was significant only in some analyses. Significant results show higher germination and seed viability (viability increase lower than germination, which indicate low proportion of dormant seeds) in seeds coming from the warmest condition. Warm target temperature increased germination, T50 and reduced proportion of viable seeds (i.e. low proportion of dormant seeds in warm target temperature). Target temperature and moisture interacted, but original and target conditions not. Wet and warm target conditions were suitable for germination of all populations. In other germination conditions, which were less suitable for germination (warm and dry), we found high proportion of viable seeds, including high proportion of dormant seeds. Total germination showed strong negative correlation with belowground biomass and number of ramets and positive correlation with aboveground biomass.

Effects of seed origin on nutrients and seed mass

Higher seed mass is commonly expected to be associated with higher concentration of seed nutrients (Wulff and Bazzaz 1992; Rees et al. 2001). Intraspecifically, few studies confirmed this relationship for nitrogen, phosphorus and sulfur contents (Vaughton and Ramsey 2001; (Obeso 2012; Kolodziejek 2017). In our study, concentrations of all nutrients correlated with seed mass, however, only starch and fructans positively. As expected, only carbohydrates contributed positively to seed mass as they have higher molecular weight than nitrogen and phosphorus. Concentration of nitrogen and phosphorus negatively correlated with seed mass. The possible explanation of our results is that the same amount of nitrogen and phosphorus is more diluted in larger seeds. This explanation was confirmed in our data as the relationship with seed mass became non-significant for nutrient content.

We found significant differences between populations both in the concentration and content of nitrogen with the highest values in seeds coming from the coldest and the wettest conditions and marginally significant differences between populations. All plants performed well in the experimental garden, and it is thus unlikely that quality of the seeds would be affected by unequal performance of some of the plants in the garden. In line with our results, (Bu et al. 2018) demonstrated higher content of nitrogen in seeds from the highest altitude i.e. in the coldest and the wettest conditions at interspecific level. Their results are based on seeds collected directly in the field Possible explanation may be that these conditions are not the most suitable for seed recruitment (Gardarin et al. 2011; Walder and Erschbamer 2015) and species invest the energy to the specific seed nutrients to supported seed establishment before they develop the root system. The higher nitrogen content of seeds from specific conditions might derive from adaptive selection (Bu et al. 2018). We did not find any differences between mother plants within populations. Only those mother plants, which sufficiently invest to seed nutrients and/or can accumulate the nutrients quickly grow on study localities and simultaneously they do not invest and/or accumulate more than it is necessary for their specific locality. Another possible explanation may be that plants from higher elevations accumulate more nitrogen in biomass and can thus invest more also into the seeds. It was demonstrated that content of nitrogen in leaves of plants in the field increased with increasing altitude of plant origin (Körner and Cochrane 1985; Friend, Woodward and Switsur 1989; Körner 1999; Oleksyn et al. 2002) and Münzbergová et al. (2020) illustrated the same trend in plants of different origin cultivated in a common garden. They suggest that this may be because plants need accumulate the nutrients quickly in the usually severe environment with short vegetation period (Körner 1999). These results maty thus explain the higher nutrient content in the seeds of plants from higher elevations.

Differences in content of nutrients in seeds could be caused by differences in soil compositions (Lukac et al. 2010). Our localities of maternal plant origin were chosen to ensure that bedrock was as similar as possible and the seeds come from plants cultivated in a uniform common garden. We thus expect that effect of soil composition is much less important than the climate itself.

In line with previous studies (Winn and Gross 1993; Wulff and Bazzaz 1992; Zas and Sampedro 2015; Gorden et al. 2016), we found higher seed mass in plants originating from warmer and wetter conditions. It is thus possible that ongoing changes in temperature and precipitation will have impact on seed mass, which could further affect for instance dispersal, predation, seed-bank persistent or seedling establishment.

Effect of seed mass, original and target conditions on germination

Many previous studies suggested that larger seeds have higher and faster germination (e.g., Greipsson and Davy 1995; Münzbergová and Plačková 2010; Wu and Du 2007; (Paulů et al. 2017; Veselá et al. 2019). This is commonly attributed to higher content of nutrients, which nourish the sprout (Rees et al. 2001). Our study demonstrated that higher seed mass is caused by higher concentration only of carbohydrates not of nitrogen and phosphorus. Therefore, higher concentration of carbohydrates could contribute to higher and faster germination. However, more extensive studies are required to confirm this.

Seed mass interacted with target temperature, with the fastest and highest germination in heavy seeds exposed to warm conditions. This result could indicate that carbohydrates are more effectively used in warm target conditions. In our previous study, we showed that seed mass interacted with original conditions with higher and faster germination in seeds coming from warm and dry localities (Veselá et al. 2019). In the current study, we observed a similar trend but only for the interaction of seed mass with original temperature and not moisture. To our best knowledge, no other study tested the interaction of seed mass with original climatic conditions.

Original temperature itself significantly affected total germination and proportion of viable seeds. In line with our expectation, higher values were observed in seeds coming from warmer conditions. This trend has been observed in studies using seeds collected directly in the field (Cruz et al. 2003; Ndihokubwayo et al. 2016; Santo et al. 2015; Bauk et al. 2017; Veselá et al. submitted). In the field studies, this result could be caused by longer vegetation period in lowlands allowing seeds to properly develop (Milbau et al. 2009; Meineri et al. 2013). The same trend was detected also in plants originating from warmer conditions and then cultivated in common garden conditions (Mira et al. 2017; Veselá et al. submitted). The explanation could be that plants adapted on longer vegetation period bloom earlier and experience long vegetation period in garden as well, while plants originating from cold conditions expect late frost, bloom later and experience short vegetation period in garden (Kreyling et al. 2012; Walter et al. 2013). Another possible explanation could be the higher seed mass in seeds originating from warmer conditions as described above. Germination response of *F. rubra* was affected by original moisture, but only for T50. Similar result was found in (Veselá et al. submitted). However, both the effects of original temperature and moisture were significant only in the model including seed mass. This result could indicate that seed mass masks the effect of original conditions.

It is known that target conditions have strong impact on seed germination (e.g., Grime et al. 1981; Schütz and Rave 1999; Gardarin et al. 2011). Our results are in line with other studies demonstrating that germination increases in higher target temperatures (Gardarin et al. 2011; Walder and Erschbamer 2015, but see Veselá et al. 2019). Temperature and moisture can interact and significantly influence germination response (Rivas-Arancibia et al. 2006; Gurvich et al. 2017; Veselá et al. submitted) as in the case of *F. rubra.* Total germination and T50 was negatively affected by decreasing target moisture both in cold and warm target temperature (similarly e.g., Wen et al. 2015; Ruhl et al. 2015), but high proportion of viable seeds, indicating high proportion of dormant seeds, in all dry target conditions suggests that the seed are able to survive period of drought and germinate in wet season. Significant effects of target conditions indicate high plasticity of germination response of *F. rubra*, which may enable species to tolerate fluctuating climate conditions (Wainwright and Cleland 2013).

Phenotypic plasticity can be defined as 'the ability of one genotype to produce different phenotypes when exposed to different environmental conditions' (Pigliucci, Murren and Schlichting 2006). While this concept is common use to describe changes in plant performance, it needs to be used with caution when talking about seed germination. When seeds do not germinate and die, seed germination cannot be considered as a phenotypically plastic. In contrast, when seeds do not germinate and stay dormant, we can talk about phenotypic plasticity, as dormancy is response on environmental condition similarly as germination speed (Baskin and Baskin 2014). Our seeds thus show significant phenotypic plasticity in response to warm-dry conditions.

We expected that original and target conditions will interact similarly as in our previous study (Veselá et al. submitted), but no such interactions have been detected in the main results of this study. This could be because we did not include target moisture into the main analysis. These interactions both for total germination and proportion of viable seeds were found in additional analyses studying target moisture. Only a few studies focused on the importance of this interaction for germination (reviewed in Walck et al. 2011). These interactions indicate that plants of different origin have different ability to respond plastically to target environmental conditions and they are able to switch into dormancy in case of the conditions are not suitable for germination. This interaction can thus be considered as genetic differentiation in plasticity (*sensu* Pigliucci 2001).

Correlation of plant performance and germination response

Many studies have detected negative correlation between generative and vegetative reproduction (e.g., Cheplick 1995; Worley and Harder 1996; Ronsheim and Bever 2000; van Kleunen et al. 2002; Herben et al. 2012), while others have not (e.g., Reekie 1991; Cain and Damman 1997). Negative correlations of total germination with belowground biomass and number of ramets in our study confirm the negative correlation between generative and vegetative reproduction. Number of ramets positively correlated with proportion of viable seeds, which is in line with previous correlation, as proportion of viable seeds includes both germinated and dormant seeds. It is highly probable that in cases of high proportion of dormant seeds, growth of ramets is supported. Positive correlation of belowground biomass and T50 support the statement described above, since with slow germination (high T50), production of vegetative organs is preferred. All the results indicate that *F. rubra* shows trade-off between generative and vegetative reproduction.

In our study, target temperature affected plant performance and germination, in plants originated from the same localities and exposed to the same target temperatures. This is in line with a range of previous studies studying either plant performance (Münzbergová et al. 2017) or germination (Gardarin et al. 2011; Walder and Erschbamer 2015). This result indicates that *F. rubra* has high phenotypic plasticity both for plant performance and germination and species will probably successfully cope with ongoing climate change. We did not find effect of original climate on plant performance, despite the previous study providing plant performance data (Münzbergová et al. 2017) found this effect.. This is likely because our study used only plants cultivated under wet target conditions, while (Münzbergová et al. 2017) used complete dataset, i.e. plants cultivated under both dry and wet conditions. This result suggests that genetic differences in plant performance may be detected just under some conditions. While drier conditions seem more stressful for plant germination, these same conditions are probably less stressful for plant growth (as the plants in wet conditions suffered from water logging).

Conclusion

To our best knowledge, this is the first study demonstrating relationship between seed mass and specific seed nutrients across localities experiencing different climatic conditions. Our results indicate that higher seed mass is caused by higher content of carbohydrates. Further, it seems that higher seed mass, and probably higher content of carbohydrates, may play role in higher and faster germination in seeds coming from warmer localities and simultaneously also in higher and faster germination in warmer target conditions. As seed mass is strongly affected by climate, it is possible that ongoing climate change will have impact on seed mass, which could further affect for instance dispersal, predation, seed-bank persistent or seedling establishment. Germination response of *F. rubra*, as a dominant species of meadows, shows high proportion of germinated or dormant seeds depending on the target conditions. This suggests phenotypic plasticity of the study species. Phenotypic plasticity allows species to cope with changing climate. When the target conditions were unsuitable and germination was low, seeds stay dormant and probably they would be able to germinate in more suitable condition. Simultaneously, *F. rubra* shows a trade-off between generative and vegetative reproduction indicating that the species is able to modify investments to generative or vegetative reproduction depending on the actual conditions. The importance of target and origin for germination cannot be, however, easily predicted from their effects on growth. Both these types of variables should thus be studied in future studies to obtain an overall picture of plant performance under changing climates.

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Study 3: SEED MASS AND PLANT ORIGIN INTERACT TO DETERMINE ALPINE SPECIES GERMINATION PATTERNS ALONG AN GRADIENT

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Abstract

Ongoing changes in temperature and precipitation regime may have a strong impact on vulnerable life-history, stages such as germination, especially in alpine regions. Differences in germination patterns among species and populations may reflect their adaptation to conditions of their origin or may be determined by the phylogenetic constraints. These two effects are, however, rarely separated. All the germination patterns may also be modified by seed mass. We studied 40 populations of 14 species of Impatiens coming from different elevations in the Himalayas. Germination was tested in four target temperatures: three simulating original conditions plus a warmer climate change scenario. We also studied the combined effect of shorter stratification and warmer temperature as another possible effect of climate change. Original and target climate differences had a strong impact on total germination, but not on germination speed and seed dormancy. Seed mass and original climate interaction indicated different germination strategies in light and heavy seeds. Only seed mass was affected by phylogenetic relationships among the species, while germination response (except T30) was driven primarily by climate of origin. This study is the first to show that the effect of seed mass interacts with original climate in determining species' germination patterns under changing climate. The differences in seed mass are thus likely crucial for species' ability to adapt to novel conditions since seed mass, unlike seed germination patterns, is strongly phylogenetically constrained. Further studies exploring how seed mass modifies species' germination under changing climate are needed to confirm generalisability of these findings.

Key words: phylogeny, seed mass, reproduction characteristics, original environment, target environment, climate change, plant-climate interactions

Introduction

Ongoing as well as forecasted increases in temperature and changes in precipitation regimes as predicted (IPCC 2014) may have a strong impact on vulnerable stages in plant life-history, such as, germination, seedling emergence and seed production (Knapp et al., 2008; Walck et al., 2011; Dreesen et al., 2014) due to their strong dependency on temperature and moisture (Baskin and Baskin, 2001; Fenner and Thompson, 2005). Alpine species require special attention since they have limited possibilities to migrate to colder conditions and must therefore respond to changing climate via adaptation or phenotypic plasticity. Simultaneously, earlier snow melting caused by increasing temperatures may lead to increased seedling establishment due to the extension of the growing period (Mondoni et al. 2012). The effect of climate change on seeds and seedlings of alpine species has, however, been largely neglected (reviwed in (Briceno, Hoyle and Nicotra 2015).

The impact of environment both original (Meyer, Allen and Beckstead, 1997; Santo, Mattana and Baechetta, 2015; Seglias et al.,2018) and target environment (Grime et al., 1981; Schütz and Rave, 1999; Gardarin, Daurr and Colbach, 2011) affects germination at both interspecific (Gimenez-Benavides, Escudero and Perez-Garcia 2005) and intraspecific level (Qaderi and Cavers, 2002; Tingstad et al., 2016; Satyanti, Guja and Nicotra, 2019). Recent studies, for instance, found that populations from the warmest conditions showing the highest germination (Santo et al., 2015; Ndihokubwayo, Nguyen and Cheng, 2016; Bauk et al., 2017; Mira, Arnal and Perez-Garcia, 2017). Further, many studies demonstrated increasing germination with increasing target temperatures (Gardarin et al., 2011; Klady, Henry and Lemay, 2011; Walder and Erschbamer, 2015), but some studies detected the opposite effect (Hovenden et al., 2008; Gao et al., 2012). Only a few studies have focused on the interactions of original and target climate on species' germination (reviewed in Walck et al., 2011) though these interactions are known to strongly affect plant performance (Münzbergová et al., 2017) as well as species' germination (Veselá et al., submitted).

In nature, seeds are exposed to cold stratification, which is often necessary for dormancy breaking, especially in mountain (Meyer 1992) and temperate zone species (Baskin and Baskin 1988). Even though some seeds are able to germinate without stratification, seeds with stratification germinate more and faster (Martin, 1965; Cavieres and Arroyo, 2000; Garcia-Fernandez et al., 2015). The duration of stratification also plays an important role in species' germination. Some species germinate well after one or two months of stratification (Carta et al., 2014; Perglová et al., 2009) while others require stratification of six and more months (Schütz and Milberg, 1997; Esmaeili et al., 2009 ; Perglová et al., 2009 ; Garcia-Fernandez et al., 2015; Schütz and Milberg, 1997). Necessary stratification duration can vary not only among species but also among populations of one species, as demonstrated by Cavieres and Arroyo, 2000). Specifically, stratification duration may vary along elevation gradients, with seeds from higher altitudes requiring longer stratification than seeds from lower altitudes. The ongoing climate change may shorten the stratification period (IPCC, 2014) possibly leading to decreased germination in some species (Garcia-Fernandez et al., 2015; Carta et al., 2016b). The effects of reduced stratification on species' germination in warmer climates have, however, not yet been studied.

Germination characteristics depend not only on environmental conditions but also on seed traits such as seed mass (Wang et al., 2009; Wang et al., 2012; Liu et al., 2013; Hradilová et al., 2019; Rees et al., 2001) hypothesised that heavier seeds have higher content of nutrients and higher percentage germination. The positive effect of seed mass on germination at intraspecific level was also confirmed in studies by Navarro and Guitian (2003) and by Münzbergová and Plačková (2010) and at interspecific level by Wu and Du (2007) and Paulů, Harčariková and Münzbergová (2017). Some studies (Wang et al., 2009; Wu, Li and Du, 2011) did, however, show the opposite trend. The effects of seed mass on species' response to changing climatic conditions, however, remain to be explored.

Seed traits including seed mass are strongly associated with phylogeny ((Zhang, Du and Chen, 2004; Moles et al., 2005; Norden et al., 2009; Barak et al., 2018). Phylogeny explains a considerable part of the variance in germination among species (Bu et al., 2008; Wang et al., 2009; Xu et asl., 2014; Seglias et al., 2018) and a strong phylogenetic signal was found also in seed dormancy traits (Dayrell et al., 2017). The likely cause is that phylogeny imposes limits to variability in reproductive attributes within a clade because of similar developmental and design constraints in related species (Ackerly and Donoghue, 1995; Figueroa and Armesto, 2001). To our knowledge, there is, however, only one study focusing on the effects of within-genus phylogeny on species' germination. It is based on seven species and showed that more closely-related species had more similar germination behaviour and the authors (Carta, Hanson and Muller, 2016a) point out that this subject requires further attention. Such knowledge is likely to increase our understanding of the evolution of the requirements for species' germination.

In this study, we explored germination response and necessary stratification duration of 40 populations belonging to 14 species of the genus *Impatiens* coming from an elevation gradient in the Himalayan mountains in Nepal. The aim of our study was to answer the following questions: (i) What is the effect of seed mass and original and target conditions on species' germination and the necessary stratification duration? (ii) Does shorter stratification influence germination response to warmer conditions? (iii) Are the patterns affected by phylogenetic relationships among the species?

We hypothesised that germination will increase with increasing seed mass. It will also increase with increasing original as well as target temperature, and the original and target effects will interact with each other. Populations from warmer conditions will require shorter stratification than populations from colder conditions and reduced stratification will reduce germination in warmer temperature. Concurrently, we predict that more closely-related species will have more similar germination responses and the effects of seed mass and target and original conditions on seed germination will be thus weakened when accounting for species' phylogeny.

Material and methods

The studied species and their seed collection

We used species of the genus *Impatiens*, Balsaminaceae, for the study. Genus *Impatiens* is highly-diversified genus of annual or perennial herbs comprising over 1 000 species, commonly occurring at high altitudes (i.e., more than 1 500m above sea level), distributed in the mountains of the Old World tropics and subtropics, with only few species occurring in northern hemisphere temperate regions (Grey-Wilson, 1980; Yuan et al., 2004; Janssens et al., 2009). One of the biodiversity hotspots of the genus is found in the eastern Himalayas and south-east Asia (Song, Yuan and Kupfer, 2003; Yuan et al., 2004; Yu et al.,2016) - the region of our study. The species occupy diverse habitats such as forest understory, roadside ditches, valleys, abandoned fields, stream banks and seepages (Yu et al., 2016). By contrast with many other evelational gradients showing increased precipitation with increasing elevation (Sevruk, 1997; Gouvras, Sakellariou and Xystrakis, 2009; Soomro et al., 2019), in our system, there is a strong negative correlation of altitude with precipitation ($r = -0.735$, using Pearson's correlation coefficient).

In total, we used seeds collected from 40 populations, with seeds from 24 populations collected in 2016 (September – October) and 16 populations in 2017 (September – October) belonging to 14 species (a list of species and locations of their collection are provided in 'Supporting information 1'). Seeds collected in 2017 came from different populations than seeds collected in 2016, but often belonged to the same species (Supporting information 1).

Populations were distributed along an elevation gradient (1 100m – 3 700m a.s.l.) representing populations originating from different climates. which is a commonly-used method in this type of study (e.g., Cavieres and Arroyo, 2000; Gimenez-Benavides, 2005; Tingstad et al., 2016). Seeds in each population were collected from five maternal plants. This collection was driven by the size of the populations, some of which are very small. Thus, five maternal plants sufficiently represent variation in population. Seeds from each maternal plant were kept separately during storage and germination tests. After collection (in both years), the seeds were stored for five months before the beginning of the experiments at ambient moisture level (approximately 55%) and at room temperature (approximately 15 °C). For each population, we recorded the year of collection and the origin of the population (altitude, longitude, latitude) and the determined weight of one thousand seeds. Further, we determined original temperature and original precipitation, which were derived from the WorldClim database (Hijmans et al. 2005) according to the co-ordinates of the populations. We used mean temperatures from March to June since that represents the pre-monsoon period when most *Impatiens* species germinate and start to grow.

Germination tests

Undamaged, fully-developed seeds were used for the germination tests. The seeds were incubated on moist filter paper in 5cm. diameter Petri dishes. We had only a limited number of available seeds because some of the populations were very small, The number of mother plants available for collection was also limited by the fact that seeds are released quickly after seed ripening and the window of opportunity when the seeds are ripe, but the seeds are still on the plant is narrow; not many plants met this condition. Thus, for each population and growth chamber (four growth chambers, see below), we established five replicates (each replicate corresponding to one mother plant) with 12 seeds on each Petri dish. We thus have 20 Petri dishes for each population. In cases when we had fewer seeds available, we used less than 12 seeds per Petri dish, but never less than six.

First, the seeds on Petri dishes were exposed to cold stratification (5°C). When at least one seed on 30% of Petri dishes germinated (i.e., at least one seed from at least six Petri dishes). All Petri dishes of that population were transferred to growth chambers (described below) and the time was recorded as the necessary stratification duration. Germinated seeds were recorded and removed every week. The seed was considered germinated if the radicle was visible to the naked eye. Weekly, we also removed all the rotten seeds from the dishes. When no seed of any population germinated for three subsequent weeks, the experiment was terminated. Healthy-looking un-germinated seeds were tested for viability by the tetrazolium chloride method (Cottrell 1947).

We used four combinations of germination conditions in the growth chambers, further referred to as target conditions. The target conditions were established with respect to the range of temperatures during the day in the original localities: (1) the coldest regime (further referred to as 6/17.5°C on the basis of minimum and maximum temperatures) - mean temperature from March to June at 2 700m a.s.l., (i.e., in the altitude representing median of upper altitudinal limits of *Impatiens* species in Nepal); (2) an intermediate cold regime (further referred to as 9/20°C) - mean temperature from March to June in 2 250m a.s.l. (i.e., in the altitude representing the median of centres of altitudinal ranges of *Impatiens* species in Nepal); (3) an intermediate warm regime (further referred to as 12/22.5°C) - mean temperature from March to June at 1800m a.s.l. (i.e., in the altitude representing median of the lowest altitudinal limits of *Impatiens* species in Nepal); (4) the warmest regime (further referred to as 15/25°C) - mean temperature from March to June in 1 800m a.s.l. (i.e., in the altitude representing the median of the lowest altitudinal range of *Impatiens* species in Nepal), in 2050 as predicted by the global climate model MIROC5 at RCP8.5 (Tatebe et al., 2012).

Information on the altitudinal ranges of *Impatiens* species in Nepal was obtained from the Annotated Checklist of the Flowering Plants of Nepal (http://www.efloras.org/flora_page.aspx?flora_id=110), which is an updated online version of (Press et al., 2000). Temperature data were obtained from WorldClim database (Hijmans et al. 2005). Data on mean temperatures in particular altitudes were obtained from slopes of correlations between the altitudes and the mean temperatures for particular data points along four valleys in Central and East Nepal where our seed collections took place. We used mean temperatures from March to June since it represents the pre-monsoon period (see above). The course of the temperatures during the day was modelled based on mean, minimum and maximum temperatures. The lowest day temperatures were from 3 a.m. to 5 a.m. The highest day temperatures were from 12 noon to 2 p.m. For all details see ‘Supporting information 2’. For all the regimes, the same daylength and radiation were used, i.e., 13 hours of light (with intensity 250 μmol m^{-2} s^{-1}) and 11 hours of dark. Petri dishes were regularly watered with demineralised water.

For seeds collected in 2017, only three growth chambers were available for our experiments due to technical constraints. For these seeds we used the same regimes as in the previous year, i.e., the coldest (6/17.5 °C), intermediate temperatures (12/22.5 °C) and the warmest (15/25 °C). We did not, however, use the intermediate cold regime (9/20°C). Instead, we added one additional germination regime with all the seeds which had been stratified for only one month and then transferred them to the growth chamber with the warmest regime (15/25°C). This temperature sequence simulated one possible impact of climate change, i.e., the shortening of stratification and a warmer climate. This regime was not used in the main statistical analyses presented in Table 1 and was only compared with seeds kept under stratification until 30% germination and subsequently moved to the warmest regime (i.e., 15/25°C).

Data analysis

The dependent variables in our analyses were: total germination, time to 30% germination (T30), germination index (GI - the ratio of the germination percentage and speed), seed dormancy, seed viability and the necessary stratification duration. Total germination was expressed as the proportion of germinated seeds from all seeds in one Petri dish over the whole period. We used T30 instead of the more commonly used T50 because of low germination rates in some treatment combinations (Benech-Arnold and Sánchez, 2004). T30 was calculated according to the method devised by Coolbear, Francis and Grierson (1984) and modified by Farooq et al. (2005). The germination index was calculated as described in the Association of Official Seed Analysts (AOSA 1983). GI resp. T30 cannot be calculated when 0 resp. 0 or 100% seeds of all the seeds in a Petri dish germinate and corresponding cases were thus excluded from this calculation. Seed dormancy was defined as the proportion of seeds, which were found to be viable after tetrazolium chloride application. Seed viability was the sum of the proportion of germinated and dormant seeds. The necessary stratification duration is described above (the time when at least one seed on 30% of the Petri dishes germinated). Pair-wise correlation matrix of the variables, based on Pearson's correlation coefficient, is presented in 'Supporting information 3'. Because of the strong correlation of GI and seed viability with total germination and the dormant seeds ($r \geq 0.577$), we did not use the seed GI and seed viability in further analyses.

We tested the effect of target temperature (temperature in the growth chambers), original temperature, original precipitation, seed mass and all their interactions on all dependent variables. To assess whether seed mass is affected by original climate, we tested the effect of original temperature, original precipitation and their interaction on seed mass. All the tests were done using generalised, linear mixed-effect models as implemented in the lme4 package in R (Bates et al., 2015) with population as a random factor. We assumed binomial distribution of total germination and seed dormancy (information on the number of germinating/dormant and non-germinating/non-dormant seeds linked using c-bind function in R). After log-transformation, T30 and necessary stratification duration followed Gaussian distribution. No transformation was necessary for seed mass. In all analyses, the year of seed collection, longitude, latitude and the interaction of longitude and latitude were used as covariates.

For the seeds collected in 2017, we did not study the germination in one of the target temperatures (intermediate cold – 9/20°C) owing to technical constraints; we repeated all the tests after excluding this regime from the total dataset. The results of this analysis are presented only in 'Supporting information 4' since, in most cases, they were in line with results presented in Table 1.

To compare germination of seeds which experienced shorter stratification with seeds stratified until germination in the warmest regime, we used generalised, linear mixed-effect models with population as a random factor, as described above. Since no dormant seeds were found in the warmest regime (i.e., 15/25°C), the effect on the number of dormant seeds was not tested in this case.,

To assess the effect of phylogenetic relationships among the species on the patterns observed, we used ITS-based phylogeny of the plant group, developed for the purpose of another study (Líblová et al, in preparation). A phylogenetic distance matrix describing the relationships between the species was broken down into its eigenvectors using PCoA as suggested by Diniz et al. (1998) and Desdevises et al. (2003) using the R-package 'ape' (Paradis et al. 2004). The first two eigenvectors explained 76 % of variability in the data.

Their effect was tested on all dependent variables and seed mass. In cases where eigenvectors had a significant effect (eigenvectors had high values), they were included as co-variables in the above-described models in order to correct for phylogenetic autocorrelation and to compare the effects of phylogeny to the effects of original and target environments.

Results

Effect of original and target environment and seed mass

All the germination characteristics were independent of original temperature, original precipitation and their interaction with the exception of necessary stratification duration (Table 1). Necessary stratification duration lengthened with increasing original temperature. The effect of original temperature on the necessary stratification duration also interacted with original precipitation; with the necessary stratification duration being the highest in seeds coming from warm and dry localities and the lowest in seeds coming from cold and wet localities (Fig 2).

All the germination characteristics were affected by the target temperature (Table 1), but with low explanatory power (Fig 1). T30 increased and total germination and seed dormancy decreased with increasing target temperature.

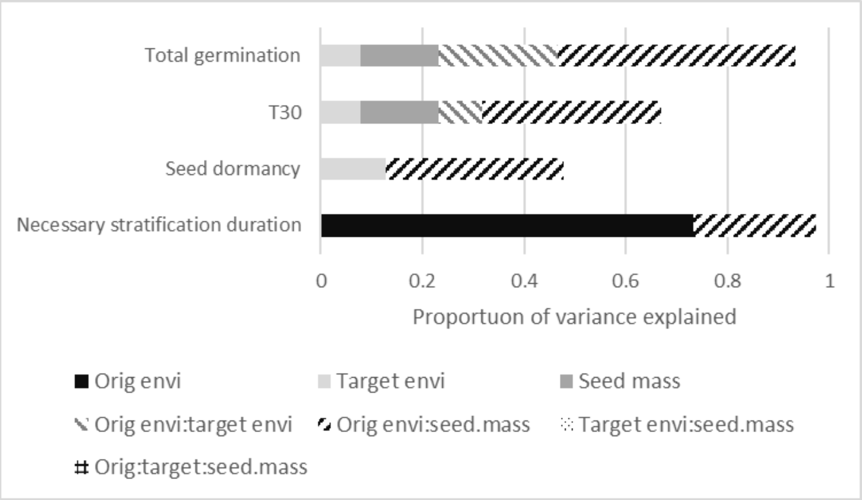


Fig 1. Proportion of variance explained by original environment (including both temperature and precipitation), target temperature, seed mass and their interaction on total germination, germination speed (T30), seed dormancy and necessary stratification duration. Interaction of target environment and seeds mass and triple interaction of original environment, target environment and seed mass are not figured in the graph as proportion of variance explained was very low.

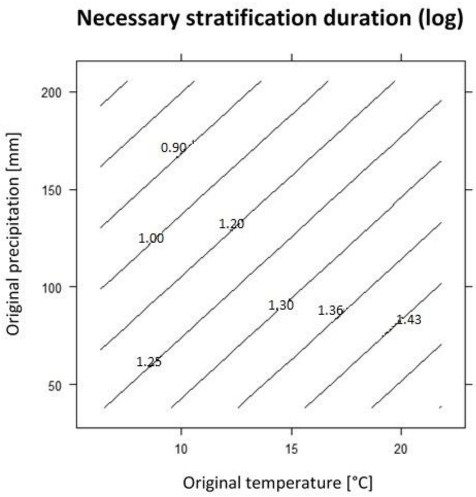


Fig 2. The effect of original temperature (°C) and original precipitation (mm) on necessary stratification duration in weeks (log). Increasing numbers in the graph indicate longer necessary stratification duration. Diagonal lines indicate equal dependency of necessary stratification duration on original temperature and original precipitation.

Total germination and T30 were also significantly affected by seed mass (Table 1) with heavier seeds germinating more but not as quickly as lighter seeds. The explanatory power of seed mass was similar to that of target temperature (Fig 1). Seed mass was significantly affected by original temperature (F = 15.34, p <0.001), original precipitation (F = 19.622, p <0.001) and their interaction (F = 31.56, p = <0.001).

Total germination and T30 were also affected by the interaction between original precipitation and target temperature (Table 1). Total germination was highest in seeds coming from the wettest localities exposed to intermediate cold conditions (9/20 °C) and lowest in seeds coming from the driest localities exposed to intermediate warm conditions (12/22.5 °C). By contrast, T30 was the highest in seeds coming from the wettest localities exposed to the coldest conditions (6/17.5 °C) and the lowest in seeds coming from the wettest localities exposed to the warmest conditions (15/25 °C).

Seed mass significantly interacted with original temperature and also with original precipitation in its effects on total germination, seed dormancy and necessary stratification duration (Table 1), with a high proportion of variance explained in total germination and seed dormancy (Fig 1). Total germination and seed dormancy were the highest in heavy seeds coming from the warmest localities and the lowest in light seeds coming from the coldest localities. Total germination was the highest in heavy seeds coming from the driest localities and the lowest in light seeds coming from the wettest localities and the germination was more dependent on seed mass than on original precipitation. Seed dormancy was the highest in heavy seeds coming from the warmest localities and the lowest in light seeds coming from the coldest localities. Necessary stratification duration increased with increasing seed mass and original precipitation.

Total germination was also affected by the triple interaction of original temperature, original precipitation and target temperature with quite a high proportion of variance explained (Fig 1). In all target temperatures (with the exception of intermediate warm conditions - 12/22.5 °C), total germination increased with increasing original temperature and decreasing original precipitation. In intermediate warm conditions (12/22.5 °C), germination increased with decreasing original precipitation and original temperature. Total germination in these target conditions was more dependent on original precipitation than on original temperature, while in the three other target conditions, germination was affected equally by both variables. The highest germination was observed in the coldest target conditions, i.e., 6/17.5 °C (Fig 3).

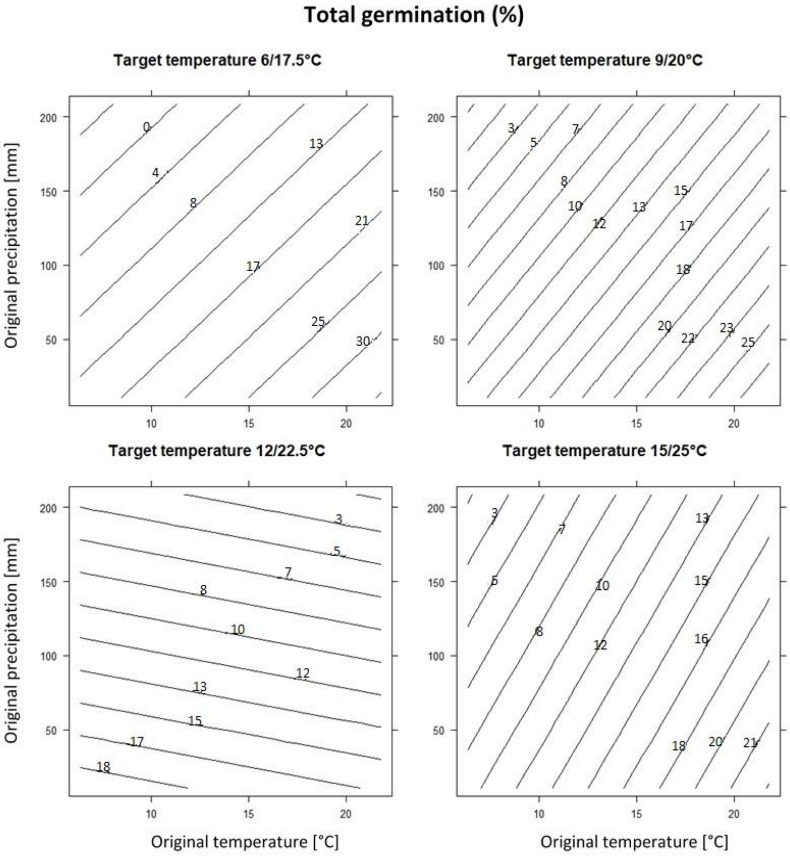
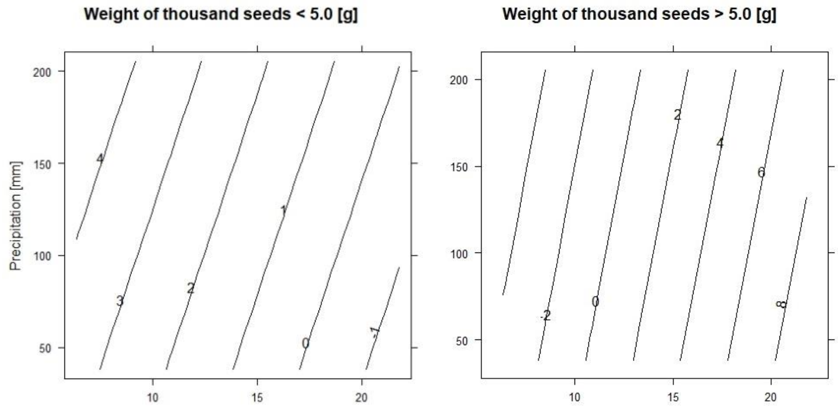


Fig 3. The effect of target temperature, original temperate (°C) and original precipitation (mm) on total germination (%). Increasing numbers in the graph indicate higher total germination. Diagonal lines indicate equal dependency of total germination on original temperature and original precipitation.

Total germination and T30 were significantly affected by the interaction of original temperature, original precipitation and seed mass (Table 1) with high explanatory power both in total germination and in T30 (Fig 1). Lighter seeds coming from colder and wetter localities germinated in high numbers and relatively quickly. By contrast, heavier seeds germinated in high numbers, when they came from warmer and drier localities. Fast germination was found in seeds coming from warmer and wetter localities and was more dependent on original temperature than on original precipitation (Fig 4).

Total germination (%)



T30 (log)

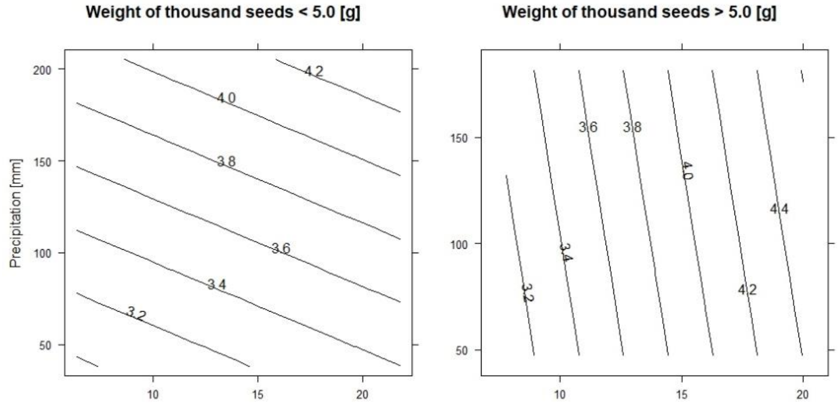


Fig 4. The effect of seed mass (g), original temperature (°C) and original precipitation (mm) on total germination (%) and germination speed (T30) (log). For the graphs, we divided population according to the weight of one thousand seeds to seeds < 5.0g and ≥ 5.0 g since the maximum seed mass was 9.8 g. Increasing numbers in the graph indicate higher total germination resp. T30. Diagonal lines indicate equal dependency of total germination resp. T30 on original temperature and original precipitation.

Significant triple interaction between seed mass, original temperature and original precipitation (Table 1) also indicated that the stratification duration increased in lighter seeds with decreasing original temperature and original precipitation, and the stratification duration was more dependent on original precipitation than on original temperature. The necessary stratification duration in heavy seeds increased with increasing original temperature and decreasing original precipitation and the stratification duration was more dependent on original temperature than on original precipitation (Fig 5).

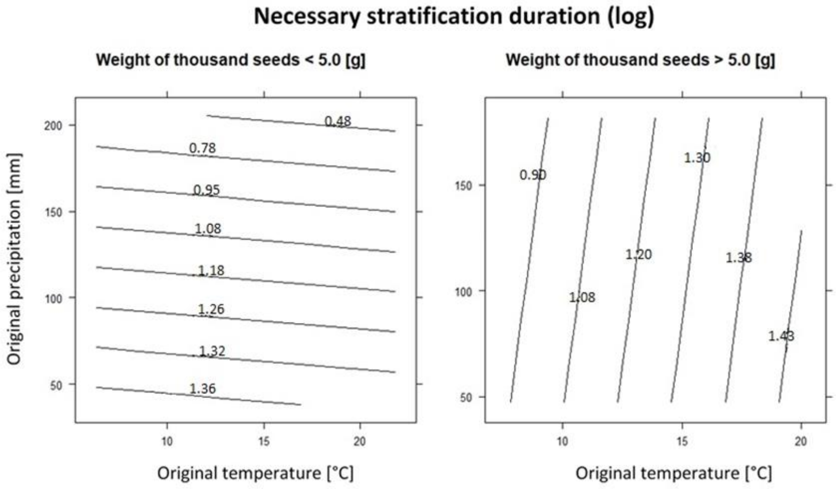


Fig 5. The effect of seed mass (g), original temperature (°C) and original precipitation (mm) on necessary stratification duration in weeks (log). For the graphs, we divided the populations according to the weight of one thousand seeds between < 5.0g and > 5.0g as the maximum was 9.8g. Increasing numbers in the graph indicate longer necessary stratification duration. Diagonal lines indicate equal dependency of necessary stratification duration on original temperature and original precipitation.

The effect of shorter stratification and warm temperature

We found significantly higher total germination (F=3.82, p=0.051) and lower T30 (F=10.06, p=0.003) in seeds kept under stratification until 30% germination and warmer temperature than in seeds exposed to shorter stratification and warmer temperature.

The effect of phylogeny

Total germination, seed dormancy and necessary stratification duration were not significantly affected by phylogeny (all p-values ≥ 0.091). T30 was significantly affected only by phylogenetic axis 2 (F=6.30, p=0.031) and seed mass was significantly influenced by both phylogenetic axes (F=39.55, p<0.001 and F=16.19, p<0.001, respectively).

After accounting for phylogeny, all previously significant predictors of T30 became non-significant (Table 1). By contrast, since all the other predictors were not already significant prior to accounting for phylogeny (all p-values ≥ 0.246), the results for seed mass remained unaffected by consideration of species' phylogeny.

Table 1. The effect of original temperature, original precipitation, seed mass and target temperature on total germination, seed dormancy, germination speed (T30) and necessary stratification duration assessed using generalised linear mixed-effects models with population used as a random factor. Year, longitude, latitude and their interaction were used as covariates in the tests. N.t. indicates not tested. Significant values (≤ 0.05) are in bold. * indicates results becoming non-significant after including phylogeny.

	Total germination		**T30**		**Seed dormancy**		**Necessary stratification duration**	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
OTemp	0.63	0.673	2.47	0.083	0.01	0.899	**4.94**	**0.033**
OPrecip	0.58	0.698	1.73	0.182	0.60	0.701	**13.85**	**0.001**
TTemp	**5.98**	**0.015**	**17.14**	**<0.001***	**3.76**	**0.049**	N.t.	
Seed mass	**11.55**	**<0.001**	**16.56**	**<0.001***	0.27	0.860	0.75	0.387
OTemp: OPrecip	0.11	0.729	1.47	0.179	1.41	0.171	**12.28**	**0.001**
OTemp: TTemp	0.39	0.239	2.10	0.209	0.24	0.682	N.t.	
OPrecip: TTemp	**4.04**	**0.035**	**9.53**	**0.014***	1.26	0.235	N.t.	
OTemp: Seed mass	**13.43**	**<0.001**	0.73	0.559	**3.74**	**0.042**	0.30	0.585
OPrecip: Seed mass	**17.50**	**<0.001**	2.20	0.153	**6.44**	**0.018**	**5.94**	**0.015**
TTemp: Seed mass	0.22	0.632	2.36	0.119	1.52	0.274	N.t.	
OTemp: OPrecip: TTemp	**13.72**	**<0.001**	2.98	0.070	2.26	0.121	N.t.	
OTemp: OPrecip: Seed mass	**4.64**	**0.024**	**29.61**	**<0.001***	0.04	0.896	**4.35**	**0.038**
Orig.temp: Target temp: Seed mass	2.08	0.106	0.09	0.902	1.62	0.159	N.t.	
OPrecip: TTemp: Seed mass	0.95	0.297	0.19	0.635	2.97	0.070	N.t.	
OTemp: OPrecip: TTemp: Seed mass	0.01	0.935	3.07	0.069	3.06	0.051	N.t.	

Discussion

The germination characteristics were affected by seed mass and climate with only limited effects of phylogeny, with the highest variation being attributable to the interaction between original conditions and seed mass. Light seeds were more affected by original precipitation than heavy seeds. Reducing target temperature increased germination even in seeds from populations in lower altitudes. A shorter stratification duration followed by warmer temperature reduced total germination and increased germination speed (T30). This suggests that climate change could potentially affect both the timing of germination and the overall rate of recruitment from seeds. Phylogeny significantly affected only seed mass and germination speed.

Despite a range of previous studies demonstrating that germination depends on original conditions (e.g., Meyer et al., 1997; Santo et al., 2015; Mira et al., 2017), original environment affected only the necessary stratification duration in our study. Populations coming from colder localities required longer stratification (similarly in (Navarro and Guitian 2003). Our results indicate that the necessary stratification duration also depends on precipitation. In our study, highlands have lower precipitation than lowlands. In higher altitudes the cold period lasts longer than in lowlands and species are adapted to this. Correspondingly, seeds are probably waiting for a period with sufficient moisture. As insufficiency of water is more dangerous for seeds and seedling in warm conditions (Baskin and Baskin 2001), this effect is strongest where there is a combination of low precipitation and a warm temperature. The lack of a significant main effect of original climate on the other germination characteristics is in line with (Chamorro, Luna and Moreno 2013). In our system, it may be explained by many significant interactions between original and target conditions, and original conditions and seed mass as discussed below.

Target temperature influenced all studied germination characteristics as demonstrated previously (e.g., Grime et al., 1981; Schütz et al, 1999; Gardarin et al., 2011). In contrast to Gardarin et al., (2011), Ooi et al., (2012) and Walder and Erschbamer (2015), total germination decreased and did not increase with increasing target temperature. T30 showed the opposite trend, i.e., seeds germinated faster in warmer conditions, which is in line with Walder and Erschbamer (2015). Fast germination does not, however, have to be advantageous in all cases, as strong competition - especially for light - can occur between seedlings (Baskin, Baskin and Parr 1986). Perglová et al., (2009) demonstrated that *Impatiens* species are able to form short-term persistent seed banks (*sensu* Thompson, 1993). We found the highest number of dormant seeds in the coldest target temperature. The species probably form short-term persistent seed banks in cold rather than in warm conditions i.e., in the highlands than in the lowlands. This result, together with the results of germination, indicate that cold, alpine conditions are the most suitable for *Impatiens* seedling recruitment.

The effects of original and target conditions interacted in their effect on seed germination. This is in line with our previous results from a different system, (Veselá et al., submitted) and the study of Bauk et al. (2017). Although interaction between target and original conditions can show real species' germination response to a changing climate

(Veselá et al., submitted), these types of studies are still very rare. Such knowledge is necessary for predicting the effects of climate change on the abundance and distribution of species (Butler, Wheeler and Stabler, 2012; Davila, Tellez and Lira, 2013; Aragon-Gastelum et al., 2017). From our results, it seems that *Impatiens* will be probably forced to migrate to higher altitudes. Although the highest total germination was recorded in seeds coming from warm and dry localities in almost all target conditions, total germination under predicted future temperature was the lowest. The highest germination was observed in the coldest target temperature. This result is different from our previous study, Veselá et al. (submitted), suggesting that *Anthoxanthum* species, dominant grass species, will likely profit from the expected climate change when the timing of germination and rain coincide.

In line with studies of Navarro and Guitian (2003), Wu and Du (2007) and Münzbergová and Plačková (2010), we found that heavier seeds germinate more than lighter ones. This could be caused by a higher content of nutrients (Rees et al., 2001). To the best of our knowledge, our results are the first to show that the effects of seed mass interaction with original climatic conditions. Heavy seeds followed the above-mentioned trend that seeds coming from the warm and dry localities have the highest germination and germinate fast. By contrast, light seeds have the highest germination when they come from cold and wet localities. Germination is initiated by absorption of water (Fenner and Thompson 2005) and light seeds probably require higher water content in the soil than heavy seeds. This is obvious from results of germination and necessary stratification duration. Necessary stratification duration is the shortest in seeds coming from the wet localities, and stratification duration in light-seeded species is more dependent on precipitation than on temperature. Heavy seeds require only short stratification when coming from wet and cold localities. When compared with precipitation, temperature is, however, a more important determinant for the necessary stratification duration. It seems that future changes (mainly in temperature and less in precipitation) will be crucial for heavy-seeded species, while changes both in temperature and in precipitation will play a role in recruitment of light-seeded species.

The effect of shorter stratification and warm temperature

With ongoing climate change, the plants will probably face a combination of shorter stratification and warmer temperatures during the vegetation period (IPCC 2014).

Shortening of stratification can lead to lower germination (Garcia-Fernandez et al., 2015; Carta et al., 2016b). Correspondingly, warmer climate can increase germination (Gardarin et al., 2011; Ooi et al., 2012; Walder and Erschbamer, 2015; Veselá et al., submitted). Shorter stratification can also bring potential advantage to alpine species in terms of a longer vegetation period due to reduced snowfall and earlier snowmelt, resulting in a longer vegetation period (Beniston 2012; (Pederson, Betancourt and McCabe, 2013; Sanchez-Bayo and Green 2013). Our results suggest that such temperature changes will lead to faster but reduced germination. Since this is the first study providing such data, more experiments are needed to confirm this. In any case, these results indicate that both changes in temperature and stratification need to be considered in order to obtain reliable insights into species' response to future, climatic changes.

The effect of phylogeny

Seed traits, dormancy patterns, and germination responses have ancient origins and, therefore, phylogenetic relationships remain an important part of understanding how they vary (Forbis, Floyd and de Queiroz, 2002; Donohue et al., 2010; Willis et al., 2014; Dayrell et al. 2017). In line with previous studies (Zhang et al., 2004; Moles et al., 2005 ; Nordern et al., 2009; Barak et al., 2018), seed mass was strongly affected by phylogenetic relationships. Since it was not affected by the original environment, seed mass of *Impatiens* seems to result from constraints over the long-standing evolution of the genus.

Absence of the effect of phylogeny on total germination, seed dormancy and necessary stratification duration indicates that these germination characteristics are driven by environmental conditions rather than by phylogeny. Germination response is probably a result of species' adaptation to their original conditions. Germination speed was the only germination characteristic related to phylogeny. Due to the existence of phylogenetic niche conservatism (Westoby, Leishman and Lord, 1995; Leishman, Westoby and Jurado, 1995), it is not possible to distinguish whether the germination speed of *Impatiens* is the result of environment, or whether phylogenetic constraints are more important in determining these patterns. Carta et al. (2016a) found phylogenetic signal in germination speed within one genus; this subject, however, requires further attention.

Conclusion

This study demonstrates the importance of including the interaction of original and target environment in climate change studies. Concomitantly, it highlights the strong dependency of necessary stratification duration on original climate. Predicted climate change represented by a shorter stratification period followed by warmer temperature will negatively affect species' germination. Germination of the *Impatiens* species will, therefore, probably be negatively affected by climate change forcing the species to migrate to higher altitudes. Germination response of *Impatiens*, with the exception of germination speed (T30), is driven by environmental conditions rather than by phylogeny, which indicates that germination behaviour will change with changing conditions. Seed mass is affected both by phylogeny and climatic conditions, thus climate change can have impact on it as well. Heavy seeds germinate best and fastest when they come from warm and dry localities, while light seeds germinate best when they come from cold and wet localities. It seems that future changes both in temperature and precipitation will affect *Impatiens'* germination, but the effects will differ between heavy- and light-seeded species. Since seed mass strongly affected the species' ability to adapt to their original conditions, consideration of seed mass is crucial in order to predict accurately future germination behaviour of the species. The effects of seed mass on species' germination patterns thus need to receive more attention in future studies.

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Study 4: ARE THERE SYSTEMATIC DIFFERENCES IN GERMINATION BETWEEN RARE AND COMMON SPECIES? A CASE STUDY FROM CENTRAL EUROPEAN MOUNTAINS

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Abstract

Understanding the factors responsible for species rarity is key for designing effective management strategies aimed at long-term conservation of species. Most such studies investigate plant size, competitive vigour or habitat requirements, but studies that explore more complex characteristics of species directly related to different stages of the plant life cycle are much less common. Germination is the most critical stage in the life cycle of many plants and is the primary source of variation in the regeneration niche. However, only a few authors have studied differences in germination requirements between common and rare congeners, and none have studied these requirements for large sets of species. The aim of this study was to compare seed mass, germination requirements and proportions of germinated seeds of rare and common congeners across a large set of species. We analysed seed mass, proportions of germinated seeds in specific conditions and total proportions of germinated seeds of 18 congeneric pairs of rare and common plant species. We tested different lengths of cold stratification, constant or alternating temperatures, and different light conditions. Seed mass of rare and common congeners was not significantly different. The results indicated that after cold stratification, the proportions of germinated seeds were higher in rare than in common species. In common species, the proportions of germinated seeds were significantly higher during cold stratification than those of rare species. Rare species had higher proportions of germinated seeds than those of their common congeners, and total proportions of germinated seeds were influenced by seed mass and species rarity. Rare species were apparently well adapted to local conditions in the mountains and the inability to germinate was clearly not a cause for their rarity. The study provides unique data on proportions of germinated seeds over a wide spectrum of species and conditions.

Key words: commonness, Krkonoše Mts., rarity, reproduction characteristics, seed size, temperature requirements

Introduction

The reason some species have a highly restricted geographic range while closely related species have widespread distributions has long been a subject of study (e.g., Brown, Stevens and Kaufman 1996; Webb and Gaston 2003; Gabrielová et al. 2013). Many studies highlight the differences between rare and common species and attempt to determine the causes of species rarity. For example, rare species often occur at higher altitudes (e.g., McDonald and Cowling 1995; Kessler 2000) and have reduced colonization abilities (e.g., (Fiedler 1987; Byers and Meagher 1997), in addition to reduced competitive vigour (e.g., Lavergne, Garnier and Debussche 2003; Gabrielová et al. 2013) and increased susceptibility to predispersal seed predation (Combs, Lambert and Reichard 2013). Rare species also have less aboveground biomass (e.g., Pilgrim, Crawley and Dolphin 2004; Gabrielová et al. 2013), short duration of flowering (e.g., Gustafsson 1994; Gabrielová et al. 2013), small size and low number of flowers (Lavergne et al. 2004) and low number of seeds (e.g., Lavergne et al. 2004; Pilgrim et al. 2004). However, the results of such studies are not always consistent (for exceptions, see e.g., Hegde and Ellstrand 1999; Lloyd, Lee and Wilson 2002; Lavergne et al. 2004; Gabrielová et al. 2013; Vazačová and Münzbergová 2014). Thus, many studies compare simple characteristics between rare and common species, which are data that can often be easily retrieved from various databases. Studies that explore more complex species characteristics directly related to different stages of the life cycle or to the life cycle as a whole are much less common (e.g., Münzbergová 2005; Münzbergová 2013).

Germination is one of the most critical stages in the life cycle of plants (Donohue 2005) and is the primary source of variation in the regeneration niche (Pearson et al. 2002). However, only a few authors have studied differences in germination between common and rare congeners (e.g., Brown, Enright and Miller 2003; Simon and Hay 2003; Ramirez-Padilla and Valverde 2005; Mattana, Daws and Bacchetta 2010; Hewitt et al. 2015). Brown et al. (2003) showed that rare *Acacia* species germinated only in one specific temperature treatment, whereas the common species germinated across a broad range of temperatures. However, Simon and Hay (2003) showed that rare species of *Mimosa* had the highest proportions of germinated seeds compared with those of three common *Mimosa* species. Other studies indicate that the proportions of germinated seeds are similar in rare and common species in most cases, with exceptions when exposed to specific environmental conditions such as low water potential (Ramirez-Padilla and Valverde 2005) and shade (Hewitt et al. 2015). Mattana et al. (2010) showed that proportions of germinated seeds in common species are significantly higher than those in rare species at 5 °C, whereas rare species preferred alternating temperatures.

Although most of these studies indicate that common species generally germinate in higher percentages and tolerate wider ranges of conditions than rare species, all these authors studied the differences in only a few closely related species (the maximum is four species). To properly understand whether germination requirements are related to species rarity, comparative studies are required that examine large sets of species. However, such studies have not been conducted, although aspects of reproductive biology and requirements for seed germination may play important roles as causal factors for limited abundance or restricted species distribution ranges (Fiedler 1987).

Comparative studies of species rarity and commonness provide a suitable alternative for assessing whether processes or traits are characteristic of species with narrow distributions. The results of such studies have the potential to provide useful information for the conservation and management of rare species (Murray et al. 2002). Although large comparative studies exploring germination requirements as possible drivers of species rarity have not been conducted, germination is a useful predictor of other species characteristics. For example, germination percentage and seedling establishment can help to explain the invasiveness of a species (Moravcová et al. 2010; Moravcová et al. 2015). Brändle et al. (2003) and Qi et al. (2014) demonstrated that differences in germination percentage could also explain differences in the distributional ranges of weeds. Therefore, germination may also be a factor that contributes to explaining species rarity.

In this study, we conducted a comparative analysis of germination requirements and proportions of germinated seeds in 18 congeneric pairs of rare and common plant species occurring in the Krkonoše Mts. in the Czech Republic. Because germination depends on seed mass (Wu, Li and Du 2011; Liu et al. 2013), we also explored differences in seed mass between the rare and common species. The aim of our study was to answer the following questions: i) Do rare and common species differ in terms of seed mass? ii) Do rare and common species differ in germination response to temperature and light? iii) Do rare and common species have differences in proportions of germinated seeds? iv) Do differences in seed mass explain the possible effects of species rarity on germination? v) Do rare and common species have differences in the range of germination conditions? We predict that common species will have higher seed mass and that rare species will germinate only at one specific temperature, likely after cold stratification, and under light. We also predict that rare species will have lower proportions of germinated seeds than their common congeners and that differences in seed mass will explain the differences in proportions of germinated seeds between rare and common species. Simultaneously, we expect that common species will germinate in a broader range of conditions.

Materials and methods

Study area

Seed material for the study was collected in the Krkonoše Mts. (Czech Republic). The altitude of the mountains ranges from 400 to 1600 m, and the climate is cold-humid alpine with mean annual precipitation ranging from 800 to 1200 mm. Mean annual temperature ranges from 0 to 6 °C, and mean January and July temperatures range from -4.2 to -7.2 °C and 8.3 to 14 °C, respectively. Snow occurs each year from November to March.

Study species

We studied 24 congeneric pairs (Table 3), with each pair composed of one rare species of the Krkonoše Mts. (rare species) and one common species with a widespread geographic distribution (common species). Rare species were species included on the Black and Red lists of vascular plants of the Krkonoše Mts. (Štursa et al. 2009). The genetic bank of the Krkonoše Mts. national park administration (represented by one co-author, L. Harčariková) has authorization for manipulations with the seeds of these rare species. For this study, we preferred rare species that were important for this region and produced sufficient seeds. Common species were selected based on occurrence in habitats similar to those of the rare species and were assessed according to their Ellenberg indicator values (Ellenberg et al. 1992). We identified 18 common species for 24 rare species; therefore, some pairs shared the same common species, resulting in only 18 fully independent pairs. We explored the effects of this dependency as described below. Comparisons of closely related rare and common species are better than comparisons of unrelated species, because with this approach, biases associated with phylogenetic distance and habitat affinities of the compared species are minimized (e.g., Pyšek et al. 2003; Burns 2004; Tremlová and Münzbergová 2007; Gravuer et al. 2008).

Seed collection and seed mass

Seeds were collected in the natural habitats of species between 2006 and 2015 (from April to October), and seeds for each species were collected from at least two populations (separated by at least 5 km) per species from at least 10 maternal plants per population. Seeds from all maternal plants were mixed and populations were kept separately. All seeds of rare species were collected in the Krkonoše Mts., whereas seeds of common species were collected in the Krkonoše Mts. and in nearby surroundings. Seeds of some species were collected in multiple years. After collection, seeds were stored at ambient moisture (approximately 55%) at room temperature (approximately 20 °C) for 1 to 6 months before the beginning of the experiments. For each population, we recorded storage length, year of collection and origin of the population. We tested the effects of storage length, year of collection, and origin and altitude of the population on proportions of germinated seeds and did not identify any systematic effects ($p > 0.48$, for all factors). Therefore, these data were not considered further. All seeds were tested in the same growth chambers (Heraeus BK6160).

For each species, we determined the weight of one thousand seeds by counting 4 × 100 seeds. These seeds were dried in a desiccator (one month) and weighed on an analytical balance (according to recommendations of International Seed Testing Association, 2005). For determinations of seed weights and the germination tests, we used only undamaged, mature seeds, which were assessed by naked eye and touch. The weighed seeds were not used for germination tests.

Germination tests

Proportions of germinated mature seeds were determined under laboratory conditions. We used a total of 22 temperature/light regimes. For each species and germination regime, four replicates of 50 seeds were incubated on three layers of moist filter paper in 9-cm diameter Petri dishes. The regimes simulated the sequence of temperatures during the year after seed maturation, and the regimes differed in the light/temperature values, sequence and also duration of the stages of a given sequence. The sequence of conditions in the different regimes is shown in Table 1.

Table 1. The sequences of conditions in each regime of germination tests. A, B, C, D, 2Month-8Month: different types of germination tests. regimes 2Month-8Month are called according the length of stratification. m: month; w: week, D: dark, L: light

Regime	Light	20 °C 12/12h	20 °C 24h dark	5 °C 24h dark	5°/18 °C 8/16h	20 °C 12/12h	20 °C 24h dark
A	L	1m	-	-	2m	1m	-
	D	-	1m	-	2m	-	1m
B	L	1w		3m	2m	1m	-
	D	-	1w	3m	2m	-	1m
C	L	1w	-	5m	2m	1m	-
	D	-	1w	5m	2m	-	1m
D	L	1m	-	4m	-	1m	-
	D	-	1m	4m	-	-	1m
2Month	L	1w	-	2m	-	1m	-
	D	-	1w	2m	-	-	1m
3Month	L	1w	-	3m	-	1m	
	D	-	1w	3m	-	-	1m
4Month	L	1w	-	4m	-	1m	-
	D	-	1w	4m	-	-	1m
5Month	L	1w	-	5m	-	1m	-
	D	-	1w	5m	-	-	1m
6Month	L	1w	-	6m	-	1m	
	D	-	1w	6m	-	-	1m
7Month	L	1w	-	7m	-	1m	-
	D	-	1w	7m	-	-	1m
8Month	L	1w	-	8m	-	1m	-
	D	-	1w	8m	-	-	1m

According to many studies (e.g., Grime et al. 1981; Baskin and Baskin 1988; Spindelbock et al. 2013; Iakovoglou and Radoglou 2015), some species require an increase in temperature only during germination or only before cold stratification. Therefore, the first temperature to which the seeds were placed was 20 °C, but for different durations. The light conditions for the seeds during this temperature period were either 24 h of darkness or 12/12 h dark/light. Cold stratification followed, or in regime A, alternating temperatures for 2 months (Table 1). The alternating temperatures simulated conditions in spring under a light regime of 8/16 h dark/light. We tested different lengths of cold stratification that varied from 2 to 8 months, because the length of the cold period in the Krkonoše Mts. varies at different altitudes and also between years. The cold stratification (5 °C) simulated conditions under snow; therefore, seeds were in 24 h of darkness during this period. Regime D was similar to regime A, with the length of warm stratification also 1 month, but the warming was followed by cold stratification for 4 months. The temperature regimes B and C began with 1 week of warm stratification followed by 3 months (regime B) and 5 months (regime C) of cold stratification. Then, the seeds were placed in alternating temperatures for 2 months. The temperature regimes 2Month-8Month started with 1 week of warm stratification, followed by cold stratification that continued for 2 to 8 months. These temperature regimes did not include alternating temperatures. A warm stratification at 20 °C was the final stage of each temperature regime (Table 1).

The seeds were inspected weekly, and germinated seeds were counted and removed. A seed was considered germinated when the radicle was visible to the naked eye. We used the data from all regimes to express total proportions of germinated seeds. We used the data from specific temperature conditions across all tested regimes to express proportions of germinated seeds under specific temperature conditions: i) 20 °C, light without stratification; ii) 20 °C, dark without stratification; iii) 5 °C (2-4 months), 24 h darkness; iv) 5 °C (5-8 months), 24 h darkness; v) 5/18 °C, 8/16 h dark/light; vi) 20 °C, light after cold stratification; and vii) 20 °C, dark after cold stratification. Twenty-four hours of darkness defined dark, and 12/12 h dark/light defined light.

Data analyses

We tested differences in seed mass between rare and common congeners and species pairs by ANOVA. In all analyses, seed mass was log-transformed to ensure homogeneity of variance (Leishman and Westoby 1994).Total proportion of germinated seeds was defined as the number of germinated seeds for the entire monitored period irrespective of conditions. The results for the proportions of germinated seeds were analysed by ANOVA. We tested the effect of species pair and species rarity (independent variables) on the total proportions of germinated seeds and on the proportions of germinated seeds in specific temperature/light conditions, with proportional germination in one Petri dish representing one replicate (dependent variable). We used the same approach to test the effects of seed mass and the interaction of seed mass and species rarity on the total proportions of germinated seeds and proportions of germinated seeds in specific temperature/light conditions. Because the results did not change in all categories after incorporating seed mass into the model, we present results only with seed mass. Based on Pearson's correlation coefficient, the proportions of germinated seeds at 20 °C in the light strongly correlated with the proportions of germinated seeds at 20 °C in the dark (Supporting information 4A); therefore, for statistical analyses, we only used proportions of germinated seeds in the light at 20 °C.

To assess whether the rare and common species differed in the range of conditions favourable to germination, we counted the number of regimes in which common species had higher proportions of germinated seeds than those of rare congeners and vice versa as a dependent variable and tested the effects of species pair and species rarity on the values by ANOVA.

Twelve pairs had the same common species for two or three rare species, and therefore, the data for these pairs were not independent. Thus, all analyses were performed with only one of the pairs with the same common species. We also removed the *Sorbus aucuparia-S. sudetica* pair from this set of analyses, because *Sorbus* species were the only trees in the data set and *S. sudetica* has extremely heavy seeds; thus, we analysed only 17 pairs. Then, we repeated all the analyses with all 24 pairs. Data were analysed using R i386 3.1.1 (R Development Core Team 2011).

Results

Differences in seed mass

Seed mass was not significantly different ($F_{1,827} = 1.43$, $p = 0.231$) between rare and common congeners for the 17 pairs. However, after including all pairs (24 pairs), rare species had significantly heavier seeds than those of common species ($F_{1,817} = 6.11$, $p = 0.013$).

Table 2. The results of comparing rare and common congeners in total proportions of germinated seeds and in detailed categories light/temperature conditions. Significant values (≤0.05) are in bold. The results of effect of pair are presented in Supporting information 4D. ↑ - rare species had higher proportions of germinated seeds than common congeners, ↓ common species had lower proportions of germinated seeds than rare congeners. Df error=1125 for 24 pairs, Df error=835 for 17 pairs.

Dataset	Factor		**Total germination**	**20 °C**	**5 °C (2-4m)**	**5 °C (5-8m)**	**5/18 °C**	**20 °C light after cold**	**20 °C dark after cold**
24 pairs	Seed mass	F	**25.48**	0.004	0.53	**4.90**	2.85	0.30	1.62
		P	**<0.001 ↑**	0.95	0.466	**0.028↑**	0.092	0.579	0.203
	Rarity	F	**51.44**	**6.40**	**90.26**	0.04	**9.58**	2.72	0.15
		P	**<0.001 ↑**	**0.011 ↑**	**<0.001↓**	0.827	**0.002 ↑**	0.099	0.694
	Seed mass*rarity	F	**8.45**	**13.98**	0.04	1.53	1.90	<0.001	1.13
		P	**0.003**	**<0.001**	0.244	0.217	0.168	0.991	0.318
17 pairs	Seed mass	F	**23.16**	0.005	0.18	3.55	1.14	0.35	1.16
		P	**<0.001 ↑**	0.939	0.672	0.062	0.301	0.552	0.281
	Rarity	F	**19.51**	0.18	**57.05**	0.11	**25.06**	0.83	2.31
		P	**<0.001 ↑**	0.663	**<0.001↓**	0.730	**<0.001↑**	0.361	0.128
	Seed mass*rarity	F	**7.24**	**5.47**	0.02	1.04	0.01	0.06	0.17
		P	**0.007**	**0.019**	0.888	0.311	0.901	0.791	0.663

Effects of temperature and light

Proportions of germinated seeds did not differ significantly between common and rare species in conditions of 20 °C without stratification, in the long cold stratification, at 20 °C and light after cold stratification and at 20 °C and darkness after cold stratification (Table 2). However, the proportions of germinated seeds in rare species were significantly higher than those in common species under alternating temperatures (Table 2). The largest difference was in the pair *Carex nigra-C. atterina*, with proportions of germinated seeds of 2% and 60%, respectively (Fig. 1, Table 3). By contrast, the proportions of germinated seeds of common species at 5 °C for 2-4 months were significantly higher than those of rare species under the same regime. The largest difference was observed for the pair *Luzula campestris-L. spicata*, with proportions of germinated seeds of 97% and 0%, respectively (Fig. 1, Table 3). Proportions of germinated seeds between pairs were significantly different in all categories (Table 2).

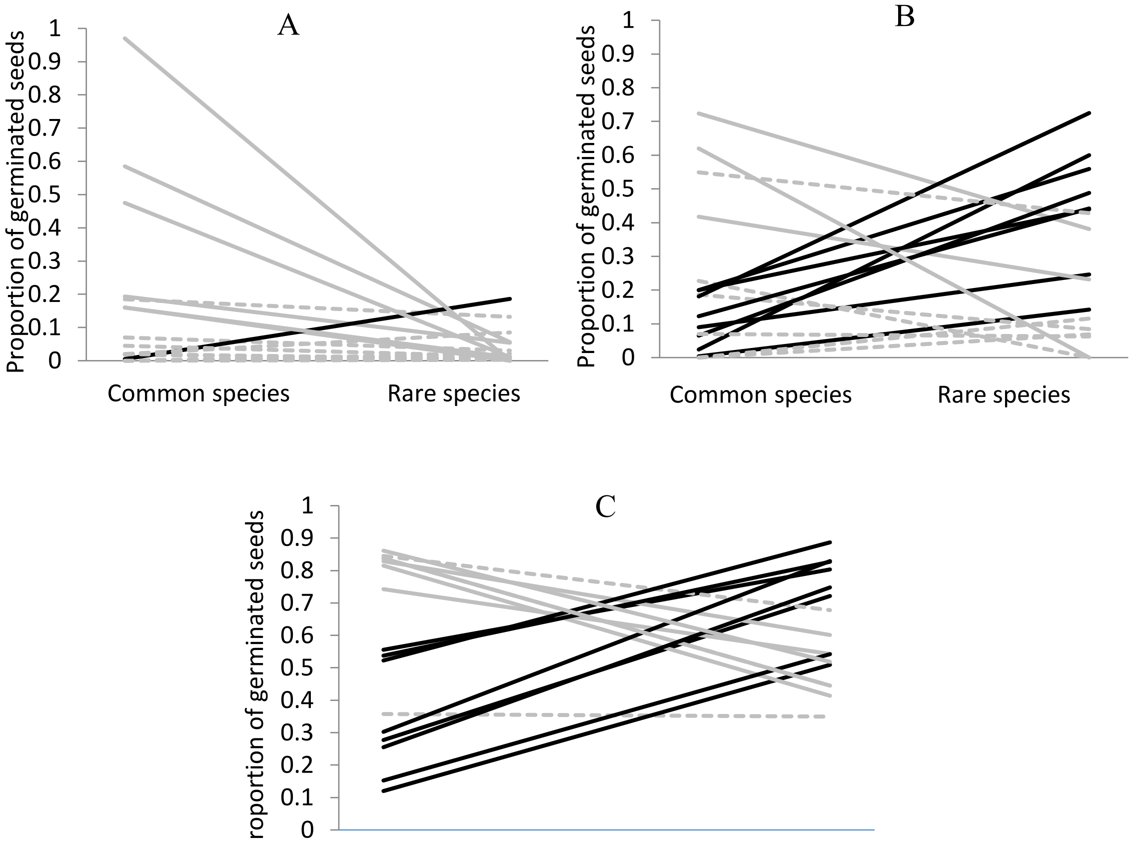

Fig. 1. Proportions of germinated seeds common and rare congeners in A) in cold stratification (5 °C), B) at alternating temperatures (5/18 °C), C total proportions of germinated seeds. The black lines indicate that rare species had higher proportions of germinated seeds than common one, the grey lines indicate that common species had higher proportions of germinated seeds than the rare species and the dashed line indicates non-significant results.

The results of analyses with all pairs were consistent with the results described above, with exception of proportions of germinated seeds at 20 °C without cold stratification (Table 2). The proportions of germinated seeds of rare species were significantly higher than those of their common congeners (Supporting information 4B, Table 3). The largest difference was observed for the pair *Hieracium murorum–H. decipiens*, with proportions of germinated seeds of 12% and 75%, respectively. Mean proportions of germinated seeds for each species in each tested regime are presented in Supporting information 4C.

Differences in total proportions of germinated seeds

Total proportions of germinated seeds (germination irrespective of conditions) of common species were significantly lower than those of rare species, and the proportions of germinated seeds were significantly different between species pairs (Table 2). The difference in proportions of germinated seeds was also significant in the test with duplicates of common species (Table 2). Of the 24 pairs, proportions of germinated seeds of 16 rare species were higher than those of their common congeners (Table 2). The pairs with the highest proportions of germinated seeds for the rare species compared with those of the common one were *Primula elatior-P. minima* (25% and 75%, respectively), *Carex nigra-C. atterina* (24% and 72%, respectively) and *Cirsium vulgare-C. acaule* (8% and 51%, respectively).

The pair with the lowest proportion of germinated seeds for the rare species compared with that of the common one was *Luzula campestris-L. spicata* with 84% and 45%, respectively. In the pairs *Campanula patula-C. bohemica*, *Hieracium lachenalii-H. tubulosum, Hieracium lachenalii-H. pedunculare* and *Luzula campestris-L. sudetica*, no significant differences in proportions of germinated seeds between rare and common species were detected.

The lowest total proportion of germinated seeds was in the common species *Cirsium vulgare* (8%). Among the rare species, the lowest proportion of germinated seeds was observed in *Campanula bohemica* (35%). By contrast, the highest proportions of germinated seeds were found in the common species *Luzula campestris* (84%) and the rare species *Hypericum humifusum* (88%).

The effect of seed mass was significant for total proportions of germinated seeds. The results also showed a significant interaction between rarity and seed mass for total proportions of germinated seeds and proportions of germinated seeds at 20 °C without stratification (Table 2). Specifically, proportions of germinated seeds of common species decreased with seed mass, whereas in rare species, the proportions germinated were unaffected or increased slightly with increasing seed mass (Fig. 2). Species with light seeds, whether rare or common, had comparable total proportions of germinated seeds. Proportions of germinated seeds of common species at 20 °C without stratification decreased with increasing seed mass, whereas proportions of germinated seeds of rare species increased with seed size.

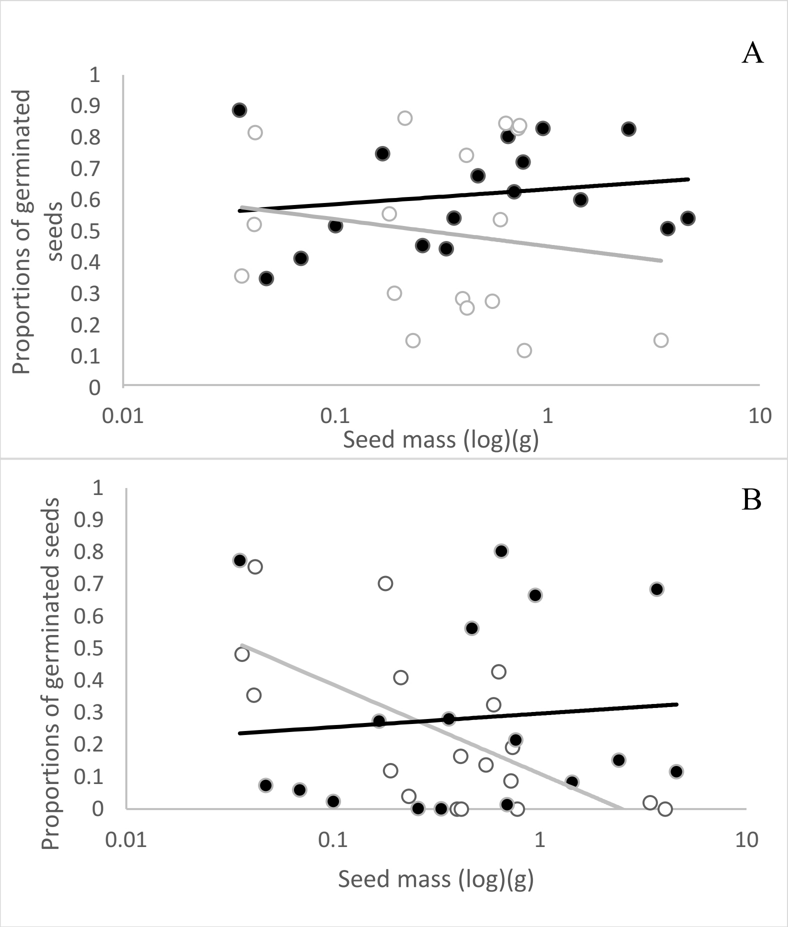

Fig. 2. Effect of seed mass and species rarity (17 pairs) to A) total proportions of germinated seeds and B) proportions of germinated seeds at 20 °C light without stratification. ● rare species ○ common

The results with duplicates and *Sorbus* spp. included were consistent with the results described above with two exceptions (Table 2). We found a significant effects of seed mass on proportions of germinated seeds at 5 °C (5-8 months), with heavier seeds having higher proportions of germinated seeds than those of lighter seeds. Additionally, the results were significantly different for the interaction between rarity and seed mass at 20 °C without stratification, with the proportions of germinated seeds of rare species unaffected or slightly decreased (Fig. 2).

Range of conditions suitable for germination

The range of germination conditions did not differ significantly between rare and common species, either for the 17 pairs ($F_{1,19} = 1.53$, $p = 0.217$) or the 24 pairs ($F_{1,14} = 0.15$, $p = 0.695$). Therefore, compared with their common congeners, rare species did not require specific conditions for germination.

Table 3. Summary table of results for each pair. ↑ rare species had higher proportions of germinated seeds than common congeners, ↓ common species had lower proportions of germinated seeds than rare congeners, ns – non-significant. Gray-marked species were removed for analysis without duplicates and *Sorbus* spp.

Pairs	**Total germination**	**20 °C without stratification**	**5 °C (2-4m)**	**5/18 °C**
Campanula patula-C.bohemica	ns	↓	ns	↑
Carex nigra-C.aterrina	↑	ns	ns	↑
Carex nigra-C.capillaris	↑	ns	ns	ns
Carex nigra-C.davalliana	↑	ns	ns	↑
Cirsium vulgare-C.acaule	↑	↑	ns	↑
Dianthus carthusianorum subsp. carthusianorum-D.superbus subsp.alpestris	↑	ns	ns	ns
Dianthus carthusianorum subsp. carthusianorum-D.superbus subsp.superbus	↑	↑	↓	↓
Eriophorum angustifolium-E.latifolium	↑	ns	↑	↑
Galium saxatile-G.sudeticum	ns	ns	↓	ns
Geum urbanum-G.montanum	↓	ns	ns	↓
Gnaphalium uliginosum-G.supinum	ns	↓	↓	ns
Hieracium lachenalii-H.pedunculare	↓	ns	↓	↓
Hieracium lachenalii-H.tubulosum	ns	↑	ns	↓
Hieracium murorum-H.bifidum	↑	↑	ns	ns
Hieracium murorum-H.decipiens	↑	↑	ns	ns
Hypericum maculatum-H.humifusum	↑	↑	↓	↑
Hypochaeris radicata-H.uniflora	↑	↓	ns	↑
Knautia arvensis subsp. arvensis-K.arvensis subsp. pseudolongifolia	↑	ns	ns	↑
Luzula campestris-L.spicata	ns	↓	↓	ns
Luzula campestris-L.sudetica	ns	↑	↓	↓
Poa annua-Poa laxa	↑	ns	ns	↑

Primula elatior-P.minima	↑	↑	ns	ns
Sorbus aucuparia-S.sudetica	↑	ns	ns	ns
Veronica chamaedrys-V.bellidioides	↓	↓	↓	↓

Discussion

Differences in seed mass

We did not find significant differences in seed mass between rare and common congeners. However, in analyses with duplicates and *Sorbus* spp. included, this difference was significant and rare species had heavier seeds than those of their common congeners, most likely because of a bias due to the heavy seeds of *S. sudetica.* Lavergne et al. (2004) and Gabrielová et al. (2013) also found that seed mass was not significantly different between rare and common species. We expected higher germination of common species and simultaneously that common species would have heavier seeds than those of their rare congeners, because heavier and larger seeds can have more storage and nutrient compounds than lighter ones, which can nourish seedlings (Rees et al. 2001). The absence of a difference in seed mass could be because seed mass is an evolutionarily stable trait, with variation that is more associated with a high taxonomic level such as the family, and therefore, seed mass is similar between species from the same genus (Leishman et al., 2000). However, empirical observations do not necessarily support this hypothesis. According to Brown et al. (2003), rare species had lighter seeds than those of their common congeners, whereas in the study of Hewitt et al. (2015), rare species had heavier seeds than those of their common congeners. However, all these authors studied the differences in only a few closely related species, and the contrasting results in their studies and lack of patterns in our study could be because the patterns are largely species-pair specific.

Effects of temperature and light

We did not detect any differences in proportions of germinated seeds between common and rare species at 20 °C without stratification in analyses without duplicates and *Sorbus* spp. When pairs with common species duplicates were included, rare species had significantly higher proportions of germinated seeds than those of their common congeners. Proportions of germinated seeds of rare and common species in conditions without stratification are comparable because most species of the temperate zone require cold stratification for breaking dormancy (Baskin and Baskin 1988), and therefore, only a few species germinate early after maturation of seeds. Germination early after maturation is most likely not dependent on species rarity but is a species characteristic of dormancy of the seeds.

Rare species had higher proportions of germinated seeds than those of their common congeners in alternating temperatures after cold stratification. Consistent with this result, Gkika et al. (2013) showed that rare species germinated in higher percentage in alternating temperatures. Additionally, Brown et al. (2003) showed that rare species germinated only at one specific temperature treatment, which can be explained because rare species usually have a narrower niche and therefore more specific germination requirements. In our data set, rare species were restricted to the mountains, whereas the common species were more widespread. Germination after cold stratification in rare species could therefore be an adaption to germination in spring, after winter, i.e., the mean temperature of five consecutive days exceeds 5 °C, and the seedlings have a chance to successfully establish even in the extreme mountain environments (Meyer 1992). Germination at low temperatures in mountain species may be risky because of the danger of late frosts, which can damage the seedlings.

This conclusion is also consistent with the result that common species had higher proportions of germinated seeds than those of their rare congeners during the period of cold stratification in our study and also in Mattana et al. (2010). Early germination in common species can give them a competitive advantage, and therefore, early germination could be one of the reasons for the broader distribution of these species (Grime et al. 1981; Baskin, Baskin and Parr 1986; Radford and Cousens 2000; Skalová, Moravcová and Pyšek 2011).

Differences in total proportions of germinated seeds

In contrast to our expectation, rare species had higher proportions of germinated seeds than those of their common congeners. This result is also in contrast to the study of Ramirez-Padilla and Valverde (2005) showing higher proportions of germinated seeds in common than in rare species. However, the result is consistent with the study of Simon and Hay (2003) showing that rare *Mimosa setossima* had higher proportions of germinated seeds than those of three congeners. Lastly, Hewitt et al. (2015) suggested that proportions of germinated seeds of rare and common species do not differ. Higher proportions of germinated seeds in common than in rare species might be expected, because high proportions of germinated seeds theoretically lead to an increase in seedling numbers and therefore larger populations.

However, rare species often produce fewer seeds than those of their common congeners (e.g., Murray et al. 2002; Lavergne et al. 2004; Pilgrim et al. 2004; Hewitt et al. 2015). Higher proportions of germinated seeds in rare than in common species could therefore be explained by higher production of seeds of lower quality in common species. To confirm this explanation, further studies of seed production and quality are required.

Another possible explanation for this result could be that common species have dormant seeds, which remain in the soil seed bank (Brenchley 1918; Chippindale and Milton 1934; Araki and Washitani 2000) and therefore do not germinate immediately. However, rare species can also develop persistent soil seed banks (e.g., Bucharová, Brabec and Münzbergová 2012) and persistent soil seed banks are often composed of small seeds (Thompson, Band and Hodgson 1993). In this study, we did not detect any differences in seed mass between rare and common congeners. To confirm this explanation, further studies of the rare and common species in soil seed banks are required.

Effects of seed mass and species rarity

Heavy seeds had higher total proportions of germinated seeds than those of light seeds in our study, which was also found by Navarro and Guitian (2003). Higher proportions of germinated seeds of heavy-seeded than light-seeded species can be explained by higher quantities of storage and nutrient compounds, as noted previously. However, several studies, (e.g., Schütz and Rave 1999; Bu et al. 2008; Wang et al. 2009; Wu et al. 2011; Liu et al. 2013), show that light seeds had higher proportions of germinated seeds than those of heavy seeds. In our study, heavy-seeded species also had higher proportions of germinated seeds in cold stratification (5-8 months) than those of light-seeded species, but this result became non-significant when we analysed data without the duplicates and pair of *Sorbus* spp. Because *Sorbus* spp. require cold stratification for breaking dormancy (Flemion 1931; Devillez et al. 1980), and *S. sudetica* has extremely heavy seeds, the significant result in the total data set was strongly influenced by the germination of *Sorbus* spp. Therefore, we presumed that germination in cold stratification was not generally influenced by seed mass.

We also found that total proportions of germinated seeds of common species decreased with increasing seed size, whereas the proportion was unaffected or increased slightly with seed size in rare species. Increasing proportions of germinated seeds of rare species with seed mass can be explained by more storage and nutrient compounds, as previously noted. Decreasing germination with increasing seed mass in common species could be explained so that lighter seeds germinate faster (Bu et al. 2008), with faster germination contributing to wider distribution (Qi et al. 2014). Results with duplicates and *Sorbus* spp. included showed the same trends.

Results also showed that germination of rare species increased with increasing seed mass. When we analysed the data with repeated pairs, the proportions of germinated seeds of rare species decreased with increasing seed mass at 20 °C without stratification, but this result was strongly influenced by the germination of *S. sudetica*. Burke and Grime (1996) and Mattana et al. (2010) showed that heavy-seeded species germinate over a wider range of conditions, but our results showed that rare species germinated instead at restricted temperatures. From this result, rare species with heavy seeds also apparently germinated at restricted temperatures and had a marginal tendency to germinate before the cold period early after maturation of the seeds.

Limitations of the study

The seeds used for this study were stored for different lengths of time, and Perglová et al. (2009) demonstrated that length of storage affects germination percentage. However, we did not detect any systematic effect of storage duration on proportions of germinated seeds in our study, and therefore, the effect of storage was not considered in our models. However, with the use fresh seeds, the results could be slightly different, because, e.g., El-Keblawy and Al-Rawai (2006) and Leverett et al. (2016), showed that dry stored seeds have higher germination than that of fresh seeds.

In our data set, rare species were restricted to the mountains, whereas the common species were more widespread, which could contribute to the result that rare species were adapted to mountain conditions, whereas the common species were not. For a better comparison than that of this study, the germination response should be examined for congeners with similar ranges in altitude, although this type of study may not be possible because common species are defined as species with wider ranges than those of rare species (Gaston 1994).

Conclusions

To conclude, this study is the first to compare proportions of germinated seeds of rare and common congeners across a large set of species, providing new insights into the importance of germination in determining species rarity. The results indicated that an inability to germinate clearly did not cause species rarity in the Krkonoše Mts., and that rare species were apparently well adapted to local conditions. To design appropriate management for rare species, further studies describing processes and traits that are characteristic of species with narrow distributions are required. Total seed production may be one of the traits identified as potentially interesting based on our results.

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SUPPORTING INFORMATION STUDY 1

Supporting information 1A. Pair correlation matrix showing Pearson correlation coefficient among the dependent variables. Significant values (≤ 0.05) are in bold.

	Proportion of dormant seeds	GI	T30	Germination percentage	Seeds viability
Proportion of dormant seeds	1	-0.013	0.039	**-0.219**	**0.606**
GI		1	0.035	0.008	0.019
T30			1	-0.062	-0.022
Germination percentage				1	**0.643**
Seeds viability					1

Supporting information 1B. Effect of maternal environment, original (O) and target conditions (T) on germination percentage, time to 30% germination (T30referred to as germination speed in the text), germination index (GI) and proportion of dormant seeds tested in mixed effects models with population of *A. alpinum* used as a random factor. Significant values (≤ 0.05) are in bold. N = 960, df = 1.

	Germination percentage		**T30**		**GI**		**Proportion of dormant seeds**	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Mat. Envi	0.01	0.963	2.76	0.097	0.36	0.551	0.21	0.648
Original temperature	2.78	0.132	0.03	0.872	2.14	0.148	**21.27**	**<0.001**
Original moisture	1.79	0.217	0.89	0.766	0.31	0.579	**22.05**	**<0.001**
Target moisture	**12.96**	**<0.001**	0.83	0.288	1.97	0.161	1.54	0.215
Target temperature	**20.95**	**<0.001**	0.09	0.925	1.99	0.158	**15.29**	**<0.001**
Stratification	0.40	0.525	2.37	0.128	2.50	0.115	1.75	0.186
Mat. Envi: OTemp	0.02	0.900	1.91	0.167	0.31	0.580	3.15	0.076
Mat. Envi: OMoist	0.63	0.427	1.85	0.175	0.00	0.948	0.98	0.322
Mat. Envi: TTemp	0.07	0.789	0.61	0.435	0.43	0.511	3.68	0.055
Mat. Envi: TMoist	0.02	0.894	0.01	0.968	0.76	0.383	**5.36**	**0.021**
Mat. Envi: Stratification	0.32	0.568	0.20	0.652	2.72	0.099	3.77	0.053
OMoist: OTemp	1.19	0.306	1.95	0.164	0.28	0.596	**16.43**	**<0.001**
TMoist: TTemp	**22.24**	**<0.001**	1.33	0.331	1.46	0.227	**10.26**	**0.001**
OMoist: TMoist	**7.79**	**0.005**	1.29	0.264	0.11	0.743	**9.43**	**0.002**
OMoist: TTemp	**21.80**	**<0.001**	2.01	0.156	0.14	0.710	**10.44**	**0.001**
OTemp: TMoist	**10.80**	**0.001**	1.32	0.254	1.68	0.196	**4.05**	**0.045**
OTemp: TTemp	**8.50**	**0.004**	1.31	0.253	1.85	0.175	**9.30**	**0.002**

	Germination percentage		**T30**		**GI**		**Proportion of dormant seeds**	
Stratification: OTemp	0.76	0.384	0.93	0.339	2.09	0.149	0.53	0.465
Stratification: OMoist	0.01	0.912	0.01	0.975	0.14	0.713	0.17	0.684
Stratification: TMoist	1.14	0.286	**9.56**	**0.002**	3.10	0.079	0.01	0.941
Stratification: TTemp	0.70	0.402	0.21	0.648	1.86	0.173	0.95	0.331
Stratification: OTemp: OMoist	0.02	0.892	0.03	0.852	0.12	0.728	0.09	0.761
Stratification: TTemp: TMoist	1.34	0.247	0.33	0.718	2.29	0.130	0.00	0.967
OTemp: OMoist: TTempe	**14.93**	**<0.001**	**9.21**	**0.004**	0.13	0.718	**7.63**	**0.006**
OTemp: OMoist: TMoist	**6.76**	**0.009**	1.43	0.290	0.10	0.757	**8.89**	**0.003**
TTemp: TMoist: OTemp	**15.26**	**<0.001**	2.01	0.121	1.27	0.260	**7.79**	**0.005**
TTemp: TMoist: OMoist	**19.20**	**<0.001**	2.14	0.119	0.08	0.775	**15.41**	**<0.001**

Supporting information 1C. Effect of maternal environment, target, origin and their interactions

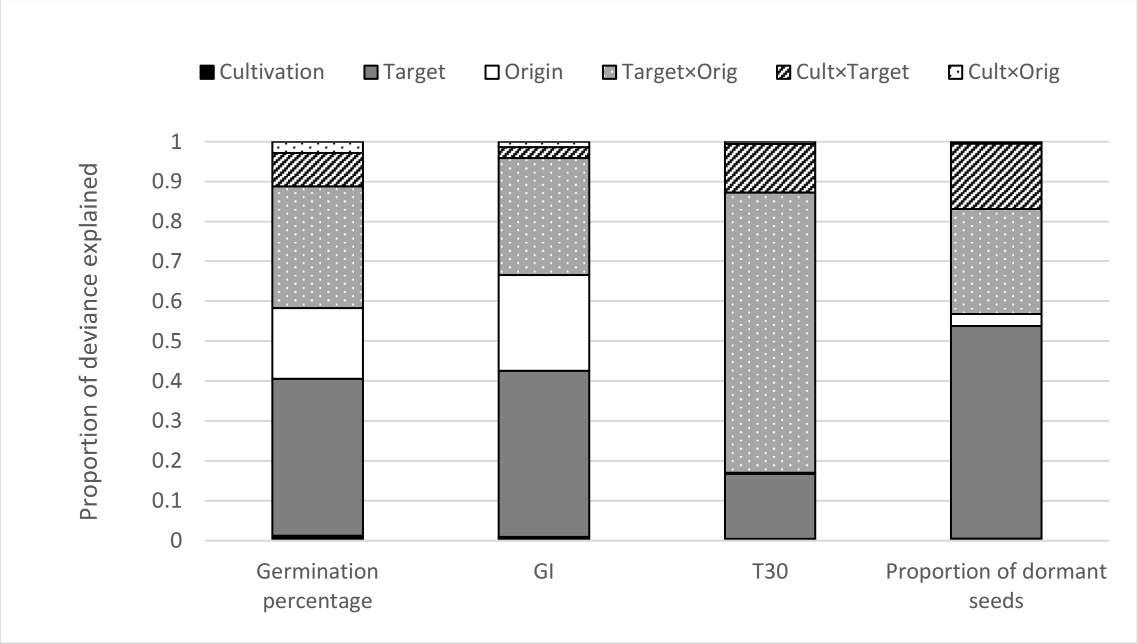
Germination percentage, T30, germination index and proportion of dormant seeds of *A. alpinum* were not significantly influenced by maternal environment. We did not find any significant interaction with maternal environment for any study variable with exception of interaction maternal environment and target moisture for proportion of dormant seeds (Supporting information 1B).

Target moisture significantly influenced germination percentage of *A. alpinum* (Supporting information 1B). Germination percentage increased with increasing moisture. Germination percentage increased with increasing target temperature. The trend was opposite in proportion of dormant seeds. Interaction of target moisture and target temperature significantly influenced germination percentage and proportion of dormant seeds (Supporting information 1B). Germination percentage and proportion of dormant seeds increased with increasing target temperature in dry target conditions. The trend was the same in germination percentage and opposite in proportion of dormant seeds in wet conditions. Target temperature had significantly impact on all variables with exception of germination speed (Supporting information 1B). Stratification and all their interactions did not significantly affect any of the dependent variables with exception of T30 (Supporting information 1B). Stratification interacted with target moisture. Target conditions had high explanatory power for all study variables (Supporting information 1D).

Both original temperature and original moisture, alone or in interaction with each other, had significant effect on proportion of dormant seeds (Supporting information 1B) but had quite low explanatory power (Supporting information 1D. Proportion of dormant seeds was higher in colder temperatures in all original moisture conditions.

Interaction of target and origin condition had high explanatory power in all dependent variables (Supporting information 1D) and significantly affected all study variables with exception of GI (Supporting information 1B). We found several triple interactions connected with original and target conditions (Supporting information 1B). With regard that these interactions showed very similar trend as interaction of moisture change and temperature change presenting in the main text, we do not describe these triple interactions in more details here.

Supporting information 1D. Proportion of deviance explained by maternal environment, origin (including temperature and moisture) and target (including temperature, moisture and stratification) environment and their interaction on germination percentage, proportion of dormant seeds and germination rate (GI and T30).



SUPPORTING INFORMATION STUDY 2

Supporting information 2A. Pair correlation matrix, based on Pearson ´s correlation coefficient, for seed mass and content and concentration of nitrogen (N), phosphorus (P), starch (St) and fructans (Fr). Significant values (≤ 0.05) are in bold.

	Seed mass	N content	N concentration	P content	P concentration	St content	St concentration	Fr content	Fr concentration
Seed mass	1	-0.1514	**-0.4531**	-0.2356	**-0.5002**	**0.8730**	**0.4265**	**0.6077**	**0.8480**
N content		1	**0.8468**	**0.3386**	**0.3844**	**0.4512**	**0.3202**	0.1763	0.1765
N concentration			1	**0.6004**	**0.6086**	0.2746	0.2403	0.1828	0.1305
P content				1	**0.3386**	0.1751	**0.3510**	0.2472	0.1284
P concentration					1	0.2621	**0.3994**	0.2830	0.2254
St content						1	**0.8730**	**0.3216**	**0.3663**
St concentration							1	0.2964	0.2961
Fr content								1	**0.9644**
Fr concentration									1

Supporting information 2B. Effect of original (O) temperature and moisture on A) nutrient content and B) nutrient concentration. Results for nutrient concentration are shown in section *Effects of seed origin on nutrients and seed mass*. Significant values (≤ 0.05) are in bold.

A	**Nitrogen**		**Phosphorus**		**Fructans**		**Starch**	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
OMoist	1.42	0.241	1.77	0.192	0.26	0.611	1.54	0.223
OTemp	0.01	0.985	0.98	0.330	0.04	0.847	3.27	0.079
Omoist:Otemp	**7.21**	**0.011**	0.12	0.734	1.22	0.277	0.22	0.640

B	**Nitrogen**		**Phosphorus**		**Fructans**		**Starch**	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
OMoist	0.01	0.979	1.05	0.311	0.11	0.744	0.16	0.686
OTemp	0.30	0.586	0.06	0.808	0.78	0.383	2.38	0.082
Omoist:Otemp	**4.37**	**0.033**	0.12	0.737	2.57	0.117	0.29	0.591

Supporting information 2C. Pair correlation matrix, based on Pearson ´s correlation coefficient, for dependent variables. Pair correlation matrix is for complete germination dataset. Significant values (≤ 0.05) and variables further used for statistical analyses are underlined.

	Prop. dormant seeds	GI	Total germination	Prop. viable seeds	T50
Prop. dormant seeds	1	**-0.861**	**-0.987**	**0.419**	**0.737**
GI		1	**0.899**	-0.087	**-0.784**
Total germination			1	-0.267	**-0.746**
Prop. viable seeds				1	-0.029
T50					1

Supporting information 2D. Effect of seed mass, original (O) and target (T) conditions on total germination, proportion of viable seeds and time to 50% germination (T50) assessed using mixed effects models with population used as a random factor. Table A represents results of two boreal and two subboreal populations (the third analysis), Table B represents results of boreal populations (the second analysis). Significant values (≤ 0.05) are in bold. * indicate significant result in the model not including seed mass. ● indicate non-significant results in the model not including seed mass

A	**Total germination**		**Prop. viable seeds**		**T50**	
	F-values	p-value	F-values	p-value	F-values	p-value
OMoist	0.22	0.603*	**28.8**	**<0.001**	0.01	0.941*
OTemp	0.72	0.400*	0.01	0.919*	0.70	0.400
TMoist	**811.22**	**<0.001**	**14.63**	**<0.001**	3.36	0.070
TTemp	**142.15**	**<0.001**	0.72	0.400	0.90	0.350
Seed mass	**36.87**	**<0.001**	**15.52**	**<0.001**	0.05	0.928
TMoist:TTemp	**27.59**	**<0.001**	**19.25**	**<0.001**	0.11	0.740
TMoist:OTemp	0.03	0.792	**8.09**	**0.002**	1.09	0.300
TMoist:Seed mass	3.06	0.058	2.67	0.075	0.78	0.380
TMoist:OMoist	0.48	0.502	**4.67**	**0.024**	1.18	0.280
OMoist:TTemp	**7.47**	**0.006**	0.14	0.208	1.75	0.190
OMoist:Seed mass	**28.65**	**<0.001**	0.64	0.442	2.56	0.110
TTemp:OTemp	0.95	0.329	**5.37**	**0.02**	0.07	0.790
TTemp:Seed mass	**4.60**	**0.015**	0.27	0.598	0.14	0.710
OTemp:Seed mass	**5.45**	**0.012**	**3.87**	**0.042**	0.14	0.710
TMoist:TTemp:OTemp	0.01	0.929	1.96	0.158*	0.43	0.520
TMoist:TTemp:Seed mass	0.11	0.814	**7.20**	**0.003**	1.40	0.240
TMoist:OTemp:Seed mass	0.49	0.511	2.32	0.126	0.64	0.420
OMoist:TMoist:TTemp	0.68	0.433*	2.11	0.138	0.47	0.490
OMoist:TMoist:Seed mass	0.02	0.882	**6.62**	**0.009**	2.01	0.160
TTemp:OTemp:Seed mass	0.08	0.893	2.32	0.126	0.03	0.870

B	**Total germination**		**Prop. viable seeds**		**T50**	
	F-values	p-value	F-values	p-value	F-values	p-value
TTemp	**43.16**	**<0.001**	1.90	0.320	3.07	0.083
TMoist	**945.48**	**<0.001**	0.07	0.902	**13.60**	**<0.001**
OMoist	0.05	0.928	1.83	0.318*	0.17	0.863
Seed mass	1.57	0.672	1.54	0.398	0.19	0.847
TTemp: TMoist	**8.21**	**<0.001**	**30.88**	**<0.001**	2.18	0.143
TTemp: OMoist	2.55	0.128	0.43	0.823	3.30	0.072
TMoist: OMoist	**10.46**	**<0.001**	**8.26**	**<0.001●**	**6.16**	**0.015●**
TTemp: Seed mass	**8.48**	**<0.001**	2.44	0.087	2.49	0.117

B	**Total germination**		**Prop. viable seeds**		**T50**	
	F-values	p-value	F-values	p-value	F-values	p-value
TMoist: Seed mass	**32.31**	**<0.001**	**5.79**	**0.001**	**8.72**	**0.004**
OMoist: Seed mass	**14.80**	**<0.001**	**5.89**	**0.018**	0.02	1.000
TTemp: TMoist:OMoist	2.36	0.201	1.29	0.263	2.51	0.116
TTemp: OMoist: Seed mass	1.57	0.683	**9.14**	**0.002**	0.03	0.874
TTemp: TMoist: Seed mass	0.00	0.949	0.49	0.431	**7.48**	**0.007**
TMoist: OMoist: Seed mass	0.01	0.926	0.00	0.892	0.04	0.832

SUPPORTING INFORMATION STUDY 3

Supporting information 3A. List of species, year and location of their collection; altitude (m a.s.l.), latitude (N) and longitude (E).

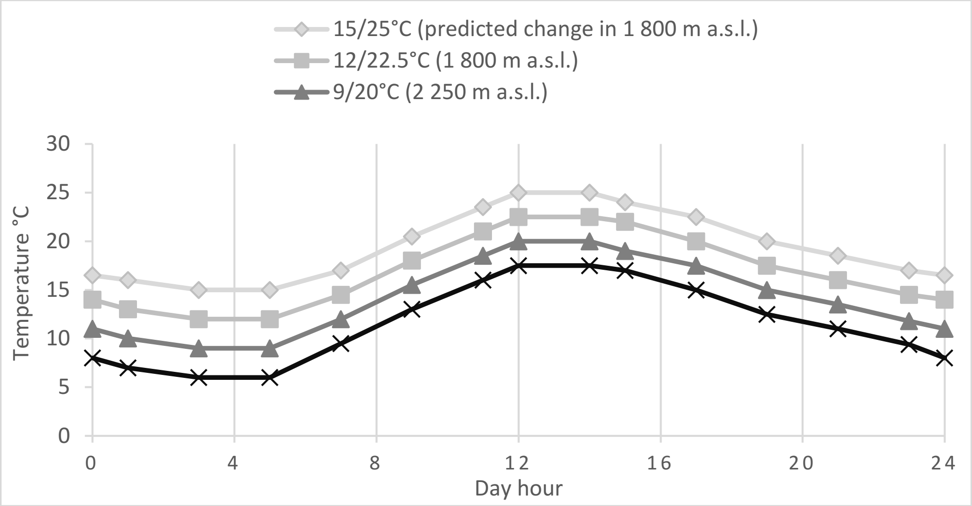
Species	**Year**	**Altitude**	**N**	**E**
Impatiens bajurensis S. Akiyama et H. Ohba	2016	1 540	29.5939	80.4573
Impatiens balsamina L.	2016	1 330	27.6838	85.2836
Impatiens bicornuta s.l. Wall.	2016	2 060	27.8141	85.3519
Impatiens bicornuta s.l. Wall.	2016	2 158	27.8031	85.4236
Impatiens bicornuta s.l. Wall.	2016	2 280	28.3691	83.7311
Impatiens bicornuta s.l. Wall.	2016	2 300	27.8120	85.3737
Impatiens bicornuta s.l. Wall.	2017	2 075	28.3824	83.8427
Impatiens bicornuta s.l. Wall.	2017	2 273	27.6573	85.2191
Impatiens bicornuta s.l. Wall.	2017	2 628	28.5292	84.3036
Impatiens bicornuta s.l. Wall.	2017	2 480	28.5256	84.3114
Impatiens cymbifera Hook. f.	2016	2 300	27.6315	87.2246
Impatiens cymbifera Hook. f.	2017	2 430	28.3824	83.8427
Impatiens devendrae Pusalkar*	2017	2 728	29.8902	80.9272
Impatiens devendrae Pusalkar*	2017	2 175	29.8627	80.9046
Impatiens discolor DC.	2016	2 356	27.6586	85.2303
Impatiens insignis DC.	2016	1 460	27.7550	85.2754
Impatiens insignis DC.	2017	1 604	27.5896	85.3792
Impatiens puberula DC.	2016	2 080	27.8141	85.3519
Impatiens racemosa DC.	2017	2 440	27.6494	85.2327
Impatiens racemosa DC.	2016	2 270	27.6561	85.2290
Impatiens racemosa DC.	2016	2 280	27.6560	85.2290
Impatiens racemosa DC.	2017	2 525	27.6652	85.2046
Impatiens racemosa DC.	2017	2 228	27.6573	85.2191
Impatiens racemosa DC.	2017	2 100	27.8081	85.3711
Impatiens racemosa DC.	2016	2 275	28.5291	84.3189
Impatiens scabrida DC.**	2016	2 180	29.2099	80.6141
Impatiens tricornis Lindl.***	2016	3 700	28.6305	84.4722
Impatiens tricornis Lindl.***	2016	1 240	28.4918	83.6508
Impatiens tricornis Lindl.***	2016	1 160	28.3138	83.7672
Impatiens tricornis Lindl.***	2017	2 688	28.5523	84.2415
Impatiens scullyi Hook. f.	2016	2 880	28.5722	84.1939
Impatiens scullyi Hook. f.	2016	2 280	28.5291	84.3189
Impatiens scullyi Hook. f.	2016	2 360	28.3978	83.7801
Impatiens scullyi Hook. f.	2017	2 688	28.5523	84.2415
Impatiens scullyi Hook. f.	2017	2 587	28.5517	84.2723
Impatiens sulcata Hook. f.	2016	2 805	28.4022	83.7011
Impatiens sulcata Hook. f.	2016	3 580	28.6696	84.0176
Impatiens sulcata Hook. f.	2016	3 540	28.8184	83.8490
Impatiens sulcata Hook. f.	2016	3 280	28.6230	84.1350
Impatiens falcifer Hook. f.	2017	2 499	27.5774	85.3995

* Sensu (Pusalkar and Singh 2010)

** Sensu (Akiyama and Ohba 2016)

*** Sensu (Akiyama and Ohba 2016). Before revision by (Akiyama and Ohba 2016) usually called *I. scabrida.*

Supporting information 3B. Supporting information 2. The courses of the temperatures during the days in the growth chambers. Degrees centigrade indicate minimum and maximum day temperatures.



Supporting information 3C. Supporting information 3. Pair correlation matrix showing Pearson correlation coefficients among the dependent variables. Significant values (≤ 0.05) and variables further used for statistical analyses are in bold.

	Total germination	GI	**Seed dormancy**	Seed viability	**T30**	**Necessary stratification duration**
Total germination	–	**0.694**	0.083	**0.964**	0.164	-0.071
GI		–	-0.288	**0.577**	**-0.505**	**-0.451**
Seed dormancy			–	**0.345**	**0.626**	**0.466**
Seed viability				–	**0.321**	0.050
T30					–	**0.590**
Necessary strat. duration						–

Supporting information 3D. Effect of original temperature (O), original precipitation (O), seed mass and target temperature (T) on total germination, seed dormancy and germination speed (T30) tested in generalized linear mixed effects models with population used as a random factor. Year, longitude, latitude and their interaction were used as covariates in the tests. Results using data including only three target temperature regimes (without 9/20°C) are presented. Significant values (≤ 0.05) are in bold.

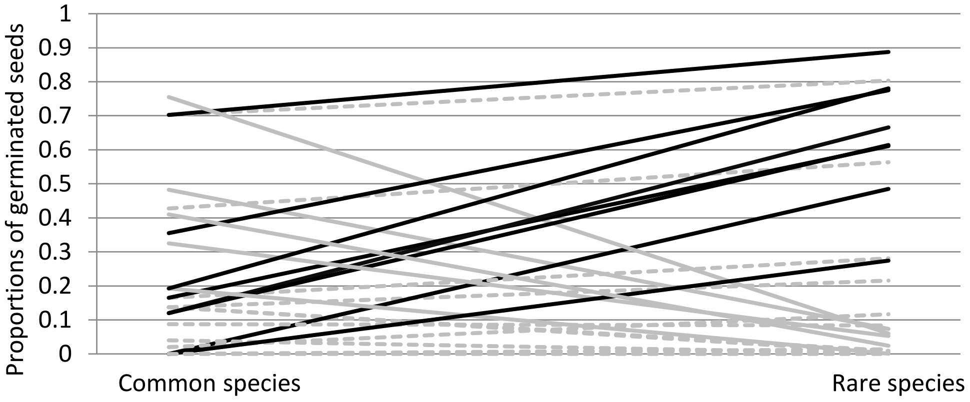
	Total germination		**T30**		**Seed dormancy**	
	F-value	p-value	F-value	p-value	F-value	p-value
OTemp	0.72	0.328	0.32	0.580	0.50	0.978
OPrec	0.56	0.412	1.03	0.322	0.68	0.613
TTemp	**5.78**	**0.011**	**3.61**	**0.031**	**5.18**	**0.008**
Seed mass	**4.25**	**0.017**	1.09	0.307	**3.27**	**0.050**
OTemp:OPrec	0.02	0.897	0.22	0.644	0.42	0.701
OTemp:TTemp	0.25	0.803	0.40	0.533	0.79	0.640
OPrec:TTemp	**3.91**	**0.044**	**3.53**	**0.033**	1.07	0.154
OTemp:Seed mass	**14.89**	**<0.001**	0.09	0.765	**3.82**	**0.047**
OPrec:Seed mass	**5.14**	**0.038**	3.33	0.062	**4.43**	**0.040**
TTemp:Seed mass	0.20	0.819	0.14	0.716	**4.06**	**0.042**
OTemp:OPrec:TTemp	**12.87**	**<0.001**	2.17	0.154	0.21	0.591
OTemp:OPrec:Seed mass	**4.73**	**0.026**	0.26	0.616	1.39	0.104
OTemp:TTemp:Seed mass	3.11	0.075	0.11	0.747	0.30	0.744
OPrec:TTemp:Seed mass	0.39	0.766	2.78	0.109	2.47	0.076
OTemp:OPrec:TTemp:Seed mass	0.11	0.738	1.40	0.246	2.50	0.078

SUPPORTING INFORMATION STUDY 4

Supporting information 4A. Correlation matrix of proportions of germinated seeds in the different temperature regimes. One data point in the analyses represents one species. Significant values (≤0.05) are in bold.

	20 °C light	20 °C dark	5 °C (2 - 4m)	5 °C (5 - 8m)	5/ 18 °C	20 °C light	20 °C dark
20 °C light	1						
20 °C dark	**0.786**	1					
5 °C (2 - 4m)	0.028	-0.057	1				
5 °C (5 - 8m)	0.015	-0.256	0.310	1			
5 °C/18°C	-0.171	-0.190	-0.090	-0.093	1		
20 °C light	-0.163	-0.106	-0.092	-0.061	0.098	1	
20 °C dark	-0.101	-0.129	0.015	-0.099	0.092	0.169	1

Supporting information 4B. Proportion of germinated seeds of common and rare congeners (24 pairs) at 20 °C light without stratification. The black lines indicate that rare species had higher proportion of germinated seeds than common one, the grey lines indicate that common species had higher proportion of germinated seeds than the rare species and the dashed line indicates non-significant results.



Supporting information 4C. Mean proportions of germinated seeds for each species in each tested regime.

Species	Rarity	Total germination rates	20°C without stratification	5°C (2-4 months)	5°C (5-8 months)	5/18°C	20°C light after stratification	20°C dark after stratification
Campanula patula	common	0.358	0.483	0.070	0.000	0.090	0.000	0.000
Campanula bohemica	rare	0.350	0.074	0.031	0.190	0.246	0.024	0.000
Carex nigra	common	0.277	0.137	0.018	0.000	0.024	0.000	0.000
Carex aterrina	rare	0.721	0.216	0.007	0.018	0.600	0.065	0.000
Carex capillaris	rare	0.539	0.005	0.001	0.000	0.054	0.180	0.000
Carex davalliana	rare	0.563	0.009	0.001	0.000	0.522	0.127	0.015
Cirsium vulgare	common	0.120	0.000	0.000	0.000	0.004	0.138	0.000
Cirsium acaule	rare	0.509	0.485	0.012	0.000	0.142	0.040	0.387
Dianthus carthusianorum subsp. carthusianorum	common	0.556	0.703	0.030	0.000	0.227	0.000	0.225
Dianthus superbus subsp. alpestris	rare	0.803	0.803	0.000	0.000	0.000	0.000	0.000
Dianthus superbus subsp. superbus	rare	0.866	0.888	0.000	0.000	0.000	0.000	0.000
Eriphorum angustifolium	common	0.285	0.000	0.000	0.000	0.200	0.321	0.000
Eriophorum latifolium	rare	0.626	0.014	0.021	0.000	0.559	0.000	0.000
Galium saxatile	common	0.845	0.428	0.585	0.255	0.000	0.000	0.000
Galium sudeticum	rare	0.678	0.563	0.056	0.000	0.069	0.040	0.000
Geum urbanum	common	0.829	0.088	0.185	0.000	0.724	0.000	0.007
Geum montanum	rare	0.601	0.084	0.132	0.000	0.381	0.196	0.118
Gnaphalium uliginosum	common	0.815	0.755	0.160	0.000	0.000	0.000	0.000
Gnaphalium supinum	rare	0.414	0.060	0.000	0.000	0.116	0.068	0.000
Hieracium lachenalii	common	0.743	0.165	0.193	0.000	0.418	0.188	0.000
Hieracium pedunculare	rare	0.543	0.281	0.054	0.000	0.232	0.000	0.000
Hieracium tubulosum	rare	0.770	0.611	0.153	0.000	0.056	0.000	0.000
Hieracium murorum	common	0.303	0.120	0.005	0.000	0.187	0.000	0.000
Hieracium bifidum	rare	0.663	0.614	0.186	0.000	0.100	0.381	0.000
Hieracium decipiens	rare	0.829	0.666	0.053	0.000	0.084	0.077	0.039
Hypericum maculatum	common	0.523	0.355	0.160	0.000	0.200	0.206	0.098
Hypericum humifusum	rare	0.887	0.775	0.010	0.000	0.439	0.000	0.000
Hypochaeris radicata	common	0.538	0.325	0.045	0.000	0.181	0.079	0.000

Hypochaeris uniflora	rare	0.827	0.053	0.013	0.005	0.725	0.164	0.050
Knautia arvensis subsp. arvensis	common	0.153	0.020	0.020	0.000	0.123	0.022	0.011
Knautia arvensis subsp. pseudolongifolia	rare	0.542	0.117	0.085	0.260	0.442	0.043	0.105
Luzula campestris	common	0.838	0.193	0.970	0.000	0.549	0.000	0.000
Luzula spicata	rare	0.445	0.001	0.000	0.074	0.428	0.167	0.000
Luzula sudetica	rare	0.842	0.780	0.099	0.000	0.063	0.002	0.000
Poa annua	common	0.151	0.040	0.020	0.000	0.065	0.137	0.003
Poa laxa	rare	0.455	0.002	0.005	0.000	0.488	0.002	0.000
Primula elatior	common	0.255	0.000	0.000	0.000	0.070	0.024	0.000
Primula minima	rare	0.748	0.274	0.003	0.000	0.063	0.357	0.000
Sorbus aucuparia	common	0.334	0.000	0.005	0.105	0.249	0.025	0.000
Sorbus sudetica	rare	0.686	0.000	0.000	0.375	0.357	0.045	0.000
Veronica chamaedrys	common	0.861	0.410	0.475	0.000	0.620	0.000	0.000
Veronica bellidioides	rare	0.518	0.024	0.011	0.363	0.000	0.170	0.200

Supporting information 4D. The results of comparing rare and common congeners in total proportions of germinated seeds and in detailed categories light-temperature conditions. Significant values (≤0.05) are in bold. ↑ - rare species had higher proportions of germinated seeds than common congeners, ↓ common species had lower proportions of germinated seeds than rare congeners. Df error=1125 for 24 pairs, Df error=835 for 17 pairs.

Dataset	Factor		**Total germination**	**20 °C**	**5 °C (2-4m)**	**5 °C (5-8m)**	**5/18 °C**	**20 °C light after cold**	**20 °C dark after cold**
24 pairs	Seed mass	**F**	**25.48**	0.004	0.53	**4.90**	2.85	0.30	1.62
		P	**<0.001** ↑	0.95	0.466	**0.028**↑	0.092	0.579	0.203
	Pair	**F**	**15.75**	**31.28**	**8.70**	**10.18**	**9.56**	**19.30**	**13.89**
		P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Rarity	**F**	**51.44**	**6.40**	**90.26**	0.04	**9.58**	2.72	0.15
		P	**<0.001** ↑	**0.011** ↑	**<0.001**↓	0.827	**0.002** ↑	0.099	0.694
	Seed mass*rarity	**F**	**8.45**	**13.98**	0.04	1.53	1.90	<0.001	1.13
		P	**0.003**	**<0.001**	0.244	0.217	0.168	0.991	0.318
17 pairs	Seed mass	**F**	**23.16**	0.005	0.18	3.55	1.14	0.35	1.16
		P	**<0.001** ↑	0.939	0.672	0.062	0.301	0.552	0.281
	Pair	**F**	**14.01**	**24.28**	**16.27**	**20.54**	**22.24**	**19.22**	**23.60**
		P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Rarity	**F**	**19.51**	0.18	**57.05**	0.11	**25.06**	0.83	2.31
		P	**<0.001** ↑	0.663	**<0.001**↓	0.730	**<0.001**↑	0.361	0.128
	Seed mass*rarity	**F**	**7.24**	**5.47**	0.02	1.04	0.01	0.06	0.17
		P	**0.007**	**0.019**	0.888	0.311	0.901	0.791	0.663