Experimental comparisons among very rare to widespread plant species of Switzerland

Inauguraldissertation
der Philosophisch-naturwissenschaftlichen Fakultät
der Universität Bern

vorgelegt von

Hugo Vincent

aus Frankreich

Leiter der Arbeit:
Prof. Dr. Markus Fischer
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“Much to learn, you still have.”

Master Yoda

Table of contents

Chapter 1

General Introduction

Most species are rare, i.e. have low abundance or small ranges, and only few are widespread (Gaston 1994, Brown et al. 1996, McGill et al. 2007). This pattern appears to be a general rule for the frequency distributions of the geographic ranges of species, probably first pointed out by Willis in his book *Age and Area* (1922), where he termed it the "hollow curve of distribution". Decades earlier, however, the question had already emerged: "A species exists on a very small territory, another extends over vast areas, why?" (de Candolle, 1855). From the earliest biogeographers of the 19th century onward, generations of ecologists and evolutionary biologists have been fascinated by this question and it has led to a large body of theoretical and empirical work on rarity.

In addition to its fundamental interest, the understanding of drivers of species rarity or commonness is also of major concern for conservation. Caused by global changes, the current species extinction rate is estimated to be a thousand times greater than the ones estimated from the fossil record (MEA 2005), indicating that the world is experiencing its sixth mass extinction (Thomas et al. 2004a, Barnosky et al. 2011). Biodiversity loss in itself is a major driver of ecosystem change, and high species diversity has been shown to be essential to maintain ecosystem services within and across ecosystems (Isbell et al. 2011, Hooper et al. 2012, van der Plas et al. 2016). Thus, preserving high biodiversity is of utmost importance.

Understanding differences in species distributions and informing conservation both require to understand whether rare and more widespread species differ from each other in their population biology. This involves plant performance at various stages of their life cycles, including the earliest ones, phenotypic and genetic variation within species, the response of plants to environmental change, and the ability to establish at new sites.

Comparison of rare and widespread species and the study of seed and germination characteristics

Trait differences in the study of rarity

An important classical element of the study of plant rarity is to compare species environmental characteristics and life-history or functional traits between rare and widespread plant species, to reveal general patterns that could drive differences in abundance and distribution between species (Kunin & Gaston 1997, Gaston 1994, Bevill & Louda 1999). For instance, it has been shown that widespread species are generally larger (Murray et al. 2002, Lavergne et al. 2003, Cornwell & Ackerly

2010, Dawson et al. 2012), produce more and bigger flowers (Lavergne et al. 2004), are more competitive and grow faster (Murray et al. 2002), and respond more positively to increased nutrient availability (Dawson et al. 2012) than related rare species. These differences have important implications for rarity as they relate directly to the fitness of individuals and populations.

Differences in traits linked to dispersal and establishment

Traits related to the ability to disperse and establish, such as seed size or germination success, might also play a role in explaining species rarity (Kunin & Gaston 1997). However, while there are several comparisons between pairs of species or for larger sets of species (e.g. Murray et al. 2002), the evidence is not conclusive, possibly because these studies did not involve the rarest species of their study areas. Alternatively, it might be that different mechanisms lead to positive relationships between rarity and seed size in some cases and to negative ones in others. Widespread species may benefit from large seeds conveying them a competitive advantage, facilitating the establishment of new populations (Leishman et al. 2000, Moles & Westoby 2004, Baraloto et al. 2005, Metz et al. 2010). On the other hand, small-seeded species are expected to produce more seeds and disperse further, they are thus likely to expand their ranges further than species with larger seeds (Guo et al. 2000, Fenner & Thompson 2005). Studying variation in seed size in a single experiment involving multiple species, from very rare to very widespread ones, might reveal whether there is a general relationship between seed size and rarity.

Other traits related to establishment have received little attention. While some studies investigated a potential link between germination characteristics and rarity and found no general pattern, these studies were mostly limited to experiments comparing few species or pairs of rare and widespread species (e.g. Brown et al. 2003, Simon & Hay 2003, but see Luna & Moreno 2010). Thus it is not clear whether germination percentages, which directly relate to the fitness of individuals and populations, or the time of germination, which can increase performance and competitive ability through early germination (Verdú & Traverset 2005, Donohue et al. 2010), differ between rare and widespread species. It is also unclear whether very rare and more widespread species differ in seed dormancy and in their responses to seed stratification. In these contexts an experiment comparing germination characteristics for a large number of species, including very rare ones and related widespread species, is missing.

In addition to the fundamental interest in studying establishment traits of rare and widespread species, this is also essential for conservation as it informs ex-situ conservation programs and also matters largely for introductions of rare species to the wild.

Genetic diversity and evolutionary potential of rare and endangered species

Rare species may have lower genetic diversity than more widespread ones

Several studies have highlighted a generally reduced performance of rare species compared with more widespread ones (e.g. Murray et al. 2002, Lavergne et al. 2003, Dawson et al. 2012). While this may reflect important drivers of rarity, it might also be a consequence of rarity. Populations of the rarest and most endangered plant species are often smaller and more isolated than those of less rare and less endangered species, and thus might have reduced genetic diversity (Gaston 1994, Gaston 2003, Leimu et al. 2006). In small and isolated populations, limited gene flow, increased inbreeding depression and genetic drift (Fisher 1930, Ellstrand & Elam 1993) lead to reduced genetic variation and fitness (Leimu et al. 2006) and eventually contribute to the extinction of these populations (Charlesworth & Charlesworth 1987, Gilpin & Soulé 1986, Frankham 1995). Although environmental change and environmental stochasticity are major drivers of population extinctions (Lande 1993, Melbourne & Hastings 2008), most species are affected by genetic factors before they go extinct (Spielman et al. 2004). Understanding the relationship between rarity and genetic diversity is therefore of great importance for conservation biology.

Evolutionary potential of rare and endangered plant species

Studies based on molecular markers support the hypothesis that rarer species have lower genetic diversity (Karron 1987, Gitzendanner & Soltis 2000, He et al. 2000, Cole 2003, Edwards et al. 2014). Molecular markers can provide relevant information for the conservation of endangered species, e.g. on population structure, genotypic diversity, mating systems and inbreeding rates (Schaal et al. 1991, Storfer 1996, Frankham et al. 2014). However, most molecular markers are selectively neutral and therefore generally not well correlated with variation in ecologically important traits (Reed & Frankham 2011). In contrast, quantitative-genetic approaches combine information on the relatedness between individuals, inferred from markers or from pedigrees, with the study of phenotypic trait variation, often of traits that are directly relevant for plant functions or fitness (Schaal et al. 1991, Storfer 1996, Petit et al. 2001). Quantitative-genetic approaches also allow the estimation of narrow-sense heritabilities of populations, which represent the amount of phenotypic variation between individuals that is due to additive genetic variation. As narrow-sense heritability reflects the proportion of variation that responds to selection, it indicates the evolutionary potential of populations (Fisher 1930, Falconer & Mackay 1996). If rare species have generally lower genetic diversity than more widespread ones, they might also have lower heritability, and might therefore be less able to adapt to potential changes in their environment.

In the context of global changes, populations of rare and endangered species may need to adapt to new environmental conditions to survive (Franks et al. 2013). Therefore knowledge on their evolutionary potential is crucial, and particularly important to inform both ex-situ and in-situ conservation measures (Cochrane et al. 2007, Menges 2008, Ensslin et al. 2015). Several studies have investigated quantitative trait variation and heritability in relation to rarity. However, these experimental studies used either only one or two rare species, e.g. Widén & Andersson (1993) and Petit et al. (2001), which does not inform on differences with more widespread species, or only a single pair of a rare and a related widespread species (Waldmann & Andersson 1998), which does not allow for general conclusions on the relationship between heritability and rarity. Whether the rarest and most endangered species generally have lower heritability, and thus lower evolutionary potential, than less rare and less endangered species, remains to be tested for a large number of species differing in their range size and threat of extinction, from very rare and threatened to widespread.

Rarity and tolerance to climate change

Adaptation and tolerance to climate change

Climate change, leading to the loss of climatically suitable area, is expected to become a major driver of species extinction (Thomas et al. 2004b, Thuiller et al. 2005, Pimm et al. 2014). To survive climate change, plant populations may migrate to keep track of favorable environmental conditions (Franks et al. 2013). However, plant migration may be limited, for instance by topographic boundaries or by habitat fragmentation (Jump & Peñuelas 2005). In addition, plant populations may also survive climate change by adapting to a new climate (Franks et al. 2013). However, to adapt in the long run populations must be able to persist for several generations, and thus they must initially be able to tolerate changes in climate. While many studies predict that species will shift their range in response to environmental modifications (e.g. Bakkenes et al. 2002, Thomas et al. 2004b), the fundamental climatic niche of species, which represents their initial ability to tolerate new climates, has rarely been taken into account in predictions of future species distributions under changed climate. To determine the fundamental climatic niche of species, experiments are needed where species are grown under a wide range of different climatic conditions.

Niche breadth hypothesis and tolerance to climate change

It is assumed that species with a greater range size, i.e. more widespread species, experience a larger range of ecological and climatic conditions, and thus are more able to tolerate different environmental conditions than species with a smaller range size, i.e. rarer species (Brown 1984, Gaston 2003). This was already suggested by Darwin (1859) in the *Origin of species*, referring to earlier studies: "Alph. de Candolle and others have shown that plants which have very wide ranges generally present varieties; and this might have been expected, as they become exposed to diverse physical conditions […]." Slatyer et al. (2013) showed in a meta-analysis that a smaller niche breadth of rare species than of widespread ones indeed seems to be a general pattern. If rare species have a smaller niche breadth than widespread ones, they are likely to be less tolerant to environmental changes than widespread species. However, the tolerance of individual populations to climate change is unknown, and we lack knowledge on whether populations of more widespread species are more tolerant to climatic variation, i.e. whether they have a larger fundamental climatic niche, than populations of more rare and endangered species do.

To our knowledge, experimental studies on the fundamental niche of rare, endangered species and widespread species are scarce (but see Brändle et al. 2003, Luna et al. 2012), and no study has linked plant rarity to the tolerance of plant populations to altered climates. This information is essential to predict the impact of the ongoing climate change on plant species extinction, and to develop relevant conservation measures focusing on species which are likely to be more affected by climate change.

Introductions of rare and endangered species to the wild

As many as half of the plant species may be threatened with extinction because of human-driven global changes (Pitman & Jørgensen 2002). To preserve endangered plant species, habitat protection and restoration are considered as the most important conservation measures (Maunder 1992). However, their efficiency may be strongly reduced by dispersal limitations due to habitat fragmentation. Thus, introductions, i.e. deliberate actions to release organisms into natural habitat, are an increasingly used conservation technique (Wolf et al. 1996, Vergeer et al. 2004, Menges 2008). Introductions aim to reinforce the size and genetic diversity of existing, often declining populations, or to establish self-sustaining populations having sufficient genetic diversity to allow adaptation to environmental changes (Wolf et al. 1996, Guerrant & Kaye 2007). They are, however, often considered as labour-intensive and unsuccessful (Maunder 1992, Guerrant & Kaye 2007, Godefroid et al. 2011, Maschinski & Haskins 2012).

Effect of genetic diversity on the success of endangered plant species introductions

Among the main factors considered to affect the success of rare and endangered plant species introductions is the genetic diversity of the introduced propagules (Vergeer et al. 2005, Menges 2008, Maschinski & Haskins 2012). High genetic diversity is likely to enhance fitness and survival of populations, by increasing the probability of having genotypes pre-adapted to future perturbations (Gamfeldt & Källström 2007, Prati et al. 2016) or by preventing inbreeding depression in small and isolated populations (Charlesworth & Charlesworth 1987, Ellstrand & Elam 1993). Nevertheless, the evidence of a positive effect of increased genetic diversity on the success of rare species introductions remains limited. Forsman (2013) found a positive relationship between genetic diversity and establishment success in a meta-analysis including nine plant studies, none of which involved rare and endangered plant species, however. Vergeer et al. (2005) and Prati et al. (2016) conducted experimental introductions in natural habitats of the endangered plants *Arnica montana* and *Ranunculus reptans*, respectively, and showed increased persistence of introduced populations of higher genetic diversity. These two studies also highlighted that performing introductions as experiments is crucial to understand factors leading to their failure or success (Guerrant & Kaye 2007, Godefroid et al. 2011). However, to our knowledge, the feasibility of introductions and the effect of increased genetic diversity on their success has not been tested yet in an experimental study in natural conditions involving several rare and endangered plant species.

This thesis

Rarity is a complex phenomenon and after nearly three centuries of scientific studies, it still appears difficult to disclose general patterns in differences between rare and widespread species. This may be due to most experimental studies investigating species differences in traits or responses to environmental factors defining rarity only coarsely as a two-level categorical variable, e.g. “rare” or “widespread”, which may not address all the complexity of species abundances and distributions, as rarity is essentially continuous (Gaston 1994). Also, this may further be due to studies often being conducted with a single, or a few, pairs of rare and widespread species. While this approach is useful to inform conservation of the study species themselves, multi-species experiments are needed to provide answers at a general level (van Kleunen et al. 2014).

This thesis aims at contributing to our understanding of differences between rare and widespread species, and to provide scientific knowledge informing conservation in the context of global changes. With more than a third of its vascular plants being threatened with extinction (Bornand et al. 2016) and a high diversity of habitats (Landolt et al. 2010, Delarze et al. 2015), Switzerland offers a great opportunity to address these questions with native species in experimental and natural conditions. To do so, we performed multi-species experiments with a large number of species from 18 plant families, differing in their rarity and in their conservation status in Switzerland, also involving very rare and critically endangered species. Further, our study species represented a great diversity of life forms, and covered a wide altitudinal gradient, thus a wide range of climates and habitat types, going for instance from low altitude marshlands to high alpine environments.

In Chapter 2, we tested whether plant species differing in their rarity, i.e. continuously distributed along a gradient of range sizes, also differ in their seed mass and germination characteristics. We weighed the seeds of 39 rare and 32 widespread species of Switzerland, germinated them and recorded seedling emergence to calculate germination percentages, time of germination and half-germination time. Additionally, seeds of the rare species received a stratification-by-cold treatment to test whether more or less rare and endangered species differ in seed dormancy and whether rarer and more endangered species benefit more from stratifying. As seed mass and germination characteristics are linked to plant fitness and establishment ability, they are relevant for understanding differences between rare and widespread species. Moreover, their study represents knowledge directly valuable for ex-situ and in-situ conservation.

In Chapter 3, we investigated the relationship between rarity and evolutionary potential of populations of 31 species differing in range size, IUCN category of threat (Moser et al. 2002) and priority for conservation in Switzerland (FOEN 2011). For each species we obtained seeds of known maternal origin and grew the plants in a common garden. We measured quantitative traits related to reproduction, resource use, size and performance, and calculated narrow-sense heritability for each species and trait. Narrow-sense heritability, i.e. the proportion of phenotypic variation that is due to additive genetic variance, represents the evolutionary potential of populations. Rare species often occur in small or isolated populations and are therefore expected to have lower genetic diversity. We thus hypothesized that the rarest and most endangered species have lower heritabilities than less rare and less endangered species, which may have important consequences for the future of populations and their conservation in the light of global changes.

In Chapter 4, we studied whether rarer species are able to tolerate climate change equally well than more widespread species. To simulate climate change, we transplanted 35 plant species, continuously distributed along a gradient of range sizes, to five botanical gardens in Switzerland differing in altitude and climatic conditions. For each species we calculated how different the climate, i.e. mean temperature and mean amount of precipitation, was between their natural habitats and the novel climate of the respective botanical garden. We investigated the relationship between rarity, climatic difference, and plant performance and survival. Rarer species are expected to have narrower niche breadth, i.e. to tolerate a narrower range of environmental conditions, than more widespread species.

Therefore rarer species might have reduced survival and performance than more widespread ones when the climatic conditions are different from their natural habitat, which would indicate a higher susceptibility and vulnerability to climate change for rare and endangered plant species.

In Chapter 5, we investigated the feasibility of introductions of rare and endangered plant species, and the effect of increased genetic diversity on their success. In the framework of the MSc thesis of Deborah Schäfer, we performed introductions of eight species threatened with extinction in Switzerland, in natural sites which were selected in collaboration with field botanists and conservation practitioners. Increased genetic diversity is expected to have a positive effect on the fitness of populations. To test whether increased genetic diversity has a positive effect on the success of the introductions, we established plots of higher genetic diversity and plots of lower genetic diversity in each introduced population. We monitored the introduced populations for two years to assess whether establishing viable new populations of endangered plant species was possible, and we studied the effect of genetic diversity on the success of the introductions.

Finally in Chapter 6, I present a summary of the most important findings of this thesis and draw general conclusions concerning differences among very rare to widespread species, highlighting threats to rare and endangered plant species in the context of global changes and information relevant for their successful conservation.

Chapter 2

Experimental germination of 71 rare and widespread species of Switzerland suggests poorer germination of rarer species

Hugo Vincent, Anne Kempel and Markus Fischer

Abstract

Little is known about potential differences in seed and germination characteristics between very rare and widespread plant species. However, seed and germination characteristics matter largely for fitness, population growth and ultimately species abundance and distribution. Moreover, scientific knowledge on the germination of rare and endangered species is essential for plant conservation, in particular for ex-situ living collections and for introductions of endangered plant species to the wild. In this study, we weighed and germinated seeds of 39 rare and 32 widespread species of Switzerland, continuously distributed along a gradient of range sizes. Additionally, seeds of the rare species received a stratification by cold treatment to test whether more or less rare species differ in seed dormancy and whether rarer species benefit more from stratifying. We monitored seedling emergence for two months, until no further seedlings emerged, and tested whether seed mass, germination percentage, time of germination and half-germination time are related to species range size. Rarer species did not have lower seed mass, time of germination and half-germination time, but they had a significantly lower germination percentage than more widespread ones (+0.05% of germinated seeds per unit of range size). While germination was enhanced by stratifying for all rare species, this was not related to their range size or their IUCN category of threat. We conclude that germination is reduced for rarer species, and that experimental germination is nevertheless possible even for very rare species, which indicates that successful ex-situ living collections and potential introductions to the wild are possible for these species.

Introduction

Investigating and understanding differences between rare and widespread species is a challenge of great interest for ecologists and evolutionary biologists, and it provides essential knowledge for the conservation of rare and endangered species in a changing world (Kunin & Gaston 1997, Gaston 2000). Many studies aimed to identify general patterns of differences between rare and widespread plant species that might drive plant rarity. It has for instance been shown that widespread species are generally larger (Murray et al. 2002, Lavergne et al. 2003, Cornwell & Ackerly 2010, Dawson et al. 2012), produce more and bigger flowers (Lavergne et al. 2004), are more competitive and grow faster (Murray et al. 2002), respond more positively to increased nutrient availability (Dawson et al. 2012) and have a broader climatic niche (Vincent et al., Chapter 4) than related rarer species.

One important question is whether rare species and widespread species differ in seed mass. Species with large seeds generally produce larger seedlings, giving them a competitive advantage (Leishman et al. 2000, Moles & Westoby 2004, Baraloto et al. 2005, Metz et al. 2010), and may allocate more resources to stress tolerance (Moles & Westoby 2004, Fenner & Thompson 2005, Muller-Landau 2010). Species with large seeds are also suggested to suffer less from conspecific negative density dependence during early recruitment (Lebrija-Trejos et al. 2016) than species with smaller seeds. On the other hand, small-seeded species are expected to produce more seeds, disperse further, persist longer in seed banks (Guo et al. 2000) and may have advantages in defense against seed predators (Fricke & Wright 2016). Differences in seed mass could therefore drive plant rarity, where widespread species might either benefit from larger seeds (conveying them a competitive advantage) or from smaller seeds (conveying them a dispersal advantage). Murray et al. (2002) reported results of 17 studies which mostly found no difference in seed size between rare and widespread species, and there was no consistent pattern between studies, possibly because these studies did not involve the rarest species of their study areas. Studying variation in seed size in a single experiment involving multiple species, from very rare to very widespread ones, might reveal whether there is a relationship between seed size and rarity.

Another important, but hardly investigated, question is whether seeds of rare species have lower germination percentages and germinate slower than seeds of more widespread species. As these traits are directly linked to the fitness of individuals and populations, they might well differ between rare and widespread plant species. Profuse germination might have positive effects on population growth, as more seedlings increase the probability of maintaining or even increasing population size, and colonizing new sites. A large germination niche, i.e. possible germination under a wide range of environmental conditions, might allow species to occupy many niches and to become more widespread than species with special germination requirements (Baker 1965). In turn, poor germination might not only be a driver of plant rarity, but also a consequence of having restricted ranges with small and isolated populations, where negative effects on plant fitness due to low genetic diversity are more likely to occur than in large populations (Ellstrand & Elam 1993, Gaston 2003, Leimu et al. 2006). Fast germination is suggested to be particularly beneficial in competitive environments (Ross & Harper 1972, Weiner 1985, Dyer et al. 2000, Smith et al. 2000, Donohue et al. 2010) and to allow plants to accumulate more resources due to a longer growing period which might positively affect fitness (Verdú & Traverset 2005). Both germination percentages and time of germination might therefore be inversely related to the rarity of species. The presence or absence of seed dormancy might also differ between rare and widespread plant species. Seed dormancy aims to reduce the risk of germination at a time where the conditions for germination and establishment are not met (Fenner & Thompson 2005, Donohue et al. 2010, Hoyle et al. 2015). Another strategy to overcome such unfavorable germination conditions is efficient dispersal in space. Seed dormancy and dispersal ability have therefore been suggested to trade off with each other (Lokesha et al. 1992, Rees 1996). If rare species are dispersal limited (Hanski et al. 1993, Gaston 2003), they might show stronger seed dormancy than widespread species. However, only few studies addressed differences in germination characteristics in the context of rarity, and found no relationship with rarity, e.g. Brown et al. (2003) comparing five Australian *Acacia* tree species and Burmeier & Jensen (2008) on the temperate species *Apium repens*. To our knowledge, the relationship between germination characteristics and rarity has never been tested in a single experimental study involving multiple temperate species.

The study of seed and germination characteristics is essential for the conservation of rare and endangered plant species. Preserving at least 75% of the threatened plant species in ex-situ collections (i.e. outside of their natural environment, for instance in seed or living collections) is one of the goals of the Global Strategy for Plant Conservation (SCBD, 2010), and the use of botanical gardens in the conservation of endangered plant species is widely recognized (Donaldson 2009). To prevent ex-situ populations from suffering from the genetic consequences of growing in small and fragmented populations in botanical gardens (Ensslin et al. 2011), large and genetically diverse living collections are needed. Ex-situ material is also used in introductions of endangered plant species to natural habitats (Guerrant & Kaye 2007). Godefroid et al. (2011) suggest that the success of such introductions can be improved with an increased knowledge of the species' biology, by using larger numbers of propagules and they recommend using seedlings rather than seeds due to potentially low recruitment. The artificial use of stratification methods, mimicking natural germination triggers to break seed dormancy, might increase the germination success of rare and endangered plant species (Donohue et al. 2010, Hoyle et al. 2015). Thus, understanding whether and how rare and widespread species differ in their seed and germination characteristics is crucial to inform managers of living ex-situ collections and to increase the success of introductions to the wild.

We compared seed and germination characteristics of 71 species of differing rarity in Switzerland, also involving very rare and endangered species of high conservation priority in Switzerland (Moser et al. 2002, Bornand et al. 2016). More widespread species might benefit from larger or smaller seed masses, and higher germination success than rarer species, which might explain why they are widespread. Rarer species might be expected to germinate less well than more widespread species, and show more pronounced seed dormancy. We also tested whether stratification of seeds improves germination success of rare and endangered plant species, and whether this depends on their range size and their IUCN category of threat. By studying many rare and widespread species in a single experiment we aimed to address the links between germination traits and plant rarity at a general scale, with a view to inform the conservation of rare and endangered species.

Material & Methods

Study species

We acquired seeds of 71 plant species from 17 plant families (see Table S1). Thirty-nine of those species are of priority for conservation in Switzerland (Moser et al. 2002, List of Priority Species at the National Level FOEN 2011) and we classified them as ‘rare’ *a priori*. Thirty-two of them are rather widespread and are not of priority for conservation in Switzerland, and we classified them as ‘widespread’. Seeds of the rare plant species were collected in the wild in 2011 (seeds of 10-20 mother plants of one population, hereafter called seed families), and seeds of the widespread species were obtained from commercial seed suppliers (Rieger-Hofmann GmbH, Germany and UFA Samen, Switzerland) or collected in the wild (seeds of *Sedum alpestre*). The rare and widespread species are congeneric or confamiliar pairs from similar habitats, and were selected with the help of botanists (Info Flora, hosted by the Botanical Gardens of Bern and Geneva, Switzerland) to represent a wide range of rarities and species habitat characteristics.

Rarity was assessed according to two categorical variables: rarity status, i.e. rare or widespread species, and IUCN category (Near Threatened (NT), Vulnerable (VU), Endangered (EN), and Critically Endangered (CR); Moser et al. 2002, FOEN 2011, Table S1). We also defined rarity as a continuous variable using the range size of the species in Switzerland. Range size was defined as the number of 10 x 10 km grid cells occupied by a given species in Switzerland (data provided by Info Flora, Bern and Geneva, Switzerland; also see Bornand 2014). We used range size in Switzerland because a continuous measure of European range sizes for our species is not yet available. However, for a subset of 48 species for which European range size is available, Swiss and European range sizes were positively correlated ($r = 0.487$, $p < 0.001$, see Text S1).

Experimental design

We assessed seed masses by weighing 25 seeds per species (and per seed family for the rare species) to the nearest 0.001g. If fewer than 25 seeds were available we weighed as many seeds as possible. We calculated the mean seed mass per species (referred to in the following as 'seed mass'). At the end of February 2012, we sowed two portions of 25 seeds for each seed family of the 39 rare species in two 0.8-L pots filled with standard potting soil (Ricoter, Aarberg, Switzerland). We watered the pots, covered them with plastic foil and placed them at 4°C for two months to receive a stratification treatment. At the beginning of April 2012 we sowed another two portions of 25 seeds for each seed family of the rare species in two pots which were not cold-stratified. For some species for which we could not obtain four times 25 seeds per seed family, we divided the number of available seeds in four equal portions. The stratification treatment allowed us to test whether species differ in their seed dormancies and whether this is related to the rarity of the species.

Additionally, for each of the 32 widespread species we sowed six portions of 25 seeds in six 0.8 L pots. We transported all pots to a greenhouse (Muri, close to Bern, Switzerland) and placed them randomly in six blocks (2 columns, 3 rows) spaced by approximately 40 centimeters. To account for spatial heterogeneity in our greenhouse, we recorded the position of the pots (defined by the number of the row and of the column). We watered the plants when needed.

We counted the number of emerged seedlings (with visible cotyledons) three times a week for a period of 57 days, until all seeds were germinated or new seedlings no longer emerged (Fig. S1). For each pot we then calculated the number of days until the first seedling emerged (time of germination), the day at which at least half of the seeds had germinated (half-germination time) and the total proportion of germinated seeds (germination percentage).

Statistical analysis

We tested whether there was a phylogenetic signal in seed mass, germination percentage, and time of germination for our 71 species using a dated phylogeny from the Daphne tree database (Durka & Michalski 2012). We used the function *phylosignal* from the package *picante* (version 1.6-2, see Blomberg et al. 2003). We found a low phylogenetic signal for seed mass ($K = 0.3856$, $p < 0.001$), germination percentage ($K = 0.1505$, $p = 0.008$) and time of germination ($K = 0.1501$, $p = 0.015$). Therefore we accounted for the relatedness between our species in our analysis. We did this by fitting species identity nested into plant family as random factor, because this allowed us to include further random terms in our statistical model, which would not have been possible with phylogenetically corrected models.

Habitat characteristics such as water availability, temperature and light availability have been shown to strongly affect seed and germination traits (Grime et al. 1981, Pons 2000, Fenner & Thompson 2005, Pakeman et al. 2008, Baskin & Baskin 2014). Because our species come from different habitats which could have potentially selected for different traits and strategies, we considered abiotic habitat characteristics to account for variation in seed and germination traits not related to species rarity. For each species, we obtained three environmental indicator values (water, temperature and light availability) from the Flora Indicativa (in the following referred to as ‘Landolt values’, see Landolt et al. 2010). Landolt values describe the optima of occurrence along these environmental gradients on a five-point scale, with five indicating the wettest, warmest and brightest conditions, respectively. We included moisture, temperature and light Landolt values as covariates in our statistical models.

To test whether rare and widespread plant species differ in their seed masses (log-transformed to approximate a normal distribution), germination percentages, times of germination, and half germination times, we first compared them with linear mixed models (*lmer*, lme4 package, version 1.1-6, Bates & Maechler 2014), including Landolt values as covariates, rarity status (rare or widespread) as explanatory variable, and species identity nested into plant family and the position of the pots in the greenhouse as the random terms.

In a second analysis we tested whether plant species differ in their seed masses, germination percentages, times of germination and half-germination times, along a gradient of range sizes, i.e. with range size in Switzerland as a continuous measure of rarity. For each of the four response variables, we analyzed the data with *lmer* models including Landolt values as covariates, range size as explanatory variable, and species identity nested into plant family and the position of the pots in the greenhouse as random terms.

The status ´rare or widespread´ of our plant species is confounded with the origin of the seeds (seeds of all rare species were collected in the wild and seeds of most of the widespread species were obtained from seed suppliers). Seeds obtained from commercial suppliers might show a higher germination percentage and faster germination (Chrobock et al. 2011) than seeds collected in wild populations, and long-term cultivation of species in gardens can affect germination characteristics (Ensslin et al. 2011). Therefore, in a third analysis we included the origin of the seeds (collected in natural populations or commercial seeds) as covariate in the *lmer* models to correct for a potential bias.

We also tested whether the stratification treatment in the subset of the rare species affected germination percentage, time of germination and half-germination time, and whether the effect of stratification was related to the rarity of the species. To describe the effect of stratification we calculated the log-response ratio (LRR) of the stratification treatment as $\mathrm{LRR} = \ln\left(\frac{\overline{x_1}}{\overline{x_2}}\right)$, where $\overline{x_1}$ is the mean germination percentage, time of germination or half-germination time, respectively, for the species that had been stratified, and $\overline{x_2}$ is the mean response value for the control pots (i.e. rare species without stratification treatment). A LRR > 0 indicates that the seeds of a species had a higher germination percentage with the stratification treatment; LRR < 0 indicates that the seeds of a species had a higher germination percentage without stratification; LRR = 0 indicates that there was no difference between stratification treatment and control. We first compared mean germination percentage, time of germination and half-germination time between the pots that had received a germination treatment and control. To test whether the LRR, i.e. the strength of the stratification effect, was related to the rarity of the species, we used *lmer* models including the LRR of the stratification treatment as response variable, range size as explanatory variable, Landolt values as covariates, and plant family as random term. To test whether the LRR of the stratification treatment differed between IUCN categories (NT: n = 10 species; VU: n = 18; EN: n = 5; CR: n= 6), we compared the mean LRR values per IUCN category using post-hoc *TukeyHSD* tests in *aov* models, including the LRR of the stratification treatment as response variable and IUCN category as explanatory variable.

For all statistical analyses, we used R (version 3.3.2, R Core Team, 2016). We simplified the full models by removing non-significant terms and we determined significances using likelihood-ratio tests comparing models with and without the factor of interest.

Results

Rare species germinated less abundantly than widespread species, and tended to have smaller seeds and to germinate more slowly than widespread species. Half-time of germination did not differ between rare and widespread species (Table 1a, Fig. 1).

When we considered rarity as a continuous variable, a significantly positive relationship between range size and germination percentage also indicated that more widespread species germinate better than rarer species (Table 1b, Fig. 2b). Seed mass, time of germination and half-germination time were not related to range size (Table 1b, Figure 2a, c, d).

Species whose seeds were collected in natural populations (i.e. rare species) had a lower seed mass than species whose seeds were obtained from commercial suppliers (Table 1c). When we accounted for the different origin of the seeds (collected in the wild or commercial seeds), the positive relationship between range size and germination percentage remained significant, suggesting that the relationship was not simply due to a confounding effect between plant rarity and seed origin. Time of germination and half-germination time were not related to range size (Table 1c).

Species originating from habitats with high light availability had higher germinated percentages, faster times of germination and half-germination times than species originating from habitats with low light

Table 1. Results summary of tests of the relationship between rarity and seed mass, germination percentage and time of germination. The linear mixed models in a) included rarity status (rare or widespread) as explanatory variable and Landolt values as covariates; in b) included range size as explanatory variable and Landolt values as covariates; in c) included range size as explanatory variable, Landolt values and origin of the seeds (collected in the wild or commercial seeds) as covariates. All models also included three random terms, species identity nested into plant family to account for the taxonomic relatedness of our species, and the position of the pots in the greenhouse. We removed all non-significant terms and give estimates only for the significant and marginally significant terms. Variances of the random terms are given for each model. Estimates related to seed mass are given x10^{-2}.

		Seed mass			Germination percentage			Time of germination			Half-germination time		
		estimate	p-value	Chi^2	estimate	p-value	Chi^2	estimate	p-value	Chi^2	estimate	p-value	Chi^2
a)	Moisture	-	0.676	0.17	-	0.561	0.34	-	0.349	0.87	-	0.639	0.22
	Temperature	-	0.479	0.49	-	0.276	1.19	-	0.166	1.19	-3.18	0.018^{*}	5.58
	Light	-	0.497	0.46	12.9	0.009^{**}	6.74	-7.16	$<0.001^{***}$	14.0	-10.7	$<0.001^{***}$	14.8
	Rarity status	-0.09	$0.052^{\cdot}$	3.77	-14.9	0.011^{*}	6.41	3.93	$0.062^{\cdot}$	3.46	-	0.319	0.99
	Family/Species		<0.001			480.8			49.83			65.13	
	Family		<0.001			309.8			38.24			-	
	Position of the pot		<0.001			3.643			0.481			-	
b)	Moisture	-	0.663	0.19	-	0.587	0.29	-	0.368	0.81	-	0.650	0.21
	Temperature	-	0.451	0.57	-	0.291	1.11	-	0.193	1.69	-3.18	0.018^{*}	5.55
	Light	-	0.331	0.94	12.7	0.011^{*}	6.42	-6.19	$<0.001^{***}$	12.59	-10.5	$<0.001^{***}$	14.3
	Range size	-	0.407	0.69	0.05	0.016^{*}	5.75	-	0.189	1.72	-	0.463	0.54
	Family/Species		<0.001			489.8			52.76			62.47	
	Family		<0.001			297.7			36.61			-	
	Position of the pot		<0.001			3.660			0.549			-	
c)	Moisture	-	0.698	0.15	-	0.573	0.38	-	0.327	0.96	-	0.632	0.22
	Temperature	-	0.433	0.61	-	0.283	1.15	-	0.146	2.11	-3.22	0.018^{*}	5.60
	Light	-	0.399	0.71	12.7	0.008^{**}	6.93	-6.18	$<0.001^{***}$	14.1	-10.0	$<0.001^{***}$	14.7
	Origin of the seeds	-0.09	0.020^{*}	5.40	-	0.33	0.95	-	0.161	1.96	-	0.486	0.48
	Range size	-	0.128	2.31	0.05	0.016^{*}	5.75	-	0.189	1.72	-	0.463	0.54
	Family/Species		<0.001			489.8			52.75			64.08	
	Family		<0.001			297.7			36.61			-	
	Position of the pot		<0.001			3.66			0.549			-	

Figure 1. Differences between rare and widespread species in their a) mean seed mass, b) mean germination percentage, c) mean time of germination and d) half-germination time, with standard errors. Rare species had lower percentages of seeds germinating than widespread species, tended to have smaller seeds and to germinate slower than widespread species. Half-germination time did not vary between rare and widespread species (see Table 1a for summaries of the related statistical models).

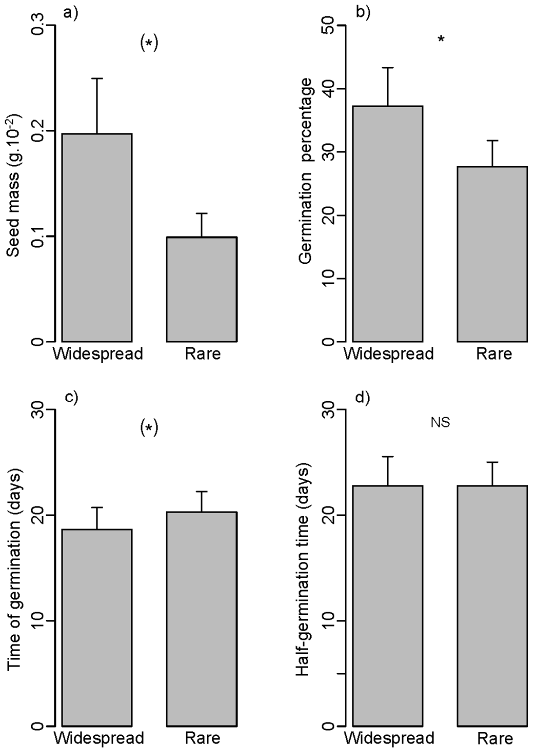
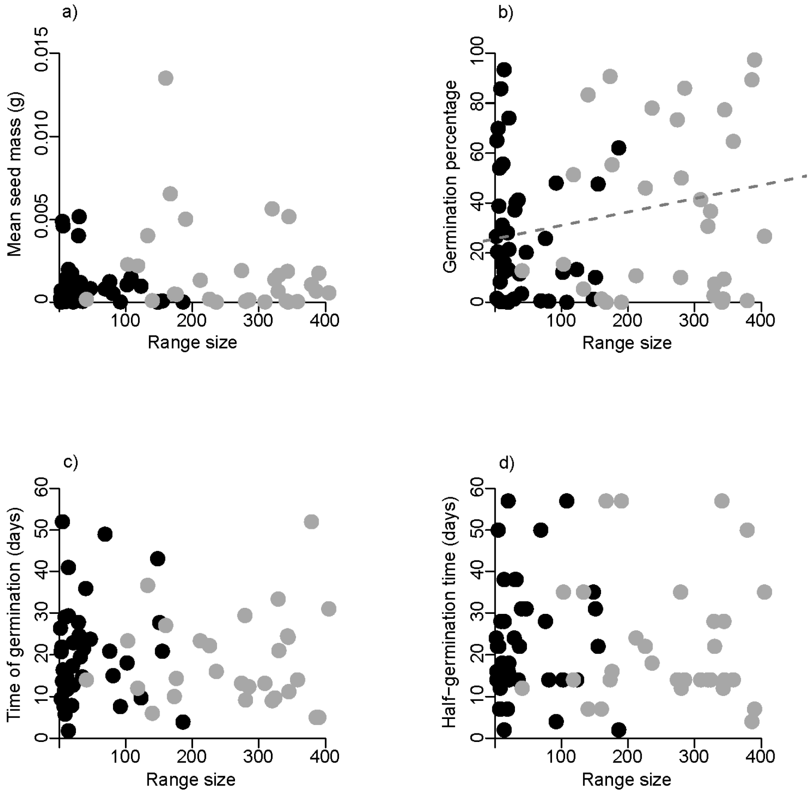


Figure 2. Relationship of species range size with a) seed mass, b) germination percentage, c) time of germination and d) half-time of germination. Each point represents the mean value for an individual species. Species whose seeds were collected in the wild are indicated with black dots, species whose seeds where purchased from commercial supplied are indicated with grey dots. The dashed line in b) indicates the positive relationship between range size and germination percentage (Table 2b).



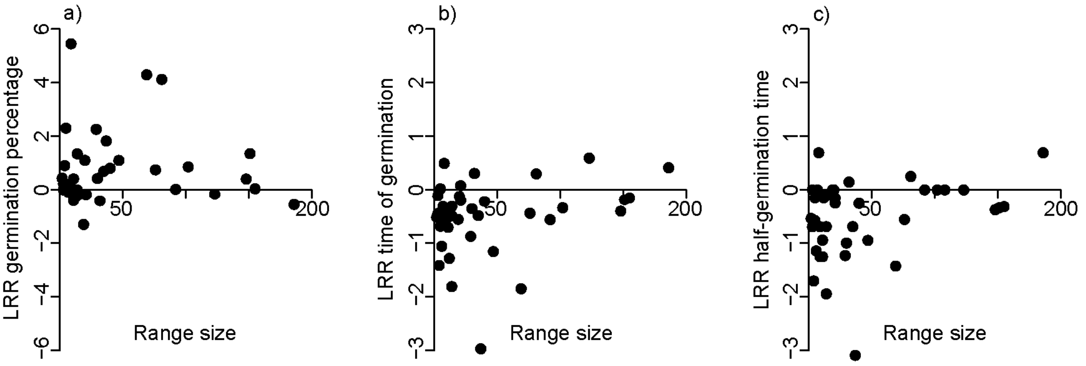
availability. Half-germination time was significantly shorter for species originating from habitats with warmer temperatures. Water availability in the species natural habitat did not affect any of the response variables (Table 1).

Seeds of the rare species that had received the stratification treatment had a higher germination percentage (t = 1.9, df = 78, p = 0.028, Fig. 3a) and germinated faster (time of germination: t = 3.0, df = 67, p = 0.002, Fig. 3b; half-germination time: t = 2.4, df = 78, p = 0.009, Fig. 3c) than control seeds, indicating a positive effect of stratification on the germination of rare species (Fig. S2). The effect of stratification on germination percentage and time of germination was not related to the range size of the rare species (Table 2, Fig. 3a, b). The effect of stratification on half-time of germination was marginally significantly affected by range size, i.e. stratification tended to shorten half-germination time more for the rarer species (Table 2, Fig. 3c). Moisture, temperature and light availability in the original habitat of the rare species were not related to the response (LRR) of any of the germination variables to the stratification treatments.

Table 2. Results summary of tests of the relationship between the effect of the stratification treatment on germination percentage, time of germination and half-germination time, respectively, and range size. Landolt values were added to the models as covariates, and plant family was added as a random term to account for the taxonomic relatedness of our species. We removed all non-significant terms and we give estimates only for the marginally significant term. Variance of the random term is given for each model.

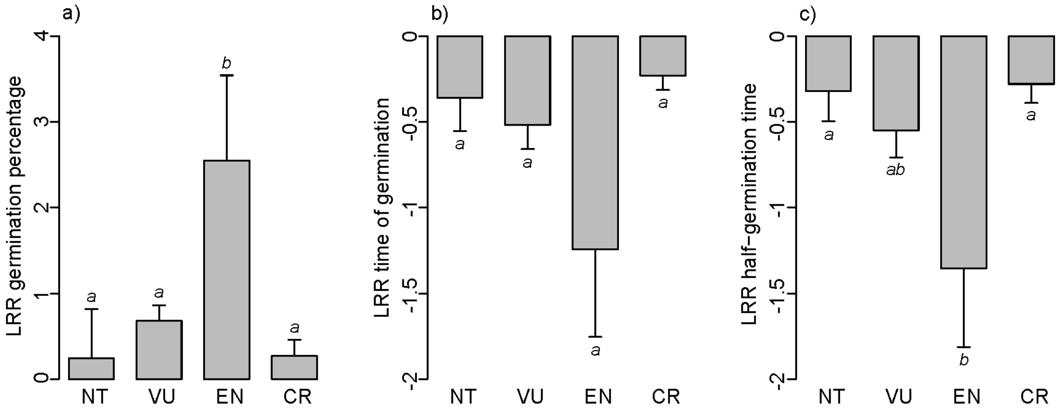
	LRR Germination percentage			LRR Time of germination			LRR Half-germination time		
	estimate	p-value	Chi2	estimate	p-value	Chi2	estimate	p-value	Chi2
Moisture	-	0.769	0.08	-	0.331	0.94	-	0.410	0.68
Temperature	-	0.591	0.29	-	0.694	0.15	-	0.173	1.86
Light	-	0.769	0.09	-	0.674	0.18	-	0.451	0.57
Range size	-	0.998	0.00	-	0.139	2.19	0.004	0.062·	3.48
Family		0.396			0.350			0.028	

Figure 3. Relationship of species range size with the effect of a cold-stratification treatment (Log Response Ratio, LRR) on a) germination percentage, b) time of germination and c) half-germination time. Each dot represents one of 39 rare species. Overall, stratification significantly increased germination percentage and decreased time of germination and half-germination time independently of range size (Table 2).



Species of the IUCN categories NT, VU and CR did not differ from each other in their response to stratification for any of the responses studied (Fig. 4). Species of the category EN showed a significantly higher germination percentage in response to stratification than species from all other categories, however (Fig. 4a) Further, species of the category EN tended to have shorter germination times and half-germination times in response to the stratification treatment than species from all other categories (Fig. 4b, c; see Table S2 for the statistics of the *TukeyHSD* tests). Overall, these results indicate that more rare or more threatened species do not benefit systematically more from stratification by cold treatment than less rare or less threatened species. However, across all rare species stratification significantly improved germination success.

Figure 4. Relationship between the IUCN status of a species and the mean effect of a cold-stratification treatment (Log Response Ratio, LRR) on a) germination percentage, b) time of germination and c) half-germination time. NT: Near Threatened, n = 10 species; VU: Vulnerable, n = 18; EN: Endangered, n = 5; CR: Critically Endangered, n= 6; different letters represent significant differences between the categories according to *TukeyHSD* tests (Table S2).



Discussion

Rare species germinate less abundantly than widespread species

Comparing seed mass and germination characteristics of 71 plant species (39 rare species and 32 widespread species in Switzerland) we found that rare species have a lower germination percentage than widespread species (Table 1a, Fig. 1b). Also, when we expressed rarity as a continuous variable, we found that species with larger range sizes germinated better than species with restricted ranges, and this relationship remained significant when we accounted for the possibly confounding origin of the seeds (Table 1b, c, Fig. 2b). Although many authors have suggested trait differences between widespread and rare species (e.g. Kunin & Gaston 1997, Murray et al. 2002), only few studies compared germination characteristics of related widespread and rare species. Of those, most found no difference in germination between rare and widespread species (Witkowski & Lamont 1997, Anderson 1980, Fiedler 1987, reviewed in Murray et al. 2002). In a data base survey, Thompson & Ceriani (2003) found no relationship between range size and the germination niche width of 175 plant species (defined as the temporal span in which these species can germinate) of the United Kingdom flora. Unfortunately, these authors did not provide the minimal and maximal range size spanned by the species in their study, leaving it open whether they also involved very rare and very widespread species. On the other hand, in an experimental study Brown et al. (2003) found lower germination for two rare species than for three related widespread species of the genus *Acacia*. The lower germination percentage of rare species in our study might reflect that rarer species often have a smaller germination niche breadth (Brown 1984, Slatyer et al. 2013, but see Thompson & Ceriani 2003) than more widespread species, i.e. special germination requirements, which might not have been met in our experiment. More widespread species, on the contrary, might be able to fulfill germination requirements in many environments. Accordingly, Brändle et al. (2003) found a positive correlation between range size and germination niche breadth of 31 weedy plant species. Another possible explanation for the overall lower germination of rare species in our study might be that rare species are more likely to carry a larger deleterious genetic load which could negatively affect their germination, as they are expected to occur in smaller and more isolated populations than more widespread species do (Charlesworth & Charlesworth 1987, Ellstrand & Elam 1993, Leimu et al. 2006).

Overall, our study, based on the large number of 71 species covering 17 plant families, suggests lower germination percentages of rare species than of widespread species as a general pattern. While we cannot disentangle whether the lower germination in rarer species reflects a cause or a consequence of plant rarity, this finding has important implications. A lower germination percentage of rarer species implies that particular attention should be paid to the germination conditions of rare plants in ex-situ cultivations, and therefore stresses the importance of studying the biology of rare plant species before developing conservation measures. Also, to increase the success of introductions, more seeds should be used for rarer species than for more widespread ones to compensate for their lower germination percentage.

No difference in seed mass and time of germination along a gradient of range sizes

In our study rare species tended to have lower seed masses than widespread species, but this difference was only marginally significant (Table 1a, Fig. 1a). Large seeds generally represent an advantage in terms of competitive ability and stress tolerance (Leishman et al. 2000, Moles & Westoby 2004, Baraloto et al. 2005, Metz et al. 2010, Muller-Landau 2010, Lebrija-Trejos 2016). The origin of the seeds (collected in natural populations or purchased from a commercial supplier) was confounded with the rarity status of our species (rare or widespread), which may even have induced the marginally significant difference in seed mass, because of potential artificial selection exerted by the commercial supplier (Chrobock et al. 2011). However, recent studies using seeds from the same commercial supplier, which produces seeds for ecological restoration, found a substantial amount of genetic differentiation and regional adaptation among different seed origins (Bucharova et al. 2016, Durka et al. 2016), suggesting that seeds provided by this company are unlikely to differ from seeds directly collected in natural populations. Nevertheless, as we cannot fully disentangle the confounded effects of seed origin and rarity status, we suggest that further studies should use seeds of the same origin. When we expressed rarity as range size, seed mass was not related to plant rarity (Table 1b, c, Fig. 2a). Murray et al. (2002) reported results of 17 studies which mostly found no difference in seed size between rare and widespread species, concordant with our findings.

Fast germination can increase plant performance both at the seedling and reproductive stages (Fenner & Thompson 2005, Verdú & Traverset 2005, Donohue et al. 2010, Baskin & Baskin 2014). In our study, seeds of rare species tended to germinate more slowly than the ones of widespread species (Table 1a, Fig. 1c), which might suggest that they are at disadvantage under strong interspecific competition or habitat heterogeneity (Dyer et al. 2000, Turkington et al. 2005). However, when rarity was expressed in terms of range size, time of germination did not differ between species of different range sizes (Table 1b, c, Fig. 2c).

Thus our results of widespread species tending to have larger seed masses and faster germination than rare species were rather inconclusive and even disappeared when rarity was express as range size. Hence we suggest that variation in seed mass and time of germination are not of high relevance for explaining rarity, and that these traits are not the most important to consider for conservation of rare and endangered plant species.

Stratification increases germination success of the rare species

Importantly, over all rare species we found strong and significant effects of the stratification treatment increasing germination percentages, reducing times of germination and half-germination times (Fig. 3). This suggests that stratification by a simple cold treatment could be used to greatly increase the germination success of rare and endangered plant species in the context of ex-situ cultivations or introductions, even if their specific dormancy-breaking requirements are not known.

As rare plants are suggested to be more limited by dispersal than more widespread plants (Hanski et al. 1993, Gaston 2003) and as good dispersal ability has been suggested to select against seed dormancy (Lokesha et al. 1992, Rees 1996) we would have expected rarer species to respond more strongly to dormancy-breaking stratification. Although we studied effects of stratification for a subset of as many as 39 rare species differing in their degree of rarity, we did not find any relationship between range size and the effect of stratification on germination percentage, time of germination and half-germination time (Table 2, Fig. 3), suggesting that very rare species do not benefit more from dormancy-breaking stratification than less rare species.

Species listed as EN benefited more from the stratification treatment than species listed as NT, VU and CR (Table S2, Fig. 4). This was likely driven by three EN species which showed particularly strong responses to stratification and might therefore not reflect an effect associated to this particular IUCN category. *Juncus castaneus* grows in subalpine habitats, with very variable humidity conditions, *Nigella arvensis* and *Polycnemum majus* grow in early succession habitats and as weeds in cultivated fields (Landolt et al. 2010, Lauber et al. 2012). High environmental variation, and the necessity of a precise timing of germination to avoid frost or competition with other species are indeed especially likely to select for seed dormancy (Fenner & Thompson 2005). Based on these considerations we do not interpret our results on enhanced stratification effects for EN species, and these three species in particular, as indication that more threatened species benefit more from stratification by cold treatment than less threatened species.

Conclusion

Our study, based on 71 species largely differing in rarity, shows that rare species have a lower germination percentage than more widespread species and we suggest that this is a general pattern. Differences in germination percentages could partly drive plant species rarity, as they are likely to directly affect the probability of establishing successful individuals and populations. However, a lower germination percentage could also be a consequence of rarity, reflecting reduced performance of rare species which are more likely to occur in small and isolated populations. To understand this relationship better, data on the size and the connectivity of the studied populations, and knowledge on the history of the species would be needed. The other germination characteristics studied here did not differ between species along a gradient of rarity. We suggest that the observed variation in these traits rather reflects adaptations to the species' habitats and their biotic and abiotic factors.

In terms of conservation our results indicate that particular attention should be paid to the germination of rare and endangered plant species, certainly by acquiring more information on their germination requirements before developing ex-situ collections and introduction strategies. We were still able to obtain a large number of seedlings even from the rarest species, and the absence of differences between rare and widespread species for the other seed and germination traits suggests that successful ex-situ cultivations and introductions from seedlings of rare plant species should very well be possible.

Acknowledgements

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Supplementary information

Text S1. To test whether species range sizes in Switzerland are correlated with the European range sizes of our study species, we used map-derived area estimates from the *Atlas Europeae* (Meusel *et al*. 1978) for all 48 of our species for which these maps were available. We assessed the number of pixels of a species for their European distribution and cross referenced these using islands, for which the exact surface values are known. Range size in Europe was significantly correlated with range size in Switzerland (r = 0.487, p < 0.001).

Meusel, H., Jäger, E.J., Rauschert, S. & Weinert, E. (1978). *Vergleichende Chorologie der zentraleuropäischen Flora*. Bd. 2, Text u. Karten. Gustav Fischer Verlag, Jena, Germany.

Table S1. List of the 39 rare and 32 widespread (indicated by *) species studied in this experiment, with their plant family, IUCN category (NT: nearly threatened, VU: vulnerable, EN: endangered, CR: critically endangered), range size in Switzerland, mean seed mass (in g.10^{-2}), mean germination percentage, mean time of germination (in days), half-germination time (in days), and three Landolt environmental indicator values (T: temperature, L: light, M: moisture). Mean germination percentage, mean time of germination and half-germination time are given for the control treatment without stratification.

Species	Family	IUCN	Range size	Seed mass	Germination percentage	Time of germination	Half-germination time	Landolt values		
								T	L	M
*Angelica sylvestris**	Apiaceae	-	343	0.186	1.3	24.5	12	3	3	4
*Astrantia major**	Apiaceae	-	190	0.503	0	NA	57	2.5	3	3.5
Eryngium alpinum	Apiaceae	VU	29	0.403	1.3	27.8	24	2	4	3
Oenanthe lachenalii	Apiaceae	CR	20	0.087	13.2	17.3	16	4.5	4	4.5
Oxytropis fetida	Apiaceae	VU	14	0.198	16	29.3	28	1	4	3
*Peucedanum cervaria**	Apiaceae	-	133	0.404	5.3	36.6	35	4	3	2
*Peucedanum palustre**	Apiaceae	-	103	0.229	15.3	23.3	35	4	3	4.5
*Pimpinella saxifraga**	Apiaceae	-	329	0.066	2.7	33.3	28	4	4	2
Seseli annuum	Apiaceae	VU	47	0.082	20.2	23.7	31	4.5	4	1
Trochiscanthes nodiflora	Apiaceae	VU	5	0.489	1	52	50	3.5	2	2
*Artemisia absinthium**	Asteraceae	-	140	0.01	83.3	6	7	4	4	2
Artemisia glacialis	Asteraceae	NT	9	0.012	85.7	5.8	7	1	5	1.5
*Artemisia umbelliformis**	Asteraceae	-	173	0.049	90.7	10	14	1	5	3
Artemisia vallesiaca	Asteraceae	NT	14	0.04	93.4	1.8	2	4.5	5	1
Bidens cernua	Asteraceae	EN	76	0.124	25.8	20.9	28	3.5	4	4.5
Bidens radiata	Asteraceae	CR	3	0.07	65	9.5	14	3.5	4	4.5
*Bidens tripartita**	Asteraceae	-	118	0.221	51.3	12	14	4	4	4
*Centaurea jacea**	Asteraceae	-	324	0.137	36.7	9.7	14	3	4	2.5
*Centaurea scabiosa**	Asteraceae	-	320	0.565	30.7	9	14	4	4	2
Centaurea valesiaca	Asteraceae	NT	19	0.174	28.1	7.9	7	4.5	4	1
*Inula conyzae**	Asteraceae	-	226	0.019	46	22.2	22	3.5	3	2.5
Inula spiraeifolia	Asteraceae	VU	4	0.021	20.4	21.8	22	4.5	3	1.5
Senecio halleri	Asteraceae	NT	12	NA	55.6	11.8	14	1	5	2
Campanula excisa	Campanulaceae	NT	21	0.002	21.4	22.9	18	1.5	4	2
*Campanula rotundifolia**	Campanulaceae	-	358	0.005	64.7	14	14	3.5	4	2

Table S1 (continued).

Species	Family	IUCN	Range size	Seed mass	Germination percentage	Time of germination	Half-germination time	Landolt values		
								T	L	M
*Chenopodium botrys**	Chenopodiaceae	-	41	0.018	12.7	14	12	5	4	1
Polycnemum majus	Chenopodiaceae	EN	69	0.081	0.6	49	50	4.5	4	1
Sedum alpestre	Crassulaceae	-	186	0.002	62.1	3.9	2	1.5	5	2
Sedum villosum	Crassulaceae	VU	92	0.002	48	7.6	4	2	4	4
Carex bicolor	Cyperaceae	NT	81	0.052	0.4	15	14	1.5	5	4
Carex bohemica	Cyperaceae	CR	2	0.024	26.6	26.4	24	4.5	4	4.5
*Carex flacca**	Cyperaceae	-	379	0.106	0.7	52	50	3	3	2.5
Carex maritima	Cyperaceae	VU	40	0.039	3.5	35.9	31	1.5	5	4
Cyperus flavescens	Cyperaceae	VU	155	0.008	47.6	20.8	22	4	4	4
Cyperus fuscus	Cyperaceae	VU	151	0.006	10	27.7	31	4	4	4.5
Ephedra helvetica	Ephedraceae	VU	6	0.463	38.8	16.4	22	4.5	5	1
*Astragalus glycyphyllos**	Fabaceae	-	279	NA	10	29.4	35	3.5	3	2
Astragalus leontinus	Fabaceae	NT	32	0.119	40.2	19.5	38	2.5	3	1
Oxytropis fetida	Fabaceae	NT	11	0.147	15.4	14.5	14	1	4	3
Trifolium fragiferum	Fabaceae	VU	123	0.097	13.1	9.7	14	4	4	3
*Trifolium pratense**	Fabaceae	-	390	0.175	97.3	5	7	3.5	4	3
*Trifolium repens**	Fabaceae	-	386	0.067	89.3	5	4	3	4	3
Trifolium saxatile	Fabaceae	VU	8	0.083	8.2	11.4	12	1.5	4	1
*Centaurium erythraea**	Gentianaceae	-	236	0.001	78	16	18	3.5	4	3.5
Centaurium pulchellum	Gentianaceae	VU	148	0.001	1.2	43	35	3.5	4	3
Gentiana schleicheri	Gentianaceae	NT	20	0.008	0	NA	57	1	5	3
*Gentiana verna**	Gentianaceae	-	341	0.009	0	NA	57	2.5	4	3
Juncus arcticus	Juncaceae	VU	35	0.006	41.2	14.7	14	1.5	5	4
*Juncus articulatus**	Juncaceae	-	344	0.002	9.3	24.2	28	3	4	4.5
*Juncus bufonius**	Juncaceae	-	309	0.002	41.3	13.2	14	3.5	4	4
Juncus castaneus	Juncaceae	EN	9	0.004	0.2	29	28	1.5	5	4
Juncus sphaerocarpus	Juncaceae	CR	3	0.001	1.6	20.7	16	4.5	4	4
*Salvia pratensis**	Lamiaceae	-	330	0.163	7.3	21	22	3.5	4	2
Stachys annua	Lamiaceae	VU	102	0.105	12	18.1	14	4	4	2.5
*Stachys recta**	Lamiaceae	-	212	0.133	10.7	23.3	24	3.5	4	1.5

Table S1 (continued).

Species	Family	IUCN	Range size	Seed mass	Germination percentage	Time of germination	Half-germination time	Landolt values		
								T	L	M
*Epilobium parviflorum**	Onagraceae	-	280	0.006	50	9.2	12	3.5	3	4
Ludwigia palustris	Onagraceae	CR	21	0.004	74	12.8	14	4.5	4	5
*Bromus erectus**	Poaceae	-	345	0.518	77.3	11.2	14	4	4	2
Cleistogenes serotina	Poaceae	VU	11	NA	31.1	15	18	4.5	4	1
*Rumex crispus**	Polygonaceae	-	274	0.191	73.3	13.2	14	3.5	4	3.5
Rumex hydrolapathum	Polygonaceae	EN	30	0.518	37.2	24.6	38	4.5	3	4.5
Rumex maritimus	Polygonaceae	CR	5	0.038	69.9	13.7	14	4	4	4
*Anemone narcissiflora**	Ranunculaceae	-	167	0.655	0	NA	57	2	4	3.5
Aquilegia alpina	Ranunculaceae	NT	108	0.148	0	NA	57	2	3	3
Nigella arvensis	Ranunculaceae	EN	37	0.065	11.4	21.5	22	4.5	4	2
*Ranunculus arvensis**	Ranunculaceae	-	160	1.35	1.3	27	7	4.5	4	2.5
*Potentilla erecta**	Rosaceae	-	405	0.057	26.7	31	35	3	3	3
*Potentilla grandiflora**	Rosaceae	-	176	0.048	55.3	14.3	16	1.5	4	2
Potentilla multifida	Rosaceae	VU	7	0.01	54	7.8	7	1	4	3
Linaria petraea	Scrophulariaceae	VU	14	0.027	12.2	40.9	38	2.5	5	2.5
*Linaria vulgaris**	Scrophulariaceae	-	285	0.015	86	12.3	14	3.5	4	2

Table S2. Relationship between the IUCN status of a species and the mean effect of a stratification treatment (Log Response Ratio, LRR) on germination percentage, time of germination and half-germination time. NT: Near Threatened, n = 10 species; VU: Vulnerable, n = 18; EN: Endangered, n = 5; CR: Critically Endangered, n= 6. Difference (diff), lower (lwr) and upper (upr) confidence intervals and the adjusted p-value are given for each pairwise comparison.

	LRR Germination percentage				LRR Time of germination				LRR Half-germination time			
	diff	lwr	upr	p-value	diff	lwr	upr	p-value	diff	lwr	upr	p-value
VU-NT	0.44	-0.97	1.85	0.835	-0.16	-0.9	0.58	0.936	-0.23	-0.92	0.47	0.813
EN-NT	2.31	0.42	4.2	0.012*	-0.88	-1.87	0.11	0.093$^{\cdot}$	-1.03	-2	-0.07	0.032*
CR-NT	0.03	-1.76	1.82	0.999	0.13	-0.81	1.07	0.982	0.04	-0.87	0.95	0.999
EN-VU	1.87	0.2	3.55	0.024*	-0.72	-1.6	0.15	0.135	-0.81	-1.7	0.09	0.089$^{\cdot}$
CR-VU	-0.41	-1.97	1.15	0.893	0.29	-0.53	1.11	0.776	0.27	-0.56	1.1	0.818
CR-EN	-2.28	-4.29	-0.27	0.021*	1.01	-0.04	2.07	0.062$^{\cdot}$	1.07	0.01	2.14	0.048*

Figure S1. Cumulative germination percentage for each of the 39 rare and 32 widespread (indicated by *) species in control pots without stratification.

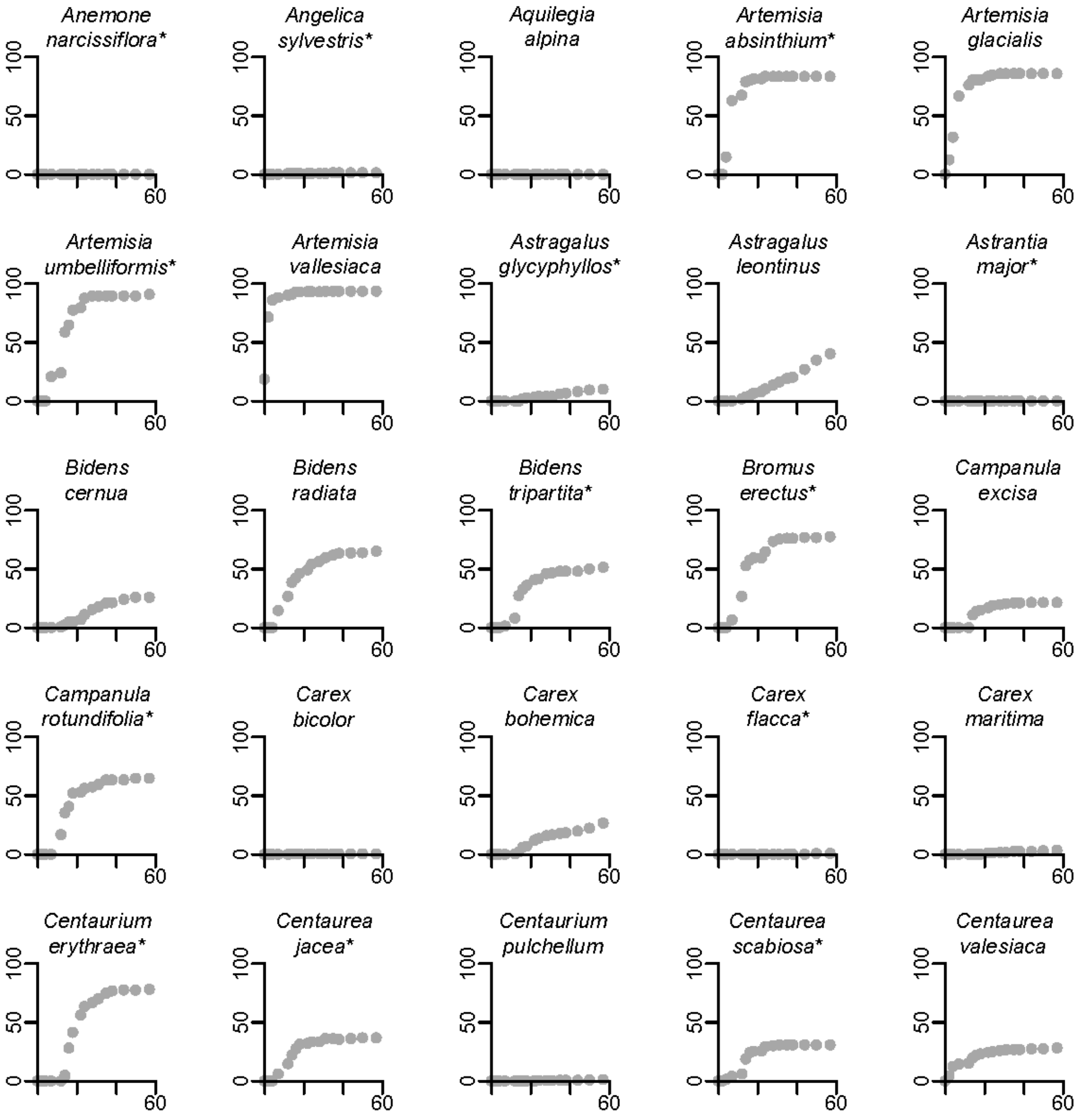


Figure S1 (continued).

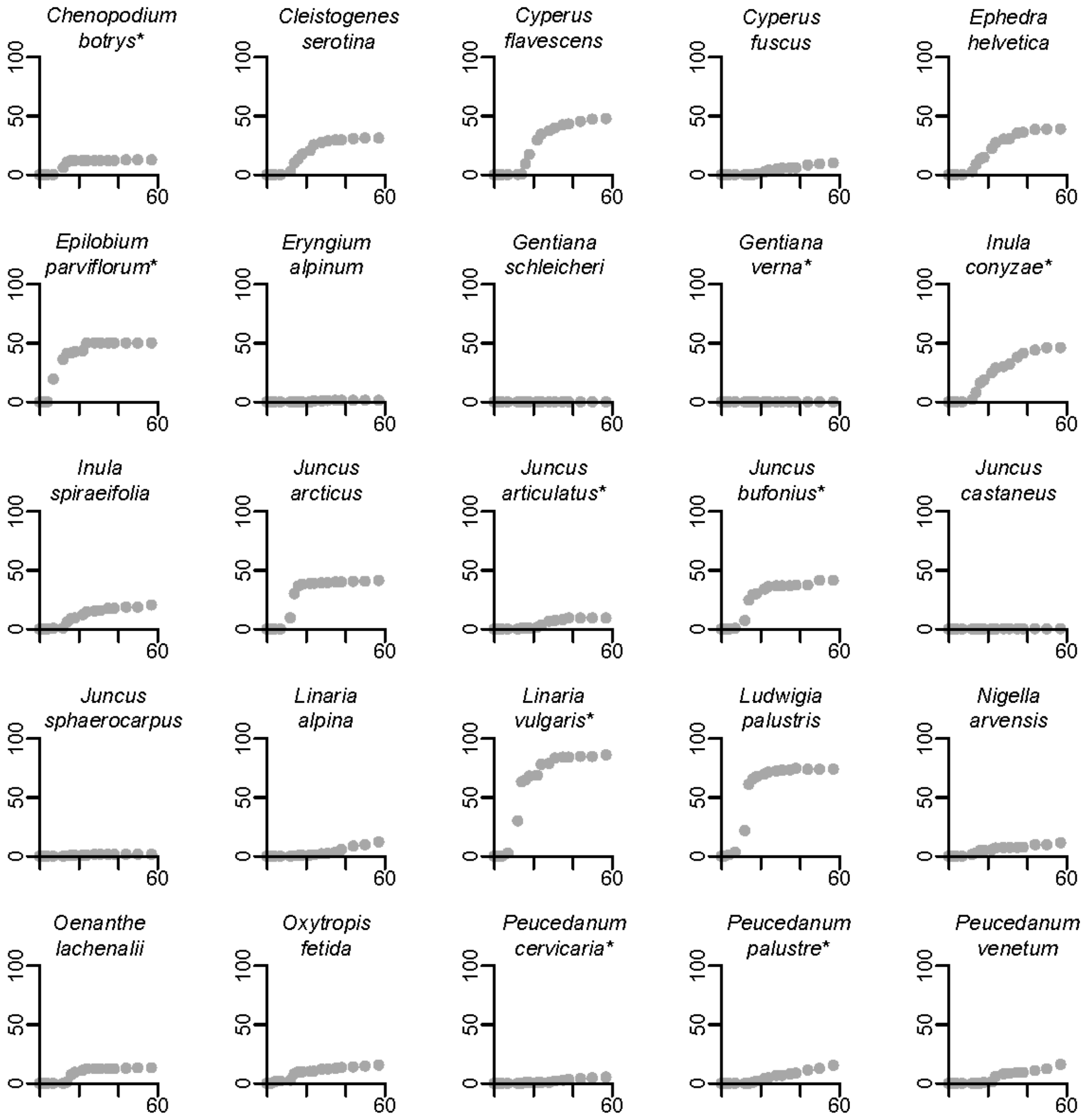


Figure S1 (continued).

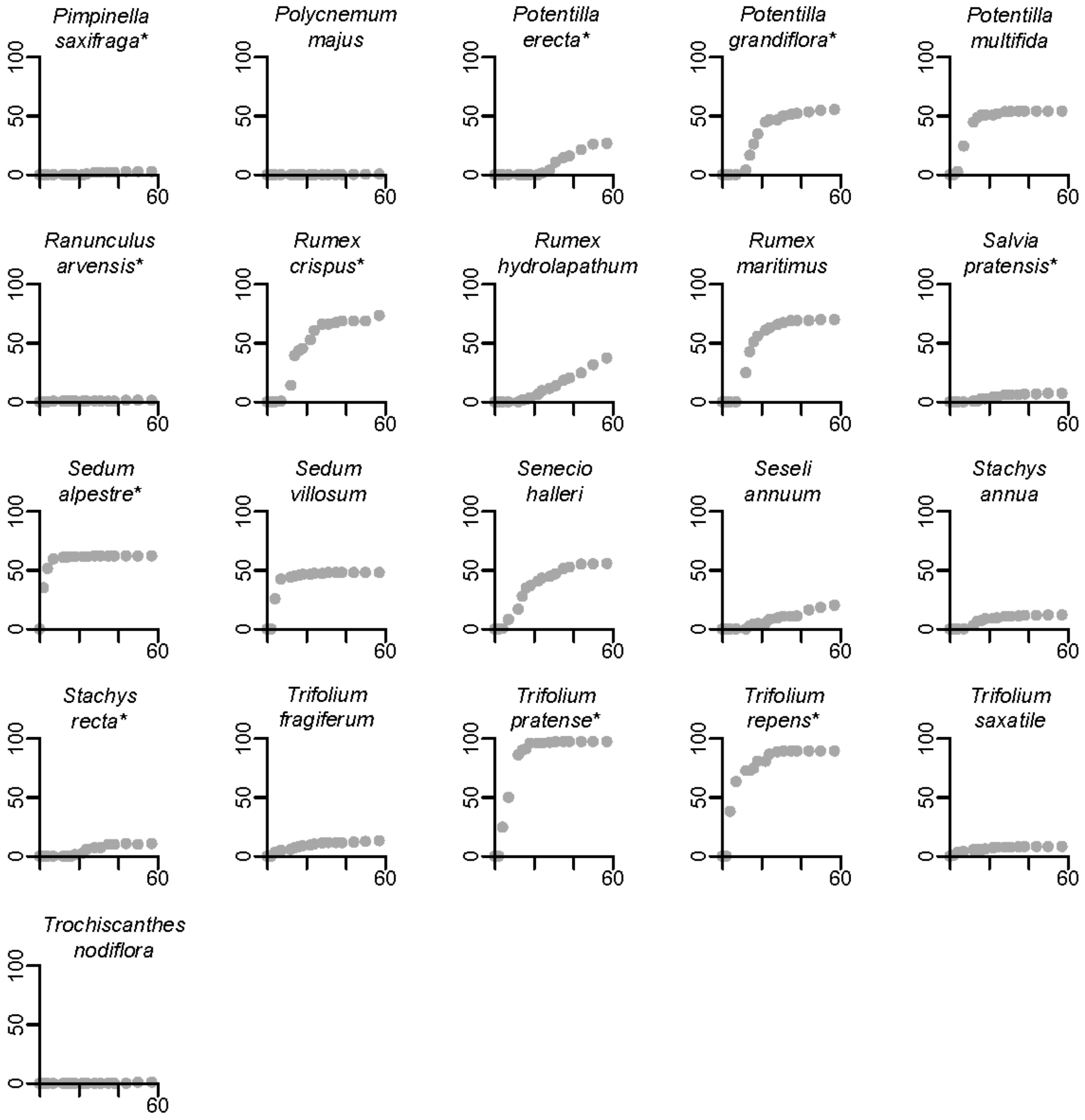


Figure S2. Cumulative germination percentage for each of the 39 rare species. The grey dots represent the control pots without stratification, the black dots represent the pots with stratification.

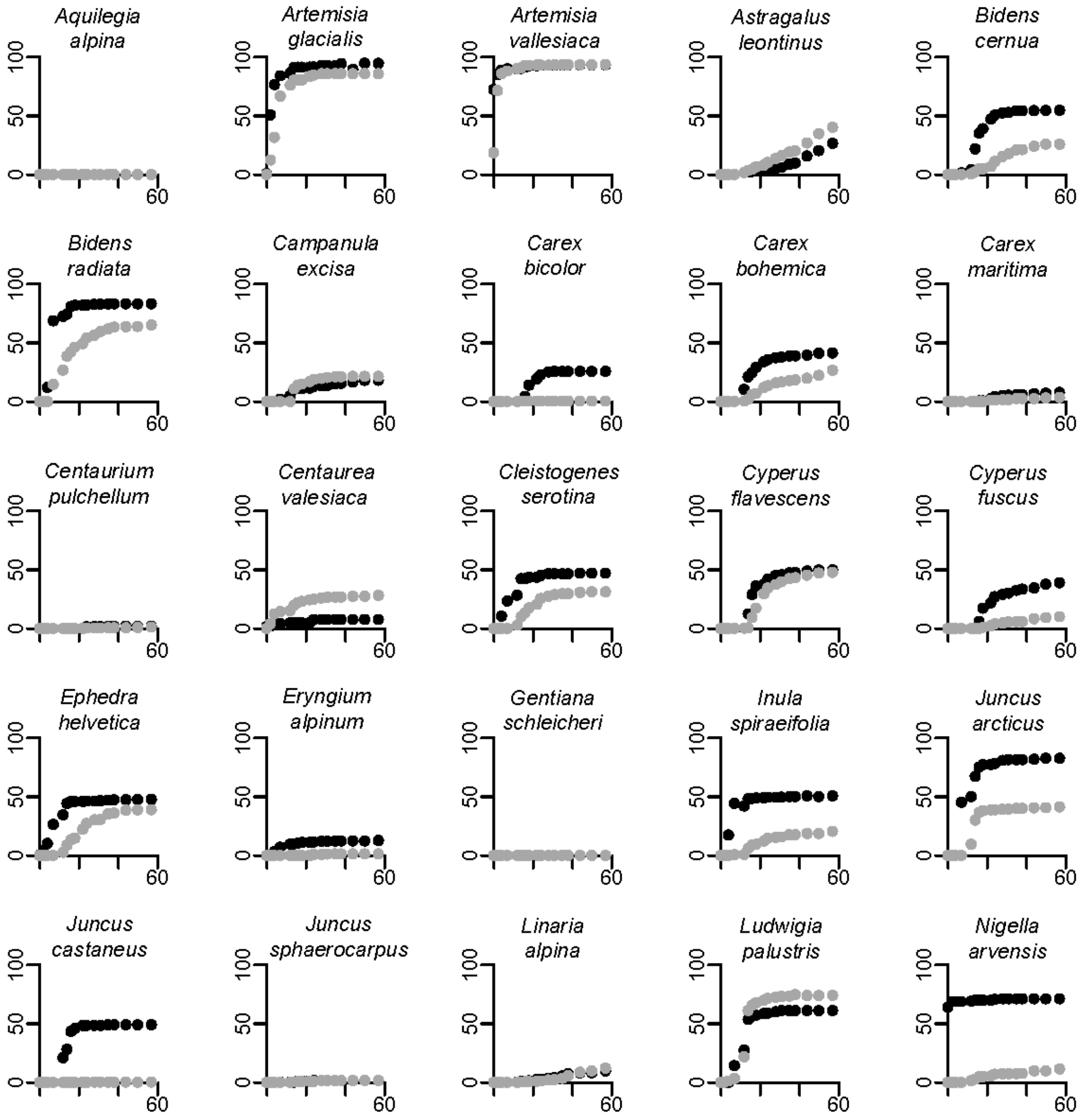
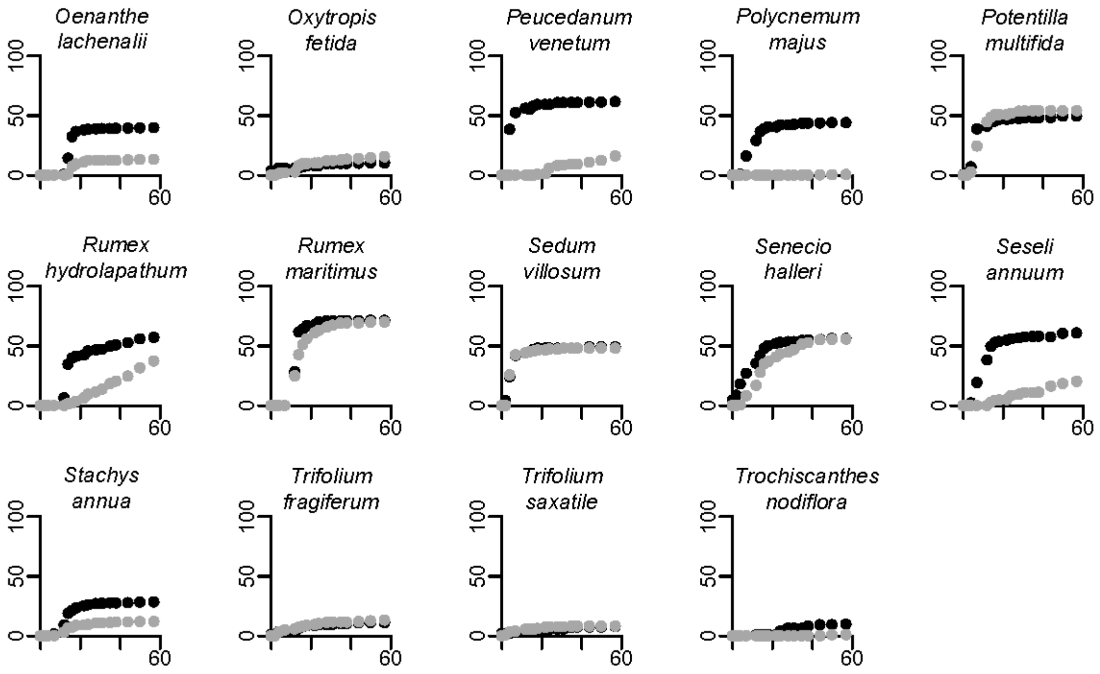


Figure S2 (continued).



Chapter 3

Experimentally assessed heritabilities of plant traits do not indicate reduced evolutionary potential of 31 rare and endangered species of Switzerland, regardless of their degree of rarity

Hugo Vincent, Anne Kempel and Markus Fischer

Abstract

Rare plant species are expected to occur in more isolated or smaller populations than widespread species, and thus in less genetically diverse populations. Accordingly, heritability of plant traits, which reflects the evolutionary potential of populations, can also be expected to be lower in populations of the rarer and more endangered species. Investigating the evolutionary potential of rare and endangered species is particularly important in the context of adaptation to future environmental changes, and for developing adequate ex-situ conservation measures or re-introductions to the wild. However, the majority of studies investigating genetic variation in rare species used molecular markers which do not relate to adaptive variation, and compared rare and widespread species, without distinguishing between different degrees of rarity and endangerment. We used a quantitative-genetic trait-variation approach to test whether rarer, including very rare and endangered ones, and more endangered plant species harbour less heritable variation than less rare and less endangered ones. We grew offspring of different seed families of 31 rare and endangered species of Switzerland in a common garden experiment from April to August 2012. The study species differed in three measures of rarity, i.e. their IUCN threat category, priority for conservation, and range size in Switzerland, ranging from very rare (minimum range size = 2 10x10 km grid cells) to widespread species (maximum range size = 155 of 431 Swiss grid cells). We measured 15 quantitative traits related to plant fitness and function and estimated narrow-sense heritabilities from genetic (between seed family) and environmental variance components. Overall mean heritability was 0.222 (95% CI = 0.169 – 0.273). We found heritabilities to be independent of all three measures of rarity and comparable to those of other rare and widespread species reported in the literature. Our results suggest that among rare and endangered species the potential to adapt to environmental changes is not necessarily reduced, not even for the particularly rare and endangered ones.

Introduction

Global biodiversity is currently experiencing unprecedented rates of global and local extinction (Pimm et al. 1995, Sala et al. 2000) and ongoing climate change, habitat loss and fragmentation, over-fertilization and pollution, biological invasions, and overexploitation pose additional threats for already endangered species by further reducing populations size and connectivity (Dirzo & Raven 2003, Helm et al. 2006). In poorly connected or small populations, as they are typical for rare and endangered species, evolutionary mechanisms such as increased inbreeding depression (i.e. reduced fitness due to mating between related individuals), genetic drift (i.e. the random fixation of alleles) and limited gene flow between-populations (Ellstrand & Elam 1993) are expected to lead to reduced genetic variation (Gilpin & Soulé 1986). A loss of genetic variation may also reduce the ability of species to adapt to environmental changes (Frankham 1999), which may compromise their ability to survive ongoing and future environmental changes (Franks et al. 2013). In a meta-analysis Spielman et al. (2004) found that most species are indeed affected by genetic factors before they go extinct. Understanding genetic variation in rare and endangered species is thus essential for conservation (Frankham 2005, Pertoldi et al. 2007).

Molecular techniques, such as the study of allozyme or DNA variation, have been widely used in recent decades to assess different aspects of genetic variation of endangered species, e.g. population differentiation, allelic richness, or inbreeding levels (Schaal et al. 1991, Frankham 1995, Frankham et al. 2014). However, most molecular markers are considered selectively neutral and variation in molecular markers is generally not well correlated with variation in ecologically important traits (Reed & Frankham 2001) and does not indicate heritable variation. Therefore, quantitative-genetic studies of variation in traits related to fitness have been advocated (Storfer 1996, Petit et al. 2001), as the evolutionary potential of populations can be inferred from measuring phenotypic variation in quantitative traits for individuals of known relatedness (Falconer & Mackay 1996, Carvajal-Rodríguez et al. 2005). The quantitative trait variation can then be separated into a component indicating additive genetic variance (V_A, the proportion of phenotypic variation determined by genetic variation) and one indicating environmental variance (V_E, the proportion of phenotypic variation determined by environmental or random variation). From these components of variance we can estimate narrow-sense heritability (h^2) of a population as the proportion of phenotypic variation between individuals that is due to additive genetic variance (Falconer & Mackay 1996, Visscher et al. 2008). Heritability is expected to vary among traits, depending on the degree to which they are under selection (fitness-related traits) and on the strength of selection (Fisher 1930, Falconer & Mackay 1996, Geber & Griffen 2003). An increase in V_A (increased differences between genotypes) increases heritability, whereas an increase in V_E (reduced differences between genotypes) decreases heritability. Heritability is considered an extremely important parameter because it reflects the proportion of variation that responds to selection (Fisher 1930) and thus the potential of populations for adaptation.

Rare and endangered species are expected to occur in more isolated, and often smaller, populations than widespread species (Gaston 2003), and are therefore likely to experience lower within-population genetic variation (Frankham 1995, Leimu et al. 2006) and thus lower V_A. Lower levels of genetic variation in rare species than in related widespread species, based on molecular markers, indeed seem to be a general pattern (Cole 2003), although differences can be small (Karron 1987, Gitzendanner & Soltis 2000). Small or isolated populations might also experience more developmental instability, i.e. phenotypic variation due to random variation in the developmental process (Fischer et al. 2000, Kiflawi et al. 2000), which would increase V_E. We can thus hypothesize that the rarest and most endangered species should have lower narrow-sense heritabilities than less rare and less endangered species, and therefore might have a lower potential for adaptation.

Previous studies investigating differences in genetic variation related to species rarity were mostly based on selectively neutral molecular data and individual studies compared pairs or small numbers of species (e.g. Karron 1991, Gitzendanner & Soltis 2000). Further, in these studies, and more generally in the literature investigating relationships between rarity and plant traits (e.g. Murray et al. 2002), rarity was defined as a binomial variable, i.e. rare versus widespread, which only captures a fraction of the variation in species distributions and abundances (Gaston 1994).

Therefore, we performed a multi-species experiment to address general patterns by growing many species simultaneously under the same conditions (van Kleunen et al. 2014). In a common garden experiment we tested whether 31 species of different degrees of rarity and endangerment, expressed as range size, category of risk of extinction and priority for conservation in Switzerland, also differ in their quantitative-genetic trait variation. Using individuals of known maternal origin we calculated the genetic and environmental components of variation and estimated narrow-sense heritabilities for ecologically relevant traits related to size, biomass, resource use and reproduction.

Material & Methods

Study species

We obtained seeds of 31 species from 15 plant families (Table 1). These species are rare and of priority for conservation in Switzerland (Moser et al. 2002, List of Priority Species at the National Level FOEN 2011). For each species, seeds were collected on up to 10 maternal plants (hereafter seeds from the same mother are called ‘seed families’) in one natural population in Switzerland. With the help of local experts and botanists (Infoflora, Botanical Gardens of Geneva and Bern, Switzerland) the species were selected to cover a wide range of habitats and different levels of rarity. Rarity and endangerment were first classified according to the Swiss Red List (Moser et al. 2002) based on the IUCN categories Near Threatened (NT), Vulnerable (VU), Endangered (EN), and Critically Endangered (CR) and second using the List of Priority Species at the National Level of Switzerland (FOEN 2011) with the priority for conservation ranging from 1 (highest priority) to 4 (lowest priority) and indicating the importance of preserving the Swiss populations of a species in a European and global context. Third, rarity was further quantified as a continuous variable, using the range size of each species in Switzerland. Range size was expressed as the number of 10 x 10 km grid cells occupied by a given species in Switzerland (data provided by Info Flora, Bern and Geneva, also see Bornand 2014). Our dataset covered a large gradient of geographical distributions, from a range size of 2 (*Carex bohemica*) to a range size of 155 (*Cyperus flavescens*) of a total of 431 10 x 10 km cells in Switzerland. We used range sizes in Switzerland because a continuous measure of European or global range sizes for our species is not yet available. Nevertheless, for a subset of 20 species, for which European range size is available, Swiss and European range sizes were positively correlated ($r = 0.66$, $p = 0.001$, Text S1).

Common garden experiment

We germinated the seeds in April 2012 and then transplanted four plants per seed family individually into 2-L pots filled with standard potting soil (Ricoter, Aarberg, Switzerland). We placed the pots in a common garden (in Muri, close to Bern, Switzerland) in four blocks. Each block contained one individual per seed family per species, and plants were randomly arranged within blocks. For most species we had seeds of at least 10 seed families and grew plants of 10 seed families in the experiment. However, due to unequal germination and some early mortality we grew plants from 9 seed families for three species and fewer than 9 for six further species; Table 1). During the experiment, plants were watered when needed.

In August 2012 we measured quantitative traits following Cornelissen et al. (2003). We counted the number of leaves, the number of stems and the number of flowers or inflorescences (for species with small flowers grouped in distinguishable inflorescences, e.g. in the Asteraceae). We measured plant height to the nearest mm; length and width of the longest leaf (hereafter called leaf length and leaf width, respectively), leaf thickness (i.e. mean leaf thickness of three randomly chosen leaves), and basal stem diameter, with a caliper to the nearest 0.1 mm; leaf greenness as a proxy for chlorophyll content (mean greenness of three randomly chosen leaves, unitless, measured with Konica Minolta Spad 502 SLA). We collected a few leaves of each plant and weighed them (to the nearest 0.1 mg), before and after drying them for 24h at 80°C to calculate leaf dry matter content (LDMC in $mg.g^{-1}$). We measured the area of the leaves (LI-COR 3100C area meter) and calculated their specific leaf area (SLA in $m^2.kg^{-1}$).

Because some of the plant species used in our experiment are very rare in Switzerland, and plants could be used for ex-situ cultivation and re-introduction purposes (e.g. chapter 5), we did not want to harvest them all to assess plant biomass. Therefore, we harvested all plants from block 1 and separated their aboveground biomass into stems, leaves and inflorescences. Aboveground biomass was dried at 80°C for 72h and weighed to the nearest 0.01 g. We assessed the linear relationships between the aboveground biomass of stems, leaves and inflorescences and all non-destructively measured traits for each species in block 1. This relationship was then used to estimate aboveground biomasses in the 3 other blocks from the non-destructively measured traits in these blocks. To do so we used multivariate imputation by chained equations (package *mice*, version 2.25, van Buuren & Groothuis-Oudshoorn 2011) and non-parametric missing value imputation (package *missForest*, version 1.4, Stekhoven & Buehlmann 2012). We compared these two approaches by calculating normalized root mean square errors (nrmse) on the complete data of block 1, and chose the *missForest* approach which showed the smallest error (*missForest* mean nrmse = 0.020, *mice* mean nrmse = 0.042).

The measured traits represented four categories: performance traits (biomass of leaves and stems), reproductive traits (biomass and number of flowers or inflorescences), resource-use traits (SLA, LDMC, greenness, thickness) and size traits (number of leaves, number of stems, stem diameter, plant height, leaf length and width).

Variance components and narrow-sense heritability

We estimated variance components for each trait and species using linear mixed models (*lmer*). For each species we ran one model per trait, using the particular trait as response variable, and seed family identity as a random term. From these models we extracted the variances between seed families (or genetic variance component V_G) and the variances within seed families (or environmental variance component V_E, i.e. the residual variance in the models; Falconer & Mackay 1996). According to our information on the mating system of the study species none of the species are obligatory selfers (Fryxell 1957, Julve 1998, Gaudeul & Till-Bottraud 2003, Razanajatovo 2016). Thus we assumed that our species are outbreeders, where it is likely that for each seed family (i.e., for each maternal plant) ovules were fertilized by different paternal plants. Hence we considered individuals of different seed families as half-siblings within each of the species. As in half-sibling experimental designs the additive genetic variance represents a quarter of the environmental variance (Petit et al. 2001) we calculated narrow-sense heritability h^2 per trait and per species as:

$$h^2 = \frac{V_A}{V_A + V_E} = \frac{4 \times V_G}{4 \times V_G + V_E}$$

Narrow-sense heritability and its variance components (see Table 1 for the mean values h^2, V_A and V_E per species) were then further analyzed.

Table 1. List of the 31 rare species of Switzerland studied in this experiment, with their plant family, number of seed families per species, IUCN category (NT: Near Threatened, VU: Vulnerable, EN: Endangered, CR: Critically Endangered), priority for conservation in Switzerland (all 31 species are considered as priority for conservation, 1 indicates the highest priority, 4 the lowest priority), range size in Switzerland (number of 10x10 km grid cells), and the mean values of heritability h^2, additive genetic variance component V_A and environmental variance component V_E.

Species	Family	Number of seed families	IUCN category	Priority for conservation	Range size	h^2	$V_A = 4V_G$	V_E
Artemisia glacialis	Asteraceae	9	NT	3	9	0.317	0.056	0.088
Artemisia vallesiaca	Asteraceae	10	NT	3	14	0.375	0.083	0.084
Astragalus leontinus	Fabaceae	10	NT	3	32	0.237	0.071	0.142
Bidens cernua	Asteraceae	10	EN	3	76	0.313	0.053	0.089
Bidens radiata	Asteraceae	10	CR	2	3	0.558	0.177	0.070
Campanula excisa	Campanulaceae	5	NT	3	21	0.041	0.012	0.349
Carex bicolor	Campanulaceae	7	NT	4	81	0.484	0.019	0.016
Carex bohemica	Cyperaceae	10	CR	2	2	0.193	0.025	0.040
Centaurea valesiaca	Asteraceae	9	NT	3	19	0.000	0.000	0.096
Cleistogenes serotina	Poaceae	4	VU	4	11	0.167	0.012	0.065
Cyperus flavescens	Cyperaceae	9	VU	4	155	0.174	0.014	0.035
Cyperus fuscus	Cyperaceae	10	VU	4	151	0.320	0.034	0.090
Ephedra helvetica	Ephedraceae	10	VU	1	6	0.140	0.021	0.056
Eryngium alpinum	Apiaceae	5	VU	2	29	0.478	0.029	0.036
Inula spiraeifolia	Asteraceae	10	VU	4	4	0.301	0.074	0.105
Juncus arcticus	Juncaceae	10	VU	2	35	0.151	0.017	0.070
Linaria alpina	Plantaginaceae	10	VU	1	14	0.183	0.057	0.227
Ludwigia palustris	Onagraceae	10	CR	2	21	0.250	0.041	0.123
Nigella arvensis	Ranunculaceae	10	EN	3	37	0.341	0.034	0.059
Oenanthe lachenalii	Apiaceae	10	CR	2	20	0.424	0.104	0.110
Oxytropis fetida	Fabaceae	10	NT	3	11	0.246	0.146	0.078
Peucedanum venetum	Apiaceae	5	VU	4	14	0.082	0.009	0.060
Polycnemum majus	Amaranthaceae	10	EN	3	69	0.056	0.011	0.175
Rumex hydrolapathum	Polygonaceae	10	EN	3	30	0.090	0.012	0.073
Rumex maritimus	Polygonaceae	10	CR	2	5	0.080	0.015	0.162
Sedum villosum	Crassulaceae	3	VU	4	92	0.172	0.026	0.084
Senecio halleri	Asteraceae	10	NT	3	12	0.040	0.003	0.048
Seseli annuum	Apiaceae	10	VU	4	47	0.064	0.004	0.064
Stachys annua	Lamiaceae	10	VU	4	102	0.172	0.012	0.123
Trifolium fragiferum	Fabaceae	9	VU	4	123	0.120	0.019	0.122
Trifolium saxatile	Fabaceae	8	VU	1	8	0.241	0.144	0.274

Phylogenetic signal in the variance of the studied traits

We tested whether there were phylogenetic signals in V_G, V_E and h^2 of the studied traits for our 31 species using a dated phylogeny from the Daphne tree database (Durka & Michalski 2012). We used the function *phylosignal* from the package *picante* (version 1.6-2, see Blomberg et al. 2003). The K values were always positive, but low (mean $K(V_G) = 0.217$, mean $K(V_E) = 0.312$ and mean $K(h^2) = 0.249$, respectively), and overall not significant (Table S1). We therefore chose to control for the relatedness of our species by using the species identity and the plant taxonomic family instead of using phylogenetic correlations.

Further statistical analysis

To test whether the measured traits differed between blocks, we used analyses of variance (*anova*) applying linear mixed models (*lmer*, lme4 package, version 1.1-6, Bates & Maechler 2014) with trait identity as the response variable, block as the explanatory variable, and seed family identity nested in species identity as the random term. As the block effect was only significant for the number of leaves, leaf greenness and biomass measurements (Table S2), we did not include block in the following analyses.

We calculated the mean overall value of h^2, i.e. the intercept value returned by the summary of an *lmer* model including h^2 as the response variable, and species nested into plant family as the random term. We then calculated a 95% profile confidence interval (function *confint*) around the overall value of h^2 to determine the relative contribution of genetic and environmental variation to h^2. A value of $h^2 > 0.5$ would indicate that $V_A > V_E$ (most of the variance for a trait is genetic) whereas a value of $h^2 < 0.5$ would indicate that $V_A < V_E$ (most of the variance for a trait is environmental).

To test whether h^2, V_G and V_E were related to species rarity, we used *lmer* models. Three species *(Artemisia vallesiaca*, *Sedum villosum* and *Stachys annua*) showed extreme variances in number of flowers, due to large differences in phenology at the time of data collection, and these extreme values had a strong effect on the distribution of residuals of the models. We therefore removed these data points and further applied a square-root transformation to V_G and V_E to meet the model assumptions, while no data transformation was needed for h^2. Data of species with missing values for h^2, V_G and V_E were removed from the analysis for seven out of fifteen traits (number of leaves: n = 25 species analyzed; number of stems: n = 15; length and width of the longest leaf: n = 24; number of stems, n = 20; number of flowers and leaf greenness: n = 22).

We analyzed h^2, V_G and V_E across all traits, and included trait identity as a fixed term in the models. The explanatory variable was rarity, defined as: a) IUCN category of threat (ordinal); b) priority for conservation in Switzerland (ordinal); or c) range size (continuous). As h^2 is expected to vary among traits (Geber & Griffen 2003) we also tested for an effect of the interaction between trait identity and rarity on h^2. Species identity nested into plant family was included as a random term to account for the relatedness of our species.

The collected data represented an opportunity to investigate the heritability of traits related to different plant functions and to plant performance on a large number of rare species. We tested whether there was a significant difference in h^2, V_G and V_E between groups of traits (performance traits, reproductive traits, resource-use traits, size traits) using *lmer* models including the identity of the group of traits as explanatory variable, and trait identity and species identity nested into plant family as random terms.

We simplified the full models by removing non-significant terms and we determined significances using likelihood-ratio tests comparing models with and without the factor of interest. All statistical analyses were performed in R (version 3.3.2, R Core Team, 2016).

Results

Mean overall heritability h^2 was 0.222 (95% CI = 0.169 – 0.273). There was no difference in h^2 between the different groups of traits ($p = 0.487$, Fig. 1).

h^2 was independent of IUCN category (Fig. 2a), priority for conservation (Fig. 2b) or range size (Fig. 2c), and there was no significant interaction between trait identity and any of the three measures of rarity and endangerment (Table 2). Moreover, neither the genetic variance component (V_G), nor the environmental variance component (V_E) were related to IUCN category (Fig. S1a, b), priority for conservation (Fig. S1c, d) or range size (Fig. S1e, f) (Table 2). There was no difference in V_G ($p = 0.305$) and V_E ($p = 0.206$) between the different groups of traits (Fig. S2a, b). These results suggest that heritability, and thus evolutionary potential, does not differ between more or less endangered species and between species of different range sizes.

Figure 1. Comparison of h^2 between groups of traits representing plant functions and plant performance measured on 31 rare species of Switzerland. There was no difference in h^2 between the groups of traits ($p = 0.487$). Shown are mean values and standard errors per category calculated from the raw data.

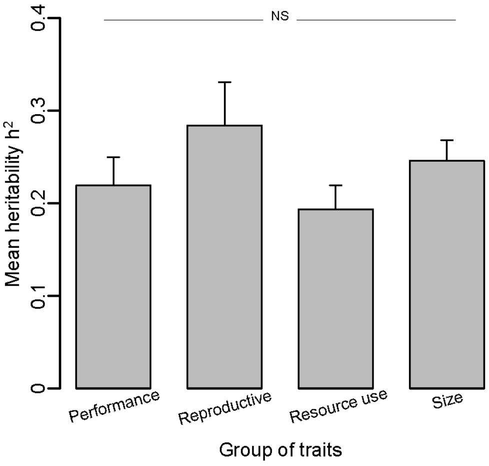


Figure 2. Comparison of mean heritability h^2 between 31 rare species of Switzerland differing in a) IUCN categories (NT: Near Threatened, VU: Vulnerable, EN: Endangered, CR: Critically Endangered); b) priority for conservation (all 31 species are considered as priority for conservation; 1 indicates the highest priority, 4 the lowest priority); and c) range size. None of the three measures of rarity affected h^2. Shown are mean values and standard errors per category (a, b) or mean values per species (c), calculated from the raw data.

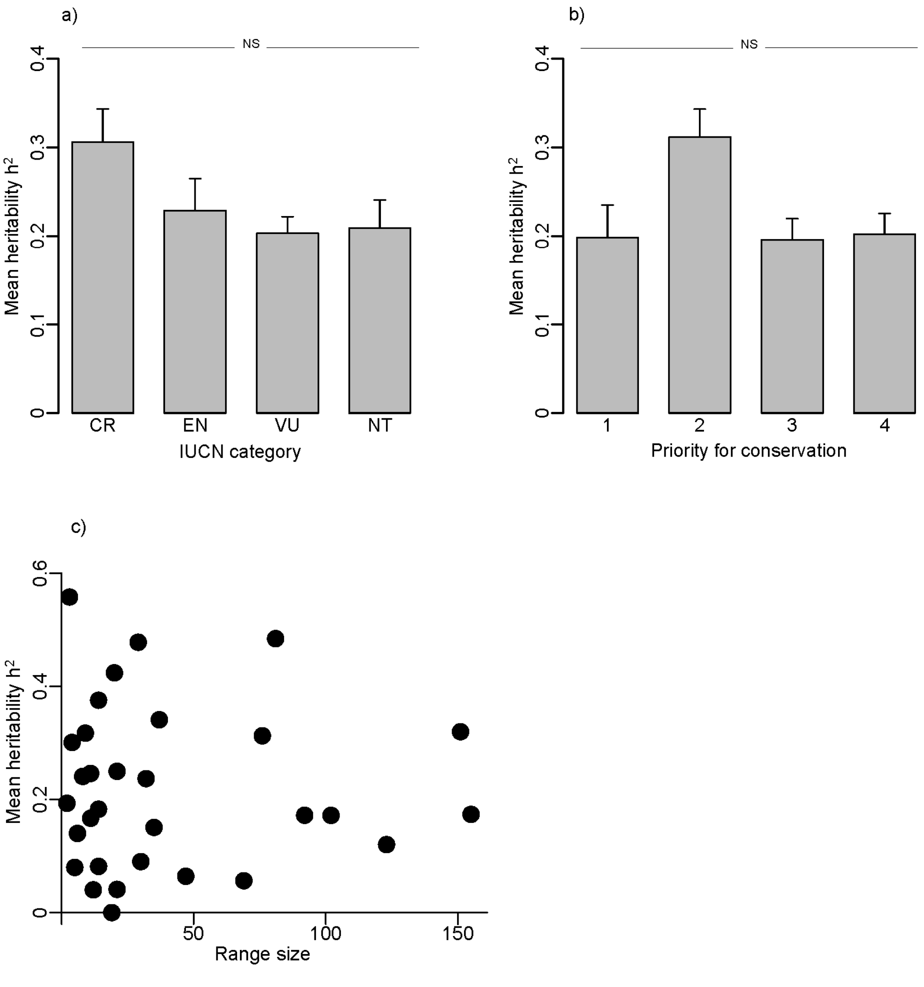


Table 2. Results of the linear mixed model testing for the relationship between heritability h^2, the variance components V_G and V_E, respectively, and rarity of 31 rare species of Switzerland. Rarity was the explanatory variable and was defined as a) IUCN category (NT: Near Threatened, VU: Vulnerable, EN: Endangered, CR: Critically Endangered); b) priority for conservation in Switzerland (all 31 species are considered as priority for conservation; 1 indicates the highest priority, 4 the lowest priority); or c) range size. All models included trait identity as fixed term, and species identity nested into plant family to account for the taxonomic relatedness of our species. We tested for the interaction between trait identity and rarity in the models with h^2 as response variable. Variances of the random terms are given for each model. V_G and V_E were square-root transformed to meet model assumptions.

		h^2		V_G		V_E	
		p-value	Chi^2	p-value	Chi^2	p-value	Chi^2
a)	Trait identity	0.013^{*}	28.4	$<0.001^{***}$	41.2	$<0.001^{***}$	102
	IUCN	0.56	2.04	0.34	3.34	0.95	0.35
	Trait : IUCN	0.40	43.7	-	-	-	-
	Family/Species	<0.001		<0.001		0.003	
	Family	<0.001		<0.001		<0.001	
b)	Trait identity	0.013^{*}	28.4	$<0.001^{***}$	40.4	$<0.001^{***}$	99.7
	Priority	0.33	3.47	0.25	4.10	$0.089^{\cdot}$	6.51
	Trait : Priority	0.23	48.5	-	-	-	-
	Family/Species	0.016		<0.001		0.002	
	Family	<0.001		<0.001		<0.001	
c)	Trait identity	0.012^{*}	28.5	$<0.001^{***}$	40.9	$<0.001^{***}$	102
	Range size	0.87	0.03	0.30	1.07	0.66	0.19
	Trait : Range size	0.74	12.9	-	-	-	-
	Family/Species	0.017		0.011		0.002	
	Family	<0.001		<0.001		<0.001	

Discussion

Overall heritability

The overall mean of heritability of 0.222 is comparable to the estimates of narrow-sense heritabilities of other rare and widespread species, as reported in field and experimental studies on rare (Waldmann & Andersson 1998, Petit et al. 2001) and widespread species (Johnson et al. 2008, Johnson et al. 2009, Blum 2016), and as summarized in a large data survey (Geber & Griffen 2003). This suggests that rarer and more endangered species do not necessarily have a lower evolutionary potential to respond to selection than less rare and even widespread species do.

The mean heritability indicates that the environmental component of variance was greater than the genetic component of variance. This could be due to some micro-environmental heterogeneity in our experimental garden, such as small scale variation in moisture or light availability, affecting plant traits via phenotypic plasticity (Nicotra et al. 2010). Moreover, it may reflect developmental instability, i.e. phenotypic variation caused by random variation in the developmental process (Siikamaki & Lammi 1998, Fischer et al. 2000). Some of the sampled Swiss populations of our species - for instance Mediterranean species (e.g. *Inula spiraeifolia*) which have their northern distribution limit in Switzerland (Broennimann et al. 2005) - are even in the periphery of their species distribution, where developmental instability may be expected to be higher than in the center (Levin 1970, Kiflawi et al. 2000).

No differences in heritability among species of different degrees of rarity and endangerment

As we found heritabilities and the genetic and environmental variance components to be independent of species range size, IUCN category or priority for conservation, our results do not support the hypothesis that the rarest and most endangered species have lower heritability, and thus lower evolutionary potential, than less rare and less endangered species.

The hypothesis of reduced genetic diversity for rare species is supported by several studies investigating genetic diversity in rare and widespread species (e.g. Karron 1987, Gitzendanner & Soltis 2000, He et al. 2000, Cole 2003, Edwards et al. 2014). Species at a higher risk of extinction are often occurring in declining, smaller and more fragmented populations (IUCN 2012), potentially leading to lower levels of genetic diversity (Ellstrand & Elam 1993, Reed 2004). Broennimann et al. (2005) reported that among 118 Swiss plant species of the highest priority for conservation, the most threatened ones have restricted geographic distributions and occur in rare habitats in Switzerland, and only four of these species had more than 200 individuals as median population size. This would suggest that the more endangered species among our study species should have had reduced genetic variation. On the other hand, heritable variation can be retained even in small populations of rare species by the effects of contrasting environmental conditions and spatial structure, as shown by Widén & Andersson (1993) for small populations of the rare *Senecio integrifolius*. Also, natural enemies can drive rapid adaptation (Agrawal et al. 2012, Züst et al. 2012) and therefore local heterogeneity in plant-herbivore interactions might maintain heritable variation. This could explain why, even though theory and some empirical work suggests lower genetic variation for rarer species, we did not find a relationship between heritability and rarity.

Further, the vast majority of the studies demonstrating a positive relationship between genetic diversity and population size are based on allozyme and DNA markers, i.e. quantified neutral molecular variation, and the link between genetic diversity and quantitative variation, thus heritability, remains controversial. A meta-analysis (Reed & Frankham 2001) showed only a weak mean correlation between quantitative and molecular measures, and no significant relationship between heritability and molecular measures. The authors attribute this to differences in the selective forces acting on molecular and quantitative measures. Therefore the importance of the quantitative-genetic approach has been emphasized by several authors, because it concerns traits that are directly related to fitness and thus gives direct information on the evolutionary potential of populations in response to selection (Frankham 1995, Storfer 1996, Petit et al. 2001, Carvajal-Rodríguez 2005). To our knowledge our study is the first to address heritabilities for many rare and endangered species of various range sizes, and the deviation of our results from expectations of reduced heritability for rarer species indicates that quantitative genetics is an essential tool to consider in conservation genetics.

The vast majority of studies investigating differences between species in traits, responses to abiotic or biotic factors or genetic variation in the context of rarity used comparisons between one or several pairs of rare and widespread congeners (e.g. references in Murray et al. 2002, Edwards et al. 2014). In contrast, we used species that are all considered rare and with a priority for conservation in Switzerland (FOEN 2011), and we characterized their rarity and endangerment with three different approaches, describing the rarity and endangerment of a given species in very different ways. Firstly, our species cover the whole range of IUCN threat categories from Critically Endangered to Near Threatened, and they therefore differ in their degree of having limited geographic distribution, and small, declining or isolated populations (Moser et al. 2002, IUCN 2012). Secondly, the study species span the whole range from the lowest to the highest priority for conservation, reflecting large differences in not only regional rarity within Switzerland but also global rarity (FOEN 2011). Finally, we used a continuous measure of rarity, i.e. range size in Switzerland, which covers a large gradient of differences in geographical distribution within this set of rare species. As we used many species, many traits and different measures of rarity and endangerment, we suggest our finding of similar heritabilities across gradients of rarity and endangerment as general pattern.

Our results indicate that the rarest and most endangered species may as well be able to adapt to future environmental changes as less rare and endangered species. However, to be able to adapt to such changes, populations must persist over several generations. The current tolerance of rare and endangered plant species to changing environmental conditions thus also needs to be addressed to inform efficient conservation measures (Chapter 4). Adaptive variation may be depleted in the process of adaptation to changing conditions, and it may be further reduced when species shift their ranges to track climate change (Buckley & Bridle 2014). Therefore, both ex-situ and in-situ conservation measures should aim at maintaining as high as possible levels of heritable variation in populations of endangered species. However, multi-generation cultivation in botanical gardens can reduce plant genetic variation and fitness, and potentially cause maladaptation to natural habitats (e.g. Ensslin et al. 2011, Lauterbach et al. 2012). To ensure the best evolutionary potential of ex-situ collections, knowledge of and particular care for heritable variation of rare and endangered species is needed

(Hamilton 1994, Cochrane et al. 2007, Ensslin et al. 2015). Moreover, high genetic variation of plant material reintroduced to the wild is thought to increase the success rates of reintroductions and translocations because it allows novel or reinforced populations to adapt to future environmental changes (Vergeer et al. 2005, Menges et al. 2008, Weeks et al. 2011). Our study suggests that material from natural populations of rare and endangered plants may serve as initial source of genetically diverse ex situ collections and introductions to the wild.

No difference in heritability between different traits of rare and endangered plant species in Switzerland

The quantitative traits measured in this study relate to plant performance, reproduction, resource use and size (Cornelissen et al. 2003). Fitness-related traits, e.g. number of flowers or biomass production, are expected to be under strong directional selection and could therefore have lower additive genetic variation (Fisher 1930, Flaconer & Mackay 1996). Alternatively, Houle (1992) suggested that fitness-related traits should have high additive genetic variation because of mutations at the large number of loci involved in their expression. We found no differences in heritability and in the variance components between groups of traits. To complete the picture, further studies on the relationship between heritability and rarity could also consider further types of traits, e.g. traits linked to secondary chemistry, which might have larger heritabilities (Geber & Griffen 2003) due to temporally or spatially heterogeneous plant-herbivore or plant-pathogen interactions. For the time being our results indicate that for rare and endangered plant species traits related to various plant functions all have the potential to respond to selection, and all to a similar degree.

Conclusion

To our knowledge, this is the first study investigating the relationship between narrow-sense heritability and rarity for many rare and endangered plant species of different degrees of rarity and endangerment. As we used many species in our experiment and three complementary methods to assess rarity and endangerment, we suggest that the independence of heritability from degree of plant rarity and endangerment is a general pattern. Our results indicate that populations of rarer and more endangered species do not necessarily have a reduced potential to respond to selection than the ones of less rare and endangered or even widespread species. This is encouraging for the establishment of ex-situ populations of rare species, as it suggest that they should be able to respond to selection as exposed for instance to environmental change. At the same time, however, the risk of rapid adaptation to the conditions of a botanical garden or other ex-situ site must be considered carefully and particular attention must be paid to maintaining high heritable genetic variation in ex-situ populations, and living collections in general. Our results also suggest that rare and endangered species may be able to adapt to novel habitats in the context of introductions to the wild and that they may have the potential to adapt to ongoing global change.

Acknowledgements

We thank Adrian Möhl for collecting the seeds in the natural populations; the numerous field assistants who helped transplanting the seedlings, growing the plants and collecting data; Eric Allan, Caterina Penone and Dani Prati for their contribution to data analysis and their helpful comments on this manuscript. This study was supported by the Federal Office of the Environment, Switzerland.

Supplementary information

Text S1. To test whether range size in Switzerland is correlated with the European range size of our study species, we used map-derived area estimates from the *Atlas Europeae* (Meusel *et al*. 1978) for the 20 species for which these maps were available. We assessed the number of pixels of a species European distribution and cross referenced these using islands, for which the exact surface values are known. Range size in Europe was correlated with range size in Switzerland (r = 0.66, p = 0.001) therefore we used the latter one, which provided us with range size data of all our species.

Meusel, H., Jäger, E.J., Rauschert, S. & Weinert, E. (1978) *Vergleichende Chorologie der zentraleuropäischen Flora*. Bd. 2, Text u. Karten. Gustav Fischer Verlag, Jena, Germany.

Table S1. Results of the analyses testing for phylogenetic signals (function *phylosignal*) in V_G, V_E and h^2 of the measured traits among the 31 rare species of Switzerland. K > 0 indicates a phylogenetic signal, if more related species were more similar to each other than expected by chance. Here, K was always positive, but overall not significant.

Trait	Phylogenetic signal	V_G	V_E	h^2
Number of leaves	K	0.121	0.338	0.304
	p-value	0.881	0.279	0.289
Number of stems	K	0.241	0.171	0.256
	p-value	0.465	0.644	0.241
Height	K	0.141	0.296	0.066
	p-value	0.619	0.148	0.990
Length leaf	K	0.371	0.306	0.368
	p-value	0.351	0.471	0.178
Width leaf	K	0.478	0.332	0.310
	p-value	0.263	0.322	0.236
Stem diameter	K	0.060	0.457	0.160
	p-value	0.832	0.104	0.578
Number flowers	K	0.39	0.408	0.149
	p-value	0.402	0.261	0.859
Leaf thickness	K	0.128	0.411	0.193
	p-value	0.935	0.066	0.767
Greenness	K	0.617	0.208	0.477
	p-value	0.148	0.808	0.129
SLA	K	0.194	0.240	0.249
	p-value	0.753	0.552	0.518
LDMC	K	0.190	0.199	0.222
	p-value	0.775	0.769	0.627
Biomass leaves	K	0.086	0.208	0.175
	p-value	0.850	0.225	0.486
Biomass flowers	K	0.110	0.532	0.211
	p-value	0.867	0.055	0.730
Biomass stems	K	0.059	0.257	0.392
	p-value	0.915	0.648	0.455
Biomass	K	0.064	0.323	0.200
	p-value	0.943	0.028*	0.354

Table S2. Results of linear mixed models testing for differences in trait values between blocks in our experimental design. There was a block effect for number of leaves, greenness and estimated biomass measures (see methods).

	Block		
Traits	df	F-value	p-value
Number of leaves	3	7.73	<0.001
Number of stems	3	0.26	0.850
Height	3	2.34	0.072
Length leaf	3	0.14	0.936
Width leaf	3	1.16	0.324
Stem diameter	3	0.84	0.471
Number of flowers	3	2.46	0.062
Thickness	3	2.49	0.058
Greenness	3	4.64	0.003
SLA	3	2.07	0.103
LDMC	3	1.49	0.214
Biomass leaves	3	6.74	<0.001
Biomass flowers	3	5.07	0.002
Biomass stems	3	5.59	<0.001
Total biomass	3	6.45	<0.001

Figure S1. Comparison of the variance components V_A (dark grey bars and dots, plotted as $4V_G$ as in the h^2 equation) and V_E (light grey bars and dots) between 31 rare species of Switzerland differing in a, b) IUCN categories (NT: Near Threatened, VU: Vulnerable, EN: Endangered, CR: Critically Endangered); c, d) priority for conservation (all 31 species are considered as priority for conservation; 1 indicates the highest priority, 4 the lowest priority); and e, f) range size. None of the three measures of rarity affected V_G and V_E (Table 2). Shown are mean values and standard errors per category (a, c) or mean values per species (e), calculated from the raw data; and the mean residuals and standard errors per category (b, d) or mean residuals per species (f) after correcting for trait identity.

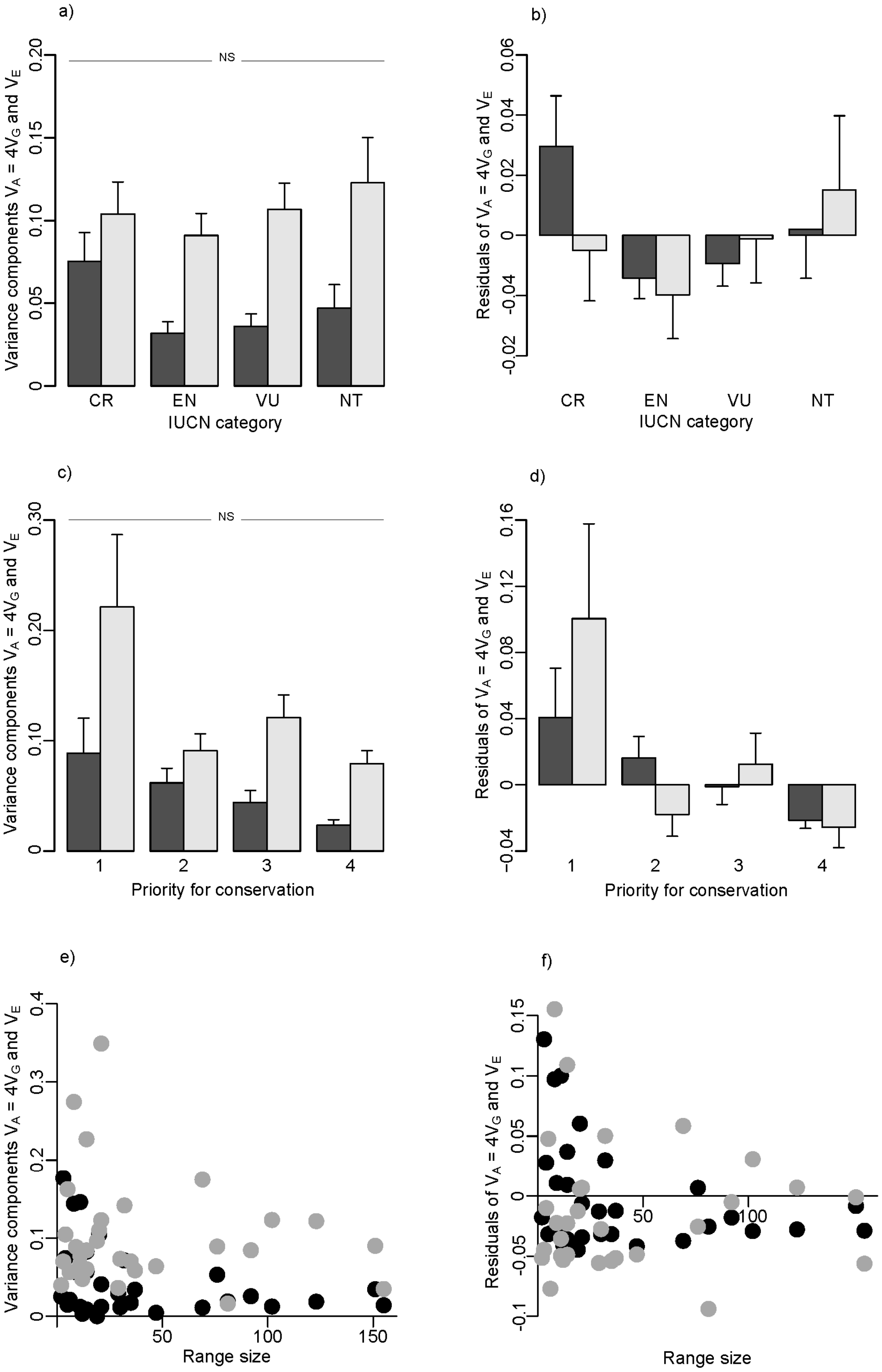
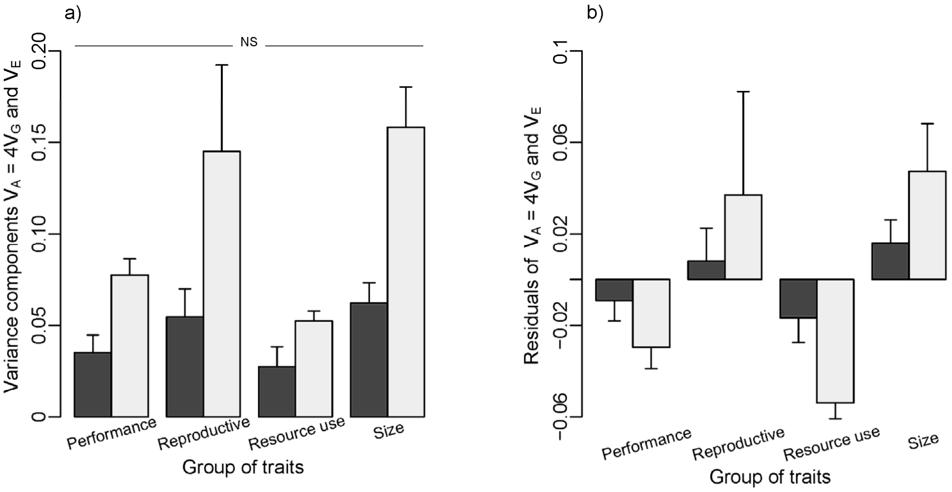


Figure S2. Comparison of V_A (dark grey bars, plotted as $4V_G$ as in the h^2 equation) and V_E (light grey bars) between group of traits representing plant functions and plant performance measured on 31 rare species of Switzerland. There was no significant difference in V_G ($p = 0.305$) and V_E ($p = 206$), respectively, between groups of traits. Shown are a) mean values and standard errors per group of traits calculated from the raw data; and b) mean residuals and standard errors per group of traits after correcting for species identity.



Chapter 4

Rare species perform worse than widespread species under changed climate

Hugo Vincent, Anne Kempel, Christophe N. Bornand and Markus Fischer

Abstract

Predicting how species, particularly rare and endangered ones, will react to climate change is a major current challenge in ecology. Rare species are expected to have a narrower niche breadth than widespread species. However, it is unknown whether they are also less able to cope with new climatic conditions. To simulate climate change, we transplanted 35 plant species varying in rarity to five botanical gardens in Switzerland, distributed along an altitudinal gradient. For each species we calculated how different the climate was between their natural habitats and the novel climate of the respective botanical garden, and investigated the relationship between rarity, climatic difference and plant performance. We found that rare species had generally lower survival and biomass production than widespread species. Moreover, fewer plants of rare species survived when the amount of precipitation differed more from the one in their natural range, indicating a higher susceptibility to climate change. Widespread species, in contrast, survived equally well under all climates and even increased their biomass under wetter or drier conditions. Our study shows that rarer species are less able to cope with changes in climate than more widespread ones, which might even benefit from these changes. Our results thus indicate that rare and endangered plant species might suffer strongly from future climate change.

Introduction

Understanding how species respond to a changing climate is one of the most important current challenges for ecologists. Particularly, information on whether rare, already endangered, and widespread species respond similarly to changes in climate is crucial. One of the most important hypotheses to explain rarity is the niche breadth hypothesis. It predicts that rare species have a smaller niche breadth, i.e. are less able to tolerate different environmental conditions, than widespread species (Brown 1984, Slatyer et al. 2013). It is therefore likely that rare species also have a lower tolerance to changes in their environment, such as changes in climate.

For plants, the predicted changes in temperature and precipitation can have profound implications for their growth and survival. An increase of 1 to 2°C in the global mean surface temperature (IPCC 2014) along with a reduction in the average amount of precipitation, and the occurrence of more extreme events such as droughts, directly impact the plants and change abiotic and biotic parameters. To survive climate change, plant populations may migrate to keep track of favorable environmental conditions, or they can tolerate and adapt to the new climate (Franks et al. 2013). However, migration may be limited, e.g. by topographic boundaries such as mountains, the increasing fragmentation of our landscapes (Jump & Peñuelas 2005), or for species with a long generation time (Aitken et al. 2008). Therefore, tolerance to climate change might be of particular importance for plants.

Many models predict that species will shift their ranges in response to climatic modifications (e.g. Bakkenes et al. 2002, Thomas et al. 2004b). They also hypothesize that a higher number of plant species will be threatened in a close future by the loss of climatically suitable areas (Thuiller et al. 2005). However, the fundamental climatic niche, i.e. the ability of species to tolerate new climates and persist in their habitat, has rarely been considered in studies predicting the future distribution of species.

Rare and widespread plant species have been shown to differ in many traits (Gaston 1994, Lavergne et al. 2004). Specifically, widespread species are generally larger (Cornwell & Ackerly 2010, Murray et al. 2002, Lavergne et al. 2003, Dawson et al. 2012), produce more and bigger flowers (Lavergne et al. 2004), are more competitive, grow faster and have more seedlings (Murray et al. 2002), and respond more positively to increased nutrient availability (Dawson et al. 2012) than rare species. However, whether rare and widespread species also differ in their tolerance to climate change is unknown, although it is likely that some species will be able to cope with new environmental conditions, whereas others will suffer if the climate becomes too different from the one they are adapted to, and that this is related to plant rarity. Hence, it is assumed that species with a greater range size, i.e. more widespread species, are more likely to occur in a wider range of habitats, and therefore to be generalists with a larger niche breadth (Brown 1984). Species with large ranges should therefore be more able to tolerate a changed climate than rare species which have smaller range sizes. Accordingly, plant species with a larger altitudinal niche breadth responded faster to climatic changes in the past, as they recolonized suitable sites after glaciation faster than species with a smaller altitudinal niche breadth (Dullinger et al. 2012). However, we lack knowledge on whether more widespread species are more tolerant to climatic variation, i.e. whether they have a larger fundamental climatic niche, than more rare and endangered species do. This information is crucial if we want to forecast the future composition of plant communities and to detect species that are particularly sensitive to climate change. Answering this question requires experimental approaches with many plant species (van Kleunen et al. 2014), which have not yet been applied in this context, however.

In this study, we tested the response of 35 plant species differing in rarity from rare and endangered to widespread species, to different climatic conditions. We used an altitudinal gradient in Switzerland, with a dryer and warmer climate at low altitudes and a wetter and colder climate at higher altitudes, to simulate climate change (Körner 2007). By transplanting the 35 plant species to five different botanical gardens along an altitudinal gradient, we were able to follow their survival and performance under various climatic conditions, which differed from the climatic conditions of their natural range. Using this experimental multi-species multi-site approach, we addressed the following specific questions: (i) Across different climatic conditions, do rare and widespread plant species generally differ in their survival and performance? (ii) Do rare and widespread plant species respond differently to changes in climatic conditions? We hypothesize that all species should perform best when the climatic conditions match the ones of their natural range. However, given that species with a small range size should have a narrower niche breadth than more widespread species (Slatyer et al. 2013), we expect rare species to be less tolerant to changes in climatic conditions, putting them at an even higher risk of extinction with climate warming.

Material & Methods

Plant species and experimental design

We obtained seeds of 35 plant species from 16 plant families (see Table S1). Twenty-four of those species were rare species with a priority for conservation in Switzerland (FOEN 2011, Moser et al. 2002), and 11 of them were species which are widespread in Switzerland. Seeds of rare plant species were collected in natural populations (one population per species) in Switzerland. Seeds of widespread species were collected in natural populations or obtained from commercial seed suppliers (Rieger-Hofmann GmbH, Germany and UFA Samen, Switzerland).

In March 2012, we germinated the seeds and planted 50 seedlings per species individually into 2-L pots filled with standard potting soil (Ricoter, Aarberg, Switzerland). Plants were then placed in a common garden (Mur, close to Bern, Switzerland) where they grew for another two months. In May 2012, we measured plant height to account for initial size differences. In June 2012 we transported the plants to five Botanical Gardens (Botanischer Garten der Universität Basel; Conservatoire et Jardin Botaniques de la Ville de Genève; La Thomasia, Pont-de-Nant; Flore-Alpe, Champex-Lac; Alpengarten, Schynige Platte) differing in altitude and climatic conditions (Table 1). In each garden, we placed, if possible, 10 pots per species (Table S2) and distributed them randomly into garden beds. In early summer 2013 we recorded the survival of the plants and collected aboveground biomass, cutting the plants at 2cm height to allow regrowth. Aboveground biomass was dried at 80°C for 72h and weighed to the nearest 0.01 g. Since watering happened only in case of severe drought, we can assume that the observed differences in plant growth between the gardens is indeed due to differences in precipitation and temperature and is not biased by the care taken by the botanical gardens.

Table 1. Location, altitude and climatic conditions of the five botanical gardens.

Botanical garden	Coordinates (CH1903)	Altitude (m)	Average annual precipitations (mm)	Average annual temperature (°C)
Basel	610797 - 267566	269.4	787.3	9.48
Geneva	500516 - 120219	372.2	909.5	9.53
Pont-de-Nant	500516 - 120219	1262.9	1451.1	5.98
Champex	574742 - 97996	1532.6	1376.9	4.19
Schynige Platte	636229 - 166947	1963.7	1630.6	1.61

Rarity and climatic variables

To obtain a continuous measure of plant rarity we used the range size of each species in Switzerland. Range size was expressed as the number of 10 x 10 km grid cells occupied by a given species in Switzerland (data provided by Info Flora, Bornand 2014). We used range size in Switzerland because a continuous measure of European range sizes for our species is not yet available. Nevertheless, for a subset of 21 species for which European range size is available, Swiss and European range size were positively correlated (r = 0.508, p < 0.001, Text S1).

For each species we calculated climatic values, which characterize the climatic conditions in the natural range of a species in Switzerland. We calculated the mean annual temperature and mean annual level of precipitation per species (Table S1) by extracting climatic information at all known locations of the species in Switzerland using precise coordinates (for complete details on the climate data, see Zimmermann & Kienast 1999). For each botanical garden we also extracted the mean annual temperature and mean annual level of precipitation (Table 1).

To define how different the climatic conditions between a botanical garden and a natural species range are, we calculated the temperature and precipitation differences by subtracting the climatic value of a species from the climatic value of a botanical garden. A negative value of a precipitation or temperature difference indicates that the climate is dryer or colder, respectively, in a botanical garden than in the species natural range.

The range size of our species was not related to the mean altitude (r = 0.01, p = 0.95) and the mean temperature (r = -0.08, p = 0.64) of their natural range. Species range size was positively related to the mean annual level of precipitation although the correlation was not very strong (r = 0.40, p = 0.02).

Statistical analysis

We tested whether species with a larger range size also occurred in a wider range of climates (i.e. whether they also have a larger climatic niche) correlating range size with the difference between the maximum and the minimum value of temperature and precipitation in a species natural range.

To test whether rare and widespread species generally differ in their survival and aboveground biomass production, we used generalized linear mixed effects models (*glmer)* with a binomial error distribution and linear mixed effects models (*lmer*) using the lme4 package (Bates et al. 2014) in R (R Core Team 2014), with the range size of the species as explanatory variable, the species identity nested into plant family (to account for taxonomy) and the botanical garden where the plants grew, as random factors. We also included the initial height of the plants as covariate, to control for initial size differences.

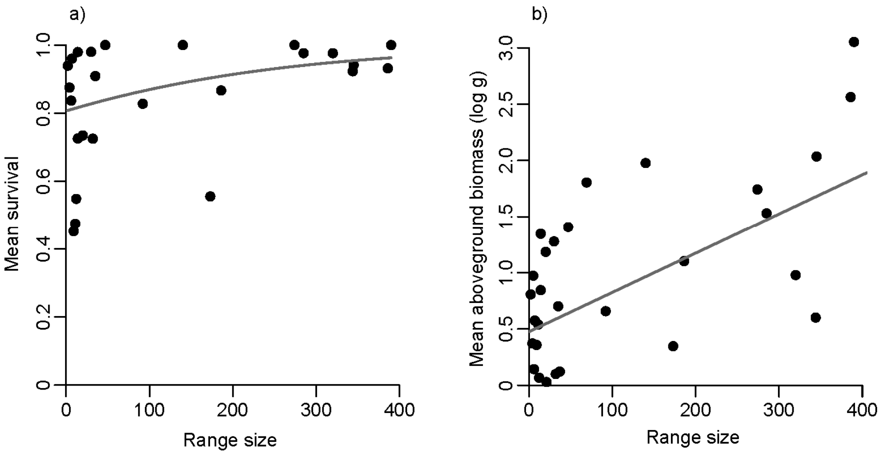
To test whether rare and widespread species respond differently in terms of their survival and aboveground biomass production to climatic differences, we used range size, temperature difference, precipitation difference, and the interaction between range size and climatic differences as explanatory variables. We also included the quadratic terms for the climatic differences as we expected a hump-shaped relationship with an optimum at a climatic difference of 0 (i.e. where the climatic conditions in a garden match the ones of a species natural range). Further, we included the interaction between the quadratic terms for the climatic differences and the range size of the species. Although the climatic variables 'temperature difference' and 'precipitation difference' were correlated with each other (r = -0.64, p < 0.001), both explained a significant part of the variation and were both kept in the model.

We simplified the full models by removing non-significant terms and we determined significances using likelihood-ratio tests comparing models with and without the factor of interest. Non-significant linear terms were kept when the corresponding interaction and quadratic terms were significant. To test for the significances of main factors, we removed higher order interactions. We log-transformed the biomass data and we scaled all continuous variables to means of zeros and standard deviations of one for an easier interpretation of the model estimates.

Results

Among species, range size was strongly correlated with the species temperature and precipitation niche breadth, i.e. with the difference between the maximum and the minimum temperature (r = 0.83, p < 0.001) and the maximum and minimum precipitation (r = 0.78, p < 0.001) in the natural range. This confirms that more widespread species occur in a wider range of climatic conditions than rarer species. Overall, species with a larger range size survived better (p = 0.049) and produced more aboveground biomass (p < 0.001) (Fig. 1), which indicates that across different climatic conditions widespread species generally performed better than rarer species.

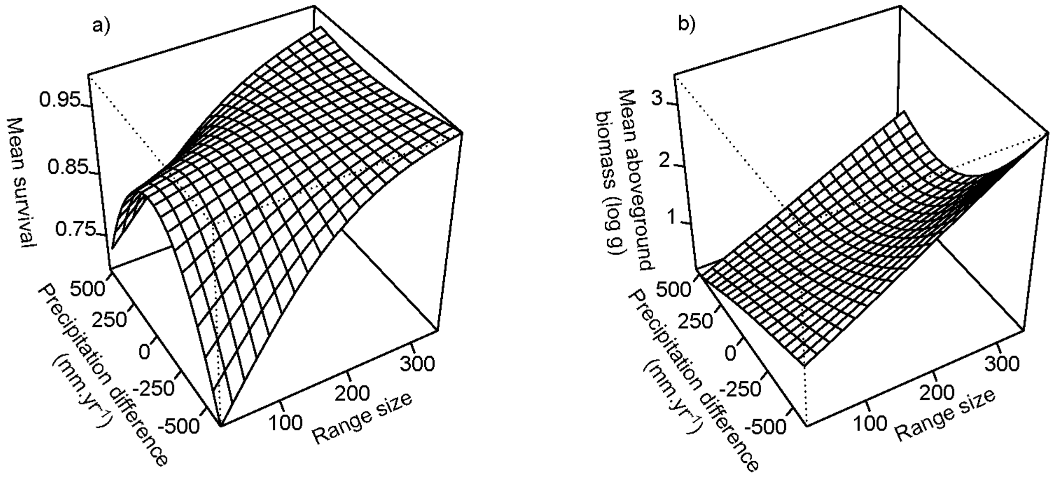
Figure 1. Effect of range size on a) mean survival, and b) mean aboveground biomass (expressed in g on a log-scale) for 35 species planted to five botanical gardens. Each point represents the mean biomass or survival per species, the line is obtained from the predicted values of the models. Range size is calculated as the number of 10x10km grid cell occupied by a given species in Switzerland. The curved line describing the relationship between range size and survival is obtained from the transformation of the binomial survival data into a continuous distribution of the probability of survival.



Survival was highest at low precipitation differences, i.e. when the climatic conditions of a garden were the most similar to a species natural ranges. This effect was only driven by rare plant species, whose survival decreased when the amount of precipitation in a garden differed from the one of their natural range (Table 2, Fig. 2a). In contrast, more widespread species were hardly affected by differences in precipitation, maintaining a high average survival in all botanical gardens (significant range size x squared precipitation difference interaction).

Aboveground biomass of rarer species was hardly affected by differences in precipitation between a botanical garden and the species natural range. Widespread species, however, produced more biomass when the conditions were drier - and thus sunnier - and when the conditions were wetter than in their natural range (Table 2, Fig 2b). This indicates that more widespread plant species are able to plastically increase their biomass in these conditions whereas rarer plant species cannot respond in such a way and show a relatively stable biomass production.

Figure 2. a) Survival and b) biomass production of 35 species in relation to precipitation difference between natural range size and botanical garden. The surfaces represent the predicted survival, respectively biomass, from the model. Biomass is expressed in g on a log-scale. A negative precipitation difference (mm.year^{-1}) indicates that the conditions in a garden are dryer than the ones in a species natural range.



All species performed worse when the temperature in a botanical garden deviated from the mean temperature of their natural range (significant squared temperature difference effect). This effect of temperature was found for survival and biomass production, and it did not differ between rare and widespread species (Table 2, Fig. 3).

Figure 3. Effect of the temperature differences (°C) on a) mean survival and b) mean aboveground biomass of 35 species planted in five botanical gardens. Each point represents the average aboveground biomass (in g on a log-scale) or survival per species per garden in 2013. The line is obtained from the predicted values of the models. To represent the effect of temperature difference, we fixed the value of precipitation difference to its mean when calculating the predicted values of the models.

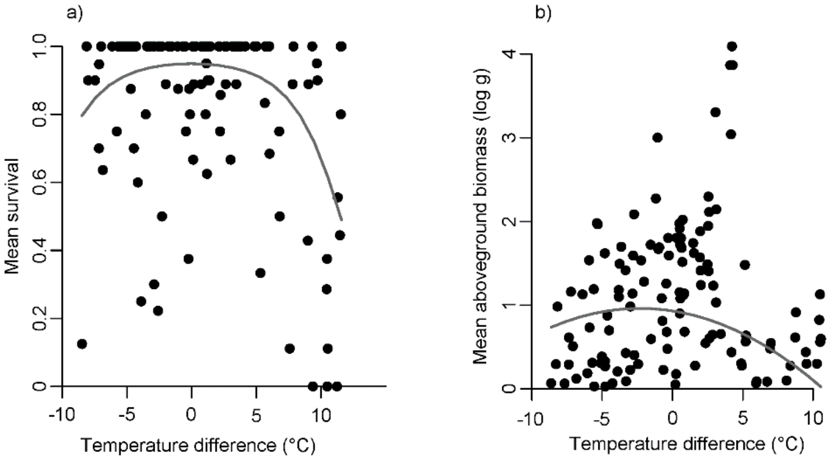


Table 2. Results of a linear mixed effects model and a generalized linear mixed effects model testing for an effect of range size, temperature difference between natural sites and botanical garden (Δ Temperature), precipitation difference (Δ Precipitation) and their interactions on biomass production and plant survival of plants of 35 species planted to five botanical gardens. We removed all non-significant terms, unless the respective quadratic or interaction term was significant. All explanatory variables are scaled. Variances of the random terms are given for each model. The parameters of the main factors were derived from models where all higher order interactions were removed.

	Biomass			Survival		
	estimate	p-value	Chi^2	estimate	p-value	Chi^2
Range	0.24	<0.001***	11.2	0.14	0.17	1.91
Δ Temperature	-0.05	0.206	1.59	-0.01	0.086	2.95
Δ Precipitations	-0.23	0.03*	11.6	-0.16	0.831	0.05
Δ Temperature^2	-0.13	<0.001***	67.8	-0.55	<0.001***	32.2
Δ Precipitations^2	0.04	0.446	0.58	-0.27	0.003**	8.85
Range x Δ Temperature	-	-	-	-	-	-
Range x Δ Precipitations	0.01	<0.001***	15	-	-	-
Range x Δ Temperature^2	-	-	-	-	-	-
Range x Δ Precipitations^2	0.09	<0.001***	26.2	0.20	0.033*	4.54
Family/Species		0.265			1.880	
Family		<0.001			<0.001	
Botanical Garden		0.016			0.924	

Discussion

Rare plant species are less tolerant to changes in climate than widespread plant species

Among the most important hypotheses to explain species rarity and commonness, the niche breadth hypothesis predicts that rare species have a narrower niche breadth than widespread species (Brown 1984, Slatyer et al. 2013), which is likely to reduce their climatic tolerance, i.e. their ability to cope with changing climatic conditions. In this study, using 35 plant species from different habitats, we experimentally showed that rare and widespread plant species respond differently to changed climate. Experimental tests of the environmental tolerance of multiple plant species as the ones we present in our study, and particularly of rare and widespread native species, are extremely rare (Slatyer et al. 2013). A few studies assessed the tolerance to different germination conditions (germination niche breadths) of rare and widespread plant species and found either a positive (Brändle et al. 2003, Luna et al. 2012), negative (Luna & Moreno 2010) or no relationship with range size (e.g. Gaston & Blackburn 2000, Thompson & Ceriani 2003). Our results, obtained two years after germination, show that species with restricted range sizes perform worse than more widespread species under a changed climate, and have indeed narrower physiological tolerances. Given the high number of plant species of different range size used in our study, we suggest that this is a general pattern.

Species with a greater range size may experience a larger range of ecological and climatic conditions, thus are expected to be adapted to a wider scope of climates (Gaston 2003) and have a higher physiological tolerance to temperature or water related stresses. Indeed, a larger niche breadth – based on the current distribution of a species – seems to be a general pattern in widespread species (Slatyer et al. 2013), and was also supported by our data (positive correlation between range size and the climatic breadth). A larger niche breadth can be either the result of many locally adapted populations (Olsson et al. 2009), or due to individuals genotypes within species and populations are ‘generalists’, and have larger environmental tolerance (Slatyer et al. 2013, Ackerly 2003). Although in our experiment we cannot entirely disentangle whether the more widespread species have a greater climatic tolerance due to locally adapted populations or individual ’generalists’, the fact that we found this pattern by placing only a few individuals into different climatic conditions indicates that widespread species are more likely to be comprised of individual generalists. However, to fully understand the influence of broad tolerance and microevolution on niche breadth, experiments simultaneously comparing the climatic tolerance of many species, populations per species, and genotypes per population are needed.

Generally, plant species in our experiment survived better and had a greater biomass when the climatic conditions of the botanical gardens were similar to the ones they experience in their natural range (Fig.

3). These results reflect the existence of a climatic niche due to physiological limitations, which is a key assumption for predicting the impact of climate change on species distributions (Wiens & Graham 2005, Pearman et al. 2008, Petitpierre et al. 2012, Lee-Yaw et al. 2016). Interestingly, the higher survival at lower precipitation differences to a species origin was only driven by rare plant species – the rarer a species the more it suffered from differences in precipitation (when conditions where either dryer or wetter than the ones at their origin). In contrast, more widespread species were not affected by precipitation differences, and showed a similarly high survival at all precipitation levels, independent of the ones of their origin (Table 2, Fig. 2a). Since climate change is expected to increase wet and dry extreme events (Knapp et al. 2008) our results suggest that species that are already threatened under the current climate will suffer most from the effects of climate change. Moreover, our results demonstrate that rarer species do indeed have a smaller fundamental niche in terms of precipitation, i.e. a lower climatic tolerance due to physiological limitations, than more widespread species.

In most cases widespread species experience a wider range of climatic conditions in their natural ranges than species with a more restricted range size. Therefore, the mean altitude, mean annual precipitation and mean temperature of the 11 species widespread in Switzerland was intermediate among those of the 25 rare species, some of which only occur in alpine or lowland regions (Figure S1). This reduced the range of data points in climatic differences for widespread species and might have affected extrapolations of our models at the extreme ends of climatic differences. To control for such potential bias, we analyzed a subset of our data by keeping only those rare species that occur within the same climatic range than our widespread species (Table S3). This analysis confirmed the effects of climatic differences and their interaction with range size found for the whole dataset. We conclude that more widespread species have a wider climatic tolerance than rarer ones, which is likely to have contributed to their larger ranges.

Aboveground biomass production of rarer species hardly changed in response to differences in precipitation. In contrast, more widespread species increased their biomass particularly when the amount of precipitation was lower than in their natural range (Table 2, Fig. 2b). Possibly, a dryer climate implies a higher number of sunny days and therefore more favorable conditions for plant growth. More widespread species therefore seem to be more able to plastically increase their biomass under favorable growing conditions, whereas rarer species seem to be less able to change their phenotypes in response to environmental variation. When precipitation was higher than in their natural range, more widespread species were also able to increase their biomass. This plastic response in more widespread species indicates that, in addition to maintaining generally high survival under different climatic conditions, widespread species were able to take advantage of both drier and wetter conditions. Widespread species have also been shown to be better able to take advantage of an increase in nutrient availability than rare species (Dawson et al. 2012) and, compared with species confined to river corridors, to better take advantage of benign conditions of non-river corridor conditions (Fischer et al. 2010). Our study therefore adds additional evidence that widespread species might be widespread as they are able to take advantage of favorable climatic and environmental conditions than species of small range size, and that this is a general pattern.

Under future climate change, with a predicted increase in extreme precipitation events (Easterling et al. 2000), our results indicate that more widespread species might better take advantage of the changing climatic conditions and potentially outcompete rarer species. This calls for developing measures to support rare species.

Rare plant species have lower survival and lower biomass than widespread plant species

Why some species are rare while others are widespread has fascinated ecologists for decades (Brown et al. 1996, Webb & Gaston 2003). Differences in species traits have repeatedly been suggested to explain the distribution and abundance of plant species in nature (Keddy 1992, Weiher et al. 1999). In our study, overall, rare plant species showed lower survival and lower biomass production than widespread plant species. This variation in the intrinsic general performance of plants could be a major driver of rarity and commonness at large spatial scales. Lower biomass of rare species has also been found in other studies (Murray et al. 2002, Lavergne et al. 2004, Cornwell & Ackerly 2010, Dawson et al. 2012) and indicates that rare species have slower growth rates (Cornelissen et al. 2003), a trait that is often attributed to slower nutrient uptake and hence lower competitive ability in productive habitats (Grime 1977). Concordantly, Dawson et al. (2012) showed that widespread species increased biomass more strongly in response to nutrient addition than rare species did. Usually, studies comparing rare and widespread species use pairs of species they had classified as rare and widespread. By using a continuous gradient of rarity and commonness with many species originating from different habitats, our approach suggests that a positive relationship between plant performance and plant range size is a general pattern. Future studies that take various aspects of rarity into account, including small and large populations of plant species differing in range size, are needed to ultimately test whether a lower general performance of species of small distribution range is a result of small population sizes and hence reduced genetic diversity (Leimu et al. 2006), or whether generally lower general fitness of such species is responsible for their small distributional ranges.

Conclusion

In this study, we used a gradient of range sizes and a gradient of climatic conditions to test whether rare and widespread plant species differ in their tolerance to climate change. We provide, based on a large number of species, experimental evidence that more widespread species indeed have larger climatic niches than rarer species. We showed that rare species not only have generally lower survival and biomass production than more widespread species but that they are also more susceptible to changed climate. On the contrary, more widespread species were not affected by a change in climate and even could take advantage of favorable growing conditions by plastically increasing their biomass. Our multi-species experiment suggests that this is a general pattern, and our results indicate that already rare and endangered species might suffer strongly from the forecasted climatic changes.

Acknowledgements

We thank Adrian Möhl and Christine Föhr for collecting the seeds in the natural populations; the teams of the Botanical Gardens of Basel, Champex, Geneva, Pont-de-Nant and Schynige Platte, for hosting the experiment and for their support; the numerous field assistants who helped harvesting the plants and collecting the data; Niklaus E. Zimmermann for providing the original climatic data; Eric Allan and Santiago Soliveres for their comments on an earlier draft of this manuscript. This study was supported by the Federal Office of the Environment, Switzerland.

Supplementary information

Text S1. To test whether range size in Switzerland is correlated with the European range size of our study species, we used map-derived area estimates from the Atlas Europeae (Meusel et al. 1978) for the 21 species for which these maps were available. We assessed the number of pixels of a species European distribution and cross-referenced these using islands, for which the exact surface values are known. Range size in Europe was significantly correlated with range size in Switzerland ($r = 0.508$, $p < 0.001$).

Meusel, H., Jäger, E. J., Rauschert, S. & Weinert, E. (1978). *Vergleichende Chorologie der zentraleuropäischen Flora. Bd. 2, Text u. Karten.* Gustav Fischer Verlag, Jena.

Table S1. List of the 24 rare and 11 widespread species (indicated by *) studied in this experiment, including their plant family, range size in Switzerland (number of 10x10 kilometers grid cells occupied by a species in Switzerland; Bornand 2014, see Methods), mean altitude, mean annual amount of precipitation and temperature of the species natural range, and the IUCN category of threat in Switzerland (LC: Least Concern; NT: Near Threatened; VU: Vulnerable; EN: Endangered; CR: Critically Endangered).

Species	Family	Range size	Mean altitude (m a.s.l.)	Mean annual precipitation (mm)	Mean annual temperature (°C)	IUCN status
Polycnemum majus	Amaranthaceae	69	608	707	9.2	EN
Oenanthe lachenalii	Apiaceae	20	422	1177	9.0	CR
Peucedanum venetum	Apiaceae	14	635	1563	9.8	VU
Seseli annuum	Apiaceae	47	768	1005	8.0	VU
*Artemisia absinthium**	Asteraceae	140	1132	907	6.4	LC
Artemisia glacialis	Asteraceae	9	2599	1291	-0.7	NT
*Artemisia umbelliformis**	Asteraceae	173	2406	1568	0.0	LC
Artemisia vallesiaca	Asteraceae	14	717	820	8.7	NT
Bidens cernua	Asteraceae	76	632	1249	8.0	EN
Bidens radiata	Asteraceae	3	495	1091	8.7	CR
*Bidens tripartita**	Asteraceae	118	478	1069	8.9	NT
*Centaurea scabiosa**	Asteraceae	320	746	1259	7.5	LC
Inula spiraeifolia	Asteraceae	4	661	1774	9.9	VU
Senecio halleri	Asteraceae	12	2463	1356	0.0	NT
*Sedum alpestre**	Crassulaceae	186	2534	1530	-1.0	LC
Sedum villosum	Crassulaceae	92	2175	1327	1.1	VU
Carex bohemica	Cyperaceae	2	429	1036	9.0	CR
Cyperus flavescens	Cyperaceae	155	374	1356	10.1	VU
Cyperus fuscus	Cyperaceae	151	433	1064	9.3	VU
Ephedra helvetica	Ephedraceae	6	612	726	9.3	VU
Astragalus leontinus	Fabaceae	32	2155	1063	1.4	NT
Stachys annua	Fabaceae	102	489	1037	8.9	VU
*Trifolium pratense**	Fabaceae	390	1193	1600	5.3	LC
*Trifolium repens**	Fabaceae	386	1178	1523	5.4	LC
Juncus arcticus	Juncaceae	35	2251	1237	0.8	VU
*Juncus articulatus**	Juncaceae	344	961	1495	6.6	LC
Ludwigia palustris	Onagraceae	21	384	1171	9.8	CR
*Linaria vulgaris**	Plantaginaceae	285	735	1270	7.5	LC
*Bromus erectus**	Poaceae	345	889	1189	6.9	LC
Cleistogenes serotina	Poaceae	11	457	1294	10.2	VU
*Rumex crispus**	Polygonaceae	274	867	1361	7.0	LC
Rumex hydrolapathum	Polygonaceae	30	448	1013	8.8	EN
Rumex maritimus	Polygonaceae	5	431	1038	9.0	CR
Nigella arvensis	Ranunculaceae	37	667	874	8.4	EN
Potentilla multifida	Rosaceae	7	2659	1307	-1.0	VU

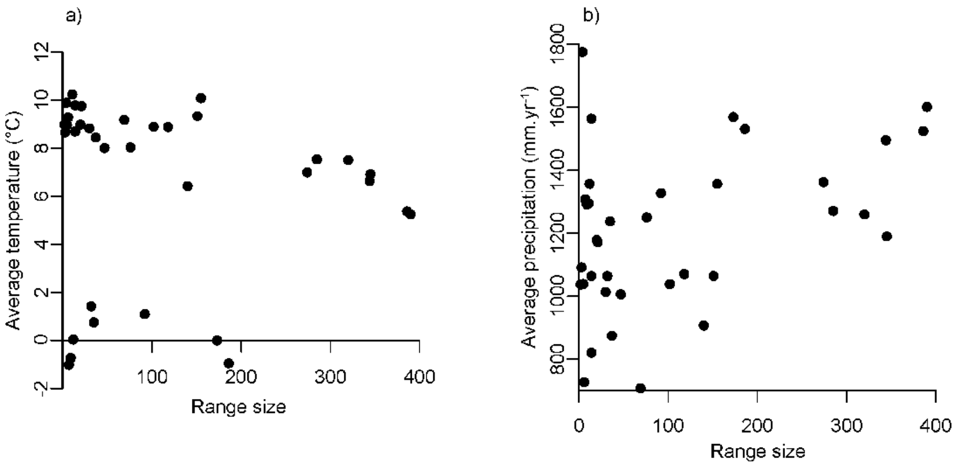
Table S2. Number of plants per species grown in each botanical garden.

	Botanical gardens				
Species	Geneva	Basel	Pont-de-Nant	Champex	Schynige Platte
Artemisia absinthium	8	7	6	7	9
Artemisia glacialis	9	8	9	7	9
Artemisia umbelliformis	9	8	4	8	8
Artemisia vallesiaca	10	10	10	10	11
Astragalus leontinus	9	7	7	9	9
Bidens cernua	10	10	10	10	10
Bidens radiata	10	10	11	10	10
Bidens tripartita	9	7	8	8	9
Bromus erectus	7	6	9	7	7
Carex bohemica	20	20	20	20	20
Centaurea scabiosa	9	8	8	8	9
Cleistogenes serotina	8	8	8	8	8
Cyperus flavescens	9	9	10	9	9
Cyperus fuscus	20	20	20	20	20
Ephedra helvetica	10	9	10	10	10
Inula spiraeifolia	10	9	10	10	10
Juncus arcticus	20	20	20	20	20
Juncus articulatus	8	9	5	9	8
Linaria vulgaris	9	8	7	9	9
Ludwigia palustris	9	10	10	9	10
Nigella arvensis	9	11	10	9	10
Oenanthe lachenalii	10	10	9	10	10
Peucedanum venetum	10	10	9	10	10
Polycnemum majus	10	10	9	10	10
Potentilla multifida	10	10	10	10	10
Rumex crispus	8	9	9	9	9
Rumex hydrolapathum	10	10	10	10	10
Rumex maritimus	10	10	10	10	10
Sedum alpestre	9	9	9	9	9
Sedum villosum	4	7	6	7	6
Senecio halleri	6	7	5	6	8
Seseli annuum	10	10	10	10	10
Stachy annua	10	9	10	10	10
Trifolium pratense	7	9	8	9	8
Trifolium repens	9	9	9	9	9

Table S3. Effect of climatic differences on the biomass production and the survival of a subset of 31 species. The rare species used in this experiment naturally occur in a wider range of climatic conditions than the widespread species used in this experiment (Fig. S1). We re-analyzed our data with a dataset including all the widespread species and a subset of 20 rare species, keeping only those which occur inside a precipitation range of 900 to 1600 mm.yr^{-1}. We considered the precipitation values to define this climatic range because it was the climatic variable which interacted with range size. The results did not differ significantly from the analysis of the entire dataset.

	Biomass			Survival		
	estimate	p-value	Chi^2	estimate	p-value	Chi^2
Range	0.19	0.006**	7.37	0.63	0.391	0.75
Δ Temperature	-0.09	0.4	0.71	1.17	0.173	1.86
Δ Precipitations	-0.28	0.017*	5.73	-	-	-
Δ Temperature^2	-0.15	<0.001***	82.8	-0.58	<0.001***	30.8
Δ Precipitations^2	0.01	0.186	1.75	-0.18	0.778	0.08
Range x Δ Temperature	-	-	-	-	-	-
Range x Δ Precipitations	0.05	<0.001***	11.4	-	-	-
Range x Δ Temperature^2	-	-	-	-	-	-
Range x Δ Precipitations^2	0.11	<0.001***	30.1	0.20	0.022*	5.22
Family/Species		0.284			17.63	
Family		<0.001			<0.001	
Botanical Garden		0.018			1.818	

Figure S1. Correlations between a) mean temperature (°C) and b) mean annual level of precipitation (mm.year^{-1}) in the natural range of our 35 species, and their range size. Widespread species showed more intermediate values than rarer species, although there was no correlation between range size and mean temperature (r = -0.08, p = 0.64), and the correlation between range size and mean annual precipitation (r = 0.40, p = 0.02) was not strong.



Chapter 5

Experimental introductions of eight rare and endangered plant species to the wild and the importance of genetic diversity for their success

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*equally contributing authors

Abstract

In the light of ongoing biodiversity losses the introduction of rare and endangered plant species to the wild has become a common conservation technique even though they are considered laborious, expensive and only rarely successful. It is broadly accepted that careful selection of suitable introduction sites matters largely for the success of introductions. Moreover, high genetic diversity among introduced plants is suggested to be important for the success of introductions. We tested whether it is possible to realize successful introductions with little effort and expenses when knowledge of experimental population biologists, field botanists and conservation practitioners is combined. We chose eight rare and endangered plant species of Switzerland, and introduced pregrown plantlets at one apparently suitable site per species. We planted 40 to 312 plants per site at two levels of genetic diversity, monoculture plots with offspring of single seed families per species and diversity plots with offspring of several seed families per species. After two years, four of the eight introductions appeared to be promising with 28 % - 78 % of the plant individuals surviving per introduction, whereas the other four introductions showed a very low survival of introduced plants (2 - 8 %). The low survival of the latter four introduced populations was likely due to drought and/or other stochastic events. In the early stages of the introductions, higher genetic diversity increased plant survival. However, this effect disappeared with time. Our study suggests that successful introduction to carefully selected sites are possible even for very rare and endangered species and that high genetic diversity can increase at least the initial short term success.

Introduction

Species diversity is lost at an unprecedented rate (Taberlet et al. 2012). Habitat loss and fragmentation have been termed the greatest drivers of the rapid decline in biodiversity (Sala et al. 2000) and measures to prevent further biodiversity loss have received considerable attention. Among those, habitat protection and restoration are the most important and most commonly used conservation methods (Maunder 1992). However, for plants, restored habitats often lack a sufficient seed bank, and dispersal of species into these sites is limited due to the fragmentation of our landscapes. To overcome these limitations, introductions, i.e. the deliberate release of organisms in a natural habitat (Wolf et al. 1996), have become an essential conservation technique (Vergeer et al. 2004, Menges 2008).

Introductions aim to ensure the long-term survival of populations, either by reinforcing the size and the genetic diversity of present populations, or by creating new self-sustaining populations in suitable habitats (Wolf et al. 1996). Despite their broad acceptance as an important tool to decrease inbreeding depression and genetic drift in declining populations and to overcome dispersal limitation (Vergeer et al. 2004), their use is discussed controversially as they are labour-intensive, expensive and often unsuccessful (Maunder 1992, Godefroid et al. 2011, Maschinski & Haskins 2012). Many factors can affect the success of rare plant species introductions, including the choice of a suitable habitat (Noël et al. 2011), the number and type (e.g. seeds, plants) of propagules introduced (Frankham et al. 2002) and the origin and genetic diversity of these propagules (Price & Waser 1979, Menges 2008, Maschinski & Haskins 2012). However, there is little scientific and specific knowledge on how these factors precisely contribute to the failure or success of introductions, as they are rarely performed as experiments (Godefroid et al. 2011). To assess the success of introductions, understanding plant population processes is required based on a sound experimental design, monitoring of individuals and rigorous data analysis. However, as collaborations between practitioners and scientific institutions appear to be very rare in plant introductions (Falk et al. 1996, Maschinski & Haskins 2012), most introductions are not performed as designed experiments and their success is not monitored quantitatively.

Many studies underline the importance of high genetic diversity for the fitness and survival of natural populations (Oostermeijer et al. 1994, Fischer & Matthies 1998). High genetic diversity increases the chance of having pre-adapted genotypes to future perturbations (Gamfeldt & Källström 2007), can lead to complementarity between different genotypes of a single species (Loreau & Hector 2001), decreases the impact of enemies such as herbivores or pathogens (Zhu et al. 2000, Tooker & Frank 2012), increases the probability of a population to adapt to changing environmental conditions (Mayr 1963, Ouborg & van Treuren 1994, Jump et al. 2009) and reduces inbreeding levels and thus the expression of inbreeding depression (Charlesworth & Charlesworth 1987, Barrett & Kohn 1991, Ellstrand & Elam 1993, Godt et al. 1996). Therefore, high genetic diversity might be crucial for successful introductions (Lesica & Allendorf 1999, Vergeer et al. 2005, Maschinski & Haskins 2012, Prati et al. 2016). There is however only limited experimental evidence for the positive effect of genetic diversity on the introduction success of rare species. For example, introductions of individuals of several seed families (i.e. seeds originating from different mother plants) of *Arnica montana* had increased survival compared to introductions of individuals of only one seed family (Vergeer et al. 2005). Moreover, reintroductions of several genotypes of the clonal *Ranunculus reptans,* a rare plant of Switzerland, were more successful than reintroductions of only single genotypes (Prati et al. 2016). Further, in a meta-analysis, Forsman (2013) found strong evidence of increased establishment success in plants and animals with higher levels of genetic and phenotypic diversity. However, of the nine studies on plants included in this meta-analysis only seven were performed in natural conditions and none of these studies introduced rare or endangered plant species. Therefore, it remains inconclusive whether high genetic diversity increases introduction success of rare plant species under natural conditions.

Switzerland harbours a species rich flora; however, about 30 % of its native vascular plant species are considered as threatened (Moser et al. 2002, Bornand et al. 2016), which is an alarming number. Due to extensive surveys of local botanists over the years, a large database on plant records (Bornand et al. 2016), as well as comprehensive knowledge on species characteristics and requirements is available for Switzerland (Landolt et al. 2010). On this basis, a list of priority species for conservation was established, taking into account both the degree to which a species is threatened and also the responsibility Switzerland has at a global level for a particular species (FOEN 2011).

For this study, we selected eight rare and endangered plant species from this list, originating from different habitats and plant families (Table 1). We then grew seedlings from field-collected seeds, and performed introductions with plantlets into natural habitats. For each species, we introduced plants at two levels of genetic diversity by either introducing offspring of only single mother plants (monoculture plots) or offspring of different mother plants (mixture plots) and compared their survival, plant height and flowering. This allowed us, on the one hand, to perform introductions with the aim to test whether establishing viable populations is possible at all with these very rare species, and on the other hand to test whether increased genetic diversity enhances the early establishment of introduced populations of rare plants.

Material & Methods

Study species and study sites

In 2011 and 2012 seeds of the eight selected rare plant species of conservation priority in Switzerland (Table 1, Moser et al. 2002, List of Priority Species at the National Level FOEN 2011) were collected from single natural populations. The seeds were harvested from 10-20 mother plants per species, and seed families (i.e. seeds originating from the same mother plant) were stored separately. In spring 2013 we sowed the seeds (separated by species and seed family) to germination trays. The seedlings were then transplanted into pots (Ø 9 cm) filled with normal potting soil (Ricoter, Aarberg, Switzerland) in a common garden (Botanical Garden of Bern, Switzerland). Between July and October 2013 we introduced each species into a separate natural site (Table S1). We selected the introduction sites for each species in collaboration with botanists and local conservation practitioners to minimize geographical distance and to maximise ecological similarity with the source populations.

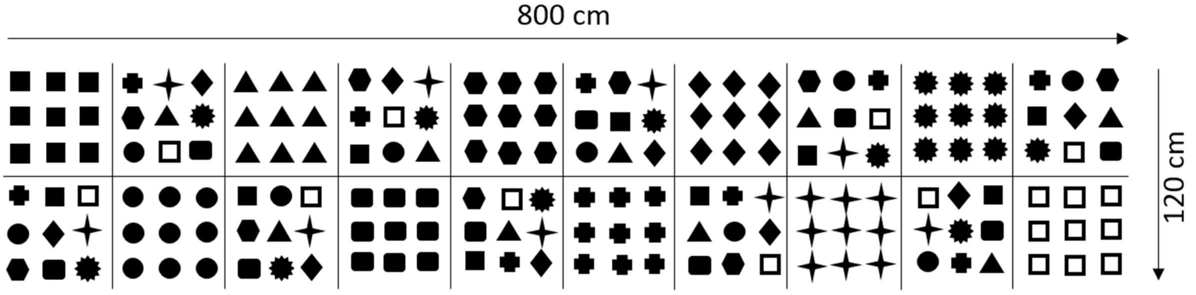
Table 1. List of the study species with their plant family, IUCN category of threat (VU: vulnerable, EN: endangered, CR: critically endangered, Red List of Switzerland, Moser et al. 2002), priority for conservation in Switzerland (1: very high, 2: high, 3: moderate, 4: low; List of National Priority Species, FOEN 2011) and their habitat preference (Lauber et al. 2012).

Species	Family	IUCN	Priority	Habitat
Allium angulosum	Amaryllidaceae	VU	4	wet meadows
Campanula cervicaria	Campanulaceae	EN	3	clay soils in shady places, changing soil humidity
Cleistogenes serotina	Poaceae	VU	4	dry grasslands
Inula helvetica	Asteraceae	VU	2	often next to bushes along water and at forests edges
Ludwigia palustris	Onagraceae	CR	2	trenches, at the margins of ponds, occasionally flooded
Oenanthe lachenalii	Apiaceae	CR	2	wet grasslands
Senecio erraticus	Asteraceae	EN	3	along moist forest paths, brooksides, and alluvial soils
Teucrium scordium	Lamiaceae	EN	3	in fens, in trenches, and on river banks

Study design

We defined two levels of genetic diversity: monoculture plots (each only with individuals of a single seed family) and mixture plots (with individuals of several seed families). We planted *n* (*n* = number of seed families per species ranging from 5 to 13) monoculture plots and *n* mixture plots per species. Each monoculture plot consisted of *n-1* individuals of one seed family. Each mixture plot consisted of a unique combination of *n-1* individuals of different seed families (Fig. 1, see Table S2 for more information on the introduction design for each species). We planted the individuals at 20 cm distance within plots and with 40 cm distance between plants of different plots. All plants were watered after planting but no additional care was provided thereafter.

Figure 1. Design of the introduction plots, example of *Allium angulosum* (180 individuals, 10 seed families, 10 monoculture plots, 10 mixture plots). Each entry represents one individual plant and each symbol represents one seed family.



Measurements

Two months after the introductions (1st season), and in spring 2014 (seven to ten months after the introductions, 2nd season), we recorded plant survival, measured the height of the introduced plants as a non-destructive estimation of plant performance, and recorded whether plants were flowering. Flowering could only be analysed for *Ludwigia palustris* and *Oenanthe lachenalii* in the 2nd season, as the other species did not flower yet or at very low numbers. In the 1st season, individuals from three species were too small to be measured with the same precision as the other species. Thus we did not record plant height for these species at this stage (Table 2).

In autumn 2015 (two years after the introductions, 3rd season) we monitored the introduced populations again. Since some species had produced offspring by sexual reproduction in the previous year and some by clonal growth, we were not always able to identify the originally planted individuals unequivocally and therefore refrained from assigning the plants to their respective seed families or treatments at this stage. For each species we counted the number of individuals, calculated population growth relative to the number of planted individuals and we counted the total number of flowering individuals.

Statistical analysis

To test whether plant performance was affected by genetic diversity, we used generalized linear mixed effect models (*glmer*) with a binomial error distribution for the survival and flowering data and linear mixed effect models (*lmer*) for the continuous plant height data (package lme4, Bates et al. 2015). We tested for the effect of genetic diversity after the 1st season and after the 2nd season. Plant height for dead individuals was set to NA to only look at the diversity effect on surviving individuals. The models included treatment (monoculture plot, mixture plot) as fixed effect, and species, seed family, and plot identity as random factors. We also included the time since transplantation (in days) as a covariate. We additionally carried out the analyses for each species separately, where we only included seed family and plot identity as random factors. We log-transformed plant height to account for non-normal distribution.

The effect of our treatment could not be analysed beyond the 2nd season (see above), as we were not able to assign the individual plants to their original treatment any longer. The data of the 3rd season therefore only serves as a measure of success of our introductions in the longer run.

All data were analysed using R, Version 3.3.2 (R Core Team 2016).

Results

Survival and population growth of introduced populations

By the 1st season (Table 2, Fig. 2) between 56 % and 100 % of the introduced individuals per species had survived and by the 2nd season between 41 % and 97 % of the introduced individuals per species had survived, indicating that all introductions were successful in the short term. After the 2nd season we found flowering individuals in the introduced populations of *Ludwigia palustris* (37 %), *Oenanthe lachenalii* (65 %) and *Senecio erraticus* (3 %). By the 3rd season four of the introduced populations had an overall population growth between 28 % and 78 % (*Senecio erraticus*, *Campanula cervicaria*, *Oenanthe lachenalii*, *Cleistogenes serotina*), while the other four populations had an overall population growth lower than 10 % (*Allium angulosum*, *Ludwigia palustris*, *Teucrium scordium*, *Inula helvetica*; Table 2, Fig. 2). We found 30 % of the individuals of *Campanula cervicaria* and 3.5 % of the individuals of *Cleistogenes serotina* flowering (Table 2) and observed clonal reproduction in the introduced populations of *Cleistogenes serotina* and *Senecio erraticus*.

Figure 2. Mean population growth (number of individuals counted relative to the number of individuals planted) of the individual species over the three measurements. 1st season was two month after the introductions, 2nd season was seven to ten month after the introduction, and 3rd season was two years after the introductions.

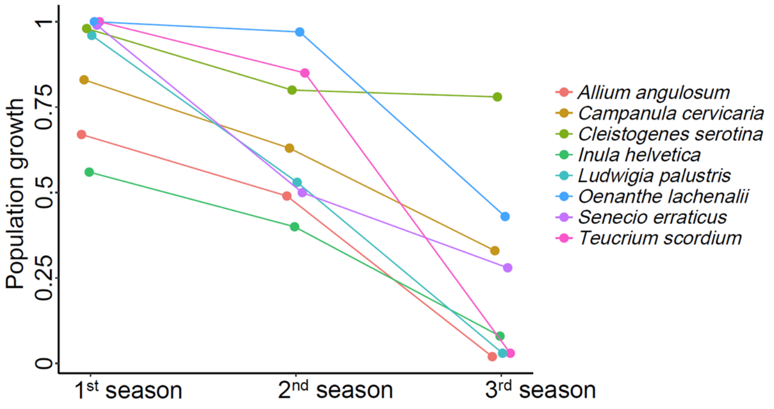
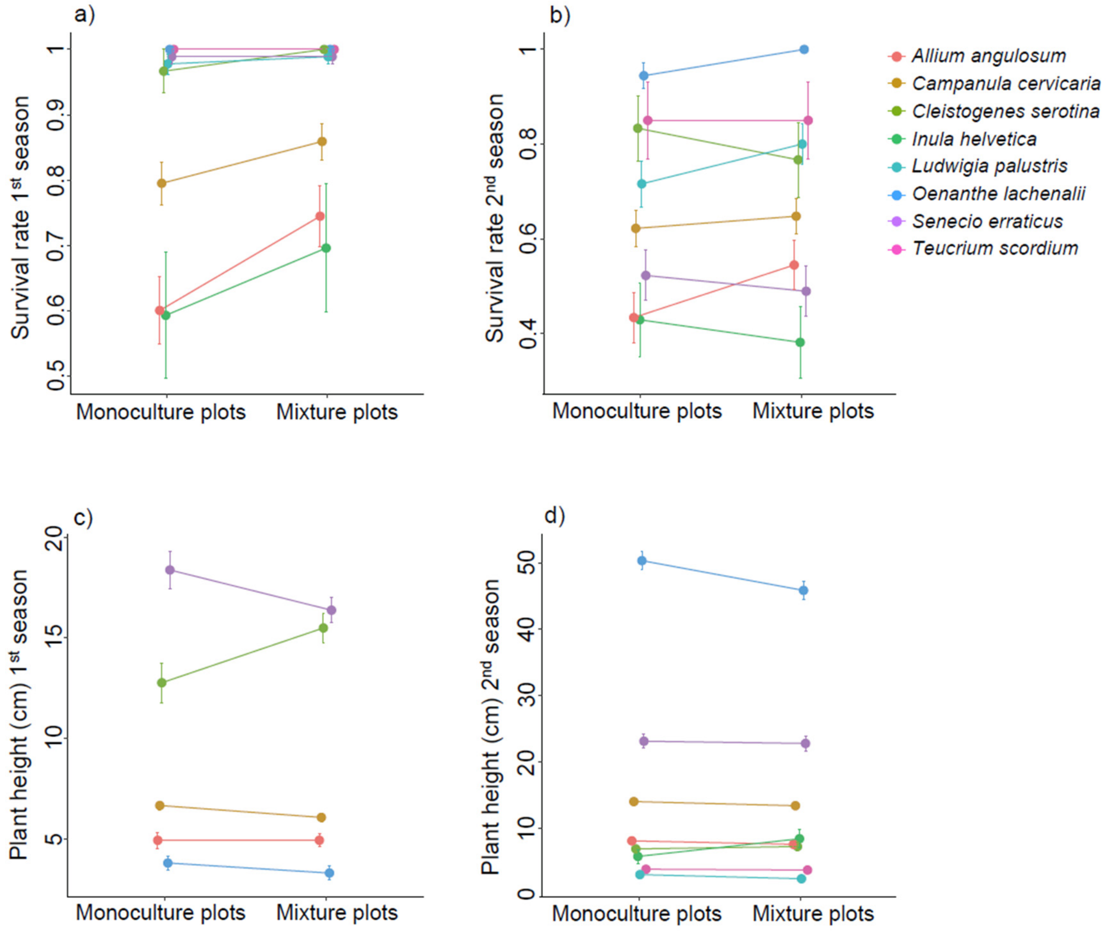


Table 2. Results of the experimental introduction of plants of eight rare species, each into a different field site. Survival denotes mean survival rate in relation to the number of introduced plants, height denotes mean plant height in cm, and flowering denotes the number of flowering individuals of the introduced populations in the 1st, 2nd, and 3rd season of the experiment.

Species	1st season			2nd season			3rd season	
	Survival	Height	Flowering	Survival	Height	Flowering	Survival	Flowering
Allium angulosum	0.67	4.9	0	0.49	7.8	0	0.02	0
Campanula cervicaria	0.83	6.3	0	0.63	13.7	0	0.33	30
Cleistogenes serotina	0.98	14.1	0	0.8	7.1	NA	0.78	2
Inula helvetica	0.56	NA	0	0.41	7	0	0.08	0
Ludwigia palustris	0.96	NA	0	0.53	2.7	66	0.03	0
Oenanthe lachenalii	1	3.5	0	0.97	48	93	0.43	NA
Senecio erraticus	0.99	17.4	0	0.5	23	5	0.28	0
Teucrium scordium	1	NA	0	0.85	3.8	0	0.03	0

Figure 3. Effect of genetic diversity on a) survival rate for the 1st and b) 2nd season, and c) plant height for the 1st and d) 2nd season of all eight plant species in monoculture plots (individuals of one seed family) and mixture plots (individuals of several seed families).



Effect of genetic diversity over all species

During the 1st season, over all species survival was by 5 % higher in the mixture plots than in the monoculture plots (p = 0.025, Fig. 3a, Table 3), while plant height was not affected by the genetic diversity treatment (Fig. 3c). By the 2nd season overall species survival (Fig. 3b) and plant height (Fig. 3d) did not differ between mixture and monoculture plots.

Effect of genetic diversity on individual species

Analysing each species separately, we found few differences in survival and plant height between monoculture and mixture plots. During the 1st season, individuals of *Allium angulosum* survived slightly better in the mixture plots (p = 0.025, Fig. S1a, Table 3). Individuals of *Cleistogenes serotina* were taller when growing in the mixture plots (p = 0.032, Fig. S1b, Table 3). However, for both species, these effects were transient and disappeared later. In the 2nd season, individuals of *Inula helvetica* were taller in the mixture plots (p = 0.098, Fig. S1c, Table 3), while individuals of *Oenanthe lachenalii* were taller in the monoculture plots (p = 0.058, Fig. S1d, Table 3).

Table 3. Outcome of linear mixed models testing for the effect of genetic diversity (seed family monoculture vs. mixture plots) on the average survival, plant height and flowering of experimentally introduced individuals of eight rare species. In the models overall species, we included time since transplantation as a covariate and genotype, species and plot identity as random terms. In the single species models, we included seed family and plot identity as random terms. Estimates are given for the significant and marginally significant terms only.

		Mean survival rate		Plant height		Flowering	
		estimate	p-value	estimate	p-value	estimate	p-value
1st season	Over all species	0.671	0.025 *	-	0.481	-	-
	Allium angulosum	0.789	0.068 $^{(*)}$	-	0.598	-	-
	Campanula cervicaria	-	0.150	-	0.173	-	-
	Cleistogenes serotina	-	0.236	2.522	0.032 *	-	-
	Ludwigia palustris	-	0.670	-	-	-	-
	Inula helvetica	-	0.598	-	-	-	-
	Oenanthe lachenalii	-	1	-	0.572	-	-
	Senecio erraticus	-	1	-	0.105	-	-
	Teucrium scordium	-	1	-	-	-	-
2nd season	Over all species	-	0.343	-	0.654	-	-
	Allium angulosum	-	0.241	-	0.512	-	-
	Campanula cervicaria	-	0.759	-	0.457	-	-
	Cleistogenes serotina	-	0.518	-	0.707	-	-
	Ludwigia palustris	-	0.337	-	0.672	-	0.915
	Inula helvetica	-	0.679	0.412	0.098 $^{(*)}$	-	-
	Oenanthe lachenalii	-	1	-4.404	0.058 $^{(*)}$	-	0.248
	Senecio erraticus	-	0.701	-	0.878	-	-
	Teucrium scordium	-	1	-	0.763	-	-

Discussion

Introductions of rare and endangered plant species

The introduction of endangered plant species has become an increasingly important conservation method, unfortunately introductions are often not successful. In a meta-analysis including 249 plant reintroductions, Godefroid et al. (2011) assessed a mean survival rate over all introductions included in their study of approximately 52 % and a flowering rate over all introductions of about 19 % after one year, and a further decrease of survival and flowering rate with time. In our study, all eight introduced populations were still alive one year after the introduction with a survival rate from 41 % to 97 %, and three introduced populations had flowering individuals. After two years, in four out of the eight introduced populations most individuals had died (survival rate < 10 %), most likely due to the very dry summer in 2015, where sites, which are normally occasionally flooded, fell dry during the whole year (*Allium angulosum, Ludwigia helvetica, Teucrium scordium*) and did not meet the species requirements any more. Two species suffered from intensive duck-grazing immediately after planting (*Allium angulosum*) or soil erosion from water running over the plot (*Inula helvetica*). Nevertheless, four out of eight introduced populations still had a survival rate between 28 % and 78 % after two years, and out of those, three introduced populations had flowering individuals or clonal reproduction. These observations reflect, on the one hand, the difficulties of meeting habitat requirements of introduced species and highlight the importance of introducing large populations to overcome environmental stochasticity such as extreme weather events or disturbances. On the other hand, they show the feasibility of our approach combining population-biological knowledge and botanical knowledge on species and habitats for introductions of very rare plant species. The success of our introduction experiment, which involved even fewer introduced plant individuals (40 to 312 per site) than recently recommended in Switzerland (about 500, Info Flora 2016), is very promising for further introductions. We suggest that future plant introductions should be accompanied by both scientists and practitioners to comprehensively address questions on species biology, habitat suitability, choice of sample sizes and genetic material of founder individuals, transplant design and data analysis.

Relevance of high genetic diversity

Initially, plants growing in plots of higher genetic diversity survived better than plants growing in plots of lower genetic diversity. This is in line with general considerations on ecological consequences of genetic diversity (Hughes et al. 2008) and it is consistent with the existing literature on the effect of genotypic variation on establishment success (Vergeer et al. 2005, Forsman 2013, Prati et al. 2016). Higher genetic diversity is expected to increase the probability of having adapted genotypes (sampling effect) and leads to increased resistance to enemies such as herbivores and pathogens (Zhu et al. 2000, Tooker & Frank 2012) or decreased intraspecific competition for resources between different genotypes (complementarity effect, Loreau & Hector 2001). The latter two mechanisms might have caused the positive diversity effect on survival that we found in the very first stage of our introductions shortly after planting, where stresses due to transplantation such as water stress, root disturbance, abiotic differences, or the encountering of new enemies such as herbivores and pathogens can be particularly drastic (South & Zwolinski 1996). We showed that genetic diversity can enhance introduction success, however, this effect might only occur at particularly stressful stages, in our case the early stage of an introduction. Accordingly, Prati et al. (2016) found a positive effect of genetic diversity on introduced populations of *Ranunculus reptans* only after a severe flood, in this case several years after the introduction.

The strength of the diversity effect on survival differed between species, partly because mortality in some species was too low to show any effect of genetic diversity. Differences in the genetic diversity effect between species might in addition reflect differences in genetic variation between source populations of our species. For very rare species monoculture and diversity plots may hardly differ from each other, because populations of rare and endangered species, such as our sampling populations, are expected to show little genetic variation within populations due to their generally small size and large degree of isolation (Gaston 2003, Leimu et al. 2006). That we still found a positive effect of genetic diversity in the very first stage of establishment underlines the importance of using genetically diverse plant material to maximise the success of introductions of endangered plant species.

In the 2nd season of our experiment, the effect of genetic diversity disappeared, suggesting that other factors such as habitat suitability and environmental stochasticity are more important for the mid-term to longer-term survival of introduced plant populations. Nevertheless, high genetic diversity seems to be beneficial under stressful conditions, and is expected to become particularly important at even longer time scales once the consequences of longer-term evolutionary processes become apparent (Vergeer et al. 2005). Future studies should thus address the interacting effects of population size and genetic diversity on introduction success and monitor the introduced populations as long as possible.

Height, which we measured as an additional parameter may indicate fitness as it is correlated with biomass production and associated with fecundity (Cornelissen et al. 2003), or it may indicate levels of competition for light (Falster & Westoby 2003). In our study, plant height generally suggested that the plants were growing healthy and it was not influenced by genetic diversity over all species. For the individual species the effect was variable. *Cleistogenes serotina* and *Inula helvetica* had taller plant individuals in the mixture plots than in the monoculture plots, either indicating better plant performance or increased intraspecific competition for light. In contrast, *Oenanthe lachenalii* had smaller individuals in the mixture plots than in the monoculture plots, either indicating poorer performance or reduced intraspecific competition for light. To assess plant performance more precisely we would have needed to measure biomass or to count seeds. However, as we did not want to interfere with the introduced plants we refrained from such measures. As different species showed varying results, in both measurements survival and height, we suggest that further studies should develop more biological knowledge for many rare and endangered plant species to disentangle the effects of specific characteristics (e.g. life form, pollination system) on introduction success.

Conclusion

We introduced populations of eight rare plant species, all threatened with extinction, transplanting a relatively low number of 40 to 312 individuals. Nevertheless, half of our introductions were promising after two years, which encourages the use of introductions to preserve rare and endangered plant species. The very low survival of four of our introduced species was most likely due to unexpected events, which made the habitats unsuitable for the species. Thus, we recommend introducing many individuals to buffer those stochastic events. Further we recommend the use of genetically diverse plant material for the introduction of rare and endangered plant species. Lastly, to increase the success of introductions and to save costs associated with unsuccessful introductions we advise close collaboration between experimental plant ecologists, field botanists and conservation practitioners at all stages of introductions, during species and site selection, also taking the likelihood of extreme events into account, when designing and setting up the experiment, and during monitoring. This will enhance our understanding of the underlying factors responsible for success or failure and will thus increase the success of future introductions of endangered plant species.

Acknowledgements

We thank Info Flora, Bern and Geneva, Switzerland, and the Botanical Garden of Bern for their practical support and their advice concerning regulations, and all federal and cantonal authorities, conservation experts, and botanists for their collaboration. We also thank Judith Hinderling, Karl Kasper, Yvonne Küenzi, Stefan Blaser, Armin Komposch, Janique Bertschi and Judith Minker for their help in the field; Daniel Prati and Eric Allan for their help with the analyses. We thank the Swiss Federal Office for the Environment for financial support.

Supporting information

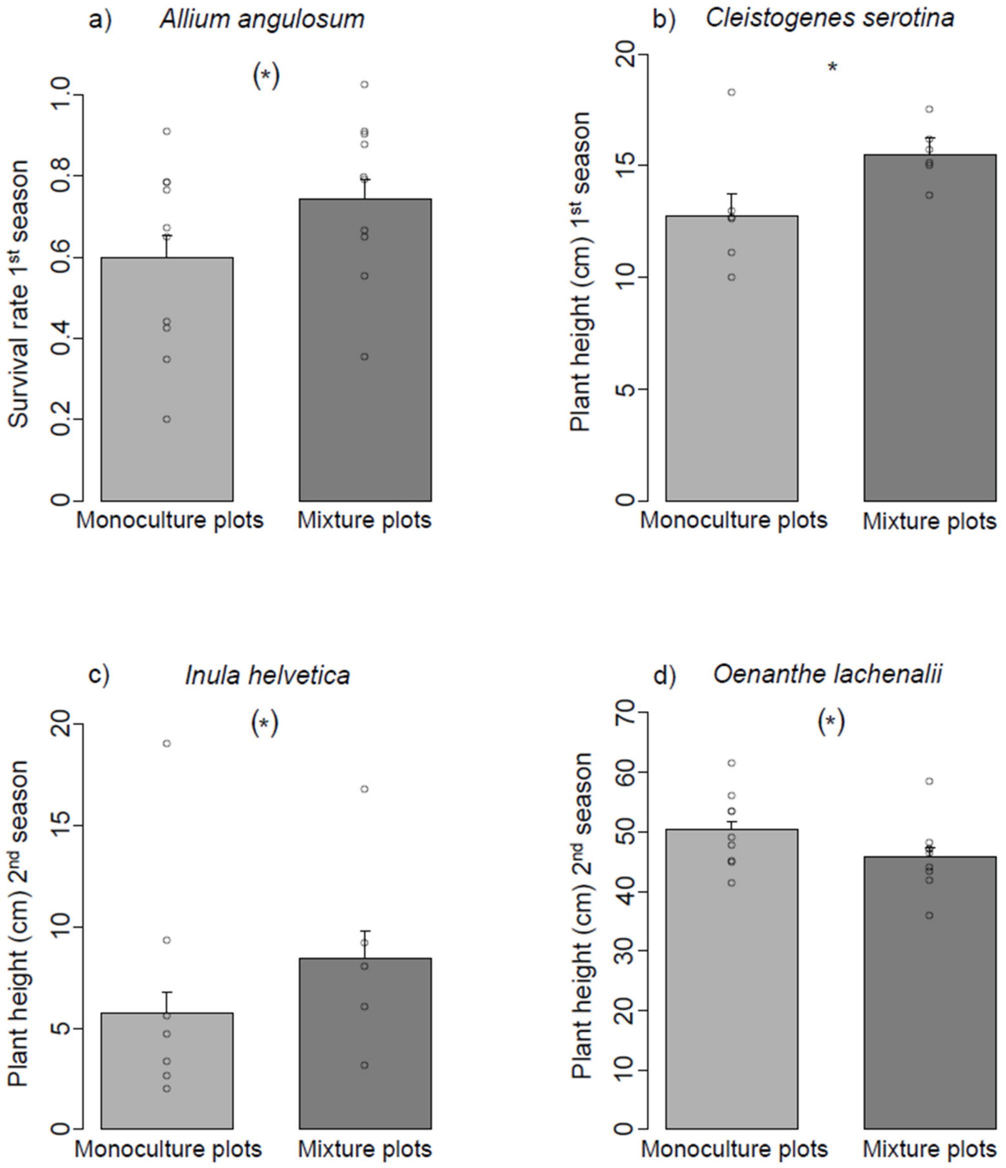
Table S1. List of the origins of the study species and the introduction locations. The abbreviations stand for different cantons in Switzerland, BE = Bern, GE = Genève, TI = Ticino, VD = Vaud, VS = Valais, ZH = Zürich.

Species	Seed origin	Year of harvest	Location of introduction
Allium angulosum	Mörigenbucht, BE	2012	Mörigenbucht, BE
Campanula cervicaria	Chlosteralp und Forst, BE	2012	Chlosteralp, BE
Cleistogenes serotina	Ayent, VS	2011	Aven, VS
Inula helvetica	Aeust am Albis, ZH	2012	Thürlerseegebiet, ZH
Ludwigia palustris	Bolle di Magadino, TI	2011	Verzasca Delta, TI
Oenanthe lachenalii	Au, ZH	2011	Au, ZH
Senecio erraticus	Aire-La-Ville, GE	2012	Commugny, VD
Teucrium scordium	Plaine des l'Orbe, VD	2012	NSG Häftli, BE

Table S2. Details on the design of the experimental species introductions.

Species	Number of seed families included	Number of plots in total	Number of individuals per plot	Total number of individuals introduced
Allium angulosum	10	20	9	180
Campanula cervicaria	13	26	12	312
Cleistogenes serotina	6	12	5	60
Inula helvetica	7	14	6	84
Ludwigia palustris	10	20	9	180
Oenanthe lachenalii	9	18	8	144
Senecio erraticus	10	20	9	180
Teucrium scordium	5	10	4	40

Figure S1. Effect of genetic diversity on mean survival rate and height in monoculture (offspring of one seed family, light grey bars) and mixture plots (offspring of several seed families, dark grey bars) for the species showing a significant or marginally significant effect of genetic diversity on survival rate or plant height. Each dot represents the mean value for a single plot. The error bars represent the standard errors.



Chapter 6

Summary and general conclusions

Most species are rare, while few are widespread (Gaston 1994, Brown et al. 1996, McGill et al. 2007). This has fascinated scientists from the time of the earliest biogeographers, and since then generations of scientists have tried to understand the drivers of species distribution and abundance. In addition to this fundamental interest in species rarity and commonness, it is also of great concern in conservation biology. Human-driven global changes such as land-cover change and associated habitat loss and fragmentation, or climate change, are threatening biodiversity, resulting in unprecedented species extinction rates (Thomas et al. 2004a, MEA 2005, Barnosky et al. 2011). Understanding why some species are rarer and more endangered than others, and the consequences of rarity for their populations is essential for developing the most efficient conservation measures.

This thesis aimed at contributing to our understanding of differences between rare and widespread species and at providing scientific knowledge informing conservation in the context of global changes. To allow us drawing general conclusions, we performed multi-species experiments (van Kleunen et al. 2014), involving dozens of plant species of various range sizes in Switzerland, from very rare and endangered to widespread.

Comparison of rare and widespread species: performance, heritability and response to climate change

A large number of studies have investigated trait differences between rare and widespread species (e.g. Murray et al. 2002, Lavergne et al. 2004, Cornwell & Ackerly 2010, Dawson et al. 2012). If traits are linked to the fitness of individuals and populations, these differences may explain why some species are rarer than others (Kunin & Gaston 1993, Gaston 1994). However, traits linked to establishment ability, which may have a direct influence on species abundance and distribution, have received little attention.

In the experiment presented in Chapter 2 we tested whether traits important for plant establishment are related to plant rarity. We weighed and germinated seeds of 32 rare and endangered and 39 widespread species of Switzerland. While seed mass and time of germination did not differ between species of different range sizes, rarer species showed significantly lower germination percentages than more widespread ones.

Based on these findings we conclude that seed mass and the timing of germination are unlikely to play an important role in the explanation of rarity. Differences in germination percentages, however, might be a cause of rarity by reducing the establishment potential of populations of rare species. Lower germination percentages might also be a consequence of rarity, as rare species are expected to occur in smaller and more isolated populations than widespread species and therefore suffer more from inbreeding depression. Based on a large number of species, our results indicate that the positive relationship between range size and germination percentages is a general pattern. In this experiment, we also tested whether rarer and more endangered species benefit more from a cold-stratification treatment than less rare and less endangered species. The effect of stratification on germination success was not related to the rarity or the endangerment of species, as overall all species benefited significantly from being cold-stratified.

Our results of Chapter 2 suggest that particular attention should be paid to the germination of rare and endangered species in ex-situ collections and introductions to the wild. It is encouraging for conservation that germinating large numbers of individuals of endangered plant species was possible, especially after applying a cold-stratification treatment to seeds of the rare and endangered plant species. Such stratification should therefore be considered for ex-situ collections and when preparing introductions.

As rare and endangered species are likely to occur in smaller and more isolated populations, they are expected to show lower levels of genetic diversity than less rare and less endangered species (Gaston 2003, Leimu et al. 2006). Several studies have demonstrated that it is indeed a general pattern that rare species are overall less genetically diverse than widespread ones (Karron 1987, Gitzendanner & Soltis 2000, He et al. 2000, Cole 2003, Edwards et al. 2014). However, these results were mostly based on molecular markers which are often selectively neutral and not well correlated to variation in ecologically important traits (Reed & Frankham 2011). Quantitative-genetic approaches, however, are based on trait variation of ecological relevant traits and allow us to estimate narrow-sense heritabilities, which represent the proportion of phenotypic variation due to additive genetic variance and hence indicate the evolutionary potential of populations (Falconer & Mackay 1996, Schaal et al. 1991, Storfer 1996, Petit et al. 2001). Whether the most rare and endangered plant species have lower heritabilities, and thus a lower evolutionary potential, than less rare species is uncertain, although it may have profound implications for the future of their populations which might be exposed to important environmental changes.

We aimed to fill this research gap in Chapter 3, by investigating heritabilities of quantitative traits linked to performance, sexual reproduction, resource use and size in 31 rare plant species differing in their range size, their IUCN category of threat and their priority for conservation in Switzerland. We found heritabilities to be independent of any of the three measures of rarity. Moreover, our estimates of heritability were comparable to those reported in the literature for other rare and even widespread species. To our knowledge, this is the first time a study assessed quantitative genetic trait variation and heritability in a single experiment involving multiple rare species. We conclude that rarer and more endangered plant species have generally similar levels of additive genetic variation, and thus similar evolutionary potential, than less rare and less endangered species.

This work has particularly important implications for the conservation of plant species. On the one hand, the risk of rapid adaptation of ex-situ collections to the environmental conditions of botanical gardens or ex-situ sites should be considered very carefully. On the other hand our results imply the positive message that rarer and more endangered plant species may be similarly able to adapt to future environmental changes than less rare or even widespread species.

Understanding how species will respond to climate change is a challenge of major interest for ecologists, and predicting climate effects on rare and endangered species is particularly crucial to develop effective conservation measures. It has been hypothesized that more widespread species have a larger niche breadth, i.e. can cope with a wider range of environmental conditions, than rarer species (Brown 1984, Gaston 2003, Slatyer et al. 2013, Bornand 2014). Consequently, widespread species should be more tolerant to changes in climatic conditions. However, no experimental study has addressed the link between plant rarity and the tolerance of plants to altered climates, which is fundamental to predict the impact of ongoing climate change on species extinction.

In Chapter 4, we filled this research gap using a multi-species experiment with 35 rare and widespread plant species varying in their range size in Switzerland, which, to simulate climate change, we planted in five botanical gardens situated at different altitudes. We investigated the relationship between species rarity, their performance and survival, and the climatic difference between the natural range of the species and the respective gardens. Our results show that rarer species generally had fewer surviving plants and produced less aboveground biomass than widespread species. Moreover, the survival of plants of rarer species was strongly reduced when precipitation levels differed from the ones of their natural range. More widespread species, in contrast, survived equally well under all climates, and were even able to increase their biomass under dryer or wetter conditions. To our knowledge our study is the first to demonstrate experimentally, with a large number of species and at different sites, that rare species do have a narrower fundamental climatic niche than widespread ones, and that species which are already rare and endangered might suffer strongly from the forecasted climate change.

These results have important implications for ecologists and biogeographers as they help explaining rarity in plant species. They also have important implications for conservation biologists and practitioners, as they will help developing relevant conservation measures focusing on species which are likely to be more affected by climate change.

Experimental introductions of rare and endangered plant species

To face the dramatic effects of human-driven global changes on plant biodiversity, the Global Strategy for Plant Conservation (SCBD 2010) recommends that not only at least three quarters of known threatened plant species should be conserved in natural populations (Target 7) and in ex-situ collections (Target 8a), but that at least a fifth of the threatened plant species should be available for recovery and restoration programs (Target 8b). In this context, endangered species introductions, i.e. the deliberate release of organisms to the wild, are a widely promoted conservation measure. However, such introductions are considered to be costly, labour intensive and often unsuccessful (Maunder 1992, Guerrant & Kaye 2007, Godefroid et al. 2011, Maschinski & Haskins 2012). Several factors can affect the success of plant species introductions, such as the number of propagules introduced, habitat suitability and genetic diversity of the introduced material (Vergeer et al. 2005, Menges 2008, Maschinski & Haskins 2012). However, as introductions of rare and endangered species are very rarely performed as experiments, scientific knowledge on the factors affecting their success is very scarce.

Hence, in Chapter 5 we investigated the feasibility and the factors affecting the success of endangered plant species introductions in a multi-species field experiment, performed in the framework of the MSc thesis of Deborah Schäfer. We introduced plants of eight rare and endangered species with a priority for conservation in Switzerland to natural habitats. For each species one habitat was carefully selected with the help of field botanists and local conservation experts, to maximize habitat similarity and minimize geographical distance to the source populations. In addition, each introduced population was established in replicate plots of two levels of genetic diversity to assess the effect of genetic diversity on the success of introductions. After two years of monitoring, four introduced populations appeared to be promising, whereas the other four were strongly and negatively affected by stochastic environmental events. Moreover, our results indicate that higher genetic diversity increased survival in the early stages of the introductions.

Despite relatively low numbers of introduced individuals, up to 312 per species, half of the experimental introductions of rare and endangered plant species were promising. We conclude from our findings that genetic diversity may increase the early success of introductions, and thus recommend the use of plant material as genetically diverse as possible. Further, we recommend introducing many individuals to buffer stochastic environmental changes that are likely to strongly affect small populations. Finally, our study highlights the importance of the collaboration between experimental scientists, field botanists and conservation practitioners to perform successful introductions of rare and endangered plant species

Final conclusions

This thesis addresses essential questions on plant species rarity, and using experimental multi-species approaches we were able to provide answers at a general scale. On the one hand my thesis provides new insights into the differences between rare and widespread species, and on the other hand it presents scientific evidence that can be directly applied to improve the efficiency of conservation measures.

This work highlights that rare and endangered plant species are particularly under threat in the context of global changes: they germinate less profusely and have a lower biomass than more widespread species, which might be a cause of their rarity and limit their ability to shift their ranges in the future. Moreover, rare and endangered plant species are likely to suffer strongly from climate change due to their smaller fundamental niches compared to more widespread species.

On the other hand, we show that rarer and more endangered plant species do not have a reduced evolutionary potential, which gives reason to hope that they will be similarly able to adapt to future environmental changes than less rare plant species. This may be possible provided that conservation efforts ensure the current persistence of their populations to allow sufficient time for adaptation. Our work further shows that introductions of endangered plant species are a promising and effective tool in the protection of plant biodiversity, especially when experimental scientists and field practitioners work together.

The use of large numbers of species, from very rare and endangered species to widespread ones, allowed us to draw general conclusions on the performance, evolutionary potential, response to environmental changes and conservation of rare plant species. I suggest that future research should continue in this direction and combine fundamental studies with applied conservation measures in multi-species experiments, to simultaneously provide scientific knowledge and support for concrete actions for the preservation of rare and endangered plant species.

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Declaration of consent

on the basis of Article 28 para. 2 of the RSL05 phil.-nat.

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I declare herewith that this thesis is my own work and that I have not used any sources other than those stated. I have indicated the adoption of quotations as well as thoughts taken from other authors as such in the thesis. I am aware that the Senate pursuant to Article 36 para. 1 lit. r of the University Act of 5 September, 1996 is authorised to revoke the title awarded on the basis of this thesis. I allow herewith inspection in this thesis.

Bern, 25.04.2017

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