Causes and consequences of the rarity of plant species

Inaugural dissertation
of the Faculty of Science,
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presented by

Sarah Audrey Bürli

from Klingnau AG, Switzerland

Supervisors of the doctoral thesis:

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“Let everything happen to you:

Beauty and terror.

Just keep going.

No feeling is final.”

Rainer-Maria Rilke

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Chapter 1

General introduction

Plant rarity

Some species are rarely encountered, while others are very abundant and widespread. This has intrigued biologists for at least a century (Darwin, 1859; Griggs, 1940; Baker, 1965; Stebbins, 1980; Gaston, 1994), and today it is even more central in biology, as around 40 percent of the world's plant species are threatened with extinction (Antonelli et al., 2020). The first necessary step towards understanding what causes a species to be rare is to define what a rare species is. Numerous attempts (e.g. Griggs, 1940; Stebbins, 1942; Drury, 1974; Smith, 1976) were made in the last decades. Most of them were based on a single characteristic of rare species (e.g. low competitive ability; Griggs, 1940). However, in 1981, a decisive step in defining plant rarity was taken with Deborah Rabinowitz's proposal of a typology to characterize species rarity based on three aspects: the local population size (i.e. local abundance), geographic range (i.e. regional distribution) and habitat specificity. Combining these aspects, she suggested eight categories of species, of which seven represent rare species. Hence, the term "rare plants" encompasses different types of plant rarity, including for example species that are consistently sparse with restricted distribution and specific habitat type and species restricted to a specific habitat but widely distributed with large populations. With this comprehensive definition of rarity, Rabinowitz (1981) defined a framework for future investigations on species rarity.

Variables to account for the three rarity aspects

It is important to consider all three aspects of plant rarity in studies, because, although related with each other (He & Gaston, 2000; Heino, 2005), they may have different implications for species biology. To evaluate regional rarity, we can calculate the range size that a species occupies at a given time. The range size can be derived from distribution maps, which are nowadays elaborated at different scale and made publicly available by several institutions (e.g. Info Flora for Switzerland, Floraweb for Germany, Siflore for France and Atlas Florae Europae for Europe). To account for the habitat specificity of a species, we can use indicator values, which describe the position of the realized ecological niche of a species along an environmental gradient (Ellenberg et al., 1991; Landolt et al., 2010). Landolt indicator values are the Swiss equivalent of the Ellenberg indicator values for Central Europe and are based on an ordinal scale from one to five. Moreover, the Landolt indicator value for dominance *in-situ* can be used to account for the local rarity of a species. Indeed, this variable describes the typical accumulation (i.e. abundance) of individuals of a species at places where it occurs. As an example, high Landolt indicator values for nutrients, moisture and dominance indicate that a species requires a rich and wet habitat and forms dense patches, i.e. is locally abundant. Thus, by using range sizes and Landolt indicator values, we can account for all three aspects of rarity defined by Rabinowitz (1981) when studying questions related to plant rarity.

Link between plant rarity and life-history strategy

Rabinowitz's (1981) three aspects of rarity, and in particular habitat specificity, typically correlate with other plant characteristics (Kempel et al., 2020; Ibanez et al., 2021), as these have evolved under the selective pressures typical for specific habitats. Depending on the environment, some characteristics may benefit species and increase their persistence, some may be neutral, while some others may be detrimental (Darwin, 1859; Kempel et al., 2011; Louthan et al., 2015). Along environmental gradients, species will thus perform and persist thanks to various characteristics. Some of these characteristics, however, trade off with each other e.g. due to limited resource availability. The environment in which a species lives, and the trade-offs it experiences, provide the context shaping its life history and resource allocation strategies (Kempel et al., 2020). Hence, many regionally rare species live in resource poor environments and are characterized by resource conservatism, whereas many common species are found in nutrient-rich environments and are adapted for high resource acquisition (Drury, 1974; Grime, 1979). Furthermore, plants from resource-poor environments exhibit inherently slower growth rates than plants from resource-rich environments (Coley et al., 1985). Therefore, if we aim to study differences between species varying in rarity and want to avoid biases due to context dependency, we need to take life-history and resource-allocation strategies into account (Murray at al. 2002; Kempel et al., 2020).

Accounting for different plant life-history strategies

One way to account for plant life history and resource allocation is to consider traits tightly linked to growth strategy. A simple measure of mean plant size after a given time of growth and under common environmental conditions provides rough information for comparing how several species allocate resources to growth. Furthermore, specific leaf area (SLA) and chlorophyll content are good candidates to account for plant resource allocation. SLA is used in the leaf economic spectrum to distinguish between plants with a fast-growing strategy (species with short-lived, nutrient-rich leaves and high SLA) and plants with a slow-growing strategy (species with long-lived, nutrient-poor leaves with low SLA; Westoby, 1998; Wright et al., 2004). SLA and chlorophyll content also provide information on allocation to defence against herbivores, which may trade off with other major aspects of resource allocation, such as growth (growth-defence trade-off: Coley et al., 1985). Grime's CSR life-strategy scheme (Grime, 1974; 2006) is another important element for accounting for plant resource allocation, as it describes the competitive ability of species for light capture. Accordingly, high competitive ability characterizes species with fast resource acquisition in productive environments, usually with short-lived leaves and high allocation to vegetative growth (Grime & Pierce, 2012).

Although many studies investigated patterns linked to plant rarity (e.g. Dawson et al., 2012; Kuglerová et al., 2019; Vincent et al., 2020), few conducted investigations taking into account the species local and regional rarity, habitat specificity, and traits linked to life history and resource allocation (e.g. Kempel et al. 2015 & 2020). Yet, this is the only way to disentangle patterns driven by the species rarity *per se* and by the species environment. This lack of comprehensive investigations precludes finding general patterns linked to plant rarity, which limits, in its turn, our general understanding of how rare and common plant species differ and what are the drivers of plant rarity.

Drivers of plant rarity

Currently, the leading cause of rarity and extinction of most species is habitat loss and fragmentation (Dirzo & Raven 2003; Laurance, 2008; Bennett & Saunders, 2010). Fragmentation divides populations into parts and leaves behind spatially disconnected patches of the remaining individuals with unsuitable habitat between them (Opdam et al., 1993). Once most of the habitat of a species is destroyed and its populations fragmented, other processes "finish the work", by further driving the species in the vortex towards rarity and ultimately extinction (Soule, 1982).

The hypothesis for causes of rarity can hence be broadly grouped into extrinsic (i.e. exerted by elements outside of the species) and intrinsic (i.e. exerted by species traits) factors: extrinsic factors encompass many aspects of global change, such as climatic change, land-use intensification, eutrophication, pollution, biological invasions and overexploitation (e.g. Dirzo & Raven, 2003; Helm et al., 2006; Enquist et al., 2019). Indeed, rare species respond less positively to environmental changes, such as nutrient and climate change, than common species do (Dawson et al., 2012; Vincent et al., 2020). Furthermore, an important hypothesis predicts that an important extrinsic driver of plant rarity may be biotic interactions ("apparency hypothesis": Cates & Orians, 1975; Feeny, 1976; Rhoades & Cates, 1976), as they were reported to alter plant abundance. For example, Kempel et al. (2015) showed that herbivore preference drives the abundance of plant species in communities. Moreover, plant-plant competition (Dawson et al., 2012) and soil biota (Gibson et al., 2010; Kempel et al., 2018) were indicated to impact rare species more than common ones.

In addition to these extrinsic factors, numerous intrinsic factors contribute to further driving plant species towards rarity: earlier hypotheses about what renders some species rare and some others common suggested that rare species might be either very young (Willis et al., 1922) and did not spread yet, or, alternatively “senescent” and are becoming extinct (Fernald, 1926). Both hypotheses have been partially refuted, as many rare species are known to be of intermediate age (Stebbins, 1980). Other studies emphasized the role of the ecological niche of species for driving plant rarity. The niche-breadth hypothesis is well-known and predicts that the geographical range size of a species increases with the width of its ecological niche (Brown, 1984; Slatyer et al., 2013). Species having narrower habitat preferences for abiotic soil parameters are rarer than species with broader preferences (Bornand, 2014; Wamelink et al., 2014). The position of the ecological niche has also been suggested to play a role in driving plant rarity (Hanski et al. 1993; Venier & Fahrig, 1996). Indeed, if the ecological requirements of a species restrict it to a habitat with low availability (i.e. marginal habitat), its distribution and local abundance will be low as well (Bornand, 2014; de Souza et al., 2022).

Further intrinsic factors linked to species ecology and morphology, reproductive biology, population dynamics, evolutionary history and genetics are also suggested to play major roles for driving plant rarity. Previous studies found that rare plant species have a lower overall reproductive effort than common plant species (Kunin & Gaston, 1993). For instance, rare plant species have reduced reproductive structures (i.e. fewer and smaller flowers) and recruitment (Lavergne et al., 2004; Vincent, 2017; Boyd et al., 2022), smaller statures and thus tend to establish less successfully (Lavergne et al., 2003 & 2004) than common species do. Rare plant species are also considered to be less competitive (Wilson & Tilman, 1993; Lauterbach et al., 2013) and have poorer dispersal ability (Normand et al., 2011; Lauterbach et al., 2013). Finally, another important hypothesis is that genetic factors play a decisive role for plant rarity. As indicated by Frankham (2005), most species become threatened when genetic processes start to play a role. Yet, rare species have a number of characteristics, such as low levels of genetic diversity and heterozygosity, long inbreeding history and high level of self-compatibility (e.g. Ellstrand & Elam, 1993; Oostermeijer et al., 2003; Charlesworth & Willis, 2009; Grossenbacher et al., 2017) that can lead them to suffer from inbreeding depression (i.e. fitness reduction of inbred individuals; Charlesworth & Charlesworth, 1987; Charlesworth & Willis, 2009) and become extinct (O’Grady et al., 2006; Allendorf et al., 2013)

If these intrinsic traits and the sensitivity to extrinsic factors are linked to the fitness of individuals and populations, these differences may further drive species towards rarity and explain why some species are rarer than others (Kunin & Gaston, 1993, Gaston, 1994; Vincent, 2017). However, despite the volume of the literature body dedicated to this research field, it is astonishing to realize that “rare” is a word describing most of the taxa in the world (Enquist et al., 2019), yet we are still lacking a full understanding of species rarity (Yates et al., 2007).

Difficulties encountered when investigating drivers of plant rarity

According to Yates et al. (2007), the number of different pathways through which a species may become rare is one of the reasons why it is complex to determine a general framework defining drivers of plant rarity and why this is currently still missing. The fact that rarity is multidimensional and that there are consequently several types of rarity (Rabinowitz 1981; Fielder & Ahouse, 1992) is another reason. To this list, I would add a third one: the difficulty to conduct multi-species experiments. Such experiments are indeed notoriously difficult to conduct, in general due to their size, and especially for rare species. Because rare species have usually low germination percentage, slow growth and specific environmental requirements (Dawson et al., 2012; Wamelink et al., 2014; Vincent, 2017; Boyd et al., 2022), conducting multi-species experiments with rare species is thus even more complex than with common ones. Although autecological studies allow detailed investigations and useful findings on specific rare species, multi-species experiments are the key to infer general patterns. Indeed, conducting autecological studies on each rare plant species is considered simply not feasible (Edwards, 1996; Murray et al., 2002). Moreover, considering that the current rate of species extinction is estimated to be a thousand times higher than the natural rate of extinction (i.e. extinction rate without humans as the primary cause; Pimm et al., 2014), we need a quicker alternative if we want to mitigate the biodiversity loss. Yet, most studies working on rare plant species have conducted experiments on one or few species and without comparison with (closely related) common species (but see Kempel et al., 2018; Vincent et al., 2020). In addition, meta-analyses can quickly reach their limits due to the wide variation in design and reported information between studies (Walker et al., 2008). It is therefore essential to conduct multi-species experiments to study drivers and consequences of plant rarity. In this thesis, I therefore performed multi-species experiments to test important hypotheses that have long been debated but still lacking robust multi-species tests.

Generalist herbivory as a driver of plant rarity

The first hypothesis we focused on is called the “apparency hypothesis” and was developed in the 1970s (Cates & Orians, 1975; Feeny, 1976; Rhoades & Cates, 1976). It stipulates that plant’s “apparency” determines its allocation to defence against herbivory. “Less apparent” (e.g. rare) plants would be less defended than apparent (e.g. common) species and if subsequently exposed to generalitst herbivores, they would be more consumed. In addition, genetic depauperation of rare species (Broennimann et al., 2005; IUCN, 2012) may contribute to lower their resistance to enemies (Gaston, 1994; Spielman et al., 2004; Laine, 2006). This hypothetical difference of allocation to defences between “apparent” and “less apparent” species has been tested in animals (Altizer et al., 2007), between plants of different successional status (Cates & Orians, 1975) and with plant pathogens (Gibson et al., 2010; Kempel et al., 2018). However, it lacks a robust test for multiple generalist herbivores and with multiple rare and common plant species. Moreover, the experiment should account for plant local and regional rarity, habitat and resource-allocation and assess both herbivore performance and preference, as plant defence may target them differentially (Kempel et al., 2015). If the results of such a rigorous test support the idea that plant rarity is a good proxy for plant investment into defence, this may suggest that rare plant species are threatened by generalist herbivory. Direct implications for conservation strategies and policies for rare plants would follow from this conclusion (see e.g. Bevill et al., 2003)

Genetic processes as a driver of plant rarity

The second hypothesis we focused on stipulates that rare plant species may be rare due to the occurrence of genetic processes with negative feedback loops in their populations (Young et al., 1996; Frankham et al., 2002; Frankham, 2005; O'Grady et al., 2006). This hypothesis stems from studies in genetic and population biology and is built on the following considerations. The allele pool within the small and isolated patches of a fragmented population (Opdam et al., 1993; Young et al., 1996; Frankham et al., 2002; Frankham, 2005) decreases relatively to the original population. By simultaneously increasing genetic drift and inbreeding (Ellstrand & Elam, 1993; Oostermeijer et al., 2003; Charlesworth & Willis, 2009), this has been shown to reduce fitness in numerous plant and animal species (i.e. inbreeding depression; Leimu et al., 2006; Charlesworth & Willis, 2009 and references therein). Population survival is therefore compromised and if this happens in many populations of a species, or if the species has only few remnant populations, this is expected to reduce species abundance and distribution. However and in opposition with this hypothesis, Angeloni et al. (2011) reported in their meta-analysis that inbreeding depression significantly increased with population size. Hence, it is still debated whether rare plant species suffer more from inbreeding depression than common species do.

To determine whether genetic processes occurring in small populations drive plant rarity, further studies examining how inbreeding impacts rare and common plant species are needed. Controlled pollinations can be used to achieve this goal, as experimental selfing, outcrossing between closely related and between distantly related individuals permits to increase and decrease inbreeding levels in plant offspring in a simple way. Furthermore, this method allows testing simultaneously another hypothesis about plant rarity. It was suggested that negative consequences of population fragmentation can be reversed by restoring gene flow between population fragments or re-establishing allelic diversity by crossing two closely-related populations of the same species ecotype (Willi et al., 2007; Frankham et al., 2011). Yet, population outcrossing can have negative consequences (e.g. Edmands & Timmerman, 2003; Tallmon et al., 2004), which are often feared and preclude the use of outcrossing in conservation, despite its potential to rescue genetically depauperate populations (Frankham et al., 2011).

Hence, it is necessary to test these hypotheses rigorously in a multi-species framework taking into account plant resource-allocation and the three aspects of rarity. The results of such a study are of fundamental interest for plant ecology and population biology and would allow informing conservation practice, e.g. on the implications of using single versus mixed origins in *ex-situ* cultures and translocations.

Plant translocation as experimental approach combining research and conservation

Drivers of plant rarity can be studied in *ex-situ* experiments, such as in greenhouses, but also in *in-situ* experiments through conservation programmes. This approach allows for proactive actions to support rare species while, at the same time, it allows testing practical implications of biological hypotheses and expanding our scientific knowledge (Bontrager et al., 2014). Translocations of native species, which are defined as the reinforcement, reintroduction, and introduction of populations (IUCN, 2013), are a common tool in species conservation worldwide. So far many programs of translocations have been carried out. However, most of these were performed for single species and without any experimental design or scientific monitoring, rendering general determinants of translocation failure or success unknown (Fischer & Lindenmayer, 2000; Godefroid et al., 2011; Fenu et al., 2019). In addition, because many translocation programmes have little success, the proportion of case studies that are not reported or are reported only in the grey literature is high, biasing the international literature towards the most successful programmes (Griffith et al., 1989; Fischer & Lindenmayer, 2000; Deredec & Courchamp, 2007; Godefroid et al., 2011). We therefore require translocation programs including several species and specifically designed and monitored to determine factors increasing establishment success, survival and fitness of the translocated populations.

Among the numerous factors known to alter translocation outcome (Godefroid et al., 2011 & 2016), genetic diversity and propagule pressure (i.e. the number of individuals released; Lockwood et al., 2005) may play a determinant role, as suggested by their positive effects on the establishment of invasive species, natural and translocated populations (Oostermeijer et al., 1994; Fischer & Matthies, 1998; Lockwood et al., 2005; Vergeer et al., 2005; Leimu et al., 2006; Robert et al., 2007; Menges, 2008; Godefroid et al., 2011; Forsman, 2014; Schäfer et al., 2020). However, there is also evidences that high genetic diversity and high propagule pressure can have negative effects on populations (Waser & Price, 1989; Marshall & Spalton, 2000; Kéry et al., 2001; Pugnaire et al., 2004; Huff et al., 2011). Furthermore, the source populations, from which translocated material originates, vary in several genetic, ecological and demographic characteristics. Thus, their identity is expected to have a considerable impact on the fitness of translocated plants and interact with other factors such as genetic diversity and propagule pressure. Considering that genetic diversity, propagule pressure and source-population identity are all central factors when planning translocations, we are lacking an inter-factorial investigation conducted for several species to determine how these factors interact with each other. Examining the consequences of such interactions for the fitness of translocated plants would advance our knowledge in plant ecology, genetic and population demography (Macnab, 1983) and, in addition, is necessary to inform conservation practitioners and policies.

This thesis

Although understanding drivers of plant rarity has made good progress in recent decades, the main reasons rendering species rare in specific contexts and determining the best conservation strategies to mitigate species extinction requires further studies (Yates et al., 2007; Ibanez et al., 2021). To test central hypotheses about plant rarity, multi-species settings are especially powerful. Multi-species experiments enable robust comparison of traits between species varying in rarity, life-history traits and strategies and allow inferring general patterns of plant rarity both across species and along ecological gradients (van Kleunen et al., 2014). In addition, if we aim to contribute to the development of a general framework defining plant rarity, we need to study species varying in all three aspects of rarity defined by Rabinowitz (1981): local abundance, regional distribution and habitat specificity. Finally, as indicated by Kempel et al. (2020), we also need to account for plant traits linked to the species resource-allocation and life-history, as this allows disentangling whether differences between rare and common plant species are driven by species rarity *per se* or rather by its environment.

In this thesis I aim to answer open and important questions on species rarity and conservation. It stands on the shoulders of many inspiring studies (e.g. Cates & Orians, 1975; Rabinowitz, 1981; Spielman et al., 2004; Vergeer et al., 2005; Robert et al., 2007; Menges, 2008; Charlesworth & Willis, 2009; Godefroid et al., 2011; Kempel et al., 2015, 2018 & 2020; Vincent, 2017; Schäfer et al., 2020). By testing long-standing and influential hypotheses about rare plants, this thesis contributes to our knowledge on the ecology of trophic interactions, population biology and conservation of rare plant species. Furthermore, by investigating factors interacting in altering the fitness of translocated plants, it contributes to best practice guidelines for rare plant conservation, with significant implications for conservation scientists and practitioners.

This thesis is divided in five chapters. After the introductory chapter, chapters two to four present three experiments, which I conducted in the framework of this thesis. These chapters are structured like research papers. Experiments were performed in Switzerland with more than 60 different herbaceous plant species in total. The species were selected to cover 16 different plant families and several habitat types and regions of Switzerland. Most importantly, species were selected to vary in local abundance, regional distribution and in resource richness of their habitat, to represent a wide variety of rarity types (Rabinowitz, 1981). A third of the selected species are (very) widespread and two thirds are rather rare to extremely rare in Switzerland. Species we considered as rare (expect one; see chapter two) are either of Swiss conservation priority (OFEV, 2019) or are considered as near-threatened or threatened by the Swiss Red List of vascular plants (Bornand et al., 2016). In addition, we accounted for resource-allocation trade-offs in plants by using mean species size, SLA, leaf chlorophyll-content (i.e. growth traits) and a variable called "competitive strategy", which is based on the plant life strategies of Landolt et al. (2010) and Grime (1979 & 2006).

In chapter two, we put the "apparency hypothesis" (Cates & Orians, 1975; Feeny, 1976; Rhoades & Cates, 1976) to the test. We assessed the performance and preference of one belowground and three aboveground generalist invertebrate herbivores from different taxonomic groups feeding on multiple plant species from Switzerland. We used 56 and 38 plant species for the performance and the preference experiment, respectively. We analysed the effects of plant local and regional rarity, habitat specificity, competitive strategy and growth traits on herbivore performance and preference and identified the most important factors determining plant defence and palatability. Moreover, we tested whether the performance of generalist herbivores is consistently related to their preference, as predicted by the "mother-knows-best" principle (Jaenik, 1978; Gripenberg et al., 2010). Lastly, we evaluated whether food plant quality is recognized in the same way by different generalist herbivore species.

In chapter three, we focused on determining the effect of selfing and outcrossing on the fitness of rare and common plant species. To do so, we performed three types of controlled hand-pollinations: selfing and outcrossing between seed families (i.e. offspring of different maternal plants), within and between populations. Using multiple fitness-related traits, we analysed how local and regional rarity, habitat specificity, competitive strategy and growth traits are shaping fitness responses to selfing and outcrossing in plants. Furthermore, we examined the results from a conservation perspective to provide recommendations for safeguarding rare plants.

In chapter four, we reintroduced four rare and threatened plant species from Switzerland following a factorial design allowing us to investigate the importance of the effect of propagule pressure, genetic diversity and source population on plant fitness. We investigated the effect of source populations in the light of their ecological distance to the reintroduction sites. By monitoring vegetative growth and reproductive structures over a two-year period, we tested how the experimental factors interact with each other in their effect on plant fitness. We also accounted for how management actions, stochastic environmental events and species habitat-type alter the early success of translocations.

Lastly, in chapter five, I summarize the main results from all chapters, make links between them and draw conclusions across the three experiments. This allows me to contribute to general recommendations for the conservation of rare and threatened plants and to identify implications for future studies. I conclude this thesis with my personal perspective.

Chapter 2

Are rare plant species less defended against herbivores? A study using multiple plant species and four above- and below-ground generalist herbivores

Sarah Bürli, Andreas Ensslin, Anne Kempel, Markus Fischer

Abstract

1. Rare plant species are suggested to be less defended against herbivores than common species, because their lower apparency might have led to the evolution of reduced defences. Moreover, their often lower levels of genetic diversity compared with common species could negatively affect their resistance against enemies. However, the hypothesis that plant defence depends on plant regional and local rarity, regardless of habitat and growth strategy, lacks evidence.

2. To test this hypothesis, we assessed the performance and preference of one belowground and three aboveground generalist invertebrate herbivores from different taxonomic groups feeding on multiple plant species from Switzerland. We accounted for differences in plant growth and competitive strategy, resource availability in the species habitat and phylogenetic relatedness.

3. We found that regionally and locally rare and common plant species did not generally differ in their defence against generalist herbivores. Moreover, and contrary to the apparency hypothesis, one herbivore species even preferred common plant species over rare ones, indicating that common species are not more defended, but tend to be less defended. We also found that all herbivore species consistently performed better on competitive plant species, although different herbivore species did not generally prefer the same plant species. The latter indicates that the use of generalist herbivores as indicators of general defence levels can be misleading.

4. *Synthesis*: Our results challenge the idea that rare plant species are less defended than common ones, and strongly suggest that the hypothesis that apparent plant species are more defended against herbivores does not hold for herbaceous plant species. Instead, our results suggest that the ability of plants to allocate resources away from defence to achieve a stronger competitive ability might have allowed plants to become locally and regionally common.

Keywords: apparency hypothesis; feeding experiment; generalist invertebrate herbivores; growth-rate hypothesis; herbivore performance; herbivore preference; plant palatability; regional and local rarity.

Declarations

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Conflict of interest: The authors declare no conflict of interest.

Introduction

The reasons why some plant species are rare, while some others are common may have important ecological implications for herbivores. Several hypotheses have been formulated about the relationship between regional and local plant rarity and the evolution of defence against herbivores. Plant species occurring at low abundances are less apparent and have a low probability to be found by generalist herbivores. They are therefore suggested to reduce their allocation to quantitative defence, which are costly and work mostly against generalist herbivores (Smilanich et al., 2016). In contrast, plant species that form dense stands are thought to invest more into defence, because they are apparent and "bound to be found" by generalist herbivores (apparency hypothesis, Cates & Orians, 1975; Feeny, 1976; Rhoades & Cates, 1976).

Similarly, regionally rare plant species often have declining populations and reduced genetic diversity (Ellstrand & Elam, 1993; Oostermeijer et al., 2003). This could negatively affect the formation of their defence (Gaston, 1994; Spielman et al., 2004). Moreover, populations of regionally rare species are usually isolated. This may limit dispersal of generalist and specialist herbivores to them, leading to fewer encounters of herbivores within their habitats (Altizer et al., 2007; Gibson et al., 2010; Smilanich et al., 2016). Such a reduced exposure to herbivores may have led to the evolution of reduced herbivore defences. Hence, regionally rare species may be more palatable than common species (Laine, 2006) if subsequently exposed to herbivores. Some of these ideas have been tested in animals (Altizer et al., 2007) or with plant pathogens (Gibson et al., 2010; Kempel et al., 2018), but we still lack a general understanding of whether plant defence depends on plant regional and local rarity.

Plants have evolved a variety of defence strategies to reduce the amount of consumed plant tissue by decreasing herbivore performance and preference (Walling, 2000; Karban & Baldwin, 2007). In addition to the variety of plant defence strategies, herbivore species differ in their host specificity (Ali & Agrawal, 2012), their feeding strategies (Strong et al., 1984) and feeding compartment (above- or belowground), which makes the assessment of plant defence a complex endeavour. An efficient way to assess plant defence is to investigate the performance and preference of generalist herbivores, which are related to herbivore fitness (tested in no-choice experiments) and feeding behavior (tested in choice or cafeteria experiments; e.g. Díaz, 2000; Kempel et al., 2015), respectively. Although herbivore fitness and feeding behavior are expected to be tightly linked in herbivorous insects ("mother-knows-best" principle: Jaenike, 1978; Gripenberg et al., 2010), it is important to assess both, as they may be affected differentially by plant defence (Kempel et al., 2015).

Hence, several studies have used generalist herbivores as indicators of plant defence and have studied the combined effects of many traits providing resistance to plants, such as nutritional quality and defence (often referred to as palatability; Schädler et al., 2003; Kempel et al., 2015; Kempel et al., 2018). While such comparative feeding assays have been commonly used to inform about differences in defence investment between invasive and native plant populations (e.g. Siemann & Rogers, 2003; Caño et al., 2009) or species (e.g. Pearson et al., 2011; Kempel et al., 2013), they have rarely been used in the context of plant rarity. Moreover, they have been confined to very few plant species (Landa & Rabinowitz, 1983; Fiedler, 1987; Baskin et al., 1997; but see Cottam, 1985; Ancheta & Heard, 2011; Kempel et al., 2020). Thus, to adequately test whether locally and regionally rare plant species differ in their defence against generalist herbivores from common and dominant (i.e. apparent) species, studies comparing the performance and preference of several generalist herbivore species differing in their feeding strategies on multiple plant species are needed.

Plant defence depends also on the growth and competitive strategy of plants and the environment in which they have evolved (Olff & Ritchie, 1998; Proulx & Mazumder, 1998; Kempel et al., 2020). Plants from resource-poor environments exhibit inherently slower growth rates than plants from resource-rich environments (Coley et al., 1985). As a consequence, slower-growing species might be less able to replace lost tissue and invest more into defences than faster-growing, more competitive species from productive environments, which typically tolerate herbivores better (Grime, 1979; Bryant et al., 1989; Díaz, 2000; Gianoli & Salgado Luarte, 2017). Hence, Coley et al. (1985) suggested that the optimal level of defence investment increases as the plant growth-rate decreases. Moreover, many common species have adaptations for high resource acquisition and fast growth, whereas regionally rare species are characterized by resource conservatism and are thus limited to resource poor environments (Drury, 1974; Grime, 1979; Kempel et al., 2020). To rigorously test for differences in generalist herbivore defence in regionally and locally rare and common plant species, it is therefore important to account for variation in plant-species growth and competitive strategy and the resource availability in the species habitat.

Here, we present a multi-species experiment where we compare the performance (no-choice) on 38 plant species and the preference (choice) on 56 plant species from Switzerland of one belowground and three aboveground generalist invertebrate herbivores from different taxonomic groups. At the same time, we accounted for differences in plant regional and local rarity, growth and competitive strategy and the resource availability in the species habitat. We used only generalist herbivores, as such a multi-species experiment is impossible to be conducted with specialists. Specifically, we addressed the following questions: 1) Do generalist herbivores differ in their performance and preference when feeding on locally and regionally rare plant species compared with common plant species? 2) Are the performance and preference of generalist herbivores affected by plant growth, competitive strategy and the resource availability in the species habitat? 3) Are the herbivore performance and preference related within and across generalist herbivore species, and do different herbivore species perceive plant defence similarly?

Material & Methods

Plant species and rarity

We selected 62 plant species from 16 families to cover a broad variety of families, rarity level, habitats, and regions of Switzerland. 19 selected species are common and 43 are rather rare to extremely rare in Switzerland (Table S1 in the Supporting Information). All rare plant species, except *Bromus squarrosus*, have either a Swiss conservation priority (OFEV, 2019) or are considered as near-threatened or threatened by the Swiss Red List of vascular plants (Bornand et al., 2016). *Bromus squarrosus* was considered rare after correspondence with A. Möhl from the National Data and Information Center of the Swiss Flora (Info Flora, https://www.infoflora.ch).

As a measure of species regional rarity, we used the maximum range size of a species calculated as the highest number of 5x5 km grid squares that it occupied during the last century in Switzerland (Bornand, 2014). We used the range size in Switzerland, because the range size of the plant species used in this study is not yet available at this resolution at the European scale. However, Vincent et al. (2020) showed that European and Swiss range size of 21 plant species are positively correlated (r=0.508, P<0.001).

As a measure of species local rarity, we used the indicator value for dominance *in-situ* (DG) according to Landolt et al. (2010). The indicator value for dominance *in-situ* describes the accumulation of plants of the same species at the place where they occur. It spans from a value of one for a plant species with scattered individuals to five for a plant species that is usually dominant.

Plant collection

Seeds of 10 seed families (i.e. seeds from 10 different maternal plants) were collected from one or two populations of the 62 selected plant species. As rare plants were collected in several regions of Switzerland due to their restricted distribution ranges, populations of common plants were selected to cover those regions as well. To break seed dormancy, Fabaceae seeds were scarified with a scalpel and seeds of other plant families were cold-stratified in pots over eight weeks in the dark at 4°C. Then, seeds were allowed to germinate in a greenhouse. After eight weeks of germination and growth, 10 seedlings *per se*ed-family and population were randomly selected and pricked out individually into pots filled with a mixture of sand and potting soil (Selmaterra, Eric Schweizer, Thun, Switzerland; ratio 1:9). Plants were watered daily or every other day and allowed to grow for three months (constant day length of 14 hours with additional light and temperature between 15-30°C). Plants were kept in the same greenhouse conditions to ensure that differences between species are not due to differences in growing conditions.

Plant habitat and growth and competitive strategy traits

To characterize the resource availability in plant habitat, we used the species indicator values for nutrients (N) and moisture (F) according to Landolt et al. (2010). Indicator values describe the realized ecological niche of a species by its position along an environmental gradient (Landolt et al., 2010; Ellenberg et al., 1991). The indicator values for nutrients and moisture characterize the nutrient content in the soil (mainly nitrogen) and the average soil moisture during the growth period of the focal species, following an ordinal scale ranging from one (very nutrient poor and dry) to five (very fertile and moist). They are the Swiss equivalent of the indicator values for nutrients and moisture according to Ellenberg et al. (1991) for Central Europe. For each species, we also defined a variable called "competitive strategy" on the basis of the species life-strategies from Landolt et al. (2010; which was partly adapted from Grime's CSR life strategies; Grime, 1979 and 2006). This variable describes the competitive ability for light of a species. To do so, we assigned the values "0", "1" and "2" to ruderal or stress-tolerator (e.g. rrr, rrs, rss or sss), competitive (e.g. crr, csr or css) and strongly competitive (e.g. ccs or ccr; Table S1) species, respectively.

We measured the size of the experimental plants as the highest stem height, or the longest leaf length including the petiole, in the cases where the plants had only a rosette. We then calculated the mean size per species. We measured the specific leaf area (here after called SLA), following the method from Cornelissen et al. (2003). We also measured the chlorophyll concentration of one leaf from five plants from different seed families per species with a chlorophyll-concentration meter (Chlorophyll Meter SPAD-502 from Konica Minolta) in SPAD (unit based on the leaf absorbance in red and near-infrared, which is proportional to the chlorophyll amount present in the leaf; https://www.konicaminolta.com). Then we averaged the SLA and chlorophyll concentration per species. High SLA and leaf chlorophyll concentrations increase plant palatability to herbivores (Coley & Barone, 1996; Poorter et al., 2004; Schuldt et al., 2012). SLA is a trait used in the leaf-economic spectrum (Westoby, 1998; Wright et al., 2004), which distinguishes between plants with a fast growth strategy (species with short-lived, nutrient-rich leaves and high SLA) and plants with a slow growth strategy (species with long-lived, nutrient-poor leaves with a low SLA). Slow-growing species are suggested to be more defended against herbivores than fast-growing species (growth-defence trade-off: Coley et al., 1985), which makes them less preferred by herbivores. A high SLA, nutrient requirement and competitive ability are known to be characteristics of fast-growing plant species (Coley et al., 1985; Poorter & Remkes, 1990; Westoby, 1998).

Herbivore species

To assess herbivore performance and preference, we used one belowground and three aboveground invertebrate generalist herbivore species. As a belowground herbivore, we used larvae of the cockchafer *Melolontha melolontha* Linnaeus 1758 (hereafter called *Melolontha*; Coleoptera: *Scarabaeidae*). This species occurs naturally all over Europe (CABI, 2018) and has been a major pest in former times. Second instar *Melolontha* larvae were collected in agricultural fields in Urmein and Bristen in Eastern and Central Switzerland. None of our plant species were collected in these two regions, so we can rule out any potential pre-adaptation of the herbivore to plant populations. Larvae were reared at 10°C in individual pots filled with a damp mix of grated carrots and soil. As aboveground herbivore, we chose the leaf-chewing caterpillars of *Spodopteralittoralis* Boisduval 1833 (hereafter called *Spodoptera*; Lepidoptera: Noctuidae). This species occurs mostly in Africa and the Middle East but also in South Europe (CABI, 2018). Neonate caterpillars from laboratory reared strains (Syngenta, Basel, Switzerland) were kept at room temperature (24±4°C) and fed *ad libitum* with maize-based artificial diet until the start of the experiment. As further aboveground herbivore, we used *Helixaspersamaxima* snails Taylor 1883 (hereafter called *Helix*; Gastropoda: *Helicidae*). This species originates from North Africa but can be found nowadays in Europe, Asia, Australia, South and North America (CABI, 2018). Pre-adult snails were bought from a commercial seller (Etis Schneckenpark, Grossgurmels, Switzerland). They were kept for 48 hours in large plastic containers (38x28x20 cm) with 2 cm of damp soil and closed with plastic wrap to water-saturate the air in the box and standardize snail water content (Ledergerber et al., 1998; Staikou, 1999) before they entered the experiment. During this time, they were fed *ad libitum* with fresh lettuce leaves for the first 24 hours and then with wet paper tissues to starve them and to standardize their gut content. As last aboveground herbivore, we used locusts of the species *Locustamigratoria* Linnaeus 1785 (hereafter called *Locusta*; Orthoptera: *Acrididae*). This species occurs naturally in Europe, Africa, Asia and Australia (CABI, 2018). Pre-adult locusts were bought from a commercial seller (Pocerias, Bürglen, Switzerland) and placed in boxes (25x15x5 cm) with sawdust and fed with egg cartons to starve them and to standardize their gut content for the last 24 hours before they entered the experiment. All four herbivore species are adequate model organisms to study herbivore performance and preference on plants because they are known to feed on a wide range of plant species (Bont et al., 2017; Kempel et al., 2015; Gomot & Pihan, 1997; Bernays & Chapman, 1977; Brown & Dewhurst, 1975). Moreover, as the three non-native herbivore species to Switzerland are unlikely to share a co-evolutionary history with any of the plant species used in this study, it is expected that their behavior reflects the general quality of the different plant species as food source.

Herbivore experiments

For all herbivores, two experiments using multiple plant species were conducted in a greenhouse: a no-choice feeding experiment, hereafter called Performance Experiment, and a pairwise choice experiment, hereafter called Preference Experiment (Fig. 1).

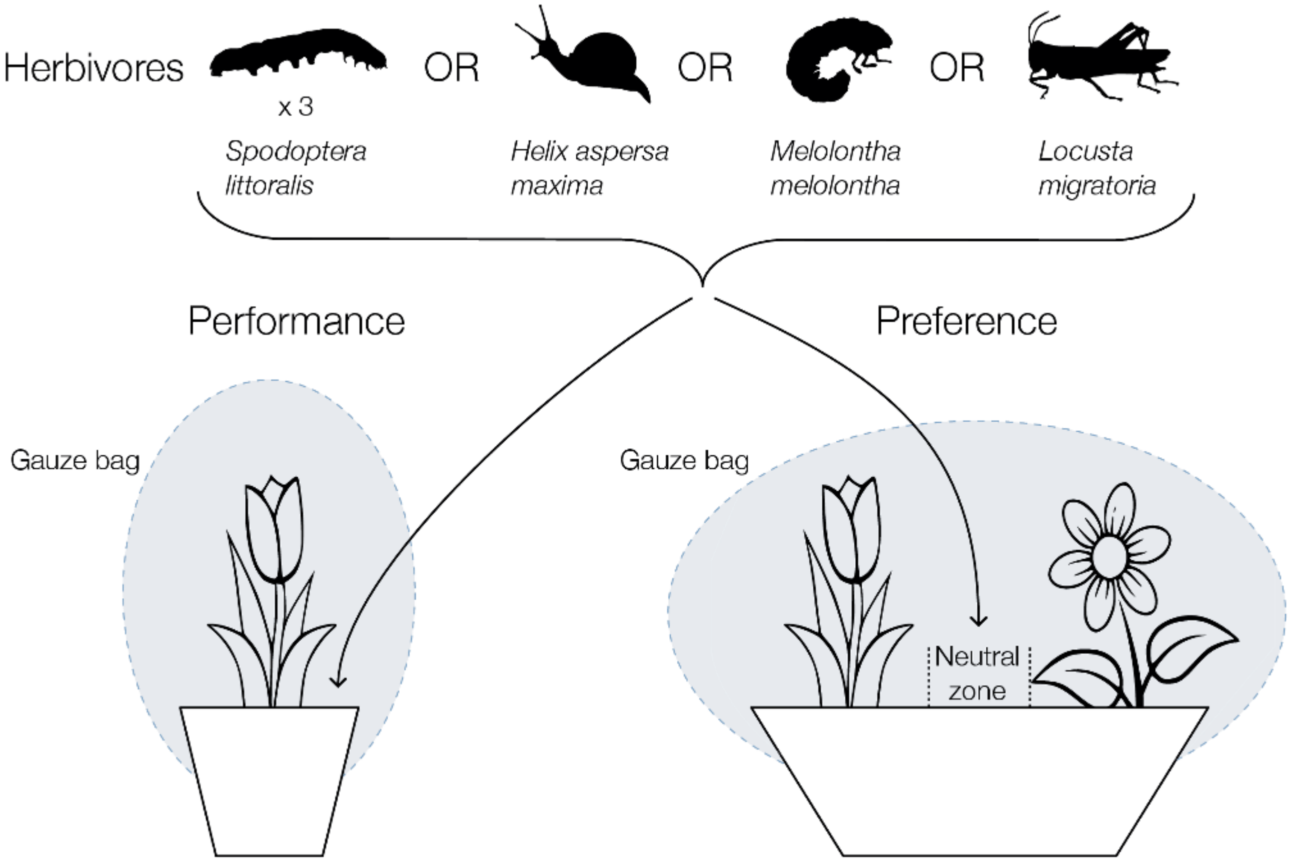

Figure 1: Sketch of the performance experiment (left) and the preference experiment (right) for four herbivore species.

Performance Experiment

For each herbivore species, one to six plants of similar size and developmental stage from three to seven different seed families per species were selected. A minimum of three and a maximum of 18 plants per plant and herbivore species were used. The difference between the number of plants and seed families per plant species is due to the variation in germination rate per seed family. We assessed the herbivore performance on a subset of 38 plant species from July to October 2018, as only seeds of this species subset were available for the performance experiment.

For the experiments with *Melolontha*, *Helix* and *Locusta*, we added one larva, one snail and one locust to each plant, respectively. For the experiment with *Spodoptera*, we proceeded to two replications, due to a high mortality rate of the caterpillars caused by a virus infection. We added two four-days old and three one-week old caterpillars to each plant for the first and the second experimental replication, respectively. Plants were then individually bagged with a gauze bag (55x30 cm) to prevent the escape of the herbivores. We allowed *Melolontha* to feed on the plants for 15 days, *Locusta* for five days, *Helix* for seven days and Spodoptera for ten and five days for the first and second experimental replication, respectively. We chose different feeding durations for the different herbivores because they differ in mobility, developmental stage, size and growth rates (summary of the experimental conditions: Table S2).

To quantify herbivore performance, we weighed the living herbivores to the nearest hundredth gram before and after the feeding (Sartorius Cubis balance MSA225P-100-DI, Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany). For each of the two experiments with *Spodoptera*, we calculated the mean weight of the caterpillars per pot before and after the feeding. We considered only herbivores that did not run out of food (i.e. the host plant was not entirely eaten) during the feeding time. A herbivore might not gain weight either because the food may be of poor nutritious quality or because it suffers from food-independent mortality or illness. As those two cases cannot be distinguished, we used only the data from herbivores gaining weight. Herbivores that died by the end of the experiments were not weighed.

Preference Experiment

We assessed the preference of the herbivores to 56 plant species from June to July 2019. Plants of the same species within the experiment were of similar size and developmental stage but from different seed families.

For each herbivore species, we conducted a series of pairwise choice tests, during which a herbivore could choose between two different plant species (Fig. 1). The two plant species for each choice test were potted together in a squared pot (i.e. experimental unit). One *Helix*, *Locusta*, *Melolontha* and three 10-12 days old *Spodoptera*, respectively, were used per experimental unit. Herbivores were positioned in the middle of the pot, not facing any plant. Pots were bagged with a gauze bag. Individuals of *Melolontha* were placed in a hole in the middle of the pot 24 hours after the plants had been potted together to allow exudates from roots to mix in the soil.

We assessed the preference of *Helix*, *Melolontha* and *Spodoptera* after 24 hours by counting the number of herbivores on each of the two plants. We refer to the number of herbivores as "goals" in analogy to football. Herbivores that stayed in the middle of the pot between the two plants, in what we called the "neutral zone", were not counted (Fig. 1). As locusts are very mobile species, we recorded the position of the locust every 5 minutes for 30 minutes and summed up the number of locust counts (goals) per plant species (giving a max of six goals per plant species).

To estimate the degree of preference, we followed a similar rule as the International Federation of Association Football (FIFA, 2014; Kempel et al., 2015). For *Spodoptera* and *Locusta*, we awarded two points (a win) to the plant species attracting at least two caterpillars or locust more than the other plant, which received zero points. Both plants received one point (a draw) when they attracted an equal number of caterpillars or locust, or when they differed only in one individual. This aims to reduce chance results. For *Helix* and *Melolontha*, we awarded two points (a win) to the species attracting the herbivore, and zero points to the species not attracting the herbivore. If the herbivore stayed in the neutral zone, each species received one point (a draw). Therefore, per experimental unit, a plant can get a maximum of 3 goals and 2 points with *Spodoptera*, 6 goals and 2 points with *Locusta* and a maximum of one goal and two points with *Helix* and *Melolontha*.

To reduce the prohibitively large number of tests that would have been required to test each possible pair of plant species ([n(n-1)]/2 tests, where n is the number of plant species; 1540 tests for 56 plant species), we did two rounds of round-robin tests (Kempel et al., 2015). We randomly assigned plant species to eight groups of six to seven species in the first round and assessed herbivore preference for each possible combination of species within each group. In the second round, we formed seven new groups of seven to eight species based on the number of points (in case of a draw, we used the number of goals to determine the species ranking) a species obtained in the first round. Then, we assessed again herbivore preference for each possible combination of species within each group. Each plant species had the same number of tests and played against preferred and less preferred species. A total number of 338, 273, 351 and 357 tests were performed to assess the preference of *Helix*, *Locusta*, *Melolontha* and *Spodoptera*, respectively. The number of tests per herbivore species varied in function of the number of available healthy herbivores and plants (plant illness and mortality increased throughout the experiments).

We then summed up the number of goals and points, separately, of all tests per plant species of both rounds and used this as an indicator of herbivore preference. Plant species with many goals and points were considered the most preferred by herbivores, whereas plant species with very few goals and points were considered the leastq preferred. Preference based on goals and points were highly correlated ($r>0.8$, $P<0.05$) within herbivore species.

Statistical analysis

To test whether plant species of different regional and local rarity, growth and competitive strategy and habitat differed in their investment to defence against generalist herbivores, we fitted two linear mixed-effect models (LMER) in R 3.5.3 (R Core Team, 2019). One model was fitted for herbivore performance and one for preference, including the four herbivore species together (lme4, lmerTest and MuMIn packages R; Bates et al., 2015; Kuznetsova et al., 2017; Barton, 2020).

Response variables of the performance and preference model were the final weight of the herbivores (or the mean final weight per experiment for *Spodoptera*) and the number of goals that herbivore species awarded to plant species, respectively. In the performance model, we included the initial weight of the herbivore and its interaction with herbivore species as a covariate. We also fitted models with weight difference and weight ratio for the performance and with the points for the preference model. However, based on the residual distribution, R^2 and AIC, we opted for the former models. The initial weight and both response variables were centred and scaled per herbivore species to standardize weight and goal number between herbivore species. As random effects, we included plant seed families nested in plant species for the performance model and plant species for the preference model.

We also included the plant species range-size (log transformed), indicator values for the dominance *in-situ*, nutrients and moisture, competitive strategy, SLA, leaf chlorophyll concentration (all coded as numeric), herbivore species and all possible two-way interactions as fixed effects (Table S3). To correct for potential effects of within plant-species variation in plant volume on herbivore performance or preference, we included the initial plant size in the performance model and the initial mean size per plant species in the preference model as additional covariates. Since no pair of highly multi-collinear variables was found (r>0.7; Fig. S1), all variables were retained in both models.

We reduced the models by using a backward stepwise procedure to remove the least significant terms (Table S3; significance threshold: P<0.05) calculated with the Satterthwaite's method of approximation (Kuznetsova et al. 2017). Random factors were kept in the model and all numeric explanatory variables were centred and scaled.

In addition, we ran models for each herbivore species separately to calculate herbivore-specific regressions for any significant plant variables. In this case, herbivore species was removed from the fixed terms and we used a linear mixed-effect model for the performance and a linear model for the preference. We also tested whether a herbivore-species performance was related to its preference. To do that, we extracted the residuals from the linear model of the herbivore final weight in function of the initial weight and ran a linear model where the residuals were explained by the herbivore preference (i.e. number of goals). We also calculated the Pearson correlation between the performance and the preference within and between herbivore species. Graphs were performed with R packages ggplot2, corrplot, effects and remef (Wickham, 2016; Wei & Simko, 2017; Fox & Weisberg, 2018; Hohenstein & Kliegl, 2020).

To test whether herbivore performance and preference were related to plant phylogeny and whether closely related plant species share similar range size and plant traits and strategy, we constructed a plant-species phylogenetic tree using the dated seed-plant phylogeny of Smith & Brown (2018). We then tested for a phylogenetic signal in the preference and performance of each herbivore species, plant trait, strategy and range size (Blomberg et al., 2003; phytools R-package, Revell, 2012). As none of the variables presented a significant phylogenetic signal (Table S4), analyses with a phylogenetic correction were not considered necessary (Carvalho et al., 2006).

Results

Performance Experiment

All four herbivore species performed better on competitive plant species (Fig. 2A, Table 1). Herbivore performance was also related to the plant SLA and indicator value for nutrients and dominance *in-situ*, however, effects varied between herbivore species (significant interaction of factors with herbivore species; Fig. 2B-D, Table 1). Herbivore performance was not related to the plant range-size, indicator value for moisture and leaf-chlorophyll concentration.

In detail, *Spodoptera* performance tended to decrease on plants originating from nutrient-rich habitats. Moreover, *Spodoptera* and *Locusta* performances were higher on dominant plant species (Fig. 2B,D). The performance of the herbivores in response to SLA differed between herbivore species: *Helix* and *Locusta* performed better on high-SLA species, while *Melolontha* and *Spodoptera* performed better on low-SLA species (Fig. 2C). Although the slopes of the herbivore-specific regressions were significantly different from each other, they were only significantly different from zero for the performance of *Locusta* in relation to SLA and the indicator value for dominance *in-situ* (Table 2). Because SLA is a proxy of plant growth-strategy, where species with high SLA usually grow faster and are more palatable, these results indicate that the performance of *Locusta* is affected by the quality of the plant as food and by plant traits linked to local abundance.

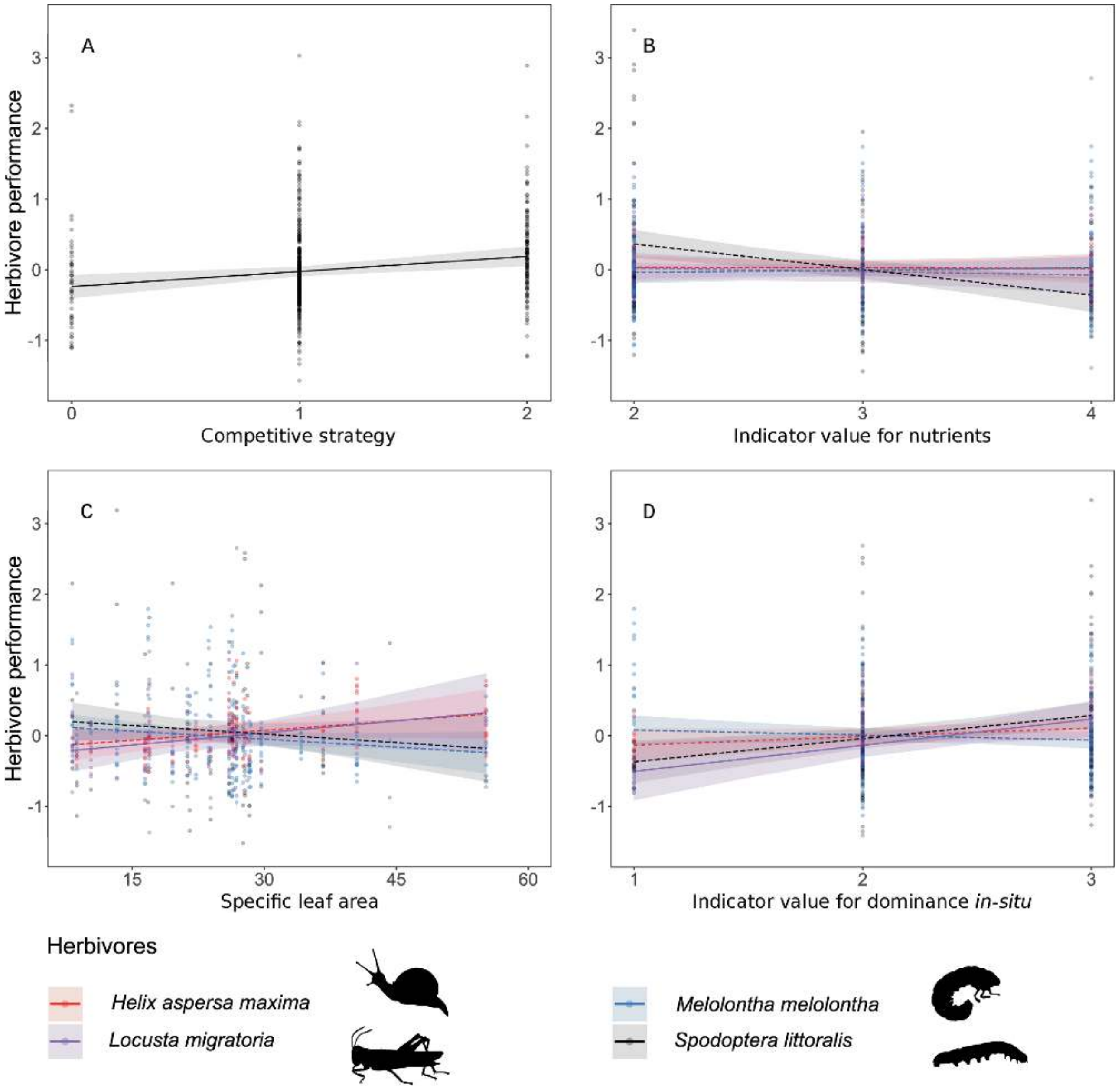

Figure 2: Relationship between herbivore performance (model estimates) and A) plant competitive strategy for all herbivores together, B) plant indicator value for nutrients, C) SLA and D) plant indicator value for dominance in-situ per herbivore species. Points show the partial residuals. Solid and dashed lines indicate significant (P<0.05) and non-significant regression lines, respectively.

Table 1: Results of linear mixed effect model testing for the effect of plant traits and strategy, indicator values and range size on the performance of the herbivore species together after model reduction. Note that the range size, chlorophyll concentration and indicator value for moisture were not retained in the final model, as they were not significant. Performance analysis was conducted on 610 observations, 212 seed families and 33 plant species. Sum Sq, DF, F, P and LRT refer to the sum of squares, degrees of freedom, F-statistic value, corresponding p value and log-likelihood ratio test statistic, respectively. Significant p values are highlighted in bold.

PERFORMANCE – all herbivores				
Fixed effects	**Sum Sq**	**DF**	**F**	***P***
Initial plant size	0.899	1	2.677	0.106
Herbivore initial weight	210.030	1	625.53	**<0.001**
Herbivore species	0.501	3	0.498	0.684
Herbivore species × Initial herbivore weight	49.171	3	48.815	**<0.001**
N	1.004	1	2.991	0.092
Competitive strategy	3.167	1	9.431	**0.005**
SLA	0.030	1	0.089	0.768
DG	2.862	1	8.523	**0.006**
N × Herbivore species	6.705	3	6.656	**<0.001**
SLA × Herbivore species	3.554	3	3.528	**0.015**
DG × Herbivore species	5.950	3	5.907	**<0.001**
Random effects	**Variance**	**DF**	**LRT**	***P***
Seed family	0.000	1	0.00	1
Plant species	0.016	1	5.392	**0.02**
Residuals	0.358			

Table 2: Estimates and corresponding p values of the herbivore-specific linear mixed effect models testing for the effect of the significant plant variables on the performance of the herbivore species separately. Significant p values are highlighted in bold.

PERFORMANCE - per herbivore species						
	N		**SLA**		**DG**	
Herbivore species	**Estimate**	***P***	**Estimate**	***P***	**Estimate**	***P***
Helix	0.017	0.757	0.067	0.155	-0.014	0.770
Locusta	-0.142	0.064	0.18	**0.012**	0.345	**<0.001**
Melolontha	-0.06	0.49	-0.082	0.205	-0.054	0.393
Spodoptera	-0.148	0.492	-0.07	0.708	0.152	0.406

Preference Experiment

The preference of the herbivores was related to the plant range-size in a herbivore species-specific way (Fig. 3, Table 3). Particularly, *Locusta* preferred common plant species. However, the preference of the other herbivore species showed no relationship with range size (Fig. 3, Table 4). The preference of the herbivores was positively related to the initial plant height (covariate), indicating that herbivores preferred plants with a large biomass. Herbivore preference was not related to SLA, leaf chlorophyll concentration and any of the indicator values of the plant species. In addition, the herbivore preference (i.e. number of goals) was generally independent of herbivore performance within and across herbivore species with the exception of the preference and performance of *Locusta* (*r*>0.6; linear model: df=1, Sum of squares=1150.0, F=10.875, *P*=0.005), the preference of *Helix* and *Locusta* and the performance of *Melolontha* and *Spodoptera* that were slightly correlated (*r*<0.6, *P*<0.05; Fig. 4, Fig. S2).

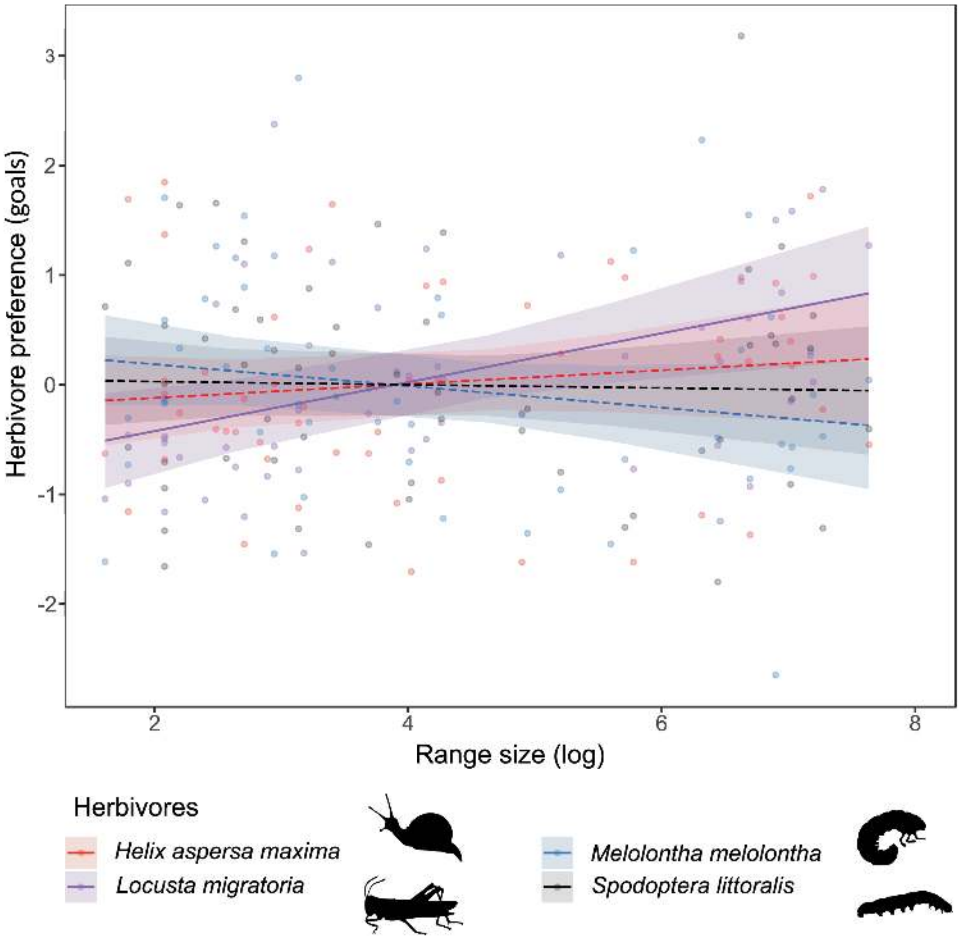

Figure 3: Relationship between the herbivore preference (model estimates based on the number of goals) and plant range-size (log transformed). Points show the partial residuals. Solid and dashed lines indicate significant (P<0.05) and non-significant regression lines, respectively.

Table 3: Results of linear mixed effect model testing for the effect of plant traits, indicator values and range size on the preference of the herbivore species together after model reduction. Note that the SLA, chlorophyll concentration, competitive strategy, indicator values for nutrients, moisture and dominance in-situ were not retained in the final model, as they were not significant. Preference analysis was conducted on 213 observations and 56 plant species. Sum Sq, DF, F, P and LRT refer to the sum of squares, degrees of freedom, F-statistic value, corresponding p value and log-likelihood ratio test statistic, respectively. Significant p values are highlighted in bold.

PREFERENCE – all herbivores				
Fixed effects	**Sum Sq**	**DF**	**F**	***P***
Initial plant size	6.972	1	8.176	**0.006**
Herbivore species	0.001	3	0.000	1
Range size (log)	0.87	1	1.020	0.317
Range size (log) × Herbivore species	10.288	3	4.022	**0.009**
Random effects	**Variance**	**DF**	**LRT**	***P***
Plant species	0.042	1	0.647	0.421
Residuals	0.853			

Table 4: Estimates and corresponding p values of the herbivore-specific linear models testing for the effect of the range size (log transformed) on the preference of the four generalist herbivore species separately. Significant p values are highlighted in bold.

PREFERENCE – per herbivore species		
	Range size (log)	
Herbivore species	**Estimate**	***P***
Helix	0.033	0.823
Locusta	0.319	**0.018**
Melolontha	-0.09	0.572
Spodoptera	0.027	0.861

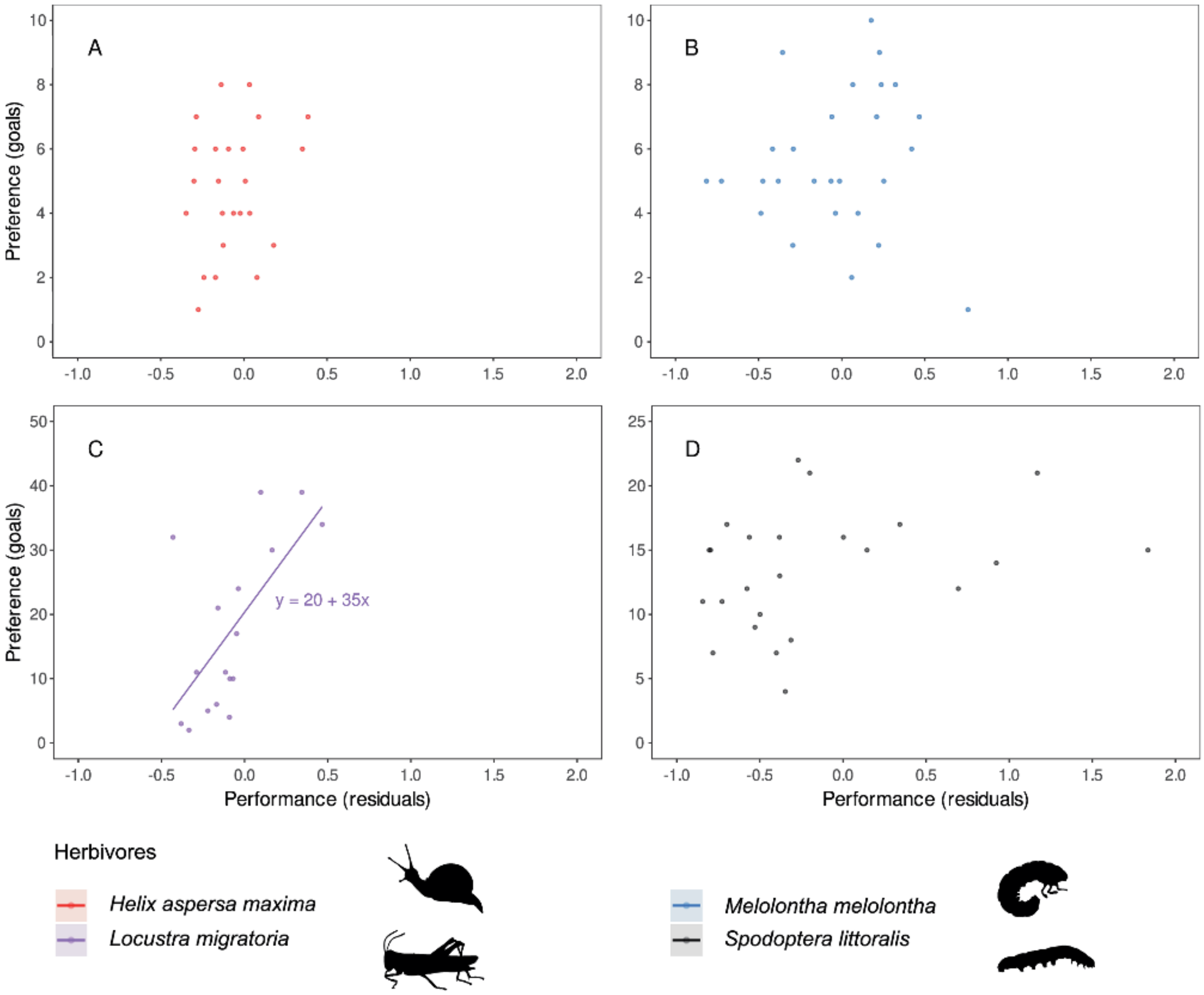

Figure 4: The relationship between herbivore preference (number of goals) and herbivore performance (residuals of the final herbivore weight adjusted for the initial weight). A point corresponds to one plant species for which the herbivore performance and preference were both assessed.

Discussion

Common plant species are not more defended against generalist herbivores than rare plant species

Our results indicate that overall generalist herbivores did not differ in their performance and preference when feeding on regionally and locally rare plant species compared with common and dominant plant species. We found these results even after accounting for plant habitat, traits and strategy known to alter plant investment into defence and thus their palatability to generalist herbivores. Hence, regionally and locally rare and common plant species in our experiment do not generally differ in their defence against generalist herbivores. This result complements the finding of Kempel et al. (2020) that there was no difference of tolerance to herbivory between 40 rare and common plant species. The few other studies that investigated the relationship between rarity and defence in plants are based on a lower number of plant species and found contrasting results: Landa & Rabinowitz (1983) showed that a generalist grasshopper preferred rare over common grasses (seven species). Similarly, Kempel et al. (2018; 19 species) and Fiedler (1987; four species) reported that rare species were more susceptible to soil biota and to leaf herbivory, respectively, than common plant species. In contrast, Leege et al. (2010) found that a common *Trillium* species tended to be more susceptible to herbivory than a rare *Trillium* species in common gardens. This suggests that studies based on few species may provide a bias of the relationship between plant rarity and defence.

In our study, only one herbivore species, *Locusta*, had a higher performance on dominant plant species and preferred regionally common over rare plant species. This contrasts with the apparency hypothesis (Feeny, 1976), which states that locally common plant species occurring in dense patches invest more into defence than non-dominant plant species, because they are more likely to be found and eaten by generalist herbivores (Cates & Orians, 1975; Southwood et al., 1986). It also contrasts with the idea that rare species have lower levels of defence due to lower genetic diversity (Spielman et al., 2004; Laine, 2006). Our results with *Locusta* indicate that locally and regionally common species invest less, rather than more, into defence. A possible explanation might be that only low investment to defence allows plants to be competitively superior and form dense patches, given that it is commonly assumed that defence is costly and trades off with growth (Coley et al., 1985; Züst & Agrawal, 2017). Such fast-growing, competitive species are often more tolerant to herbivores than less dominant species, as they can easily replace lost biomass (Gianoli & Salgado-Luarte, 2017). Similarly, fast-growing species and species more tolerant to generalist herbivores may be better able to spread and become regionally common, particularly if they are more limited by competing neighbouring plants than by herbivores. However, the weak relationship between preference and plant dominance across herbivore species may be because no highly dominant species (Landolt indicator value for dominance *in-situ* >3) were included in our study.

Our multi-plant and herbivore-species experiment therefore supports that the apparency hypothesis does not hold for herbaceous plant species. Common plant species are not more defended, but rather tend to be less defended than rarer species. The ability to allocate resources away from defence to growth might therefore be important for plants to become locally and regionally common.

Plant competitive ability is related to plant defence and palatability

Generalist herbivores preferred and performed better on competitive plant species in our study. Competitors according to Grime's CSR life-strategy scheme (Grime, 1974; 2006) are plant species with fast resource acquisition in productive environments, short-lived leaves and high allocation to vegetative growth (Grime & Pierce, 2012). These traits are also tightly linked to plant strategy against herbivores (Poorter & Remkes, 1990; Rosenthal & Kotanen, 1994; Kempel et al., 2015; Kuglerová et al., 2019), as plant growth is typically suggested to trade-off with plant defence (Coley et al., 1985; Endara & Coley, 2011; Capelli et al., 2020). Our results thus indicate that highly competitive plant species invest less into defence than less competitive species, which supports the growth-defence trade-off.

The other plant traits investigated in this study were not consistently related to herbivore performance or preference. Indeed, we found that only the nutrient-availability of a plant habitat and SLA were related to herbivore performance, but their effects varied considerably across herbivore species. Plant traits were also weakly correlated with each other, even competitive strategy and SLA, which are usually related to plant palatability. This indicates that most single plant traits are limited in explaining herbivore responses. This might be because traits evaluate only one aspect of plant palatability and different herbivore species respond differently to them as they differ in anatomy, morphology and physiology. This also explains why studies reported contrasting relationships between herbivory and plant palatability when using single traits (e.g. compare Schädler et al., 2003; Moles & Westoby, 2000; Knepp et al., 2005; Lamarre et al., 2012; Loranger et al., 2012; Kuglerová et al., 2019). A combination of traits is likely to better predict herbivore responses, which was also supported by the concept of plant-defence syndromes (Agrawal & Fishbein, 2006). That Grime's CSR scheme explained herbivore performance consistently suggests that a combination of traits representing the two axis of the plant-economic spectrum (Díaz et al., 2016), SLA and plant height, might be particularly promising candidate variables for predicting plant palatability. Nevertheless, future studies combining different plant traits, and including qualitative and quantitative defence traits, could provide further insights into the relationship between plant growth- and defence-strategy and generalist, but also specialist, herbivores, as they were reported to differ in their sensitivity to those defence types (Rhoades & Cates, 1976).

Herbivore preference is not consistently related to performance

The relationship between preference and performance in insect herbivores is suggested to be tightly linked, since females are under strong selective pressure to oviposit on plant species that maximize its offspring fitness (“mother-knows-best”: Jaenike, 1978; Gripenberg et al., 2010). Although the “mother-knows-best” principle applies for insects across two generations, Kempel et al. (2015) have reported that herbivore performance and preference for plant species were correlated within the same generation. However, herbivore preference and performance were only related in one out of four herbivore species in our study. Moreover, different herbivore species seem to perceive plant defence differently, as only the preference of the locust and the snail were positively correlated. Independence of herbivore preference and performance was also reported in other studies (Bernays, 1990; Duffy & Hay, 1991; Cronin & Abrahamson, 2001). In these cases, it was suggested that herbivores had chosen plant species on the basis of highly conservative and simple cues (e.g. taste) or traits unrelated to plant quality as food, but as shelter for example. Thus, our results contrast with two common ideas in ecology: 1) herbivore performance and preference are tightly linked together (Gripenberg et al., 2010) and 2) food plant quality is universally recognised in a similar way by different generalist herbivore species (Herms & Mattson, 1992; Pérez-Harguindeguy et al., 2003). The fact that the nutritional quality of plants is not recognized in the same way between generalists and specialists is expected (Lankau, 2007) but was not assumed between generalist species. Altogether, this suggests that the preference of generalist herbivores is mainly driven by cues not related to nutrition and thus the use of a single generalist herbivore species as universal gauge of defence is challenged, since even generalist herbivores of the same taxonomic group and feeding on the same plant organs perceive plant defence differently.

Conclusion

Our multi-species experiment suggests that regionally and locally rare plant species are not less defended against generalist herbivores than are regionally and locally common plant species. This suggests that invertebrate generalist herbivory may not be a major factor driving the distribution and abundance of rare plants. Instead, it seems that common species are slightly less defended against generalist herbivores than rare species. Likely, allocation of resources away from defence allows plants to grow vigorously, to form dense patches and to become locally and regionally abundant. Our results also show that competitive plant species are more palatable to generalist herbivores than are ruderal or stress-tolerator species. It is possible that their low investment into defence allows such plant species to become successful in productive environments. We conclude that plant apparency is a poor proxy for defence investment against generalist herbivores in herbaceous plant species. Rather, the allocation of resources to vigorous growth drives patterns of defence against generalist herbivores in plants.

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Supporting information

Table S1: Plant species included in the herbivore performance (Perf) and/or preference (Pref) experiments, with corresponding plant family, mean plant-size, mean chlorophyll-concentration in leaves (Chloro), mean SLA, competitive strategy (Comp. strat.), indicator values for moisture (F), nutrients (N) and dominance in-situ (DG) and range size.

Plant species	**Perf**	**Pref**	**Plant family**	**Plant size**	**Chloro**	**SLA**	**Comp strat.**	**F**	**N**	**DG**	**Range size**
Achillea millefolium	×	×	Asteraceae	37	6.5	8.2	cc	2	3	3	2070
Aira elegantissima	×		Poaceae	18.5	NA	NA	0	1	1	1	3
Androsace septentrionalis		×	Primulaceae	6.7	56.6	20.5	0	1	3	2	15
Arabis turrita	×	×	Brassicaceae	37	15.7	40.5	c	2	2	2	270
Arenaria grandiflora	×	×	Caryophyllaceae	12.1	4.5	55.2	c	2	2	1	8
Artemisia borealis	×	×	Asteraceae	16	11.8	13.2	c	2	2	3	11
Artemisia glacialis	×		Asteraceae	6.2	NA	NA	c	2	2	2	10
Bidens cernua		×	Asteraceae	28.8	32.2	56.9	c	5	4	1	56
Bromus squarrosus	×	×	Poaceae	58.9	43.9	34.2	c	2	4	3	55
Campanula cervicaria		×	Campanulaceae	27.1	32	30.2	c	3	3	1	25
Carpesium cernuum	×	×	Asteraceae	36.9	31.1	26.3	c	3	4	1	15
Caucalis platycarpos	×		Apiaceae	27.6	NA	NA	0	2	3	1	51
Centaurea jacea	×	×	Asteraceae	47.1	34.1	26	c	3	3	3	1128
Centaurea nigra		×	Asteraceae	27.8	37.5	31.7	c	3	3	3	30
Centaurea valesiaca	×	×	Asteraceae	44	22.1	18.6	c	1	2	3	40
Chenopodium album		×	Chenopodiaceae	72.5	51	19.8	0	2	4	2	810
Cicuta virosa		×	Apiaceae	56.4	22.6	25.9	c	5	3	1	23
Cochlearia pyrenaica	×	×	Brassicaceae	10.2	59.6	10.3	c	5	2	3	8
Crepis froelichiana	×	×	Asteraceae	7.5	43.8	22.2	c	3	2	1	5
Dactylis glomerata	×	×	Poaceae	82.1	23.9	26.7	c	3	4	3	1438
Erysimum cheiranthoides	×	×	Brassicaceae	72.1	56.4	28.5	0	4	4	2	140
Erysimum ochroleucum		×	Brassicaceae	23.9	26	27.3	c	1	2	2	6
Genista pilosa		×	Fabaceae	14.3	28.9	22.7	cc	2	1	3	23
Hypericum perforatum	×	×	Hypericaceae	64.1	14.3	28.3	c	3	3	3	1115
Hypochaeris radicata	×	×	Asteraceae	30.4	39.1	23.7	c	3	2	2	957
Hypericum richeri	×	×	Hypericaceae	43.3	37.1	26.4	c	3	3	2	24
Knautia arvensis	×	×	Dipsacaceae	44.7	35.5	21.3	cc	3	3	2	1039
Laserpitium siler		×	Apiaceae	21	36.5	17	cc	2	2	3	323
Leontodon incanus ssp. tenuiflorus	×	×	Asteraceae	19.7	37	23.9	c	2	2	2	63
Linaria vulgaris	×	×	Plantaginaceae	62.5	33.2	36.7	c	2	4	3	640
Lunaria rediviva		×	Brassicaceae	37.3	23	21.9	cc	4	4	3	182

Plant species	**Perf**	**Pref**	**Plant family**	**Plant size**	**Chloro**	**SLA**	**Comp strat.**	**F**	**N**	**DG**	**Range size**
Minuartia capillacea	×	×	Caryophyllaceae	17.4	NA	8.7	c	1	2	2	6
Origanum vulgare	×	×	Lamiaceae	54.2	22.2	16.8	c	2	3	3	992
Papaver occidentale		×	Papaveraceae	26.3	42.2	22.3	NA	NA	NA	NA	13
Poa remota	×	×	Poaceae	44.9	22.6	21.5	cc	4	3	3	19
Polycnemum majus	×	×	Chenopodiaceae	35	NA	28.1	0	1	3	2	17
Potentilla argentea		×	Rosaceae	13.2	43.9	18.5	c	2	2	2	302
Potentilla multifida	×	×	Rosaceae	22.6	25.5	16.4	cc	3	4	2	8
Prunella grandiflora		×	Lamiaceae	13.1	47.9	31.2	c	2	2	2	755
Prunella laciniata		×	Lamiaceae	16.6	43.7	26.7	c	2	2	1	72
Rorippa islandica	×	×	Brassicaceae	26.9	37.7	44.3	c	5	3	2	43
Rumex hydrolapathum	×	×	Polygonaceae	58.8	34.9	16.9	cc	5	4	2	69
Scrophularia auriculata		×	Scrophulariaceae	61.3	37.7	37.4	cc	5	4	2	12
Scrophularia nodosa	×	×	Scrophulariaceae	80.1	30.6	26.9	cc	4	4	2	801
Scutellaria alpina	×	×	Lamiaceae	25.3	36.5	27.6	c	2	2	3	19
Selinum carvifolia		×	Apiaceae	28.9	29.9	20.6	c	4	2	2	71
Senecio halleri	×	×	Asteraceae	6.8	39.8	18.8	c	2	2	2	31
Sideritis hyssopifolia	×	×	Lamiaceae	32.9	40.6	26.5	c	2	2	2	8
Silene vallesia		×	Caryophyllaceae	11.7	34.3	29.9	c	2	2	3	9
Silene viscaria		×	Caryophyllaceae	10.5	56.8	18.9	c	3	2	2	50
Silene vulgaris		×	Caryophyllaceae	57.8	44.9	34.3	c	3	2	3	1305
Taraxacum dissectum	×	×	Asteraceae	15.9	NA	NA	c	2	4	2	8
Tephroseris capitata	×		Asteraceae	20.8	NA	NA	cc	2	2	2	23
Teucrium botrys		×	Lamiaceae	21.2	21.9	28.9	0	2	2	2	134
Teucrium chamaedrys		×	Lamiaceae	20.1	39.6	23.3	c	2	2	3	554
Thlaspi rotundifolium ssp. Corymbosum	×		Brassicaceae	4.9	40.5	29.7	c	3	2	3	129
Thlaspi sylvium	×		Brassicaceae	4.5	41.3	19.6	c	4	2	2	5
Trifolium repens	×	×	Fabaceae	48.8	32.6	32.6	c	3	4	3	1334
Trifolium saxatile		×	Fabaceae	11.9	38	18	0	1	2	1	18
Veronica austriaca		×	Plantaginaceae	26.9	39.9	34.8	c	2	2	2	14
Veronica urticifolia		×	Plantaginaceae	39.6	36.5	22.3	cc	4	3	2	628
Xeranthemum inapertum	×	×	Asteraceae	52	46.8	27.8	0	1	2	2	8

Table S2: Summary of the experimental conditions for the assessment of the performance and preference of four herbivore species. Plant species nb., Nb. of herbi. and Herbi. state refer to the number of plant species used per herbivore species, the number of herbivores used per experimental unit and the age or life-stage of the herbivores when entering the experiments, respectively. Actions, Exp. duration and Measure refer to the plant and herbivore treatments, the time that a herbivore was allowed to feed on or choose a plant and the measure per herbivore species used as response variable in the analyses, respectively.

Experiment	Plant species nb.	Nb. of herbi.	Herbi. origin	Herbi. state	Actions	Exp. duration	Measure
Performance							
Helix	29	1	Park, Etis	Pre-adult	Washing Body water saturation before and after Starving + Gut standardisation	7 days	Final body weight
Locusta	23	1	Shop, Pocerias	Pre-adult	Starving + Gut standardisation	5 days	Final body weight
Melolontha	33	1	Field, Urmein	2nd instar	Gut standardisation	15 days	Final body weight
Spodoptera :	34						Mean weight of both experiments
1st experiment	33	2	Lab, Syngenta	4 days	Gut standardisation	10 days	Mean body weight
2nd experiment	28	3	Lab, Syngenta	7 days	Gut standardisation	5 days	Mean body weight
Preference							
Helix	54	1	Park, Etis	Pre-adult		24 hours	Goals & points per plant species
Locusta	49	1	Shop, Pocerias	Pre-adult		Each 5 min for 30 min	Goals & points per plant species
Melolontha	55	1	Field, Bristen	2nd instar	Potting plants 24 h before experiment	24 hours	Goals & points per plant species
Spodoptera	55	3	Lab, Syngenta	10 - 12 days		24 hours	Goals & points per plant species

Table S3: Performance and preference linear mixed-effect models for all herbivore species together. Initial models (with all terms included) were reduced using a backward stepwise procedure and significant terms (in bold) were kept in the reduced models.

PERFORMANCE MODEL	**PREFERENCE MODEL**
Herbivore final weight ~	**Goals number ~**
Initial plant size +	**Initial mean plant size +**
Herbivore initial weight +	
Herbivore species +	**Herbivore species +**
Herbivore species × Herbivore initial weight +	
Range size (log) +	**Range size (log) +**
N +	N +
F +	F +
SLA +	SLA +
Chlorophyll +	Chlorophyll +
Competitive strategy +	Competitive strategy +
DG +	DG +
Range size (log) × Herbivore species +	**Range size (log) × Herbivore species +**
Range size (log) × N +	Range size (log) × N +
Range size (log) × F +	Range size (log) × F +
Range size (log) × SLA +	Range size (log) × SLA +
Range size (log) × Chlorophyll +	Range size (log) × Chlorophyll +
Range size (log) × Competitive strategy +	Range size (log) × Competitive strategy +
Range size (log) × DG +	Range size (log) × DG +
Herbivore species × N +	Herbivore species × N +
Herbivore species × F +	Herbivore species × F +
Herbivore species × SLA +	Herbivore species × SLA +
Herbivore species × Chlorophyll +	Herbivore species × Chlorophyll +
Herbivore species × Competitive strategy +	Herbivore species × Competitive strategy +
Herbivore species × DG +	Herbivore species × DG +
1 | Plant species / Plant seed family	**1 | Plant species**

Table S4: Test for phylogenetic signal in the plant traits and strategy, indicator values, range size, performance (i.e. centred and scaled final weight) and preference (i.e. centred and scaled number of goals) of the four herbivore species. K is the test statistic and P is the corresponding p value.

Variable	**K**	***P***
Plant size	0.125	0.153
N	0.13	0.132
F	0.111	0.256
DG	0.111	0.25
Competitive strategy	0.160	0.051
Chlorophyll concentration	0.108	0.38
SLA	0.182	0.061
Range size	0.064	0.835
Performance – Final weight		
Helix	0.128	0.671
Locusta	0.073	0.832
Melolontha	0.180	0.305
Spodoptera	0.139	0.559
Preference – Goals		
Helix	0.160	0.109
Locusta	0.144	0.297
Melolontha	0.144	0.198
Spodoptera	0.134	0.242

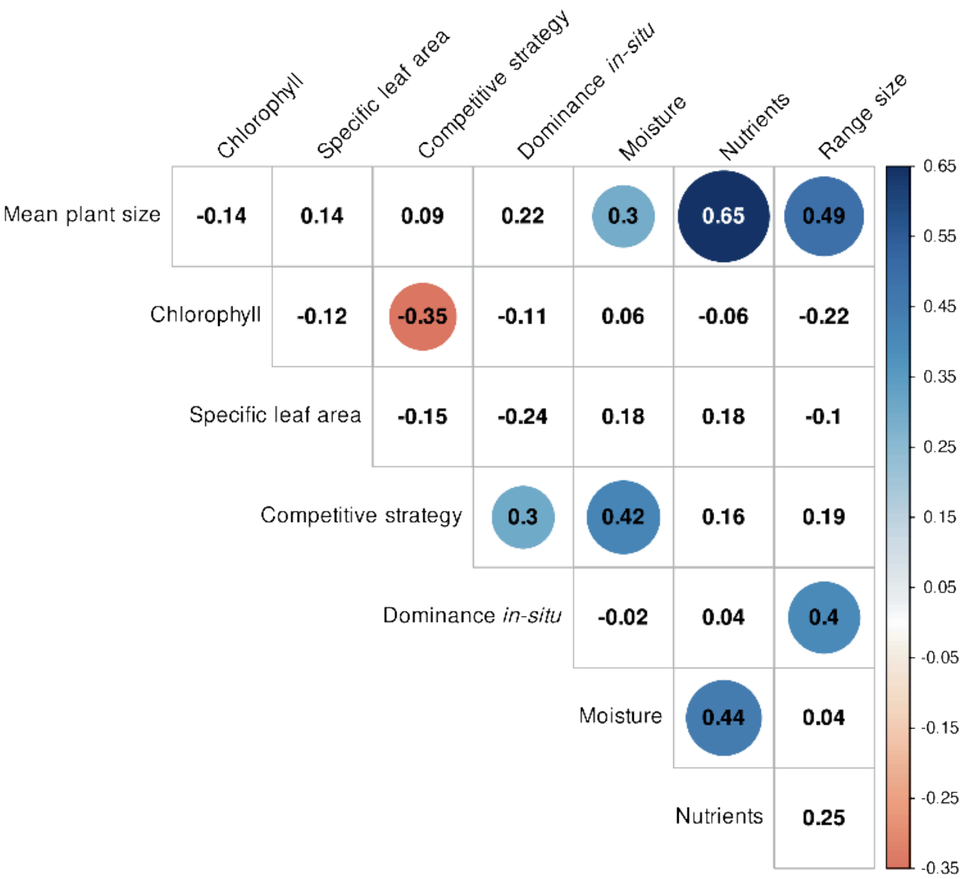
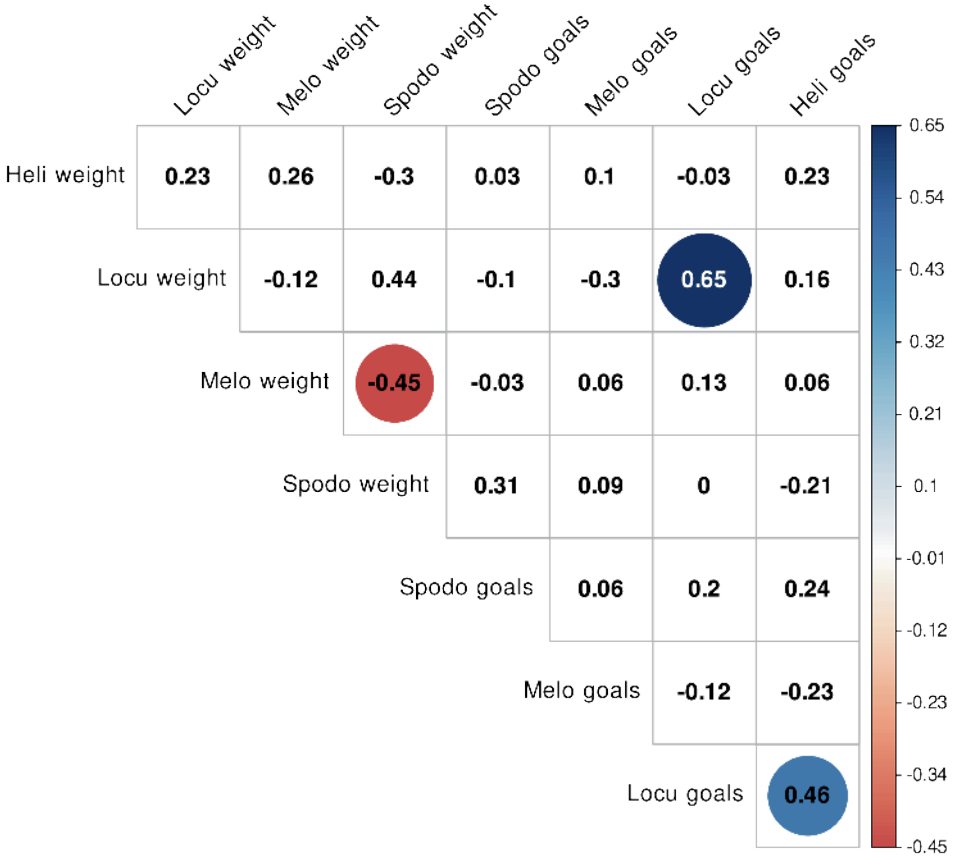


Figure S1: Pearson correlation coefficients between the plant traits and strategy, indicator values and range size. Blue and red circles correspond to positive and negative significant (P<0.05) correlations, respectively.



	Locu weight	Melo weight	Spodo weight	Spodo goals	Melo goals	Locu goals	Heli goals
Heli weight	0.23	0.26	-0.3	0.03	0.1	-0.03	0.23
Locu weight		-0.12	0.44	-0.1	-0.3	0.65	0.16
Melo weight			-0.45	-0.03	0.06	0.13	0.06
Spodo weight				0.31	0.09	0	-0.21
Spodo goals					0.06	0.2	0.24
Melo goals						-0.12	-0.23
Locu goals							0.46

Figure S2: Pearson correlation coefficients between herbivore performance (final herbivore weight adjusted for initial weight) and preference (i.e. number of goals). Blue and red circles correspond to positive and negative significant (P<0.05) correlations, respectively. Spodo, Melo, Locu, Heli refer to Spodoptera, Melolontha, Locusta and Helix, respectively.

Chapter 3

Plant rarity and resource-allocation strategy shape the outcome of selfing and between-population outcrossing in plants

Sarah Bürli, Andreas Ensslin, Markus Fischer

Abstract

1. Rare plant species are characterized by small and isolated populations. Compared with common species, they have thus been hypothesized to suffer more from selfing and to benefit more from between-population outcrossing. Alternatively, because populations of rare species typically experience long inbreeding histories and genetic drift, they may also have purged their genetic load and thus may suffer less from selfing than common species. Testing these alternative hypotheses for multiple species varying in rarity, competitive strategy, habitat characteristics and growth traits has major implications for conservation.

2. We tested whether selfing and between-population outcrossing affected five fitness-related traits for species of different regional and local rarity, competitive strategy, habitat characteristics and growth traits. To do so, we performed self-pollinations and within- and between-population cross-pollinations by hand for 16 rare and common species from seven plant families.

3. Overall we found that selfing decreased plant fitness, while between-population outcrossing increased it. Moreover, common species exhibited stronger inbreeding depression than regionally rare species, in line with purging of genetic load from regionally rare species. Despite a large variation in selfing and between-population outcrossing effects across fitness-related traits, between-population outcrossing was generally beneficial for regionally rarer species, for competitive species and for fast-growing species. This was the case across species from different types of habitat.

4. *Synthesis*: Our study demonstrates that regional rarity, competitive strategy, habitat characteristics and growth traits shape the outcome of selfing and between-population outcrossing in plants. We highlight that selfing may not represent a major threat to rare species. Moreover, as between-population outcrossing appears beneficial for plant fitness of many species, including rare ones, we suggest considering between-population outcrossing for plant-conservation activities.

Keywords: competitive strategy, fitness-related traits; growth traits; outcrossing; plant habitat; plant rarity; pollination; selfing.

Declarations

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Conflict of interest: The authors declare no conflict of interest.

Introduction

Rare plant species are typically characterized by small and isolated populations (Broennimann et al., 2005; IUCN, 2012). Such populations are expected to have reduced genetic variability and heterozygosity, due to the random loss of rare alleles (i.e. genetic drift) and several generations of inbreeding (Ellstrand & Elam, 1993; Oostermeijer et al., 2003; Charlesworth & Willis, 2009). Inbreeding, which is the production of offspring through the mating of two genetically closely related parents, increases the probability for an individual to carry recessive deleterious alleles (Charlesworth & Willis, 2009; Hedrick, 2012). This often results in inbreeding depression, i.e. a fitness reduction in inbred individuals (Charlesworth & Charlesworth, 1987; Charlesworth & Willis, 2009), which is indicated to be one of the most important factors determining the extinction of endangered species (Frankham, 2005; O'Grady et al., 2006; Allendorf et al., 2013). However, if inbreeding depression is due to (over-)dominance, natural selection may purge the population from genetic load (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987; Hedrick, 1994). Thus, rare species may have longer inbreeding histories but suffer less from inbreeding depression than common species.

Rare plant species are usually also restricted to very narrow ecological niches and their populations may be locally adapted (Slatkin, 1981; Slatyer et al., 2013; but see Leimu & Fischer, 2008). Hence, they might suffer from outbreeding depression when individuals from different populations are crossed (e.g. Edmands & Timmerman, 2003; Tallmon et al., 2004). On the other hand, between-population outcrossing could also be beneficial and serve as genetic rescue for genetically depauperate rare species (Neuwald & Templeton, 2013; McLennan et al., 2020; Zecherle et al., 2021). Indeed, outbreeding is expected to increase allelic diversity and heterozygosity and may thus reduce adverse effects of inbreeding (Charlesworth & Charlesworth, 1987; Charlesworth & Willis, 2009). Hence, rare plant species may either suffer more from inbreeding effects than common species, or they may suffer less. Moreover, rare plant species may benefit more from between-population outcrossing than common species. Testing these hypotheses is of fundamental interest and it informs conservation practice, e.g. on the implications of using single vs. mixed origins in *ex-situ* cultures, translocations, and population reinforcements.

An efficient way to test these hypotheses in plants is to conduct self- and cross-pollinations by hand and assess offspring fitness (e.g. Darwin, 1876; Stojanova et al., 2021). However, because the mutation rate varies in time and between genes (Charlesworth & Charlesworth, 1987; Angeloni et al., 2011), adverse effects of inbreeding is predicted to vary across life stages and traits. It is thus essential to assess offspring fitness in several fitness-related traits at different life stages. Moreover, to infer general patterns over a wide array of species differing in rarity (van Kleunen et al., 2014) and test for context-dependency across different trait continua and ecological niches (Kempel et al., 2020), multi-species experiments are necessary.

Plant competitive strategy, habitat characteristics and growth traits likely modulate the effects of inbreeding and outbreeding on plant fitness in the same way as rarity. Indeed, many common species are characterized by traits for competition, high resource acquisition and fast growth, whereas rare species usually grow more slowly, are less competitive and adapted to resource poor habitats (Drury, 1974; Grime, 1979; Kempel et al., 2020). Additionally, as described in Rabinowitz (1981)'s seminal work, rarity is defined by three aspects: the species distribution range at a broad scale, its local abundance and its habitat specificity. Each of these aspects are important to be considered, as they might influence the effects of inbreeding and outbreeding. Although many studies (e.g. Dudash, 1990; Hauser & Loeschcke, 1996; Leimu et al., 2008; Thiele et al., 2010; Sedlacek et al., 2012; Sandner et al., 2021; Wang, 2019) investigated effects of selfing and between-population outcrossing in plants, none, to our knowledge, has conducted a multi-species investigation, while taking into account the three aspects of rarity, competitive strategy and growth traits of the species. Hence, we still lack a general understanding of whether plant species of different local and regional rarity, competitive strategy, habitat characteristics and growth traits differ in their responses to selfing and between-population outcrossing.

Here, we present an experiment where we compare the effects of three treatments of hand-pollinations on five fitness-related traits in the offspring of 16 plant species occurring in Switzerland. At the same time we account for species differences in local and regional rarity, competition strategy, habitat nutrient-richness and moisture and growth traits. To exclude phylogenetic bias we selected eight pairs of closely related species, one regionally rare and one common per pair. We addressed the following questions: 1) Do rare and non-competitive plant species suffer more from selfing and benefit more from between-population outcrossing than common and competitive species? 2) Are effects of selfing and between-population outcrossing on plant fitness modulated by a) their habitat characteristics and b) growth traits? 3) What does this suggest for conservation interventions?

Material & Methods

Plant collection

We selected 12 pairs of congeneric or closely related rare and common herbaceous plant species from eight plant families and different habitats and regions of Switzerland. Species and their origin are given in

Table S1 in the supporting information. We defined a species as rare if it is considered as near-threatened or threatened by the Swiss Red List of vascular plants (Bornand et al., 2016) and is of Swiss conservation priority (OFEV, 2019); otherwise we considered the species as common. Seeds of ten seed families (i.e. from ten different maternal plants) were collected from two populations of each of the 24 plant species (Table S1). Populations were selected based on the availability of two populations in typical natural habitats in the western half of Switzerland. As indicators of the habitat quality we used the ecological indicator values according to Landolt et al. (2010). Populations of the same species are on average 9.67 km apart (min: 0.25 km, max: 80 km). To break seed dormancy, seeds were cold-stratified in pots over eight weeks in the dark at 4°C. Seeds were then allowed to germinate. Eight weeks after germination, ten seedlings per seed family and population were randomly selected and pricked out into single pots filled with a 1:9 mixture of sand and soil (Selmaterra, Eric Schweizer, Thun, Switzerland). Plants were watered daily or every other day and allowed to grow until flowering (constant day length of 14 hours with additional light and temperature between 15-30°C). All plants were kept in the same conditions regardless of their habitat to ensure that differences between species are not due to differences in growing conditions.

Pollination treatments

We selected five different seed families from each of the two populations per species. For each seed family we selected five plants and for each of these plants we assigned one flower to each of the three pollination treatments: self-pollination, cross-pollinations between seed families within populations and between populations. Pollinations were performed by hand. When fewer than five plants from five seed families flowered, we performed as many pollinations as the number of flowers, flowering plants and seed families permitted. However, for five species (*Campanula cervicaria*, *Erysimum ochroleucum*, *Nepeta nuda*, *Stachys recta subsp. recta* and *Veronica austriaca*), germination and flowering per population were so low that we could not do all pollination treatments or any pollination. In total, we performed 542 self-pollinations, 550 and 456 cross-pollinations between seed families and populations, respectively. The pollination experiment lasted from 2018-2020, as some species and populations did not flower in the first two years.

Flowers were emasculated prior to pollinating. For each pollination, we chose a newly opened flower with mature pistil and covered all stigmas with fresh pollen. For self-pollinations the pollen was collected from another flower of the same individual. Since the number of plants bearing pollen was limited in some populations, some plants were used as pollen-donor for several pollinations. We recorded the maternal plant height (from the ground to the tip of the longest stem) and the rank of the pollinated flower in the inflorescence by counting the number of branch intersections from ground to pollinated flower.

We placed pollinated plants into self-made mesh cages for three to five days to exclude pollinating insects. Once the fruits were ripe and about to shed seed naturally, we collected the fruits. We counted and weighed the seeds per fruit (i.e. total seed mass per fruit) to the nearest 0.01 gram (Sartorius Cubis balance MSA225P-100-DI, Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany). Aborted fruits containing non-viable seeds were recorded. We calculated the developed fruit ratio by dividing the number of developed fruits by the total number of pollinations performed per species, maternal population and pollination treatment. Since the developed fruit ratio is typically low for selfed fruits of self-incompatible species (Balogh & Barrett, 2018), we identified *Cochlearia pyrenaica*, *Linaria alpina subsp. petraea*, *Linaria vulgaris*, *Prunella grandiflora* and *Teucrium chamaedrys* as being partially self-incompatible and *Lunaria rediviva* as being probably truly self-incompatible, while all other species were self-compatible.

Germination percentage and seedling performance

To evaluate the fitness of seeds resulting from hand-pollinations, we stratified 30 seeds from each of four fruits from different seed families per pollination treatment, maternal population and species. In case of fruits having fewer than 30 seeds, we selected all seeds per fruit. We cold-stratified the seeds as described above. Four to five weeks after stratification, we counted the number of germinated seedlings per fruit and calculated the germination percentage per fruit (for a total of 401 fruits). Then we pricked out five randomly selected seedlings per fruit (or all seedlings of fruits with fewer than five seedlings) in individual pots and measured the length of their longest leaf (petiole included). To evaluate seedling performance, we measured again the length of the longest leaf of three plants per fruit after six weeks of growth in individual pots. In total, 902 seedlings were measured.

Plant rarity

As a measure of regional rarity we used the range size, defined as the highest number of 5x5 km grid squares occupied by a species in Switzerland during the last century (Bornand, 2014). We used the range size in Switzerland, as the range size of species used in this study is not yet available at this resolution at the European scale. However, Vincent et al. (2020) showed that European and Swiss range sizes of 21 plant species are quite closely and positively correlated ($R^2=0.508$, $P<0.001$). As a measure of local rarity, we used the species indicator-value for dominance *in-situ* (DG; Landolt et al., 2010; Table S1). The indicator value for dominance *in-situ* describes the accumulation (i.e. abundance) of individuals of a species at the place where they occur. It ranges from a value of one for a species with scattered individuals to five for a species that is usually dominant (Landolt et al., 2010).

Plant-habitat characteristics, competitive strategy and growth traits

To characterize plant habitat, we used the species indicator-value for nutrients (N) and moisture (F; Landolt et al., 2010; Table S1). Indicator values according to Landolt et al. (2010) describe the realized ecological niche of a species by its position along an environmental gradient following an ordinal scale ranging from one to five. Indicator values for nutrients and moisture characterize the nutrient content in the soil (mainly nitrogen) and the average soil moisture during the growth period of the species. They are the Swiss equivalent of the indicator values for nutrients and moisture according to Ellenberg for Central Europe (Ellenberg et al., 1991). For each species, we also defined a variable called “competitive strategy” based on the species life-strategy from Landolt et al. (2010; which was partly adapted from Grime’s CSR life-strategy; Grime, 1979). This variable describes the species’ competitive-ability for light capture. To do so, we assigned the values “0”, “1” and “2” to ruderal or stress-tolerator (rrr, rrs, rss or sss), competitive (crr, csr or css) and strongly competitive species (ccs or ccr; Table S1), respectively.

For ten plants per species we measured the highest stem height or longest leaf length (petiole included) if the plant had only formed a rosette. We then averaged the mean plant size per species, which is considered to be associated with plant life-history strategy (Moles & Leishman, 2008). We measured the specific leaf area (hereafter called SLA) of one leaf of each of five individuals from different seed families per species, following the method from Cornelissen et al. (2003) and averaged the values per species. SLA is a trait used in the leaf economics spectrum (Westoby, 1998; Wright et al., 2004), which distinguishes between plants with a fast-growing strategy (species with short-lived, nutrient-rich leaves and high SLA) and plants with a slow-growing strategy (species with long-lived, nutrient-poor leaves with a low SLA). A high indicator value for nutrients and a high competitive ability are also characteristics of fast-growing and common plants (Poorter & Remkes, 1990; Westoby, 1998; Murray et al., 2002).

Statistical analysis

To test whether fitness-related traits of different local and regional rarity, competitive strategy, habitat characteristics and growth traits are differently affected by selfing and within- and between- population outcrossing, we fitted a linear mixed-effect model (LMER; lme4, lmerTest and MuMIn R-packages; Bates et al., 2015; Kuznetsova et al., 2017; Barton, 2020) for each response variable: seed number, total seed mass and germination percentage per fruit, ratio of developed fruits per pollination treatment and population and seedling leaf-length after six weeks of growth. Leaf length, seed number and mass were centred and scaled per species for standardization between species. As fixed effects, we included pollination treatment, range size, indicator values for dominance *in-situ*, nutrients and moisture, competitive strategy, mean plant size SLA and the two-way interactions between pollination treatments and the other variables. As we found no strong correlation (R^2>0.7, Fig. 1; corrplot package; Wei & Simko, 2017) among explanatory variables, we tested their effects simultaneously in the models. Continuous explanatory variables were centred and scaled. Analyses were performed in R 3.6.3 (R Core Team, 2020).

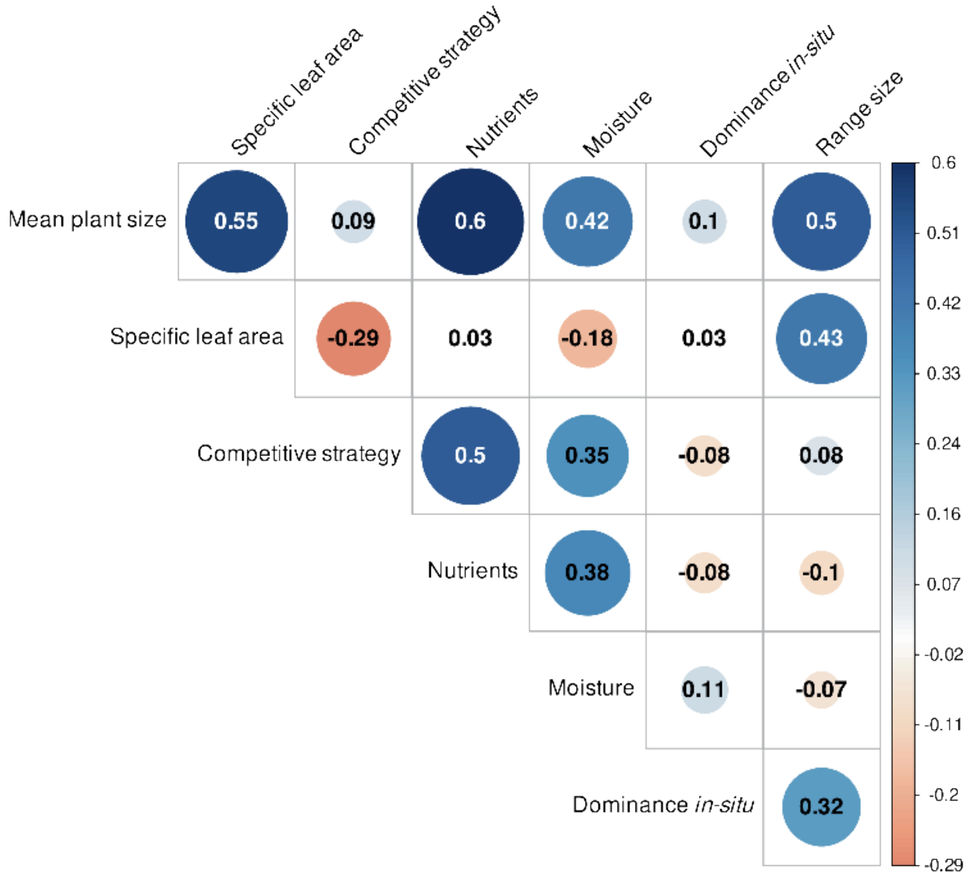

Figure 1: Pearson correlations between species traits, indicator values and range size. Cells with red and blue circle correspond to significantly negative and positive correlations (P<0.05), respectively.

Of the 24 species, we kept only species with all explanatory variables and a balanced observation number per response variable and pollination treatment in the models (Table S1). Therefore, we fitted the leaf-length model with 13 species and the other models with 16 species. To correct for the variation of seed number and mass per fruit between years, flower positions in the inflorescence and different plant size (Thompson & Rabinowitz, 1989; Moles et al., 2004; Moles & Leishman, 2008), we included pollination year, flower rank and maternal plant height as covariates in the models for germination percentage, seed number and mass. Flower rank and flowering stem length were both included in the models, since they were not highly correlated (R^2=0.320, t=10.96, df=1050, P<0.001). We tested also whether seed number and mass per fruit affected the germination percentage by adding these two terms in the germination-percentage model. Since their effect on germination percentage was not significant and weakly significant, respectively, and did not qualitatively change the results, we removed them from the model. In addition, we centred and scaled the initial seedling leaf-length per species and included it as covariate in the model for leaf length to adjust for differences in initial conditions. We included the pollination year and total pollination number per species, maternal population and pollination treatment in the model for developed fruit ratio. As random effects, we included the maternal population nested in species in all models. In addition, we nested the maternal seed family in the models for seed number and mass, the plant individual in the germination-percentage model and the pollination cross in the leaf-length model. In the models for developed fruit ratio, seed number and mass, we further nested the species in species pair. Models are shown in Tables S2-S6.

LMER models were taken as a baseline to identify significant interactions between the pollination treatments and other explanatory variables. We used a stepwise backward procedure to reduce the complexity of the LMER models and likelihood-ratio tests to calculate each interaction and factor significance (Zuur, 2009; significance threshold: $P<0.05$). To illustrate how selfing and between-population outcrossing impacted plant fitness-related traits (ggplot2 package; Wickham, 2016), we compared their effects to effects of between seed family within population outcrossing by calculating the difference between the average of each fitness-related traits for these pollination treatments. Thus, negative values indicate negative effects of selfing and between-population outcrossing compared with between seed-family outcrossing within populations, while positive values indicate benefits.

Results

Overall, selfing decreased plant fitness compared with between seed-family outcrossing, while between-population outcrossing increased it. In addition, these effects depended on regional plant rarity (i.e. range size), competitive strategy, habitat characteristics, growth traits and fitness-related traits, but not on the local plant rarity (i.e. dominance *in-situ*).

Effects of selfing and outcrossing on species of different range size and competitive strategy

Selfing had milder negative effects on the total seed mass per fruit, developed fruit ratio and seedling performance in rare plant species than in common species (Fig. 2A-D; Tables S3-6). Moreover, selfing was even more beneficial than between-population outcrossing for the germination percentage of rare species, whereas the reverse was true for the performance of rare seedlings. In contrast, selfing had generally negative effects and between-population outcrossing had positive effects on all fitness-related traits of common species.

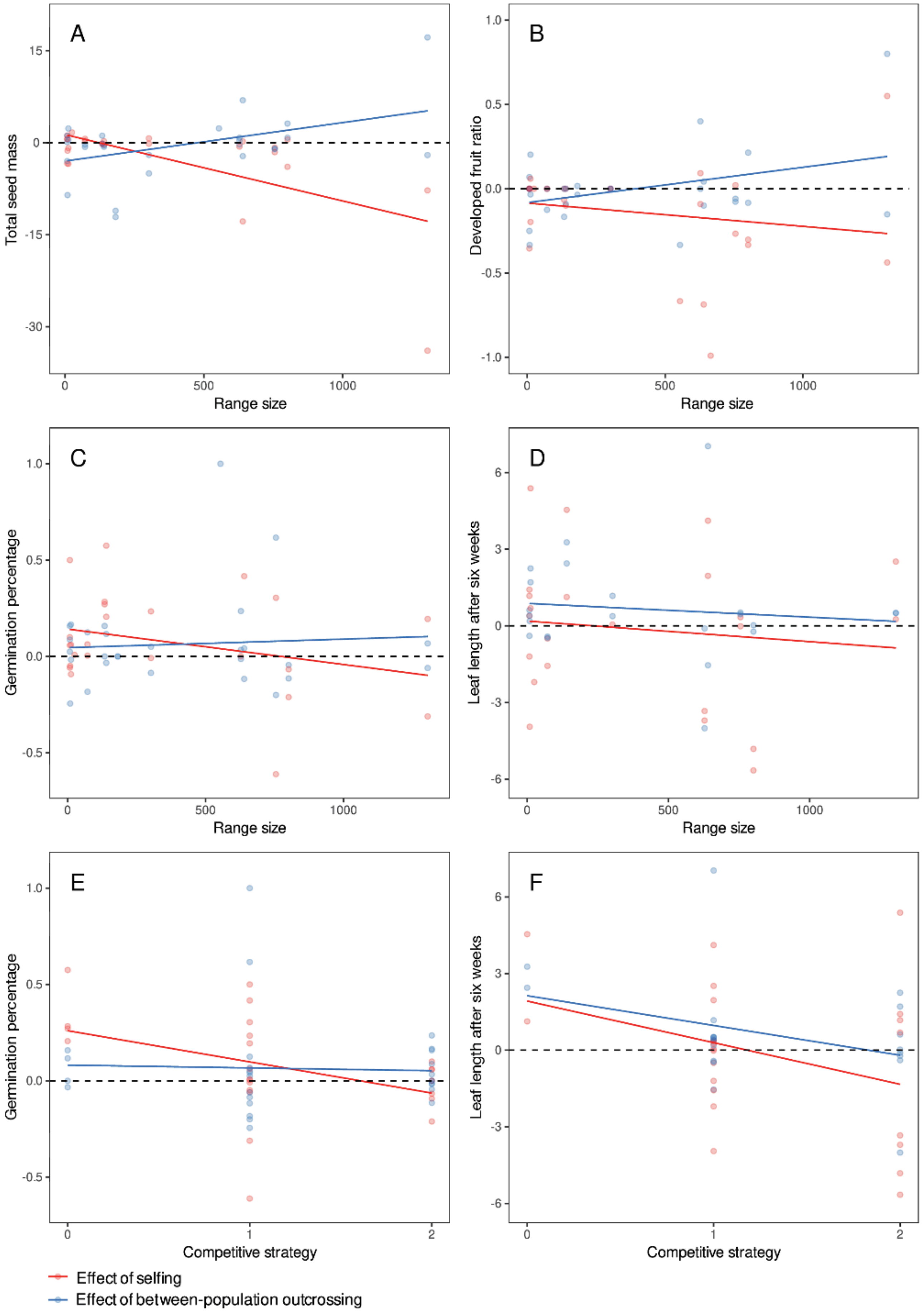
A
Total seed mass
15
0
-15
-30
0
500
1000
Range size
B
Developed fruit ratio
1.0
0.5
0.0
-0.5
-1.0
0
500
1000
Range size
C
Germination percentage
1.0
0.5
0.0
-0.5
0
500
1000
Range size
D
Leaf length after six weeks
6
3
0
-3
-6
0
500
1000
Range size
E
Germination percentage
1.0
0.5
0.0
-0.5
0
1
2
Competitive strategy
F
Leaf length after six weeks
6
3
0
-3
-6
0
1
2
Competitive strategy
Effect of selfing
Effect of between-population outcrossing

Figure 2: Relationship between total seed mass per fruit (A), developed fruit ratio per pollination treatment and population (B), germination percentage per fruit (C) and seedling leaf-length (D) with range size. Relationship between germination percentage (E) and seedling leaf-length (F) with species competitive strategy. Fitness measures were scaled and centered for analysis. Red regression lines show the selfing effect (self-pollinations – between seed-family cross-pollinations) and blue ones show the between-population outcrossing effect (between population cross-pollinations – between seed-family cross-pollinations). Points show the values per population.

Early fitness effects of selfing and outcrossing depended also on competitive strategy, i.e. for the germination percentage and seedling performance (Fig. 2E-F; Tables S5-6). Selfing had positive effects on both fitness-related traits in non-competitive species, while it had slightly negative effects in competitive species. However, between-population outcrossing was more beneficial than selfing and between seed-family outcrossing for seedling performance of non-competitive species.

Effects of selfing and outcrossing on species with different habitat characteristics

Selfing had negative effects on the seed number, developed fruit ratio and seedling performance in species from nutrient-poor habitats, while it had positive effects on seed number and seedling performance in species from nutrient-rich habitats (Fig. 3A-C, Tables S2-4 & 6). Moreover, between-population and seed-family outcrossing were both more beneficial than selfing in species from nutrient-poor habitats in all three fitness-related traits. In contrast, for species from nutrient-rich habitats, between-population outcrossing had negative effects on seed number, while it had positive effects on seedling performance.

Selfing had slightly positive effects on the seed number of species from rather dry habitats, but negative effects on the seed number of species from rather wet habitats (Fig. 3D). Moreover, selfing had slightly negative effects on total seed mass equally for species from dry and moist habitats (Fig. 3E). Between-population outcrossing had increasingly negative effects on seed number as the plant habitat became wetter, but was beneficial in seedlings regardless of the moisture-level of the habitat, where the species occur (Fig. 3D & F).

To summarize, the effects of between-population outcrossing on the seed number depended strongly on the nutrient level and moisture level of the habitat, where the species occur (Fig. 3A & D). In contrast, effects of between-population outcrossing on seed mass, developed fruit ratio and seedling performance varied less with such habitat characteristics and were generally more beneficial for plant fitness than selfing (Fig. 3B-C & E-F).

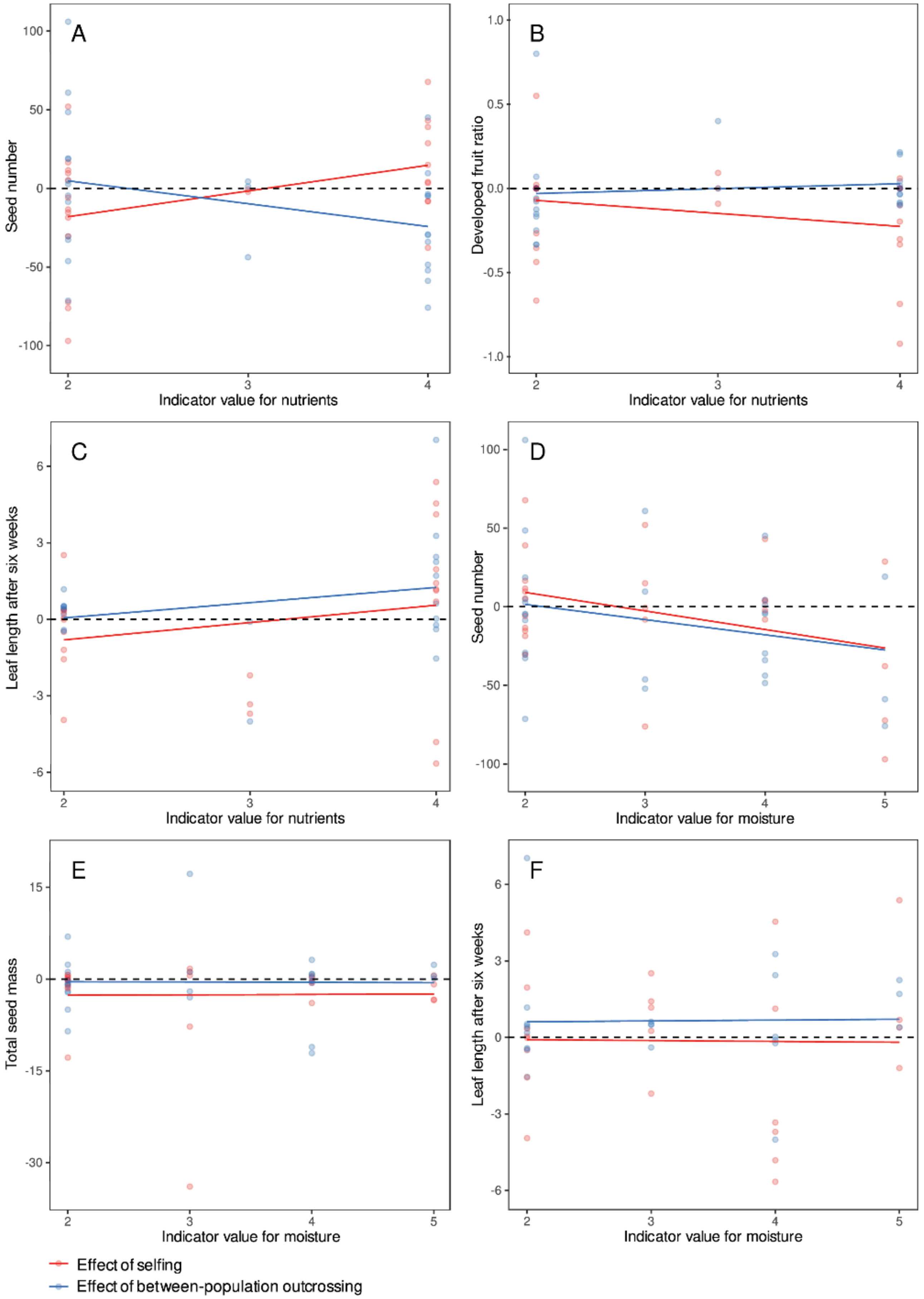
A
Seed number
100
50
0
-50
-100
2
3
4
Indicator value for nutrients
B
Developed fruit ratio
1.0
0.5
0.0
-0.5
-1.0
2
3
4
Indicator value for nutrients
C
Leaf length after six weeks
6
3
0
-3
-6
2
3
4
Indicator value for nutrients
D
Seed number
100
50
0
-50
-100
2
3
4
5
Indicator value for moisture
E
Total seed mass
15
0
-15
-30
2
3
4
5
Indicator value for moisture
F
Leaf length after six weeks
6
3
0
-3
-6
2
3
4
5
Indicator value for moisture
Effect of selfing
Effect of between-population outcrossing

Figure 3: Relationship between seed number per fruit (A), developed fruit ratio per pollination treatment and population (B) and seedling leaf-length (C) with the species indicator-value for nutrients. Relationship between seed number per fruit (D), total seed mass per fruit (E) and seedling leaf-length (F) with the species indicator-value for moisture. Red regression lines show the selfing effect (self-pollinations – between seed-family cross-pollinations within populations) and blue ones show the between-population outcrossing effect (between population cross-pollinations – between seed-family cross-pollinations within populations). Points show the values per population.

Effects of selfing and outcrossing on species of different growth traits

The effects of selfing and outcrossing depended on the SLA of the species and on fitness-related traits (Tables S2-3 & 5-6). Selfing had negative effects on seed number and seedling performance and positive effects on seed mass in low-SLA species, while the opposite was found for high-SLA species (Fig. 4A-C). Furthermore, between-population outcrossing had positive effects on seed number and negative effects on seed mass and seedling performance in low-SLA species, while the opposite was found for high-SLA species.

Selfing had slight positive effects on the germination percentage across all plant species regardless of their mean size. Selfing had slight negative effects on seedling performance only for small species, whereas for tall species, selfing had slight positive effects on seedling performance. Moreover, selfing had increasingly negative effects on the seed mass as the species became taller (Fig. 4D-F). Between-population outcrossing had negative effects on seed mass for small species, while it had beneficial effects on germination percentage for small species, and these beneficial effects were larger than those of selfing. In seedlings, between-population outcrossing was clearly beneficial compared with between seed-family outcrossing within populations, except in very small specie where both outcrossing treatments were equally beneficial (Fig. 4F).

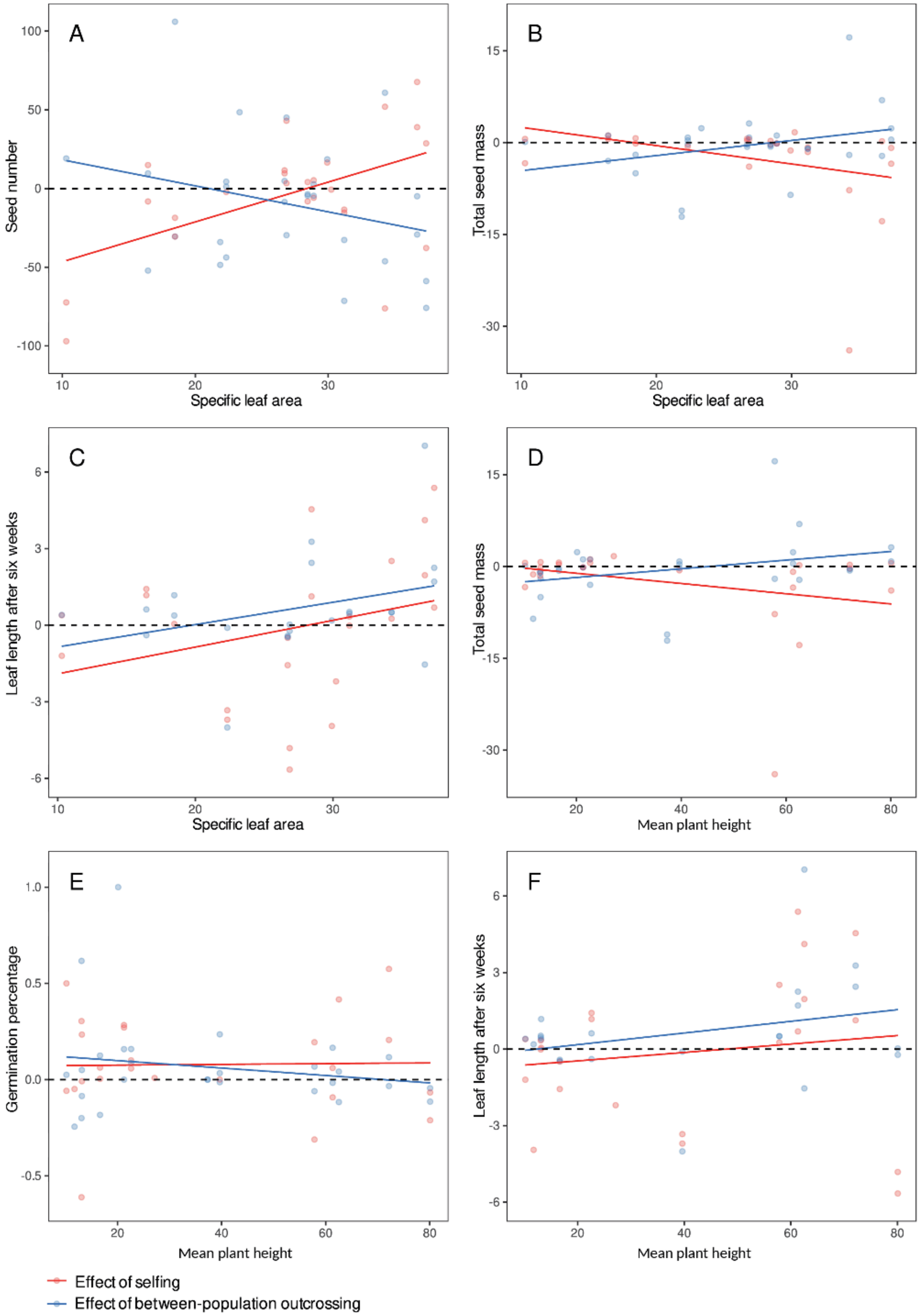
A
Seed number
100
50
0
-50
-100
10
20
30
Specific leaf area
B
Total seed mass
15
0
-15
-30
10
20
30
Specific leaf area
C
Leaf length after six weeks
6
3
0
-3
-6
10
20
30
Specific leaf area
D
Total seed mass
15
0
-15
-30
20
40
60
80
Mean plant height
E
Germination percentage
1.0
0.5
0.0
-0.5
20
40
60
80
Mean plant height
F
Leaf length after six weeks
6
3
0
-3
-6
20
40
60
80
Mean plant height
Effect of selfing
Effect of between-population outcrossing

Figure 4: Relationship of seed number (A), total seed mass per fruit (B) and seedling leaf-length (C) with SLA. Relationship of total seed mass per fruit (D), germination percentage per fruit (E) and seedling leaf-length (F) with mean size per species. Red regression lines show the selfing effect (self-pollinations – between seed-family cross-pollinations within populations) and blue ones show the between-population outcrossing effect (between population cross-pollinations – between seed-family cross-pollinations within populations). Points show the values per population.

Discussion

Interactive effects of plant rarity and competitive strategy with selfing and outcrossing

Overall, in our experiment selfing decreased plant fitness, while between-population outcrossing increased it. This is consistent with the literature (e.g. Willi & Fischer, 2005; Charlesworth & Willis, 2009; Stojanova et al., 2021), and extends it to a multi-species framework. In contrast to our alternative hypothesis, our results show that rare species did not suffer more from adverse effects of selfing than common species. Indeed, selfing had equal effects than between-population outcrossing, or was even positive for the early fitness of rare species, while it had mainly negative effects for common and competitive species (Fig. 2). Furthermore, selfing and between-population outcrossing had similar effects for non-competitive species and for rare species and similar effects for competitive and common species.

The stronger inbreeding depression found for common and competitive species than for rare and non-competitive species was likely due to genetic purging in the latter species. Populations of rare plant species, being often small and isolated, tend to have longer inbreeding histories than common ones (Ellstrand & Elam, 1993; Oostermeijer et al., 2003; Charlesworth & Willis, 2009). Thus, if inbreeding depression is due to (over-)dominance, natural selection can reduce the frequency of deleterious alleles thanks to an increased efficiency (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987). As a result, populations of rare species may recover from genetic bottlenecks and consequently suffer less from inbreeding depression than populations of common species, which did not experience such a long history of inbreeding (Barrett & Charlesworth, 1991; Swindell & Bouzat, 2006; Angeloni et al., 2011 and the references therein; Robinson et al., 2018).

Seedling performance of rare and non-competitive species benefited particularly from between-population outcrossing. This suggests that heterosis is trait-dependent and stronger after germination in rare and non-competitive seedlings, while it is prominent in all studied traits in common and competitive species. Furthermore, this suggests that the maternal populations were not locally adapted, as between-population outcrossing could have led to a disruption of locally adapted genotypes resulting in strong outbreeding depression (Waser & Price, 1989). It is possible that species life-history traits have contributed to preserve a high within-population genetic variability. For example, entomophily and perenniality, traits shared by all species included in this study (Info Flora, https://www.infoflora.ch) typically allow for long-distance gene exchange and increased reproductive opportunities (Levin & Kerster, 1974; Hamrick et al., 1979).

Conservation of rare species increasingly involves proactive interventions, such as *ex-situ* cultures, population reinforcements and translocations. Hence, the question whether to mix populations and restore gene flow between them has gained considerable attention (Stojanova et al., 2021). From our results, we conclude that between-population outcrossing appears to be the most beneficial pollination treatment for the fitness of rare species (but also for common species), as favouring pollination treatments increasing plant fitness in the later seedling stage, rather than in the earliest stages, is likely more determinant for the establishment success of translocations. Moreover, the similarity of the patterns we found in rare and non-competitive species, likely driven by the tendency of regionally rare species to be less competitive than common ones (Fig. 1; Murray et al., 2002), indicates that this is also the case for ruderal and stress-tolerator species (Grime, 1979). However, we recommend future studies to test whether between-population outcrossing increases plant fitness beyond the F1 generation, as heterosis may fade in the second generation (Tallmon et al., 2004). Also, as the maternal population pairs in this study were geographically rather close (9.67 km apart on average), we suggest caution with performing between-population outcrosses beyond this range (Fenster & Galloway, 2000).

Finally, that patterns found for regional rarity were not reflected by local rarity contrasts with our hypothesis that locally and regionally rare species may respond similarly to pollination treatments. Although related, these two aspects of plant rarity (Fig. 1; Rabinowitz, 1981) may have different implications for selfing and between-population outcrossing and thus lead to different patterns on plant fitness. Possibly, our assessment of species local rarity from the literature (Landolt et al., 2010) may have been not as precise as field observations would have been, which might possibly have obscured effects of local rarity on selfing and between-population outcrossing in our study. Hence, we recommend future studies might want to include direct field measures of local rarity (see e.g. Brändle et al., 2003), such as species abundance in communities when studying the effects of selfing and between-population outcrossing on different plant species.

Interactive effects of plant-habitat characteristics with selfing and outcrossing

In accordance with our hypothesis, we found that the effects of selfing and between-population outcrossing varied strongly depending on species-specific fitness-related traits and plant-habitat characteristics. Selfing had generally neutral or slightly negative effects on seedlings of species from rather dry habitats, while between-population outcrossing had clearly beneficial effects on seedlings of species with different habitat characteristics. Furthermore, effects of between-population outcrossing in seed mass, developed fruit ratio and seedling performance were more similar to each other (rather positive across all plant-habitat characteristics) than the mainly detrimental effects of between-population outcrossing on seed number (Fig. 3).

Inbreeding depression was predicted to be more pronounced under stress (Armbruster & Reed, 2005; Hayes et al., 2005; but see Nason & Ellstrand, 1995). For instance, Hayes et al. (2005) found that inbreeding depression increased with nutrient limitation. Moreover, Sedlacek et al. (2012) reported that with ample water, the rare *Echium wildpretii* showed no inbreeding depression, while under dry conditions, survival, but not performance, of selfed seedlings was smaller than the one outbred seedlings. Similar results have been reported in *Lychnis (Silene) flos-cuculi* by Hauser and Loeschcke (1996) and by Leimu et al. (2008). However, plants were probably not stressed in our experiment, but just originated from different habitat conditions. Hence, we did not test responses of selfed and outcrossed plants to environmental stressors but rather, whether plants from different habitat conditions had evolved different strategies to respond to selfing and between-population outcrossing. Possibly, our findings might have been different, if our experimental plants had been raised under more stressful conditions. However, recent literature questioned the triggering effect of stress on inbreeding depression (Sandner et al., 2021), suggesting that environmental variation increases phenotypic variation and subsequently increases the probability of detecting inbreeding depression.

The different pattern found between the seed number per fruit and the other fitness-related traits may be caused by decreasing maternal control over fitness-related traits with increasing plant age. Seed number per fruit depends strongly on maternal factors, such as the number of ovules per ovary and their fertility, which also depend on pollination conditions and species self-compatibility (Cilas et al., 2010). In contrast, the other fitness-related traits investigated here may be under milder maternal control. However, increasing seed number may not represent a fitness advantage for the maternal plant, as it usually trade-offs with seed mass and size (Gambín & Borrás, 2010) and thus may reduce seedling viability (Moles & Westoby, 2006).

Finally, our results demonstrated that between-population outcrossing was beneficial for most fitness-related traits, especially seedling performance, of species with contrasting habitat characteristics (except in seed number of species from nutrient-rich and moist habitats). Hence, we recommend favouring this treatment when plant material is propagated for conservation purposes, i.e. in *ex-situ* cultures for (re-)introduction to the wild.

Interactive effects of plant-growth traits with selfing and outcrossing

In accordance with our hypothesis, we found that plant SLA and mean species size influenced selfing and between-population outcrossing effects on fitness (Fig. 4). These findings are consistent with several studies reporting variation in the level of inbreeding depression between traits and life-cycle stages (e.g. Husband & Schemske, 1996; Glaettli & Goudet, 2006; Sedlacek et al., 2012). This variation is likely caused by a difference in the mutation rate over time and between genes (Charlesworth & Charlesworth, 1987; Angeloni et al., 2011). However, despite the considerable variability of species responses to selfing and between-population crossing, the fact that plants allocating fewer resources to growth (i.e. low-SLA and small species) tended to benefit from between-population outcrossing suggests that it may be the most beneficial pollination treatment to increase the fitness of slow-growing species. Therefore, we recommend favouring measures increasing genetic diversity, e.g. between-population outcrossing and mixing plants from different populations, when propagating and translocating slow-growing species.

Conclusion

Contrary to our expectations, regionally rare species did not suffer more from selfing than regionally common ones. Long inbreeding history and genetic purging in rare plant populations (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987) might explain this pattern. Moreover and despite a large variability of the effects of selfing and between-population outcrossing across fitness-related traits, we demonstrated that plant species generally benefit from between-population outcrossing. This was particularly the case in seedlings of rare, non-competitive and slow-growing species. We consider our results also useful for the conservation practice related to rare plant species. Although outbreeding depression is often feared (Frankham, et al. 2011), our study suggests that outbreeding depression is probably not a threat to plant species in general or to rare species in particular. On the contrary, it seems to be the most adequate management strategy to minimize negative effects on fitness in plants (Charlesworth & Willis, 2009). It also increases genetic diversity, which is supposed to be beneficial for species to adapt to climatic changes (Jump et al., 2009) or other global change facets. Hence, we recommend considering between-population outcrossing as useful conservation tool, when propagating plant material for conservation purposes, and when performing *ex-situ* cultures, population reinforcements and translocations.

Acknowledgements

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Supporting information

Table S1: Plant species included in the study. Each species belongs to a pair of a rare and a closely related common species. The column Analyses indicates if the species included in all models, excluded from the leaf-length model, or removed from the analyses due to low sample size (see methods). Plant family, seed-collection sites, mean plant size [cm], mean specific leaf area (SLA; [mm^2/mg]), competitive strategy, indicator values for levels of moisture (F), nutrients (N) and dominance in-situ (DG) and range size (assessed as number of monitoring areas in Switzerland) are given for each species. BE, FR, GE, NE, TI, VD, VS refer to the Swiss cantons of Bern, Fribourg, Geneva, Neuchâtel, Ticino, Vaud, Valais, respectively.

Pair	Rarity	Analyses	Species	Family	Collection sites	Plant size	SLA	Comp strat.	N	F	DG	Range size
1	rare	all	*Campanula cervicaria*	Campanulaceae	Forst (2 pop) Bern, BE	27.1	30.2	1	3	3	1	25
1	common	removed	*Campanula rotundifolia*	Campanulaceae	Le Lieu + Le Pont, VD	-	-	1	2	2	2	2504
2	rare	all	*Cochlearia pyrenaica*	Brassicaceae	Gantrisch + Eriz, BE	10.2	10.3	1	2	5	3	8
2	common	all except leaf length	*Lunaria rediviva*	Brassicaceae	Bönigen Tüscherswald + Innereriz, BE	37.3	21.9	2	4	4	3	182
3	common	all	*Erysimum cheiranthoides*	Brassicaceae	Holligen + Marzili, Bern, BE	72.1	28.5	0	4	4	2	140
3	rare	removed	*Erysimum ochroleucum*	Brassicaceae	Chasseral (2 pop), BE	23.9	27.3	1	2	1	2	6
4	rare	removed	*Linaria alpina subsp. petraea*	Plantaginaceae	Lac de Joux + Le Lieu, VD	-	-	0	2	3	2	33
4	common	all	*Linaria vulgaris*	Plantaginaceae	Güterbahnhof, Bern, BE + Visp, VS	62.5	36.7	1	4	2	3	640
5	rare	removed	*Nepeta nuda*	Lamiaceae	Nax + Suen, VS	-	-	1	3	1	2	9
5	common	removed	*Stachys recta subsp. recta*	Lamiaceae	Nax + Visperterminen, VS	-	-	1	3	3	2	622
6	rare	removed	*Papaver occidentale*	Papaveraceae	Zweisimmen (2 pop), BE	26.3	22.3	-	-	-	-	13
6	common	removed	*Papaver rhoeas*	Papaveraceae	Insel + Wylerbad, Bern, BE	-	-	0	3	2	2	785
7	common	all	*Potentilla argentea*	Rosaceae	Herbriggen + Zermatt, VS	13.2	18.5	1	2	2	2	302
7	rare	all	*Potentilla multifida*	Rosaceae	Gornergrat + Riffelberg, Zermatt, VS	22.6	16.4	2	4	3	2	8
8	common	all	*Prunella grandiflora*	Lamiaceae	Lamboing + Nods, BE	13.1	31.2	1	2	2	2	755
8	rare	all	*Prunella laciniata*	Lamiaceae	Le Landeron + Nods, NE	16.6	26.7	1	2	2	1	72
9	rare	all	*Scrophularia auriculata*	Scrophulariaceae	Choulex + Pointe à la Bise, GE	61.3	37.4	2	4	5	2	12
9	common	all	*Scrophularia nodosa*	Scrophulariaceae	Magnedens + Montagny-la-ville, FR	80.1	26.9	2	4	4	2	801
10	rare	all	*Silene vallesia*	Caryophyllaceae	Leiggru + Oberried, VS	11.7	29.9	1	2	2	3	9
10	common	all	*Silene vulgaris*	Caryophyllaceae	La Neuveville + Nods, BE	57.8	34.3	1	2	3	3	1305
11	rare	all except leaf length	*Teucrium botrys*	Lamiaceae	Bözingen (2 pop), BE	21.2	28.9	0	2	2	2	134
11	common	all except leaf length	*Teucrium chamaedrys*	Lamiaceae	Lamboing + Ligerz, BE	20.1	23.3	1	2	2	3	554
12	rare	removed	*Veronica austriaca*	Plantaginaceae	Les Jordan + Petits Michel, NE	26.9	34.8	1	2	2	2	14
12	common	all	*Veronic urticifolia*	Plantaginacea	Holzflue + Zweisimmen, BE	39.6	22.3	2	3	4	2	628

Table S2: Results of the LMER testing for the effects of pollination treatments, local and regional species rarity, SLA, mean plant size, competitive strategy, nutrient and moisture indicator values on seed number per fruit. Significances were obtained by stepwise deletion of non-significant terms and comparison of models with and without each interaction and factor using log-likelihood-ratio tests. This resulted in a minimal model containing only significant terms (in black). We kept covariables (in italics) and random factors in the model and for the latter present their standard deviations. Numbers in bold indicate statistical significance. The final model had a marginal R^2 of 0.051, and a conditional R^2 of 0.148

Seed number			
Fixed effects	**AIC**	**χ^2**	***P***
Inflorescence size	2289.37	0.08	0.777
Flower level	2266.52	17.225	0.697
Year	2288.19	0.891	0.641
Pollination treatment × SLA	2294.69	7.393	**0.025**
Pollination treatment × Nutrients	2304.24	16.943	**< 0.001**
Pollination treatment × Moisture	2302.24	14.943	**0.001**
Competitive strategy	2291.29	0.395	0.53
Plant size	2292.9	0.222	0.637
Range size	2294.68	0.098	0.755
Dominance	2296.58	0.011	0.915
Pollination treatment × Plant size	2298.57	5.099	0.078
Pollination treatment × Range size	2297.47	1.582	0.454
Pollination treatment× Competitive strategy	2299.89	1.918	0.383
Pollination treatment × Dominance	2301.97	1.732	0.421
Random effects	**SD**		
Seed family	0.13		
Population	0.293		
Species	0		
Species pair	0		
Residuals	0.952		

Table S3: Results of the LMER testing for the effects of pollination treatments, local and regional species rarity, SLA, mean plant size, competitive strategy, nutrient and moisture indicator values on total seed mass per fruit. Significances were obtained by stepwise deletion of non-significant terms and comparison of models with and without each interaction and factor using log-likelihood-ratio tests. This resulted in a minimal model containing only significant terms (in black). We kept covariables (in italics) and random factors in the model and for the latter present their standard deviations. Numbers in bold indicate statistical significance. The final model had a marginal R^2 of 0.093 and a conditional R^2 of 0.21.

	Total seed mass		
Fixed effects	**AIC**	**χ^2**	***P***
Inflorescence size	2247.7	1.237	0.266
Flower level	2233.52	27.062	0.169
Year	2246.7	2.238	0.327
Pollination treatment × Range size	2260.79	16.323	**< 0.001**
Pollination treatment × SLA	2253.9	9.437	**0.009**
Pollination treatment × Moisture	2279.36	34.898	**< 0.001**
Pollination treatment × Plant size	2260.4	15.94	**< 0.001**
Competitive strategy	2248.46	0.582	0.446
Dominance	2249.88	0.175	0.676
Nutrients	2251.71	0.079	0.779
Pollination treatment × Competitive strat.	2253.63	1.164	0.559
Pollination treatment × Dominance	2256.46	0.687	0.709
Pollination treatment × Nutrients	2259.78	0.004	0.998
Random effects	**SD**		
Seed family	0.146		
Population	0.325		
Species	0		
Species pair	0		
Residuals	0.926		

Table S4: Results of the LMER testing for the effects of pollination treatments, local and regional species rarity, SLA, mean plant size, competitive strategy, nutrient and moisture indicator values on the ratio of developed fruit per pollination treatment, population and species. Significances were obtained by stepwise deletion of non-significant terms and comparison of models with and without each interaction and factor using log-likelihood-ratio tests. This resulted in a minimal model containing only significant terms (in black). We kept covariables (in italics) and random factors in the model and for the latter present their standard deviations. Numbers in bold indicate statistical significance. The final model had a marginal R^2 of 0.464 and a conditional R^2 of 0.788.

Developed fruit ratio			
Fixed effects	**AIC**	**χ^2**	***P***
Number of performed pollinations	-5.3	0.069	0.793
Year	-3.35	4.018	0.134
Dominance	10.3	15.664	**< 0.001**
Pollination treatment × Range size	4.82	12.187	**0.002**
Pollination treatment × Nutrients	0.94	8.303	**0.016**
Plant size	-3.36	3.455	0.063
Moisture	-4.82	1.497	0.221
SLA	-4.32	2.631	0.105
Competitive strategy	-4.95	0.762	0.383
Pollination treatment × Dominance	-3.71	4.599	0.1
Pollination treatment × Moisture	-4.31	5.656	0.059
Pollination treatment × Plant size	-5.96	0.127	0.939
Pollination treatment × SLA	-2.09	0.023	0.988
Pollination treatment × Competitive strat.	1.89	0.004	0.998
Random effects	**SD**		
Populations	0.108		
Species	0		
Species pair	0.178		
Residuals	0.168		

Table S5: Results of the LMER testing for the effects of pollination treatments, local and regional species rarity, SLA, mean plant size, competitive strategy, nutrient and moisture indicator values on germination percentage per fruit. Significances were obtained by stepwise deletion of non-significant terms and comparison of models with and without each interaction and factor using log-likelihood-ratio tests. This resulted in a minimal model containing only significant terms (in black). We kept covariables (in italics) and random factors in the model and for the latter present their standard deviations. Numbers in bold indicate statistical significance. The final model had a marginal R^2 of 0.153, and a conditional R^2 of 0.725.

	Germination percentage		
Fixed effects	**AIC**	**χ^2**	**P**
Inflorescence size	67.23	1.433	0.231
Flower level	40.97	13.169	0.87
Year	64.68	0.876	0.645
Pollination treatment × Range size	72.77	8.971	**0.011**
Pollination treatment × Competitive strat.	73.63	9.826	**0.007**
Pollination treatment × Plant size	70.75	6.952	**0.031**
Dominance	67.8	1.282	0.257
Moisture	68.52	0.42	0.517
SLA	70.1	0.418	0.518
Nutrients	71.68	0.192	0.661
Pollination treatment × Moisture	73.49	1.821	0.402
Pollination treatment × SLA	75.67	3.065	0.216
Pollination treatment × Nutrients	76.6	2.348	0.309
Pollination treatment × Dominance	78.25	3.004	0.223
Random effects	**SD**		
Plant	0		
Seed family	0.034		
Population	0.064		
Species	0.315		
Residuals	0.224		

Table S6: Results of the LMER testing for the effects of pollination treatments, local and regional species rarity, SLA, mean plant size, competitive strategy, nutrient and moisture indicator values on seedling leaf-length after six weeks of growth. Significances were obtained by stepwise deletion of non-significant terms and comparison of models with and without each interaction and factor using log-likelihood-ratio tests. This resulted in a minimal model containing only significant terms (in black). We kept covariables (in italics) and random factors in the model and for the latter present their standard deviations. Numbers in bold indicate statistical significance. The final model had a marginal R^2 of 0.138 and a conditional R^2 of 0.494.

	Leaf length		
Fixed effects	**AIC**	**χ^2**	***P***
Initial leaf length	1806.99	50.439	**< 0.001**
Pollination treatment × Range size	1763.69	9.146	**0.01**
Pollination treatment × SLA	1761.05	6.506	**0.039**
Pollination treatment × Nutrients	1775.71	21.164	**< 0.001**
Pollination treatment × Moisture	1764.68	10.134	**0.006**
Pollination treatment × Competitive strat.	1768.27	13.725	**0.001**
Pollination treatment × Plant size	1773.18	18.632	**< 0.001**
Dominance	1758.55	0	1
Pollination treatment × Dominance	1760.55	2.093	0.351
Random effects	**SD**		
Fruit	0.538		
Plant	0		
Population	0.228		
Species	0		
Residuals	0.751		

Chapter 4

How genetic diversity, propagule pressure and origin shape the fitness of rare and threatened plants in reintroductions

Sarah Bürli, Andreas Ensslin, Markus Fischer

Abstract

1. Genetic diversity and propagule pressure have been suggested to be important determinants for translocation success. Genetic diversity is expected to increase plant resistance to enemies, resilience, adaptability and inter-individual resource-partitioning, while propagule pressure is expected to buffer against stochasticity. Alternatively, higher genetic diversity and propagule pressure may also result to maladaptation and increased intra-specific competition and plant-enemy attraction. Moreover, these effects may vary with source-population traits, such as ecological similarity with the translocation site.

2. To disentangle these hypotheses, we reintroduced four threatened herbaceous species in Switzerland at two levels of genetic diversity and propagule pressure. We used material from single and mixed source populations and introduced smaller or larger numbers of plants. We assessed the effects of genetic diversity and propagule pressure on several fitness components, while accounting for source-population identity.

3. Two of the four reintroductions had high survival percentages, while the others had a high mortality due to extreme environmental events. We found that genetic diversity and source-population identity interacted and were important determinants of early plant fitness. Effects of genetic diversity became positive and negative dependently of the ecological distance between source and target site. We suggest that negative effects of high genetic diversity could be due to dilution of local adaptations. Last, high propagule pressure affected the fitness of only one species and reduced it, possibly due to more negative biotic interactions.

4. *Synthesis*: Our study shows that genetic diversity, source-population identity and, to a lesser extent, propagule pressure, affect fitness of reintroduced plants. Our results show that the success of reintroductions depends on multiple factors and indicate that conservation practitioners should maximize ecological similarity of translocation site and source populations and plant translocated individuals in numerous but small plots.

Keywords: conservation, fitness, genetic diversity, propagule pressure, rare and threatened plant species, reintroduction, source population, translocation.

Declarations

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Introduction

To mitigate current worldwide plant extinction (Jackson & Kennedy, 2009), several strategies, such as habitat protection and restoration, *ex-situ* cultures and population translocations, are needed. Population translocations (i.e. reinforcement, reintroduction and introduction; IUCN, 2013) have been suggested as a powerful conservation tool for threatened species (e.g. Zimmer et al., 2019; Nebot et al., 2020; van Rossum & Le Pajolec, 2021; Munclinger et al., 2022). However, they are labour-intensive, costly and often have little success (Godefroid et al., 2011; Drayton & Primack, 2012). In part, the low success rate of translocations of threatened plants has been attributed to their weaker germination and fitness and smaller reproductive structures (Godefroid et al., 2016; Vincent, 2017; Boyd et al., 2022) compared with common species. It is therefore crucial to assess how the translocation success can be increased to support the survival of rare and threatened species.

For a given species, the factors determining translocation success can be conceptual, methodological, biological and ecological (Godefroid et al., 2011 & 2016). Among the biological and ecological factors, genetic diversity and propagule pressure (i.e. number of individuals released; Lockwood et al., 2005) are considered especially important in population and invasion biology (Oostermeijer et al., 1994; Fischer & Matthies, 1998; Lockwood et al., 2005; Leimu et al., 2006; Forsman, 2014). Studies on species translocations also reported positive effects of genetic diversity and propagule pressure (Vergeer et al., 2005; Robert et al., 2007; Menges, 2008; Godefroid et al., 2011; Schäfer et al., 2020). High genetic diversity and associated complementarity between genotypes may notably promote resource partitioning (Loreau & Hector, 2001), resistance to species antagonists (Spielman et al., 2004), genetic pre-adapation to future climate and disturbances or evolutionary resilience (Gamfeldt & Källström, 2007; Jump et al., 2009; Weeks et al., 2011). High propagule pressure reduces extinction probability of populations, as it buffers against stochasticity (Forsman, 2014).

However, genetic diversity and propagule pressure may also have negative effects on the fitness of translocations. Propagule pressure may increase intra-specific competition or the attraction and transmission of plant antagonists (Kéry et al., 2001 and the references therein). The increase of genetic diversity by mixing populations may decrease translocation success in the short term, as translocated individuals in mixtures may be less adapted locally than translocated individuals from individual populations (Joshi et al., 2001; Leimu et al., 2008; Hamann et al., 2016). Moreover, mixing populations might disrupt local adaptation, possibly resulting in outbreeding depression among offspring generations (Frankham et al., 2011).

In addition to genetic diversity and propagule pressure, the choice of the source populations is also considered important for translocations (Noël et al., 2011). Ecological distance to the translocation site, population-specific breeding-system, genetic load and (effective) size are indeed all known to affect population persistence (e.g. Newman & Pilson, 1997; Paschke et al., 2002; Weekley et al., 2002; Reed, 2005; Robert et al., 2007; Menges, 2008; Godefroid et al., 2011; Noël et al., 2011; Albrecht & Maschinski, 2012; Maschinski et al., 2012 & 2013).

Testing how genetic diversity and propagule pressure interact in affecting the fitness of translocated plants, while considering the source-population identity, is critical to improve translocation success and conservation measures. However, in this context there is only limited experimental evidence, because few conservation programs used hypothesis-driven approaches with scientific monitoring (Fischer & Lindenmayer, 2000; Godefroid et al., 2011; Fenu et al., 2019). Moreover, even fewer conservation programs were designed to investigate interactions between factors (Earnhardt, 1999; Robert et al., 2007; van Rossum & Le Pajolec, 2021). Therefore and although some studies have used mixtures of seed families or populations to examine the effects of genetic diversity on translocation outcomes (e.g. Vergeer et al., 2005; Zavodna et al., 2015; Schäfer et al., 2020; Munclinger et al., 2022), we are not aware of any study that tested interacting effects of population mixing and propagule pressure with several plant species. Yet, including several species allows increasing generalities of the results (van Kleunen et al., 2014). Furthermore, assessing translocation success on the basis of multiple plant fitness components, which has not often been done (but see e.g. Jusaitis, 2005; Caughlin et al., 2019; Schäfer et al., 2020), allows an increase in statistical power.

Here, we tested how genetic diversity and propagule pressure are interacting to shape early plant fitness in four reintroductions of herbaceous and threatened plant species in Switzerland. We manipulated genetic diversity, by using two source populations per species, and propagule pressure in a full-factorial design and studied plant survival, performance and reproduction for two years after transplantation. Specifically, we addressed the following questions: 1) How do genetic diversity and propagule pressure affect the fitness of reintroduced individuals ? 2) How do the effects of these factors interact with each other? 3) How do the source-population identity, environment and management of the reintroduction site affect the early reintroduction success of plants? We discuss our results with respect to plant population biology and in the context of conservation practice.

Material & Methods

Plant species

To perform reintroductions, i.e. to establish new populations within historical species ranges (https://www.infoflora.ch/fr/conservation-des-especes/introduction.html), we initially selected eight herbaceous plant species from different families, occurring in different habitats and considered as priority for conservation in Switzerland (OFEV, 2019): *Baldellia ranunculoides* (L.) Parl. (Alismataceae), *Campanula cervicaria* L. (Campanulaceae), *Cicuta virosa* L. (Apiaceae), *Poa remota* Forselles (Poaceae), *Prunella laciniata* L. (Lamiaceae), *Saxifraga granulata* L. (Saxifragaceae), *Teucrium botrys* L. (Lamiaceae) and *Veronica austriaca* L. (Plantaginaceae). However, due to very low percentage of germination and seedling survival in four species, we could only reintroduce *C. cervicaria*, *C. virosa*, *P. remota* and *P. laciniata*. Henceforth, we refer only to these four species.

Campanula cervicaria is an endangered monocarpic hemicryptophyte species of the margins of *Geranion sanguinei* communities (i.e. xerothermophilous nutrient-poor woodland edges) and grows on moderately moist and neutral soil in semi-open and shaded environments with low competition. *Cicuta virosa* is an endangered geophyte and characteristic species of *Phalaridion* communities (i.e. terrestrial reedbed) and grows in flooded and semi-shaded areas, such as calm water, with weakly acidic soil. *Poa remota* is a vulnerable perennial hemicryptophyte species of *Fraxinion* communities (i.e. damp ashwood) and grows on very moist and neutral soils of semi-shaded areas with low competition. *Prunella laciniata* is a vulnerable perennial hemicryptophyte and characteristic species of *Mesobromion* communities (i.e. medium-dry grassland) and grows on moderately dry, neutral and infertile soil in open and well-lit areas (Landolt et al., 2010; Delarze et al., 2015; Bornand et al., 2016).

Seed collection and germination

To modify genetic diversity in reintroductions, seeds of seven to 12 seed families (i.e. from different maternal plants) were collected from two remnant populations of each species in the Swiss Canton of Bern (Table S1). To break seed dormancy, seeds were cold-stratified in pots filled with moist soil over eight weeks at 4°C in the dark. Then seeds were allowed to germinate in the same pots in a greenhouse (constant day length of 14 hours with additional light and temperature between 15-30°C). We watered them daily. After five weeks of germination and growth, we pricked out seedlings of *C. cervicaria*, *P. remota* and *C. virosa* into individual pots (diameter and depth: 7cm) filled with standard soil (RICOTER soil n°167, Switzerland) inoculated with 1% of soil collected at the reintroduction site. This was done to preadapt the plants to their reintroduction site and microbiota and reduce a potential reintroduction shock. Because they grew more slowly than the other species, *P. laciniata* seedlings were pricked out after seven weeks of germination and growth following the same procedure as for the other species. Dead seedlings were replaced with seedlings from the same seed family, if available, or with seedlings from another seed family of the same population. After one month of growth in individual pots in the greenhouse, potted plants were placed in outdoor beds. We shaded plants on hot days and watered them daily or every other day, according to need. After two months of growth in individual pots, we fertilized *C. cervicaria* and *P. remota* plants by providing 10ml of potassium-rich Plantaktiv Type-K (Hauert, Switzerland) diluted at 0.05% in water, as recommended for plants from low nutrient habitats. We did not fertilize *P. laciniata* and *C. virosa* plants, because they had already grown large and obviously did not need it.

Experimental design

We reintroduced each species to one adequate site. To test for the effects of genetic diversity and propagule pressure on the fitness of the reintroduced individuals, we used the material from two source populations of each species and a full factorial design of six square plots composed of all combinations of two genetic diversity and propagule-pressure levels (Fig. 1). For the high-genetic diversity plots, we mixed individuals from both source populations. For half of the plots with low genetic diversity we used individuals from the first source population only, while for the other half we used individuals from the second source population only. To vary the propagule pressure per plot, we planted four times as many plants in high propagule-pressure plots than in low propagule-pressure plots (see next paragraphs for the plant numbers per plot and species). To keep propagule density constant across plots, high propagule-pressure plots were also four times larger than low propagule-pressure plots.

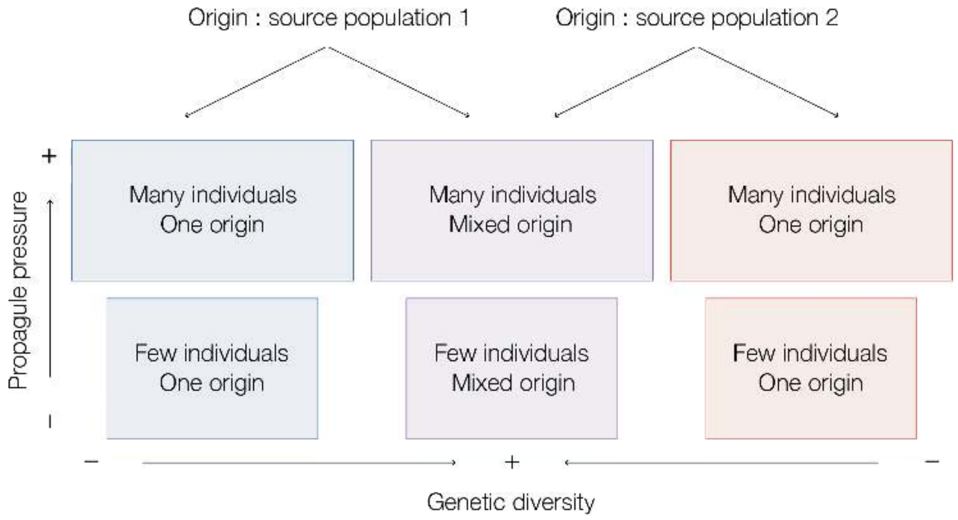

Figure 1: Sketch of the experimental design to test for the effect of two levels of genetic diversity and propagule pressure on the reintroduction success of threatened plant species.

Reintroductions

For each species we selected a suitable reintroduction site within their historical range within the Swiss Canton of Bern (A. Möhl and C. Käsermann, personal communication). As potential reintroduction site we selected the one with vegetation most closely matching the indicator values according to Landolt et al. (2010) of the focal species. Then we sought and obtained permission for reintroduction from site owners and cantonal authorities. The source populations were located within 15 km of the selected reintroduction sites, except for the Inkwil population of *C. virosa*, which is located at 25 km from its reintroduction site, because there was no suitable site closer to the two only remnant populations of this species in this region. To reduce competition and facilitate plant establishment, we mowed the reintroduction sites of *C. virosa* and *P. laciniata* and weeded the reintroduction sites of *C. cervicaria* and *P. remota* shortly before transplantation. We reintroduced plants at the selected sites in autumn (October 2019) to prevent reintroduced plants from suffering summer drought. Plants were planted 20 cm apart within plots. To prevent livestock from grazing *C. cervicaria* and *P. laciniata*, we fenced the reintroduction sites of both species. We also scattered dog hairs around the reintroduction site of *C. cervicaria* to repel roe deers, which are abundant in that region. We recorded the position of each planted individual within the plots and measured the length of the longest leaf (i.e. from the plant base to the tip of the longest leaf) to account for initial size. We watered the reintroduction plots of *C. cervicaria* and *P. laciniata*, because the soil was very dry when transplanting.

In total, we reintroduced 450 *C. cervicaria* and *P. laciniata* individuals and 525 *P. remota* and *C. cicuta* individuals (a total of 1950 individuals). High and low propagule-pressure plots consisted of 120 and 30 individuals for the two former species and of 140 and 35 for the two latter species, respectively.

Monitoring

To assess the fitness of the reintroduced individuals, we visited each reintroduction site four times in spring and autumn 2020 and 2021. For each individual, we recorded its survival and whether it was flowering or had flowered. To assess reproductive fitness, we counted the number of inflorescences per individual. To assess recruitment, we counted the number of new basal rosettes of *C. cervicaria*, *C. virosa* and *P. laciniata* within the plots and the number of new clonal individuals (i.e. connected by a stolon to the maternal plant) of *P. remota*. At each monitoring, we removed competitive and tall species, mainly *Rubus* species and *Hedera helix*, from *C. cervicaria* and *P. remota* reintroduction sites and we mowed the *C. virosa* reintroduction site in both winters of the monitored period. The *P. laciniata* reintroduction-site did not receive any management treatment within the monitored period.

Vegetation records

We took vegetation records at the reintroduction sites in spring and autumn 2020 and at the source-population sites in autumn 2020 (and in autumn 2021 for the Brünnliacher source population of *C. virosa*, which was mown in autumn 2020). For each site, we recorded the presence and cover percentage of all plant species in two plots of 2m^2 located on either side of the focal population. We also determined the size of the source populations by counting or estimating the number of individuals (Table S1).

For each vegetation record, we calculated the weighted and unweighted indicator values for temperature, continentality, light, moisture, reaction, and nutrients according to Landolt et al. (2010) using Vegedaz (https://www.wsl.ch/de/services-und-produkte/software-websites-und-apps/vegedaz.html). We then calculated the mean weighted and unweighted indicator values per site. To assess how ecologically close the reintroduction site per species is from the source-population sites, we followed the method from Noël et al. (2011). We performed a Principal Component Analysis (PCA) on the weighted and unweighted indicator values, separately. In both cases, the first two PC axes explained 94% of the data variance. We then calculated the ecological distance between the reintroduction and source-population sites as the Euclidean distance using the formula:

$D = \sqrt{[(PC1_R - PC1_S)^2 + (PC2_R - PC2_S)^2]}$, where PCx_R and PCx_S are the values of the principal component axes for the reintroduction and source-population sites, respectively. Since the ecological distances based on the weighted and unweighted indicator values were very similar, we use only the former in statistical analyses (Table S1).

Statistical analyses

To evaluate the effect of the plot genetic diversity, propagule pressure on the fitness of the reintroduced individuals and how it varied in function of the source-population identity, we used Aster models (Geyer et al., 2007; Shaw et al., 2008 & 2015; aster package of R: Geyer, 2014; R Core Team, 2020). Aster models are designed to jointly analyse life-history variables (i.e. fitness components) with different probability distributions over multiple years. By using a graphical model, they correctly account for the dependence of each fitness component on an earlier component to assess the overall fitness and the effects of the predictor variables on it. The graphical models for *C. cervicaria* and *P. laciniata* included the individual survival from the planting day to the fourth monitoring, whether a plant flowered and its number of inflorescences at the second and fourth monitoring (i.e. autumn monitoring; Fig. 2A). For *P. remota*, as no individual flowered at any monitoring, the graphical model included only the individual survival from the planting day to the fourth monitoring (Fig. 2B). Due to an extreme flooding event during early summer 2021, all individuals in the three most downstream plots were taken away by the water. We therefore took only into account the subset of the individuals from the three most upstream plots for the Aster analysis, as the most downstream individuals died due to a catastrophic event and not due to our predictor variables. For *C. virosa*, as flowering happened only in autumn 2020 and in very few individuals (ten out of 525 individuals), flowering was not included in the model due to insufficient statistical power. We did not take into account data from the fourth monitoring, as an exceptionally long and intense flooding period in the reintroduction site caused the death of the majority of the individuals at the fourth monitoring. Hence, the graphical model included the individual survival from the first to the third monitoring (Fig. 2C). Since the number of new rosettes and clonal individuals was very low in all species, we could not take into account recruitment of the species in the sites. We modelled survival and flowering variables as Bernoulli variables and the number of inflorescences as zero-truncated Poisson variable.

Because variable effects propagate over the whole life history in Aster models, they yield estimates of unconditional mean individual fitness through the entire monitored period. The operational measure of fitness in *C. cervicaria* and *P. laciniata* was the expected number of inflorescences per individual from planting to the fourth monitoring, which takes into account the number of inflorescences and the probability to flower and survive. In *C. virosa* and *P. remota*, the operational measure of fitness was the unconditional expected survival per individual from planting to the third and fourth monitoring (i.e. spring and autumn 2021), respectively.

Unconditional Aster models estimate the relationship between the operational measure of fitness and predictor variables (Shaw et al., 2008). In Aster models, we included the genetic diversity (i.e. high vs low) and propagule pressure (i.e. high vs. low) of the plot in which each individual was situated and each individual source population. For *C. cervicaria*, *P. laciniata* and *C. virosa*, we also included the two- and three-way interactions between the predictors. For *P. remota*, we could test only for the main predictor effects, since not all plots (and therefore not all predictor combinations) could be taken into account. To account for environmental gradients within the reintroduction site and the fact that larger plants may have an increased probability to survival and flower, we included as covariate the plant position within the reintroduction site and the length of its longest leaf on planting day in all Aster models.

To identify the most important predictors for the individual fitness, we performed a stepwise backward model reduction using ANOVA. Expected values of the operational measure of fitness and their standard deviation for each significant predictor interactions and main effects were then calculated for a typical individual situated in the centre of the reintroduction site and having a mean initial leaf length. Expected fitness values as function of the significant predictor interactions and main effects were visualized using the R package ggplot2 (Wickham, 2016).

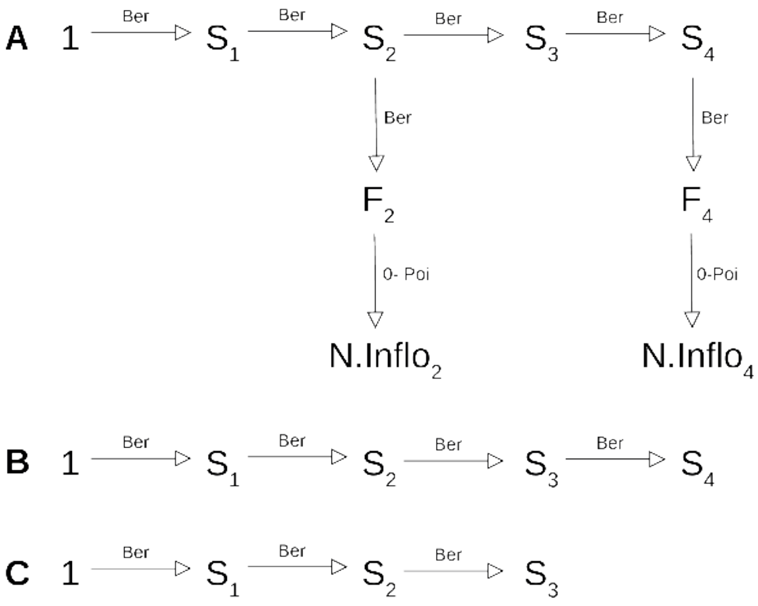

Figure 2: Graphs for Aster data of Campanula cervicaria and Prunella laciniata (A), Poa remota (B) and Cicuta virosa (C). Arrows go from parent nodes to child nodes. Nodes are labelled by their associated variables. The root node is associated with the constant variable 1. S_i *and* F_i *are the survival and flowering status at first monitoring+i, respectively. Flowering never occurred in spring (monitoring 1 and 3). N. Inflo*$_i$ *is the inflorescence count at the first monitoring+i, respectively. The* S_i *and* F_i *are Bernoulli conditional (Ber) on their parent variables being one, and zero otherwise. The N. Inflo*$_i$ *is zero-truncated Poisson conditional (0-Poi) on their parent variables being one and zero otherwise.*

Results

Survival and flowering in species reintroduction

Overall the reintroduced individuals of *Campanula cervicaria* and *Prunella laciniata* survived quite well (Fig. 3A-B). In autumn 2021 (i.e. two years since the plantation), the mean survival percentage across all plots was 0.7 for *C. cervicaria* and 0.58 for *P. laciniata*. In both species, the number of living individuals decreased slowly in all plots and over the whole monitored period. 72% and 50% of the planted individuals of *P. laciniata*, and 4% and 67% of the ones of *C. cervicaria* flowered in autumn 2020 and 2021, respectively.

In *Poa remota*, 13% of plants survived across all plots by autumn 2021 (Fig. 3C). From spring 2021, we found no living individual in three plots, the ones situated most downstream, due to a major river flooding. In contrast, in the three remaining plots (i.e. the most upstream plots), the mean survival percentage was very high (0.79 of survival by autumn 2021). No individuals flowered throughout our monitoring period.

In *Cicuta virosa*, 41% of the planted individuals survived by spring 2020 (Fig. 3D). Thereafter, the number of living individuals decreased slowly until the summer of 2021, when it decreased drastically due to a major flood. By autumn 2021, only 6 of the 525 individuals survived. The flowering percentage was 2% and 0% in autumn 2020 and 2021, respectively.

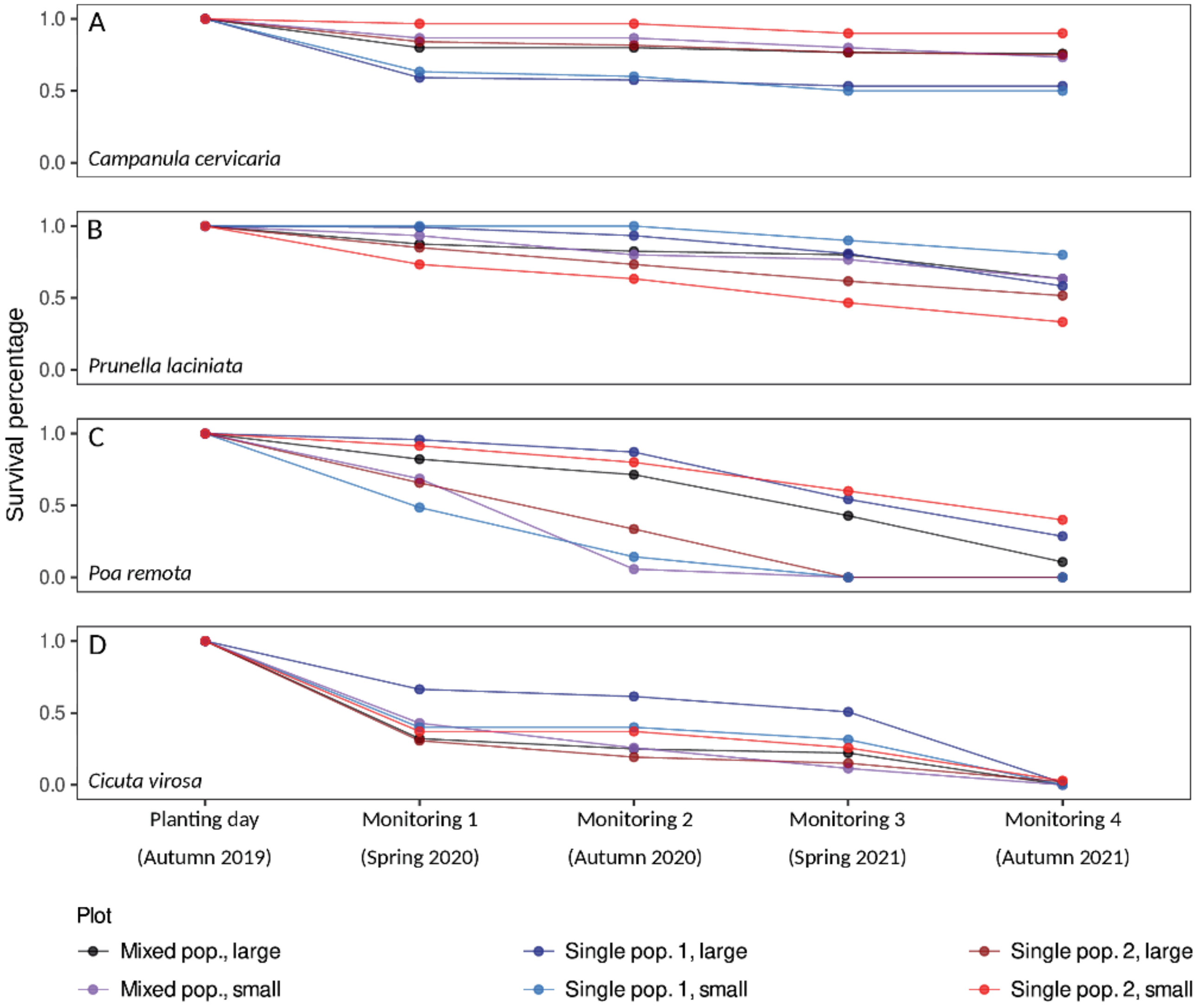

Figure 3: Survival percentage per plot for each species reintroduction. We planted the reintroduced populations in October 2019 and then monitored four times the reintroductions in spring and autumn 2020 and 2021. The plot legend refers to the experimental design (see Fig. 1).

Effects of genetic diversity, propagule pressure and source population on the fitness of plants of *Campanula cervicaria* and *Prunella laciniata*

We found clear differences in mean fitness (i.e. expected total inflorescence count produced over two years per individual) among plot treatments in *C. cervicaria* and *P. laciniata* (Table 1). In *C. cervicaria*, fitness varied significantly with genetic diversity, propagule pressure and source population (Fig. 4A). Overall, higher genetic diversity had a positive effect on the fitness of individuals from Brünnliacher, and a negative effect on the fitness of individuals from Forst. Higher propagule pressure had an overall negative effect on the fitness of individuals from both source populations of *C. cervicaria*. However, at low genetic diversity, higher propagule pressure decreased the fitness of individuals from Forst, whereas it increased the fitness of individuals from Brünnliacher. At high genetic diversity, the propagule pressure decreased the fitness of individuals from both populations. Furthermore, at low genetic diversity, the fitness of individuals from Brünnliacher was much lower than the one of individuals from Forst. In contrast, at high genetic diversity, the fitness of individuals from Brünnliacher was slightly higher than the one of individuals from Forst.

In *P. laciniata*, mean plant fitness varied significantly with genetic diversity and source population (Fig. 4B). Overall, higher genetic diversity had a positive effect on the fitness of individuals from both populations, but to different degrees. While the fitness of individuals from Nods was much lower in low genetic diversity plots than in high genetic diversity plots, the fitness of individuals from Le Landeron in low genetic diversity plots was almost as high as in high genetic diversity plots.

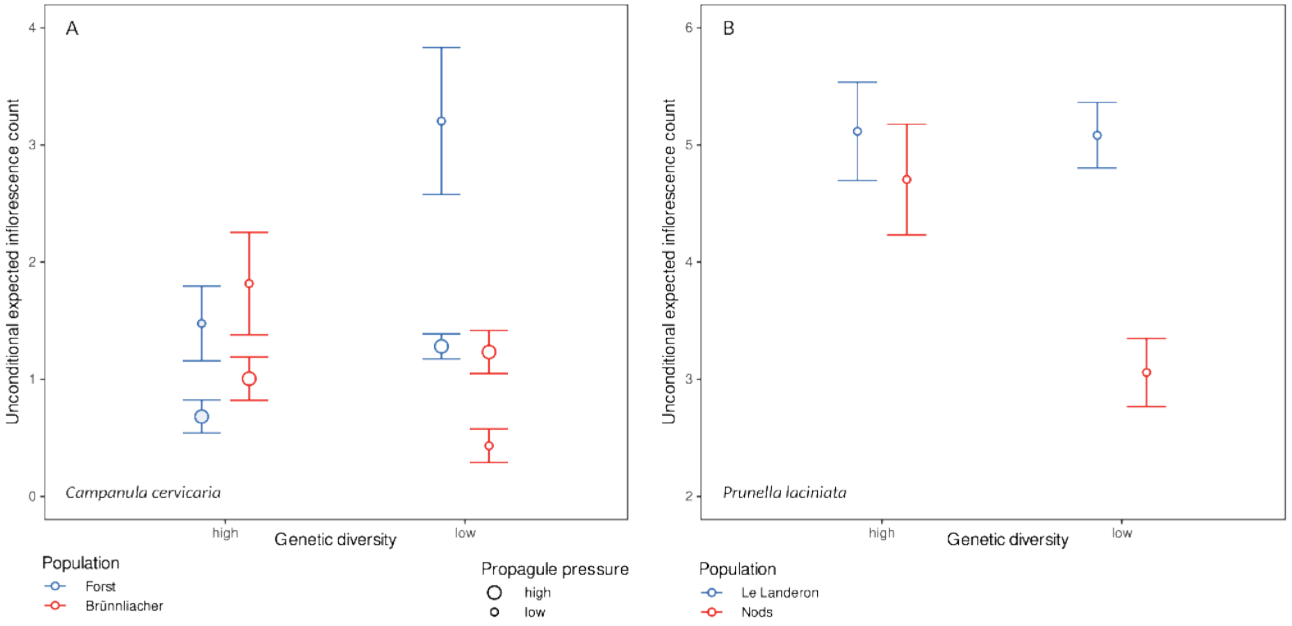

Figure 4: Predicted values and 95% confidence intervals for mean fitness quantified as expected number of inflorescences produced over two years for a typical individual (i.e. in the centre of the reintroduction site and with mean initial leaf length) of Campanula cervicaria as a function of genetic diversity, propagule pressure and source population (A) and of Prunella laciniata as a function of genetic diversity and source population (B). The populations in blue are the ecologically closer ones to the reintroduction sites.

Table 1: Reduction of the Aster model for Campanula cervicaria and Prunella laciniata using ANOVA. Residual and test degrees of freedom (df), deviance and corresponding p value are given. P values in bold are significant at the threshold of 0.05.

	Campanula cervicaria				***Prunella laciniata***			
Term	**Residual df**	**Test df**	**Deviance**	***P***	**Residual df**	**Test df**	**Deviance**	***P***
Life-history effects and Interactions - Base	24				24			
Propagule pressure × Genetic diversity × Population @ Nb inflorescences	23	1	8.998	**0.003**	23	1	0.709	0.4
Propagule pressure × Genetic diversity @ Nb inflorescences	-	-	-	-	22	1	0.000	0.982
Propagule pressure × Population @ Nb inflorescences	-	-	-	-	21	1	0.018	0.894
Genetic diversity × Population @ Nb inflorescences	-	-	-	-	20	1	5.584	**0.018**
Propagule pressure @ Nb inflorescences	-	-	-	-	21	1	2.733	0.098
Genetic diversity @ Nb inflorescences	-	-	-	-	-	-	-	-
Population @ Nb inflorescences	-	-	-	-	-	-	-	-
Quantitative effects - Base	24				20			
Initial size	21	3	87.423	**<0.001**	17	3	328.42	**<0.001**
Spatial effect	18	6	24.953	**<0.001**	14	6	29.934	**<0.001**

Effects of genetic diversity, propagule pressure and source population on the fitness of plants of *Cicuta virosa* and *Poa remota*

We found that mean fitness (i.e. expected survival over two years) was lower in plots of higher genetic diversity in *P. remota* (Fig. 5A; Table 2). In *C. virosa*, mean fitness (i.e. expected survival over one and a half years) varied significantly with genetic diversity and source population (Fig. 5B; Table 2). Here, the fitness of individuals from Inkwil was much higher in low genetic diversity plots than in high genetic diversity plots, while the fitness of individuals from Wengimoos did not vary across genetic diversity plots.

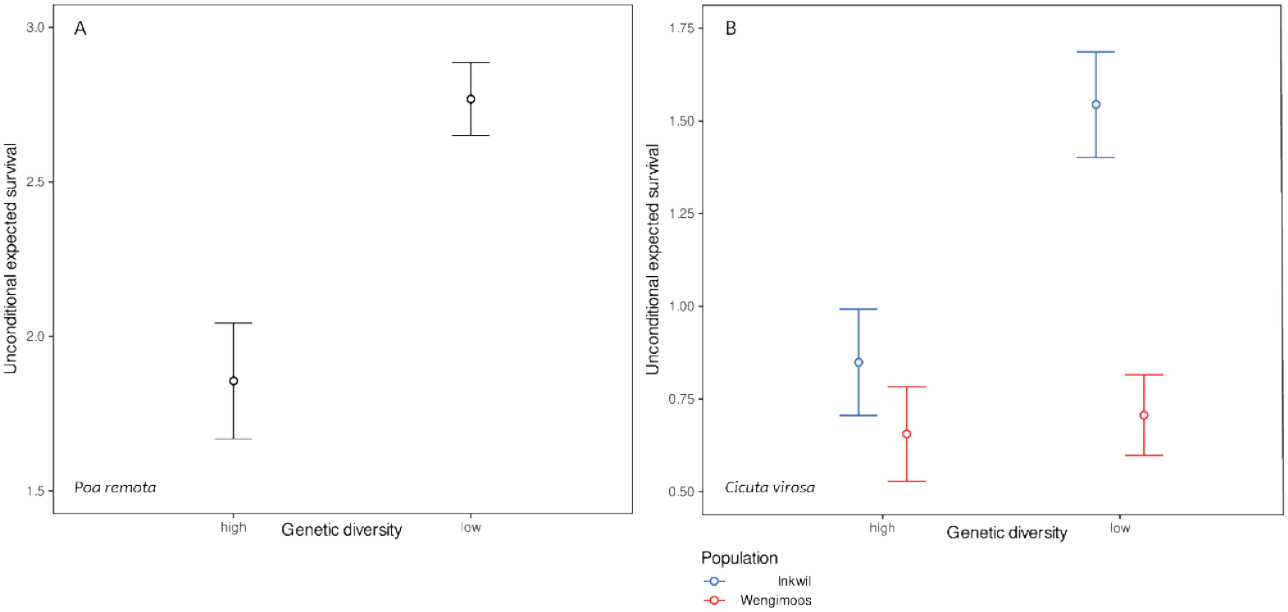

Figure 5: Predicted values and 95% confidence intervals for mean fitness quantified as expected survival over two years or one and a half years, respectively, for a typical individual (i.e. in the centre of the reintroduction site and with mean initial leaf length) of Poa remota as a function of genetic diversity (A) and of Cicuta virosa as a function of genetic diversity and source population (B), respectively. The population in blue is the one ecologically closer to the reintroduction site.

Table 2: Reduction of the Aster model for Cicuta virosa and Poa remota using ANOVA. Residual and test degrees of freedom (df), deviance and corresponding p value are given. P values in bold are significant at the threshold of 0.05.

	Poa remota				***Cicuta virosa***			
Term	**Residual df**	**Test df**	**Deviance**	***P***	**Residual df**	**Test df**	**Deviance**	***P***
Life-history effects and Interactions - Base	10				13			
Propagule pressure × Genetic diversity × Population @ Survival	-	-	-	-	12	1	0.394	0.530
Propagule pressure × Genetic diversity @ Survival	-	-	-	-	11	1	1.583	0.208
Propagule pressure × Population @ Survival	-	-	-	-	10	1	2.003	0.157
Genetic diversity × Population @ Survival	-	-	-	-	9	1	3.888	**0.049**
Propagule pressure @ Survival	9	1	3.351	0.067	10	1	0.271	0.603
Genetic diversity @ Survival	8	1	9.463	**0.002**	-	-	-	-
Population @ Survival	8	1	3.098	0.078	-	-	-	-
Quantitative effects - Base	8				9			
Initial size	7	1	11.566	**<0.001**	8	1	78.598	**<0.001**
Spatial effect	6	2	4.469	0.107	7	2	5.49	0.064

Discussion

Effects of genetic diversity on plant fitness

The effects of genetic diversity varied considerably among species and source populations in our study. Genetic diversity had a positive effect on the fitness of individuals from Brünnliacher of *Campanula cervicaria* and from both populations of *Prunella laciniata* (Fig. 4). In contrast, it had a negative effect on the fitness of individuals from Forst of *Campanula cervicaria* and from both populations of *Cicuta virosa* and *Poa remota* (Fig. 5). High genetic diversity has been suggested to increase fitness in many species and natural populations (e.g. Oostermeijer et al., 1994; Fischer & Matthies, 1998; Madsen et al., 1999; Stokstad, 2005; Willi et al., 2007; Forsman, 2014 and references therein). Hence, it was proposed as important characteristic for source-population selection for translocations (Godefroid et al. 2016). Moreover, several studies reported positive impacts on the fitness of translocated plants when seed families or source populations were mixed (e.g. Vergeer et al., 2005; Raabová et al., 2009; Godefroid et al. 2011; Schäfer et al., 2020; van Rossum & le Pajolec 2021; but see Kaulfuß & Reisch, 2017). The positive effects of high genetic diversity we found in some cases are in line with these studies.

However, in most cases and consistently for all source populations of *C. virosa* and *P. remota*, genetic diversity had negative effects. Interestingly, this appeared in individuals from those of the five source populations (of the eight included in our study) which were ecologically closest to their reintroduction site. In contrast, the three source populations whose reintroduced individuals benefited from high genetic diversity were the most ecologically distant ones from their reintroduction site (Table S1). This suggests that the further a source population is ecologically distant from a reintroduction site, the more positive is the effect of increasing genetic diversity in our study. At the same time, it suggests an interaction between genetic diversity and ecological similarity in their effects on fitness, where effects of genetic diversity turn negative if source population and target site are very similar ecologically.

Negative effects of high genetic diversity in our study cannot be due to outbreeding depression (see Waser & Price, 1989), because our analysis accounts for F0 individuals only. However, population mixing can also result in reduced mean fitness of translocated individuals, if the individuals of one translocated population are poorly adapted to the abiotic and biotic conditions at the translocation site. Indeed, a fitness reduction in the F0 generation due to mismatch between local adaptation and new environmental conditions was reported by Berggren et al. (2016) in their reciprocal translocation experiment of pike eggs. In addition to local maladaptation, non-genetic maternal effects may also reduce the fitness of translocated individuals at translocation sites ecologically different from source populations (Rossiter, 1996; Heath et al., 1999).

The two species (*P. remota* and *C. virosa*) for which we consistently found a negative effect of genetic diversity for all source populations were those with the lowest reintroduction success. Although genetic diversity is considered as particularly important for the resilience of populations under stochastic events (Mashinski et al. 2013), the flooding events, which impacted our two wetland species, may have been too severe for triggering a positive effect of genetic diversity. In the case of the reintroduction of *P. remota*, half of the plots were even completely swept away during the flood of the second year. In contrast, in the two reintroductions considered as successful at the end of our two-year monitoring (*C. cervicaria* and *P. laciniata*), genetic diversity played a positive role in three out of four cases, while in only one case it might have diluted the local adaptation of one of the source populations.

Our study highlights the challenge of creating translocated populations which combine high ecological and evolutionary potential and a high degree of local adaptation. Investigating whether the genetic diversity effect becomes more positive in recruited individuals and overcomes the short term negative effects of reduced local adaptation is important (Tallmon et al., 2004; Jump et al., 2009; Li et al., 2018). We recommend that population mixing should be planned very carefully and should not involve populations which are too ecologically distant from a translocation site. Because ecological similarity is likely to be more decisive for translocation fitness than geographical proximity *per se* (Raabová et al., 2007; Maschinski et al., 2012 & 2013), we encourage future studies to compare vegetation records at the source populations and potential translocation sites to select the ecologically closest sites available for translocation. Moreover, as the remaining populations of very rare species may not reflect the optimal habitat for a species any longer (Fowler et al., 2012; Roncal et al., 2012), we suggest to additionally consider ecological indicator values of the species themselves and compare them to the mean ecological indicator values of target sites, as we did in our study.

Effects of propagule pressure on plant fitness

Higher propagule pressure reduced the fitness of translocated plants of *C. cervicaria*. Although the effect varied with genetic diversity and source population (Fig. 4A), individual fitness was generally lower in high propagule-pressure plots than in low propagule-pressure plots. This contrasts with the expectation that the higher the propagule pressure, the larger the reintroduction success, as suggested by meta-analyses on species translocations (Godefroid et al., 2011; Guerrant & Fiedler, 2004) as well as insights from invasion ecology (Lockwood et al., 2005). Possibly, lower intra-specific competition in low propagule-pressure plots than in high propagule-pressure plots may have caused this result (see Ries et al., 2004). A second possibility is that plant antagonists, such as specialist herbivores and pathogens, may have been more attracted to and more numerous in high propagule-pressure plots than in low propagule-pressure plots

(Kéry et al., 2001 and the references therein). Thus, plant antagonists may inflict higher damage to plants in high propagule-pressure plots and reduce their fitness. We can rule out aboveground herbivores as agents of such density dependent effects, as we documented herbivory levels above ground and found them unrelated to propagule pressure (not reported in Results). Alternatively, soil biota may have had negative density dependent effects, which are considered highly relevant for plant population dynamics (see Janzen-Connell hypothesis: Janzen, 1970; Connell, 1971; and e.g. Antonovics & Levin, 1980; Newton & Jolliffe, 1998; Bagchi et al., 2010; Konno et al., 2011; Yao et al., 2020; but see Teste & Laliberté, 2021).

As higher propagule pressure did not interact with genetic diversity to increase the fitness of translocated plants, and as we found a negative effect of higher propagule pressure on the reintroduction of one species, we recommend that future translocations should consider planting individuals in small but numerous plots, instead of few very large plots to avoid potential negative density-dependent effects.

Further considerations on the reintroduction of rare and threatened plant species

Our results suggest that environmental filtering was low in the reintroduction sites of *C. cervicaria* and *P. laciniata*, as indicated by high survival and flowering percentages over the monitored period. This indicates that the sites we had selected for reintroducing these species indeed were particularly suitable and that our management actions (i.e. fencing, spreading dog hairs and weeding) likely improved early reintroduction success (see also Jusaitis, 2005; Godefroid et al., 2011).

In contrast, high mortality in *P. remota* and *C. virosa* throughout the monitored period indicated intense environmental filtering in spite of the care given to our experiment, reminding us that translocated populations, like other small populations, are subject to environmental stochasticity (Lubow, 1996; Colas et al., 1997). In spring and summer 2021, the exceptionally heavy precipitations in Switzerland (MétéoSuisse, 2022) overflowed numerous areas, including our reintroduction sites of *P. remota* and *C. virosa*. For *P. remota*, the high mortality already at the first monitoring was mainly due to sudden water-level increases due to precipitations and subsequent changes in the course of the river next to the reintroduction site. In summer 2021, we observed more than one metre higher water levels than usual. This swept away any remaining individuals in the three more downstream plots. Moreover, many individuals in the more upstream plots showed damages due to flooding and subsequent mud cover. For this species our study design and the power to analyse interactive effects of genetic diversity and propagule pressure were compromised. In *C. virosa*, the low survival percentage at the first monitoring (Fig. 3) rather suggests that this species suffered from transplantation shock, despite the inoculation of the seedling potting-soil with soil from the reintroduction site. In fact this species is likely to be particularly sensitive to transplantation, as even slight damages to its fusiform taproot may kill the whole plant (personal communication with gardeners of the Botanical Garden of the University of Bern). Furthermore, aerenchyma, an adaptation for growth in flooded habitats, pulled some plants not yet fully anchored in the soil to the water surface when the site was first flooded a few weeks after planting, leading to lost plants, which we found dried and uprooted at the first monitoring. Possibly, planting individuals through a Hessian mesh fixed on the ground might improve the reintroduction of wetland species with taproots. Finally, the exceptional flood in summer 2021 (MétéoSuisse, 2022) most likely washed more nutrients from the surrounding agricultural fields to the reintroduction site than during regular annual floods. This might have favoured faster-growing and more competitive species than *C. virosa*, e.g. highly competitive *Carex* species, which eventually outcompeted *C. virosa* (Jusaitis, 2005) and completed a drastic population decline. These considerations highlight the fact that translocations of wetland species are especially difficult, notably due to the dynamics of their habitats (Abeli et al., 2014), and encourages future studies to investigate how wetland-species translocations can be improved by various practical measures.

Conclusion

We showed that genetic diversity can play an important role in rare plant reintroductions, if it does not involve material from ecologically distant source populations not adapted to a targeted reintroduction site. Thus, we recommend to match reintroduction and source sites very carefully according to their ecological similarity and material from several sites should only be mixed if they are ecologically very similar. As high propagule pressure seemed not to play a major role in our reintroductions and as its potential positive effects might be offset by negative density-dependent biotic interactions at small scale, we recommend translocating plants in small but numerous plots.

The severe losses in our wetland reintroductions due to unexpected flooding events reflect that the number of plants usually translocated (often in the low hundreds) is too small to overcome detrimental events under strong environmental stochasticity (Lubow, 1996; Reed, 2005; Robert et al., 2007; Godefroid et al., 2011). Our study underlines that this seems to be the case even if the translocation site is selected very carefully. Especially wetland species, which typically undergo strong environmental fluctuations, may need to be supported by additional measures, such as fixing transplants until they are firmly rooted. Finally, the strong species and habitat-dependency (see also Silcock et al., 2019) of our results underlines the complexity and challenge of creating sustainable new populations of rare plant species.

Acknowledgements

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Supporting information

Table S1: List of the reintroduced plant species. Plant family, source populations (i.e. seed-collection sites), source population size (i.e. number of mature individuals), number of seed families from which seedlings were reintroduced and ecological distance to the species reintroduction-site (i.e. Euclidean distance calculated with weighted Landolt indicator values; see Material & Methods) are given for each species. BE refers to the canton of Bern in Switzerland.

Species	**Family**	**Source population**	**Size of source population**	**Number of seed families**	**Ecological distance**
Campanula cervicaria	Campanulaceae	Brünnliacher, BE	20	7	0.141
		Forst, BE	15	9	0.049
Cicuta virosa	Apiaceae	Inkwil, BE	250	8	0.032
		Wengimoos, BE	100	11	0.048
Poa remota	Poaceae	Bern, BE	20	12	0.026
		Station 4, Bern, BE	40	8	0.064
Prunella laciniata	Lamiaceae	Le Landeron, BE	35	10	0.077
		Nods, BE	15	9	0.075

Chapter 5

Summary and general conclusions

The reasons why some plant species are rare, while some others are not, is a long-standing concern for biologists (e.g. Rabinowitz, 1981; Kunin & Gaston, 1993; Wamelink et al., 2014). A better understanding of drivers of plant rarity is central to understanding the distribution of species in space and time and is crucial for conservation practitioners to identify approaches to protect and support rare and threatened species. However, despite a large literature body dedicated to this question (e.g. Griggs, 1940; Stebbins, 1942; Drury, 1974; Smith, 1976; Pearson & Dawson, 2003; Kempel et al., 2018), no general framework defining drivers of plant rarity currently exists (Yates et al., 2007).

Once habitats are destroyed and fragmented, several factors are suggested to drive species further towards rarity and ultimately to extinction. If intrinsic species traits and sensitivity to extrinsic factors induce a difference of fitness between species, these traits and factors may reduce the abundance and distribution of some species, rendering them rare, while allowing some others to be more common. One of the most debated hypothesis about how rare and common plant species differ from each other is called the “apparency hypothesis” (Cates & Orians, 1975; Feeny, 1976; Rhoades & Cates, 1976). It suggests that “less apparent” plant species allocate less in defence than common species and thus are more consumed by generalist herbivores, which could ultimately render plants rare. Another important hypothesis about species rarity suggests that rarity appears due to genetic processes occurring in small populations—in particular repetitive inbreeding—that increase the extinction risk of rare species (Frankham, 2005; O’Grady et al., 2006; Allendorf et al., 2013).

To support species that have become rare and threatened, conservation activities such as population translocations can help reduce the likelihood of species extinction. For translocations of rare plants, genetic diversity, propagule pressure (Lockwood et al., 2005) and source-population identity were hypothesized to be determinant factors (Vergeer et al., 2005; Robert et al., 2007; Menges, 2008; Godefroid et al., 2011; Noël et al., 2011; Schäfer et al., 2020), as they affect population establishment and persistence (e.g. Loreau & Hector, 2001; Spielman et al., 2004; Gamfeldt & Källström, 2007; Jump et al., 2009; Noël et al., 2011; Weeks et al., 2011; Maschinski et al., 2012 & 2013; Forsman, 2014). However, rigorous tests, which use multiple plant species differing in rarity, of the hypotheses about plant-herbivore interactions, about genetic processes as drivers of plant rarity and about the interactive effects of genetic diversity, propagule pressure and source-population identity on translocated plants are missing.

With this thesis, I aim to 1) contribute to our scientific knowledge of the processes that determine plant rarity in a world where habitats are destroyed and fragmented and 2) contribute to improving conservation strategies for threatened plant species. To this end, in chapters two and three, we conducted greenhouse experiments to test the “apparency hypothesis” and inbreeding effects in plants. In chapter four, we performed rare species reintroductions, using an experimental design and scientific monitoring, to test the effect of genetic diversity, propagule pressure and source-population identity on reintroduction success. For all three chapters we designed multi-species experiments to specifically test the hypotheses for species varying in the three aspects of rarity defined by Rabinowitz (1981; i.e. regional and local rarity, and habitat specificity), while also taking into account the species competitive strategy and growth traits.

Experimental tests of plant-rarity drivers

In chapter two, to test the “apparency hypothesis” (Cates & Orians, 1975; Feeny, 1976; Rhoades & Cates, 1976), we assessed the performance and the preference of one belowground and three aboveground invertebrate generalist herbivores feeding on multiple plant species. We then analysed how plant local and regional rarity, growth traits, competitive strategy and habitat specificity altered herbivore weight gain and plant choice. The main result of this study is that the “apparency hypothesis” does not hold for herbaceous plant species, as locally and regionally rare plant species are slightly more defended than common species against generalist herbivores. Hence, this study challenges the idea that rare plant species are more palatable for invertebrate generalist herbivores and that the latter act as a plant-rarity driver. This indicates that plant rarity may be a poor proxy of defence investment in plant species. Instead, our results suggest that a plant’s growth strategy, and not its rarity per se, predicts plant palatability to generalist herbivores. A high resource allocation to growth renders plants more palatable to generalist herbivores, but it allows plants to be highly competitive and possibly increase their abundance and distribution.

In chapter three, to disentangle alternative hypotheses about the effects of selfing and outcrossing on rare and common plant species, we performed pollinations by hand: self-pollinations and cross-pollinations between seed families (i.e. offspring of different maternal plants), within and between populations of 16 rare and common species. We then analysed how selfing and between-population outcrossing, compared with between seed-family outcrossing within populations, interacted with local and regional plant rarity, competitive strategy, growth traits and habitat specificity in altering plant fitness. The study challenges the idea that rare plant species suffer more from inbreeding than common species do, as selfing was usually more detrimental for the fitness of common plant species than of rare ones. This pattern is likely explained by the fact that populations of rare plant species experienced longer inbreeding histories and thus quite some of their genetic load was purged by natural selection (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987; Ellstrand & Elam, 1993; Oostermeijer et al., 2003; Charlesworth & Willis, 2009). In addition, purging may be more efficient in small populations, making them less impacted by inbreeding in the long run than more common plant species (Angeloni et al., 2011). As rare plants were also not generally less fit after inbreeding, we conclude that inbreeding is likely not a main driver of plant rarity.

By challenging two important hypotheses about drivers of plant rarity, our two first experiments call for additional multi-species experiments testing these hypotheses in further regions and with further species to provide support to our conclusions. They also open the horizon for further studies to investigate other hypotheses about the causes of plant rarity. Moreover, by demonstrating that regional and local rarity, competitive ability, growth traits and habitat specificity alter plant allocation to defence against herbivores or the response to selfing and outcrossing, these two experiments highlight the necessity to account for plant context-dependency. In this sense, it supports Kempel et al.'s (2020) claim that the inconsistencies between studies investigating differences between rare and common plant species may stem from the variation of traits leading to plant success along ecological gradients. Finding general across-species patterns may therefore require a high number of species to overcome strong variation between them. Moreover, future studies in this field need to consider the context dependency of the relationships between plant rarity, life-history and resource allocation strategies.

In addition to context-dependency, plant stress can directly affect inbreeding depression and the allocation to defence against herbivores. Several studies reported that inbreeding depression increased with stress (Hauser & Loeschcke, 1996; Armbruster & Reed, 2005; Hayes et al., 2005; Leimu et al., 2008) and that the direction of the relationship between inbreeding depression and population size is expected to change under stressful environmental conditions (Frankham et al., 2002, Armbruster & Reed, 2005). Other studies showed that plant-plant interactions are altered by stress (Bertness & Callaway, 1994; Luo et al., 2010; Boughton et al., 2011) and herbivory intensity varies with environmental stress (Louda & Collinge, 1992; Huberty & Deno, 2004; Suárez-Vidal, et al., 2019; Gely et al., 2020; Hamann et al., 2021). It is often assumed that field conditions are more stressful for plants than greenhouse conditions are (but see Angeloni et al., 2011). Therefore, future studies beyond this thesis might want to compare the impact of generalist herbivores and inbreeding levels in experiments conducted in the greenhouse and in the field.

In the case of the test of the "apparency hypothesis" (Cates & Orians, 1975; Feeny, 1976; Rhoades & Cates, 1976), we expect that studies conducted in the field might obtain slightly different results than ours. At least three factors could cause such differences. First, the local abundance of each plant species can be directly assessed in the field without having to rely on an indirect index describing plant dominance (i.e.

Landolt indicator value for dominance *in-situ*; Landolt et al., 2010). Second, the interactions between and within herbivore species can influence herbivore preference and performance (Cronin & Abrahamson, 2001). Besides, it remains an open question whether the use of generalist herbivores as indicators of general defence levels is misleading or whether generalist herbivores can be used to “measure” the universal quality of plants as food. Few species may not reflect accurately plant defence, but I hypothesize that a larger number of herbivores species spanning across feeding compartments (i.e. feeding on different plant organs), guilds and phylogeny taken together may better reflect plant defence (Herms & Mattson, 1992; Pérez-Harguindeguy et al., 2003). Third, multi-trophic interactions, e.g. between herbivores and predators, are also expected to affect herbivore preference and performance. For example, in the field, herbivores may prefer to feed on plants on which they can hide more efficiently to avoid being predated themselves (Duffy & Hay, 1991). Altogether these factors may alter the relationship between herbivore performance, preference, plant rarity and life-history strategy. Concerning the effects of selfing and outcrossing on fitness in rare and common plant species, a comparison between an *in-situ* and an ex-situ experiment would allow investigating the association between environmental quality and genetic effects on fitness (Angeloni et al., 2011). The nature of this association appears to be variable and complex (see e.g. Johnston, 1992; Crnokrak & Roff, 1999; Armbruster & Reed, 2005) and therefore there is still no scientific consensus on this subject.

Experimental test of translocation strategies

To face the current and global biodiversity crisis, translocations of threatened plant species were suggested to be a powerful conservation tool (e.g. Zimmer et al., 2019; Nebot et al., 2020; van Rossum & Le Pajolec, 2021; Munclinger et al., 2022). However, their efficiency is discussed controversially due to their high costs and low success rates (Godefroid et al., 2011; Drayton & Primack, 2012). Numerous factors are known to alter plant fitness in translocated and small populations (e.g. Godefroid et al., 2011 & 2016), making the development of best practice protocols a challenging task. In chapter four, we thus focused on investigating the effects of three factors considered to be key determinants for translocation success: genetic diversity, propagule pressure and origin of the translocated material (e.g. Vergeer et al., 2005; Robert et al., 2007; Menges, 2008; Godefroid et al., 2011; Noël et al., 2011; Schäfer et al., 2020). To do so, we reintroduced four rare and threatened plant species with material from two source populations per species by following a six-block factorial design with two levels of genetic diversity and two levels of propagule pressure. Using data of a two-year monitored period, vegetation records and so-called Aster models (Geyer et al., 2007), we investigated how these factors interact with each other in altering plant fitness. We accounted also for effects of the ecological similarity between the source populations and the translocation site, and of management actions and stochastic environmental events on the outcome of the translocations.

Our study shows that early fitness of translocated plants depended on their genetic diversity, source-population identity and, to a lesser extent, on propagule pressure. Contrasting with earlier findings (e.g. Vergeer et al., 2005; Raabová et al., 2009; Godefroid et al., 2011; Schäfer et al., 2020; van Rossum & Le Pajolec, 2021), we found that genetic diversity effects were mostly negative and likely depended on the ecological similarity between the source populations and the translocation site. Maladaptation to the translocation site might have offset positive effects of population mixing in the short term in case plants from one population were more adapted to the site than from the other. Propagule pressure seemed not to play a major role in our reintroductions and its effects were mainly negative. A possible explanation is that negative density-dependent biotic interactions could have counteracted potential positive effects of high propagule pressure. Even though the number of plants reintroduced was higher than in many other translocation efforts (e.g. Godefroid et al., 2011; Noël et al., 2011), it was not large enough to buffer against major stochastic environmental events in our wetland species. Overall, our reintroduction study showed important interactions between genetic diversity, source-population identity and propagule pressure in their effects on translocation success. Moreover, it illustrates the challenge of performing successful translocations of rare species, especially for the ones living in dynamic habitats, such as wetlands (Abeli et al., 2014).

Implications for conservation practitioners

The results of all three experiments conducted in this thesis have implications for plant conservation. Therefore, by taking them together, we can contribute to general recommendations informing conservation practitioners with the aim of increasing the efficiency of conservation strategies and of rising attention towards better conservation policy.

Firstly, we recommend choosing translocation sites to maximize ecological similarity with the source populations. Favouring ecological similarity may be more important than favouring geographical proximity (Raabová et al., 2007; Noël et al., 2011; Maschinski et al., 2012 & 2013). Finding the ecologically closest site involves conducting vegetation records or assessing abiotic and environmental parameters at the potential sites and comparing them before plant translocation.

Secondly, we recommend that translocations preferably should use plants from several source populations, as between-population outcrossing is beneficial for many threatened and rare species (chapter three). However, plants from the source populations have to be well adapted to the translocation site, as indicated by ecological similarity between source and translocation site.

Thirdly, for wetland species (especially the ones having aerenchyma and taproots), whose conservation is particularly difficult due to the dynamic nature of their habitat (Abeli et al., 2014), we strongly recommend translocating plants into the wild by securing them in the ground against flooding damage. E.g. a Hessian mesh attached to the ground might be a good solution, as it helps plants not yet well rooted to stay in the ground even when flooded. In addition, we suggest translocating plants to different microhabitats within translocation sites. In the case of wetland plants this might involve different distances from the water to mitigate impacts of water-level variations.

Fourthly, based on our reintroduction outcome and following the suggestion of many authors (e.g. Pavlik, 1996; Whitlock, 2000; McGlaughlin et al., 2002; Reed, 2005), we suggest increasing the number of translocated plants to at least a thousand to reduce adverse effects of stochasticity. However, we recommend planting them in numerous but small plots, as this might decrease intra-specific competition (Ries et al., 2004), attraction and transmission of plant enemies among translocated plants (Kéry et al., 2001). Regarding the latter, we suggest future studies to experimentally test whether the diversity and density of plant enemies increases with the size of translocation plots. Another interesting translocation strategy to test would be the use of common and competitive neighbouring species planted near translocated rare species. Competitive species could serve as a “bait” for plant enemies, thanks to their high palatability (chapter two), and thus indirectly increase the fitness of translocated species by reducing plant enemy impact. Some studies have investigated the role of shelter and nurse plants in facilitating the survival and growth of focal species (e.g. Bugnon et al., 1997; Ervin, 2005; Bontrager et al., 2014), notably by reducing herbivory (García & Obeso, 2003; Gómez Aparicio et al., 2008). However, to date, this has not been tested in translocation studies. In such a test, the competitive neighbouring species should be from a different growth form than the translocated species to lower interspecific competition (Anthelme & Michalet, 2009).

Finally, we recommend monitoring translocated populations as precisely as possible at the individual level and over the long term, as this is the best way to assess which strategies allow their self-sustainable establishment (Primack, 1996; Godefroid et al., 2011). We encourage future translocation programs to analyse translocation success at the individual level, as this increases the statistical power for analysing multifactorial studies. In this context, Aster analyses (Geyer et al., 2007), which generalize survival analyses and generalized linear models, represent a novel and powerful tool, yet not so often used in conservation, to assess factor effects on combined variables contributing to fitness over multiple years.

Further implications for future studies

One of the most critical points to go beyond this thesis and advancing towards a general framework defining drivers of plant rarity is to perform trans-generational experiments. In conservation, trans-generational monitoring of translocations are the key to ultimately consider whether they are successful or not. The study of the effects of genetic diversity and origin on the fitness of translocated plant over the long term is necessary to test whether genetic rescue has taken place (Willi et al., 2007), whether inbreeding depression on reproduction has not occurred (Tallmon et al., 2004) and to assess how the effects of factors of interest change over time. Lastly, investigating propagule-pressure effects over the long term would allow determining translocated population dynamics.

In genetics and pollination ecology, trans-generational studies would allow studying the effects of selfing and outcrossing on plant fitness beyond the second generation. This is particularly relevant as it has been indicated that heterosis may fade and outbreeding depression may only become visible after the second generation (Tallmon et al., 2004). In epigenetics and ecology of trophic interactions, trans-generational studies would be interesting to investigate e.g. how maternal plants induce anti-herbivore defence in their offspring (Agrawal, 2002; Holeski et al., 2012). Moreover, trans-generational studies conducted in a multi-species framework may answer the first open question that Holeski et al. (2012) defined in their review “How taxonomically widespread is trans-generational induction [of plant defence] and is it linked to particular plant life-history strategies?”.

Finally, it is very likely that the abundance and distribution of species are generally shaped by a complex interaction between several factors. Hence, we call future studies to design experiments using both multi-species and trans-generational approaches to simultaneously test multiple factors of interest and their interactions. Although this will increase the size and complexity of experiments, it would contribute to our understanding of the interplay between factors shaping the pathways toward plant rarity. The one and only global factor explaining why some plants are rare and others not has not yet been discovered and probably does not exist.

Final thoughts and personal perspective

The traditional way to investigate plant rarity in autecology, by testing hypotheses on a single species, contrasts with the more recent way to address questions at a larger scale, such as based on meta-analyses and multi-species experiments (van Kleunen et al., 2014). This transition from a specific to a more general scale is interesting as it is symptomatic of a more integrative way of asking and answering research questions about our world. Metaphysically, this reflects to me both the beauty that biologists often find in the details and intricacies of life and the fascination that physicists have for simple and elegant, yet comprehensive, general principles of nature (McArthur, 2021). However, the traditional and the more modern ways are surely complementary, as they allow examining different aspects of life. Moreover, both pathways towards finding beauty trigger a greater sense of the value of our environment and the need to protect it.

I would like to conclude with the notion that this thesis highlights the never-ending work of researchers. The hypotheses we tested and the questions we answered lead indeed to new hypotheses and questions deserving to be tested and answered to push the limits of the knowledge we have of our world. Some may see this as discouraging, others welcome the certainty of always having enough questions to satisfy their curiosity and marvel at the beauty of our world.

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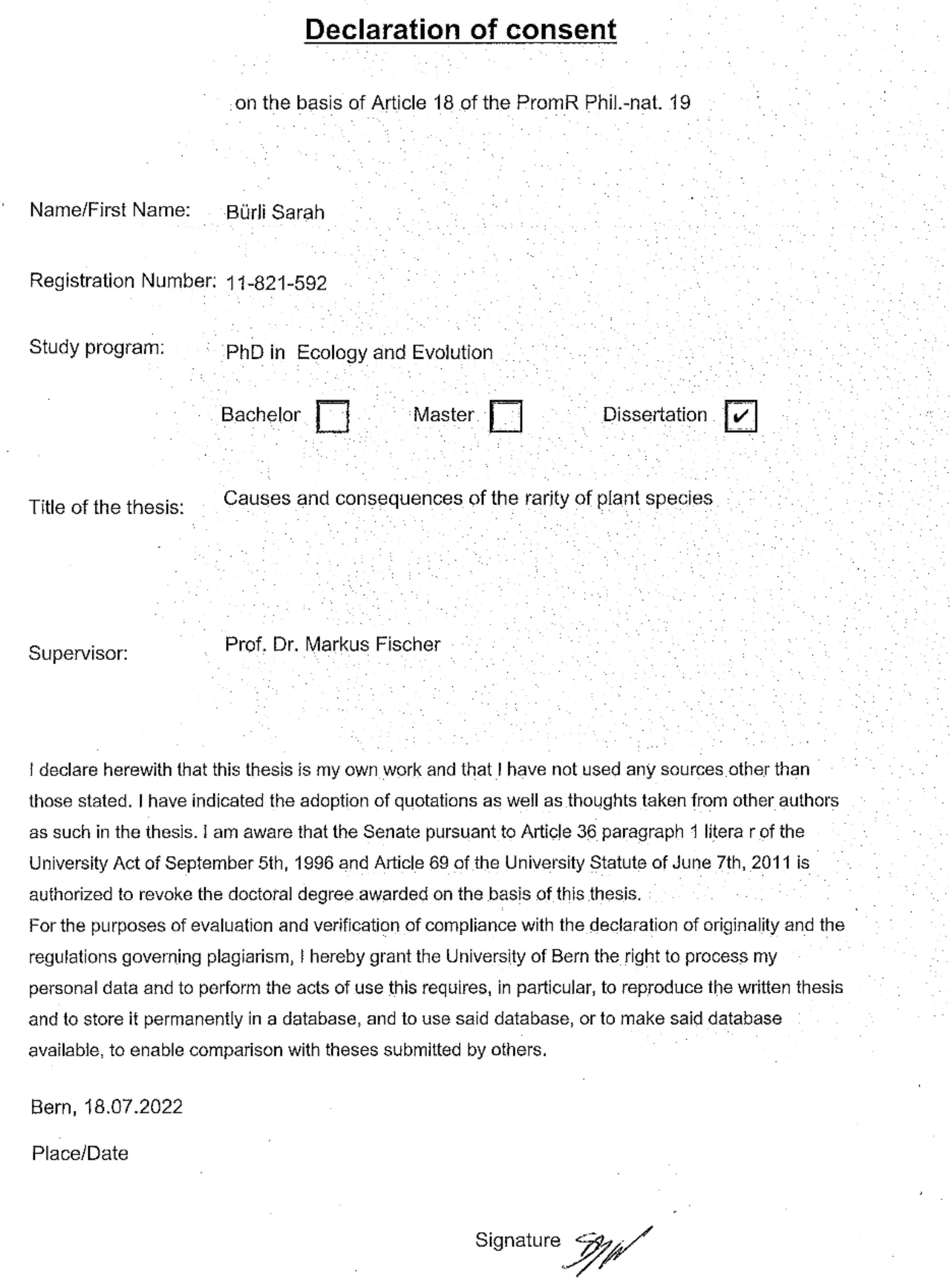

Declaration of consent

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