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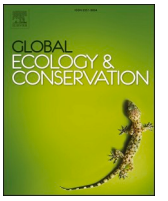

Original research article

Rarity and life-history strategies shape inbreeding and outbreeding effects on early plant fitness

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ABSTRACT

Local abundance and regional distribution are two aspects of a species' rarity. They are suggested to differentially alter genetic processes in plants: Locally rare species are hypothesized to suffer less from inbreeding and outbreeding than locally common species, thanks to genetic purging through long inbreeding histories and weaker local adaptations, respectively. Regionally rare species are hypothesized to be more susceptible to outbreeding, but less to inbreeding, compared to regionally common ones, due to small and declining range size. While this has major implications for plant conservation practices, we lack evidences and general understanding on how breeding effects on a plant's early life fitness are related to its local and regional rarity. To investigate effects of inbreeding and outbreeding on plants' early fitness, we performed self-, within- and between-population pollinations in eight pairs of closely related species differing in regional and local rarity. To avoid biases due to context dependency, we took species competitive ability, habitat resource-richness and resource-allocation strategy into account in the analyses. We then tested how inbreeding and outbreeding affected five fruit-, seed- and seedling-related traits. Inbreeding did not generally have more negative effects on early fitness of regionally rare and non-competitive species than on regionally common and competitive ones. Outbreeding was generally beneficial to early fitness of plant species across the gradients of regional rarity, competitive ability and habitat resource-richness. Our results show that outbreeding may be beneficial to the early fitness of plant species, including rare and non-competitive ones and may be considered for conservation strategies.

1. Introduction

The study of the impact of genetic processes on fitness of rare and endangered plant species is of fundamental interest for ecology and conservation. In- and outbreeding in particular are pivotal processes informing conservation practices, e.g. on the implications of meta-population networks and the use of single vs. mixed origins in *ex-situ* cultures, population translocations and reinforcements (Godefroid et al., 2011). However, species rarity is articulated on (at least) two spatial scales: Some plant species form small and scattered populations over a wide geographical range, while others form dense populations restricted to a narrow range (Rabinowitz, 1981). Thus, while both species can be considered as rare, the local rarity (for the first species) and the regional rarity (for the second)

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are two different aspects of a species rarity. The former refers to its local abundance, i.e. the typical size of its populations, and the latter to its large-scale distribution range, i.e. its range size (Fig. 1). The scale difference and low level of interdependence (Rabinowitz, 1981) between these aspects suggest that genetic processes may differ in their impacts on plant fitness in function of a species' local and regional rarity.

Locally rare species typically have reduced genetic variability and heterozygosity in their populations. This is caused by the random loss of rare alleles (i.e. genetic drift) and several generations of inbreeding (Ellstrand and Elam, 1993; Oostermeijer et al., 2003; Charlesworth and Willis, 2009). Inbreeding is the production of offspring through the mating of two genetically closely related parents. It increases the individual probability to carry recessive deleterious alleles (Charlesworth and Willis, 2009; Hedrick, 2012) and often results in inbreeding depression, i.e. a fitness reduction in inbred individuals (Charlesworth and Charlesworth, 1987; Charlesworth and Willis, 2009). Inbreeding depression is considered 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 of its genetic load (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987; Hedrick, 1994). Locally rare species are thus expected to have slightly negative effects of inbreeding (mild inbreeding depression), if any, thanks to purging through long inbreeding histories, while locally common species are expected to have stronger negative effects of inbreeding (severe inbreeding depression). Regionally rare species are characterized by restricted distribution ranges and low population number (Rabinowitz, 1981), and may therefore also have undergone genetic purging. Thus, the pattern of inbreeding effects between regionally rare and common species is expected to be similar to that between locally rare and common species (Fig. 1).

In contrast to inbreeding depression, outbreeding depression is a reduction in offspring fitness that can occur when distantly related individuals, e.g. from different populations, are crossed. This is due to the disruption of co-adapted gene complexes when they are segregated and recombined after mating (Turelli et al., 2001). Ongoing habitat destruction and fragmentation increase population isolation, interrupting gene flow between the populations, which eventually leads to the evolution of local adaptations. It has been reported that large populations of plants are more likely to carry local adaptations than small populations (Leimu and Fischer, 2008). Hence, locally common plant species may suffer from stronger negative outbreeding effects than locally rare plant species, assuming that the latter are less locally adapted. In contrast, regionally rare species are believed to be more susceptible to negative outbreeding effects than regionally common species, due to their often declining distribution range resulting in isolated and potentially more locally adapted, yet large, populations (Fischer and Matthies, 1997; Fig. 1). Studies reporting negative effects of population crosses on fitness in some species (reviewed in Edmands, 2007) have triggered a general fear of outbreeding depression (Frankham et al., 2011). However, it is indicated to happen under specific circumstances, i.e. when crossed populations are from different species, have fixed chromosomal differences, had no gene exchange for several centuries, or occur in different habitats (Frankham et al., 2011). Moreover, outbreeding may be beneficial for genetically depauperate species, as it increases allelic diversity and heterozygosity and may thus

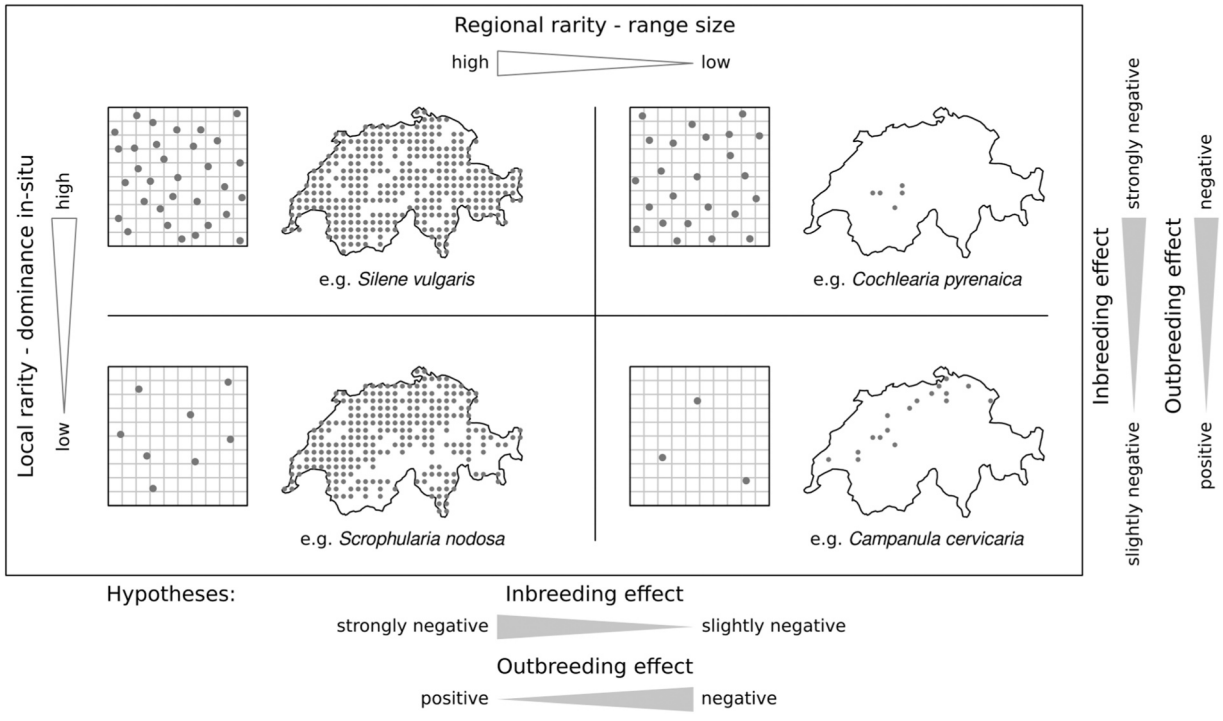

Fig. 1. Hypothetical patterns of inbreeding and outbreeding effects in plant species, given their local and regional rarity, e.g. (clockwise from top-left): Silene vulgaris: regionally and locally common; Cochlearia pyrenaica: regionally rare but locally common; Scrophularia nodosa: regionally rare but locally common; Campanula cervicaria: regionally and locally rare.

Table 1

Plant species used in the study with detailed information concerning their rarity, inclusion in statistical models, traits and characteristics, seed collection sites and population location. Pair, Rarity and Family indicate respectively the number of the pair of phylogenetically related species to which the species belongs, whether the species is considered rare or common within its pair and the plant family to which it belongs. Analyses indicates if the species is included in all models, excluded from the leaf-length model, or removed from the analyses due to low sample size (see Material and methods section). Mean plant size (Plant size; [cm]), mean specific leaf area (SLA; [mm2/mg]), competitive ability (Comp. ability), indicator values for levels of nutrients (N), moisture (F) and dominance in-situ (a measure of plant local rarity; DG; indicator values can take an integer value between 1 and 5), and range size (a measure of plant regional rarity, assessed as the number of monitoring areas in Switzerland in which the species is present) are the plant species traits, indicator values and rarity variables used in this study as described in the Materials and methods section. Collection sites indicate the location of the two populations whose seeds were collected for this study. Ind. nb indicates the number of individuals within the first and the second collected population per species and Dist. indicates the distance between the collected populations of a species [km]. BE, FR, GE, NE, TI, VD, VS refer to the Swiss cantons of Bern, Fribourg, Geneva, Neuchâtel, Ticino, Vaud, Valais, respectively.

Species	Pair	Rarity	Family	Analyses	Plant size	SLA	Comp. ability	N	F	DG	Range size	Collection sites	Ind. nb	Dist.
Campanula cervicaria	1	rare	Campanulaceae	all	27.1	30.2	1	3	3	1	25	Forst (2 pop), Bern, BE	40; 15	0.5
Campanula rotundifolia	1	common	Campanulaceae	removed	-	-	1	2	2	2	2504	Le Lieu + Le Pont, VD	100; 20	4
Cochlearia pyrenaica	2	rare	Brassicaceae	all	10.2	10.3	1	2	5	3	8	Gantrisch + Eriz, BE	1000;80	32
Lunaria rediviva	2	common	Brassicaceae	all except leaf length	37.3	21.9	2	4	4	3	182	Bönigen Tüscherswald + Innereriz, BE	100; 300	12
Erysimum cheiranthoides	3	common	Brassicaceae	all	72.1	28.5	0	4	4	2	140	Holligen + Marzili, Bern, BE	30; 30	2
Erysimum ochroleucum	3	rare	Brassicaceae	removed	23.9	27.3	1	2	1	2	6	Chasseral (2 pop), BE	35; 250	0.5
Linaria alpina subsp. petraea	4	rare	Plantaginaceae	removed	-	-	0	2	3	2	33	Lac de Joux + Le Lieu, VD	25; 50	0.25
Linaria vulgaris	4	common	Plantaginaceae	all	62.5	36.7	1	4	2	3	640	Güterbahnhof, Bern, BE + Visp, VS	100; 80	80
Nepeta nuda	5	rare	Lamiaceae	removed	-	-	1	3	1	2	9	Nax + Suen, VS	40; 50	5
Stachys recta subsp. recta	5	common	Lamiaceae	removed	-	-	1	3	3	2	622	Nax + Visperterminen, VS	25; 30	37
Papaver occidentale	6	rare	Papaveraceae	removed	26.3	22.3	-	-	-	-	13	Zweisimmen (2 pop), BE	100; 500	0.5
Papaver rhoeas	6	common	Papaveraceae	removed	-	-	0	3	2	2	785	Insel + Wylerbad, Bern, BE	30; 40	1
Potentilla argentea	7	common	Rosaceae	all	13.2	18.5	1	2	2	2	302	Herbriggen + Zermatt, VS	20; 30	0.3
Potentilla multifida	7	rare	Rosaceae	all	22.6	16.4	2	4	3	2	8	Gornergrat + Riffelberg, Zermatt, VS	50; 50	3
Prunella grandiflora	8	common	Lamiaceae	all	13.1	31.2	1	2	2	2	755	Lamboing + Nods, BE	60; 30	4
Prunella laciniata	8	rare	Lamiaceae	all	16.6	26.7	1	2	2	1	72	Le Landeron + Nods, NE	35; 15	6
Scrophularia auriculata	9	rare	Scrophulariaceae	all	61.3	37.4	2	4	5	2	12	Choulex + Pointe à la Bise, GE	15; 10	4
Scrophularia nodosa	9	common	Scrophulariaceae	all	80.1	26.9	2	4	4	2	801	Magnedens + Montagny-la-ville, FR	40; 20	10
Silene vallesia	10	rare	Caryophyllaceae	all	11.7	29.9	1	2	2	3	9	Leiggru + Oberried, VS	20; 50	12
Silene vulgaris	10	common	Caryophyllaceae	all	57.8	34.3	1	2	3	3	1305	La Neuveville + Nods, BE	30; 80	5
Teucrium botrys	11	rare	Lamiaceae	all except leaf length	21.2	28.9	0	2	2	2	134	Bözingen (2 pop), BE	25; 40	0.4
Teucrium chamaedrys	11	common	Lamiaceae	all except leaf length	20.1	23.3	1	2	2	3	554	Lamboing + Ligerz, BE	100; 25	4
Veronica austriaca	12	rare	Plantaginaceae	removed	26.9	34.8	1	2	2	2	14	Les Jordan + Petits Michel, NE	35; 20	1
Veronica urticifolia	12	common	Plantaginacea	all	39.6	22.3	2	3	4	2	628	Holzflue + Zweisimmen, BE	50; 25	0.6

reduce adverse effects of inbreeding (Charlesworth and Charlesworth, 1987; Charlesworth and Willis, 2009; Neuwald and Templeton, 2013; McLennan et al., 2020; Zecherle et al., 2021). While evidence that plants often exhibit negative inbreeding effects but suffer seldom from negative outbreeding effects accumulates (Edmands, 2007; Frankham et al., 2011), our understanding of how these effects on plant fitness relate to a plant's local and regional rarity, as well as whether locally and regionally rare plant species respond similarly to inbreeding and outbreeding, remains very limited.

Next to the local and regional rarity of a species, its competitive ability, habitat resource-richness and resource-allocation strategy likely also modulate the effects of genetic processes on plant fitness. Indeed, a species' environment and the trade-offs it faces provide the context shaping its life history strategy (Kempel et al., 2020). Many common species are characterized by traits for competition, high resource acquisition and fast growth and are found in resource-rich habitats, whereas rare species are usually less competitive, grow more slowly and are adapted to resource-poor habitats (Drury, 1974; Grime, 1979; Coley et al., 1985; Kempel et al., 2020). Plants from resource-limited habitats may be purged more effectively of their genetic load than plants from resource-richer habitats, as inbreeding depression may be more strongly expressed under stress (Armbruster and Reed, 2005; Hayes et al., 2005; but see Sandner et al., 2021). Hence, plants from resource-rich habitats could still carry the majority of their genetic load and suffer more from inbreeding depression when inbred than plants from resource-limited habitats. Therefore, if we aim to study differences between species varying in local and regional rarity and want to avoid biases due to context dependency, we need to take the species habitat resource-richness and resource-allocation strategies into account (Murray et al., 2002; Kempel et al., 2020).

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 essential. When working with rare species, a problem scientists often deal with is the inherently low number of populations, constraining the number of experimental replicates at the population level. Yet, multi-species experiments allow to obtain typical values and differences between comparator groups of species, rather than precise values per population that require numerous replicates at the population level. Although many studies (e.g. Dudash, 1990; Hauser and Loeschcke, 1996; Leimu et al., 2008; Thiele et al., 2010; Sedlacek et al., 2012; Wang, 2019; Sandner et al., 2021) investigated breeding effects in plants, none, to our knowledge, has conducted a multi-species investigation, while taking into account multiple aspects of rarity, habitat and resource-allocation strategy. An efficient way to uncover general breeding patterns in plants is to conduct self- and cross-pollinations and assess offspring fitness (e.g. Darwin, 1876; Stojanova et al., 2021). Because the mutation rate varies in time and between genes (Charlesworth and 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.

Here, we present an experiment where we assess the effects of inbreeding and outbreeding on five traits related to early life-stage fitness in the offspring of 16 plant species occurring in Switzerland by performing self- and cross-pollinations. At the same time we account for species differences in local and regional rarity, competition strategy, habitat nutrient-richness and moisture-level, specific leaf area (SLA) and mean plant size. To exclude phylogenetic bias, we selected eight pairs of closely related species, each including one regionally rare and one common species varying in local rarity. We addressed the following questions: 1) How is the fitness of regionally and locally rare plant species altered by inbreeding and outbreeding compared to regionally and locally common species? 2) Are effects of inbreeding and oubtreeding on plant fitness modulated by a) a plant's competitive ability, b) habitat resource-richness and c) resource-allocation strategy? 3) What recommendations for plant conservation can be drawn from the results?

2. Material and methods

2.1. Plant collection

We initially selected 12 pairs of congeneric or con-familiar rare and common herbaceous plant species from eight families and different habitats and regions of Switzerland. Species and their origin are given in Table 1. We defined a species as rare if it has an IUCN threat-status in the Swiss Red List of vascular plants (Bornand et al., 2016, including the near-threatened status) and a conservation priority in Switzerland (OFEV, 2019); otherwise we considered the species as common. Although each plant population has its own and unique history, the rare and common species we selected form two coherent groups of species: the distribution of all included rare species has declined in the last decades in Switzerland, while it is not the case for common species. As a result, most of the rare plants of this study are found on the Red List of Switzerland (Bornand et al., 2016). The classification of rare and common was used to select the target species, while for the analyses of rarity we used a continuous variable of range size in Switzerland (see Section 2.4). Seeds of ten seed families (i.e. from ten different maternal plants) were collected randomly from two populations of each of the 24 plant species. Population size (i.e. individual number per population) and distance between populations per species in km were recorded (Table 1). Populations in typical natural habitats in the western half of Switzerland were selected. As indicators of the species' habitat quality we used the ecological indicator values according to Landolt et al. (2010), which describe the realized ecological niche of a species by its position along an environmental gradient following an ordinal scale ranging from one to five.

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 left to grow in a greenhouse until flowering under 14-hour daylight with additional light, temperature between 15 and 30°C and daily watering. 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.

2.2. Pollination treatments

For the pollination treatments we selected five seed families per population and species. From each seed family we selected five plants and on each of these plants we assigned one flower to each of the three pollination treatments: self-pollination, between seed-family cross-pollination and between-population cross-pollination. When fewer than five plants from five seed families flowered, we performed as many hand pollinations as the number of flowers, flowering plants and seed families permitted. However, for five of the 24 species (*Campanula cervicaria, Erysimum ochroleucum, Nepeta nuda, Stachys recta subsp. recta* and *Veronica austriaca),* germination and flowering per population were so low that we were not able to perform all or any pollination treatments. In total, we performed 542 self-pollinations, 550 and 456 cross-pollinations between seed families and populations, respectively. The pollination experiment lasted from 2018 to 2020, as some species and populations did not flower in the first two years.

Flowers were emasculated before being pollinated. 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 the ground to the pollinated flower.

We placed pollinated plants into mesh cages for three to five days to prevent any pollination by insects that may have entered the greenhouse. When ripe we collected the fruits. We counted and weighed the seeds per fruit to the nearest 0.01 g (Sartorius Cubis balance MSA225P-100-DI, Sartorius Lab Instruments GmbH & Co. KG, Germany). Aborted fruits containing non-viable seeds were identified and recorded thanks to their abnormal appearance, as they are much smaller and not fully developed compared to normal fruits of the same species. We calculated the developed fruit ratio by dividing the number of developed fruits by the total number of pollinations performed per pollination treatment, maternal population and species. As the ratio of developed fruits is generally low for selfed fruits of self-incompatible species (Balogh and Barrett, 2018), we considered partially and strictly self-incompatible species as having a ratio of developed selfed fruits to developed outcrossed fruits ≤ 0.5 and equal to zero, respectively. Thus, *Cochlearia pyrenaica, Linaria alpina subsp. petraea, Linaria vulgaris, Prunella grandiflora* and *Teucrium chamaedrys* were identified as partially self-incompatible and *Lunaria rediviva* as strictly self-incompatible, while all other species were identified as self-compatible. This finding and the fact that all species were entomophilous (Info Flora, https://www.infoflora.ch) lead us to consider that *L. rediviva* has a strict outcrossing strategy, while all the other species have a mixed mating strategy.

2.3. Germination percentage and seedling performance

To evaluate further fitness-related traits in seeds resulting from hand pollinations, we stratified 30 seeds (or all seeds when less than 30) from each of four fruits from different seed families per pollination treatment, maternal population and species. 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 (or all seedlings when fewer than five) per fruit 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.

2.4. Plant rarity

As a measure of regional rarity we used the range size, defined as the maximum number of 5×5 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 was 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 (including two identical species to ours) are positively correlated (R^2=0.508, P<0.001). All species included in our study also occur outside Switzerland, with species that are regionally rare in Switzerland generally having a lower number of occurrences in a smaller number of countries than Swiss regionally common species (species maps in GBIF.org, 2023; see also Bornand, 2014 and Slatyer et al., 2013). As a measure of local rarity, we used the species indicator-value for dominance *in-situ* (DG; Landolt et al., 2010; Table 1). This indicator value describes the accumulation (i.e. abundance) of individuals of a species at the place where it occurs. 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). It is directly related to the typical population size of the species where the conditions are the most suitable and is generally comparable from one species to another. Local rarity correlated positively with the maternal population size of the species included in this study (r=0.34, P=0.01).

2.5. Habitat resource-richness, competitive ability and resource-allocation strategy of plants

To characterize the resource-richness of the plant habitat and position each species in a resource-richness gradient, we used the species indicator-value for nutrients (N) and moisture (F; Landolt et al., 2010; Table 1). Indicator values for nutrients and moisture characterize the nutrient content in the soil (mainly nitrogen) and the average soil moisture during the growth period where the species is most frequently present, respectively. 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 ability” 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 1), respectively.

For ten non-flowering individuals from different seed families per species we measured the plant size as its maximal height or length. All individuals were approximately the same age. We then averaged the mean plant size per species, which is a trait associated with plant life-history and resource-allocation strategy (Moles and Leishman, 2008). We measured the specific leaf area (hereafter called SLA) of one leaf of each of five individuals 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 and high SLA leaves) and plants with a slow-growing strategy (species with long-lived and low SLA leaves). A high indicator value for nutrients and a high competitive ability are also characteristic of fast-growing and regionally and locally common plants (Poorter and Remkes, 1990; Westoby, 1998; Murray et al., 2002). These variables reflect profound differences of adaptive strategy related to competitive ability, life history and resource allocation among species and are used here to investigate how they modulate effects of inbreeding and outbreeding on plant fitness.

2.6. Statistical analysis

To identify significant interactive effects between pollination treatments and plant local and regional rarity, competitive ability, habitat resource-richness and resource-allocation strategy on fitness-related traits, 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 of the following response variables: 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 centered and scaled per species for standardization between species (this was unnecessary for the two other variables, as they are percentage and ratio). Residuals of all five models followed a Gaussian distribution. As fixed effects, we included pollination treatment, range size, indicator values for dominance *in-situ,* nutrients and moisture, competitive ability, 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. A.1 in Appendix; corrplot package; Wei and Simko, 2017) among explanatory variables, we tested their effects simultaneously in the models. We also tested the effect of the population size as a local rarity variable. It was significant in the model for developed fruit ratio. However, because this effect was driven by only one species (*Cochlearia pyrenaica*), we consider it as not reliable and reported it only in Appendix (Fig. A.2). With the exception of pollination treatment, all explanatory variables were centered and scaled. Analyses were performed in R 3.6.3 (R Core Team, 2020).

Of the 24 species, we kept only species with all explanatory variables and a balanced observation number between pollination treatments in the models (contingency table in Appendix: Table A.1). Thus, 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, the flower position in the inflorescence and different maternal plant size (Thompson and Rabinowitz, 1989; Moles et al., 2004; Moles and 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). In addition, we centered and scaled the seedling leaf-length per species at the pricking-out time and included it as covariate in the model for leaf length to correct for differences in initial conditions. We included the pollination year and total pollination number per maternal population, species 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. We then used a stepwise backward regression to reduce the complexity of the LMER models by dropping the non-significant terms. We used likelihood-ratio tests to calculate the significance of each term by comparing the models with and without it (Zuur et al., 2009; significance threshold: P<0.05).

Cross-pollination between seed-families was considered as the typical pollination strategy for all species, since they all have a mixed mating or strict outcrossing strategy (see Section 2.2). Self-pollination and between-population cross-pollination were consequently considered as the pollination treatments testing for inbreeding and outbreeding, respectively. To measure breeding effects on fitness-related traits of plants differing in variables of local and regional rarity, competitive ability, habitat resource-richness and resource-allocation strategy, we calculated the coefficient of inbreeding or outbreeding depression as the [(mean fitness-related trait for self-pollination or between-population cross-pollination/mean fitness-related trait for between seed-family cross-pollination)-1], respectively (Edmands, 2007). The resulting coefficients are therefore standardized responses to inbreeding and outbreeding and reflect the interactive effects of the pollination treatment and plant variable on a fitness-related trait. We then plotted these values against the variables that were found to be significant in the interaction with the pollination-treatment variable. We draw monotonic linear regression lines to indicate significant relationships in the analyses, but we have refrained from indicating confidence intervals as they would not represent the uncertainty of the effects of the lmer models (ggplot2 package; Wickham, 2016). Thus, the position of the lines below and above zero indicates negative and positive effects of inbreeding and outbreeding, respectively, on a plant fitness-related trait in relation to range size, competitive strategy, indicator value for nutrient and moisture, SLA and mean plant size. We chose monotonic regression over non-monotonic regression because it is less sensitive to data local distribution and sparsity, resulting in a more accurate and robust capture of the relationship between coefficients of inbreeding and outbreeding depression and our explanatory variables.

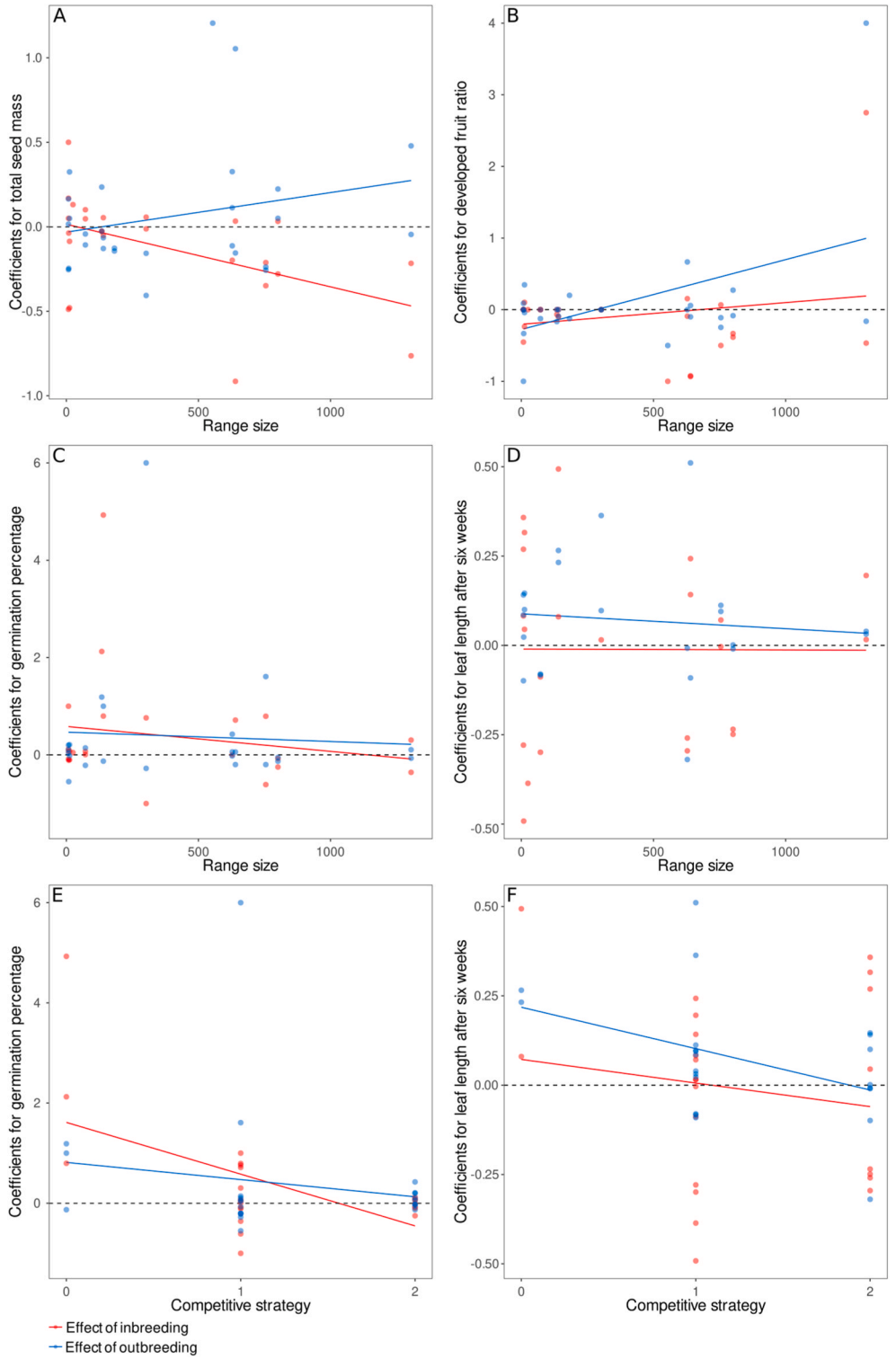

Fig. 2. Relationship between range size and coefficients of inbreeding and outbreeding depression for (A) total seed mass per fruit, (B) developed fruit ratio per pollination treatment and population, (C) germination percentage per fruit, and (D) seedling leaf-length. Relationship between species competitive ability and coefficients of inbreeding and outbreeding depression for (E) germination percentage per fruit, and (F) seedling leaf-length. Red regression lines show effects of inbreeding and blue ones show effects of outbreeding. Regression lines that fall below and above zero indicate negative and positive effects of inbreeding and outbreeding, respectively. Inbreeding and outbreeding coefficients (y-axes), range size and competitive strategy (x-axes) are unitless.

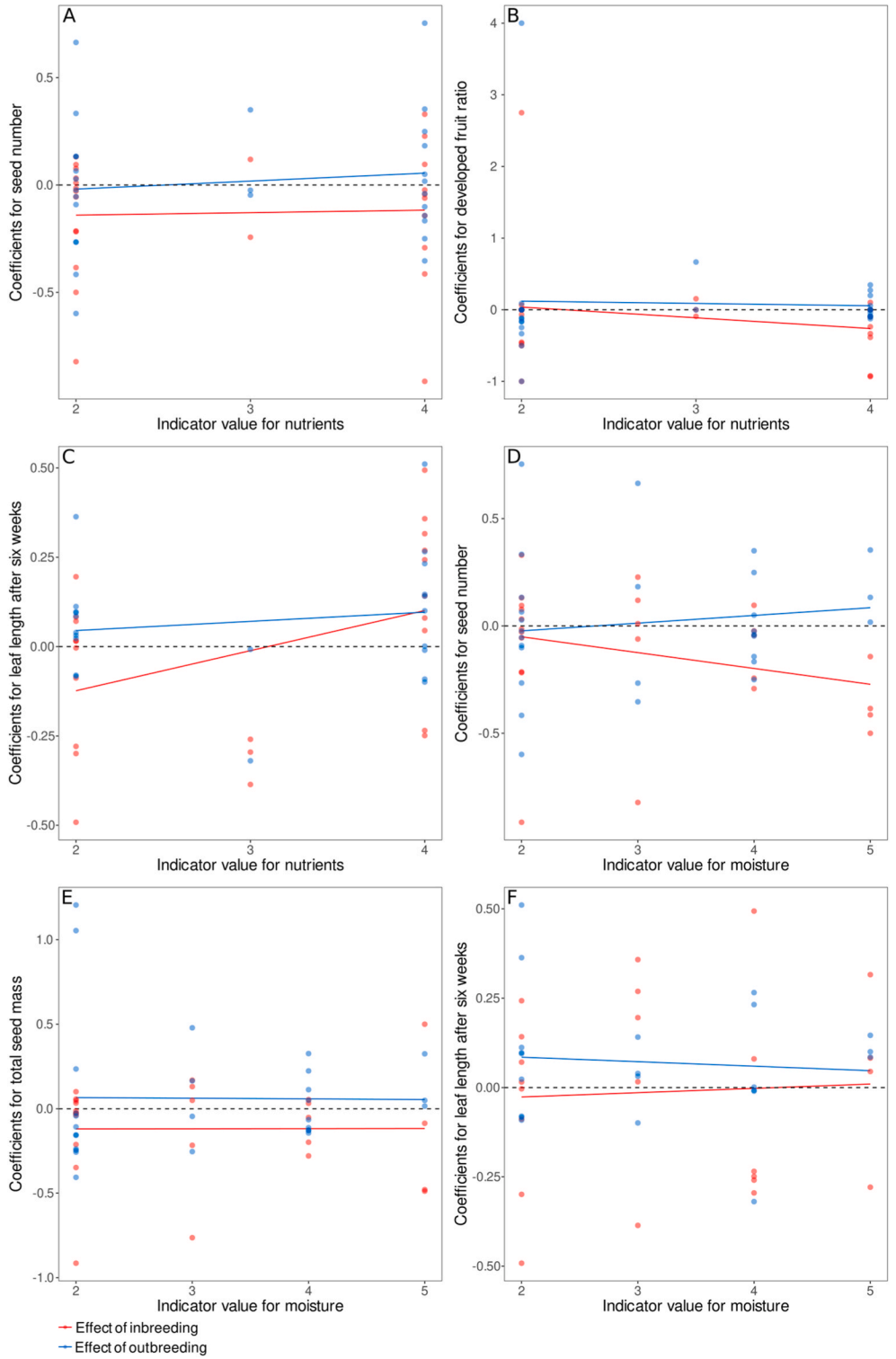

Fig. 3. Relationship between species indicator-value for nutrients and coefficients of inbreeding and outbreeding depression for (A) seed number per fruit, (B) developed fruit ratio per pollination treatment and population, and (C) seedling leaf-length. Relationship between species indicator-value for moisture and coefficients of inbreeding and outbreeding depression for (D) seed number per fruit, (E) total seed mass per fruit, and (F) seedling leaf-length. Red regression lines show effects of inbreeding and blue ones show effects of outbreeding. Regression lines that fall below and above zero indicate negative and positive effects of inbreeding and outbreeding, respectively. Inbreeding and outbreeding coefficients (y-axes) and indicator values for nutrients and moisture (x-axes) are unitless.

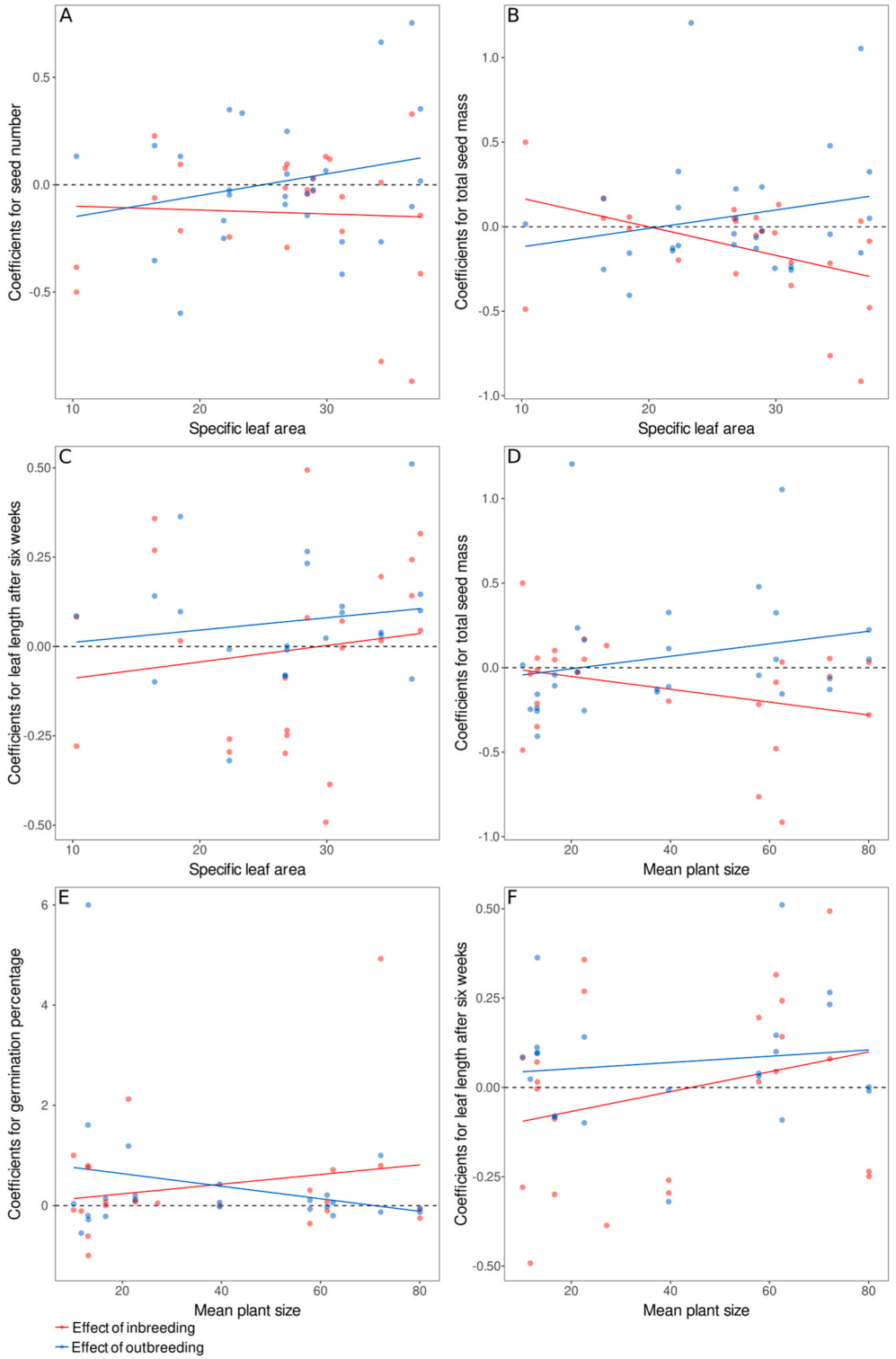

Fig. 4. Relationship between specific leaf are and coefficients of inbreeding and outbreeding depression for (A) seed number, (B) total seed mass per fruit, and (C) seedling leaf-length. Relationship between mean size per species and coefficients of inbreeding and outbreeding depression for (D) total seed mass per fruit, (E) germination percentage per fruit, and (F) seedling leaf-length. Red regression lines show effects of inbreeding and blue ones show effects of outbreeding. Regression lines that fall below and above zero indicate negative and positive effects of inbreeding and outbreeding, respectively. Inbreeding and outbreeding coefficients (y-axes) are unitless. Units of specific leaf area and mean plant size (x-axes) are [mm^2/mg] and [cm], respectively.

3. Results

Breeding effects varied as a function of the species regional rarity, competitive ability, habitat nutrient-richness and moisture, SLA and mean plant size and between fitness-related traits. However, we could not confirm any association between local rarity (i.e. indicator-value for dominance *in-situ* and population size, see Section 2.6) and breeding effects on plant fitness (Tables A.2-A.6 in Appendix).

3.1. Breeding effects on plants of different regional rarity and competitive ability

Inbreeding effects on the total seed mass per fruit became increasingly negative with increasing range size (Fig. 2A). For the developed fruit ratio, inbreeding effects shifted from slightly negative to slightly positive with increasing range size (Fig. 2B), while they were decreasingly positive on the germination percentage (Fig. 2C). For the seedling performance (i.e. leaf length after 6 weeks), inbreeding effects were slightly negative within the range of regional rarity covered by our species (Fig. 2D). In contrast, outbreeding effects on total seed mass and developed fruit ratio shifted from near neutral and slightly negative, respectively, to positive with increasing range size (Fig. 2A-B). For the germination percentage and seedling performance, outbreeding had positive effects within the range of regional rarity (Fig. 2C-D).

Inbreeding effects on the germination percentage and seedling performance shifted from positive to negative with increasing plant competitive-ability (Fig. 2E-F). Outbreeding effects were decreasingly positive on the germination percentage and seedling performance with increasing plant competitive-ability (Fig. 2E-F).

3.2. Breeding effects on plants of different habitat resource-richness

Inbreeding effects were constantly and increasingly negative on the seed number and developed fruit ratio, respectively, with increasing nutrient richness (Fig. 3A-B), while they shifted from negative to positive for the seedling performance (Fig. 3C). Outbreeding effects were near neutral on the seed number and developed fruit ratio and positive for the seedling performance within the range of nutrient richness covered by the habitat of our species (Fig. 3A-C).

Inbreeding effects on the seed number became increasingly negative with increasing moisture level in the species habitat (Fig. 3D), while they were constantly negative on the total seed mass and near neutral on the seedling performance (Fig. 3E-F). Outbreeding effects were positive on the seed number, total seed mass and seedling performance within the range of moisture level, except on the seed number of species from dry habitats, where they were near neutral (Fig. 3D-F).

3.3. Breeding effects on plants of different resource-allocation strategy

Inbreeding effects on the seed number were negative within the SLA range of our species (Fig. 4A). For total seed mass, they shifted from positive to negative with increasing SLA, while the opposite pattern was found on the seedling performance (Fig. 4B-C). Outbreeding effects shifted from negative to positive with increasing SLA on seed number and total seed mass (Fig. 4A-B) and were increasingly positive on seedling performance (Fig. 4C).

Inbreeding effects were increasingly negative and positive on the seed mass and the germination percentage, respectively, with increasing mean plant size (Fig. 4D-E). For seedling performance, inbreeding effects shifted from negative to positive with increasing mean plant size (Fig. 4F). Effects of outbreeding shifted from near neutral to positive with increasing mean plant size on the total seed mass (Fig. 4D), while the opposite was found on the germination percentage (Fig. 4E). For the seedling performance, outbreeding effects were positive within the range of mean plant size (Fig. 4F).

4. Discussion

4.1. Stronger negative inbreeding effects in common and competitive plants

In our study, regional rarity modulated the effects of inbreeding and outbreeding on the early life fitness of plants. We found that regionally rare species did not generally suffer more from adverse effects of inbreeding than common species. Moreover, non-competitive and rare species and competitive and common species were similarly affected by inbreeding and outbreeding on the germination percentage and seedling performance (Fig. 2).

The stronger negative inbreeding effects found in most traits of regionally common and competitive species compared with regionally rare and non-competitive species is likely due to genetic purging in the latter. Populations of rare plant species tend to have a longer inbreeding history than common species (Ellstrand and Elam, 1993; Oostermeijer et al., 2003; Charlesworth and Willis, 2009), making it likely that they have undergone inbreeding depression. If inbreeding depression is due to (over-)dominance, natural selection may reduce the frequency of deleterious alleles (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987). As a result, populations of rare species may consequently suffer less from negative inbreeding effects than populations of common species, which did not experience such a long history of inbreeding (Barrett and Charlesworth, 1991; Swindell and Bouzat, 2006; Robinson et al., 2018). However, these results contrast with Angeloni et al. (2011)'s finding that the magnitude of inbreeding depression did not vary with plant regional rarity. Although regional rarity and competitive strategy were not strongly correlated in our study (Fig. A.1), regionally rare species tend to be also less competitive than common ones (Murray et al., 2002). This could explain the similar effect of

inbreeding found on regionally rare and non-competitive species in our study.

Outbreeding had major positive effects on the seedling performance in regionally rare and non-competitive species and had generally beneficial effects on all early life traits studied in common and competitive species. This indicates that heterosis (positive outbreeding effects on fitness) may be quite common, while trait-dependent and stronger after germination than before, in F1 of rare and non-competitive species. Investigating the persistence of outbreeding effects beyond the F1 generation in regionally rare and non-competitive vs. common and competitive species remains a gap for future studies. Furthermore, this suggests that the maternal populations included here were likely not locally adapted or only weakly so, as otherwise between-population cross-pollinations would have led to a disruption of locally adapted genotypes resulting in outbreeding depression (Waser and Price, 1989). It is possible that short distances between populations in some of the species and traits like entomophily and perenniality (traits shared by all species included in this study; https://www.infoflora.ch), which increase gene flow and reproductive opportunities (Levin and Kerster, 1974; Hamrick et al., 1979), have prevented the evolution of local adaptation in populations (Linhart and Grant, 1996). It is also possible that local adaptations are infrequent, as suggested by Leimu and Fischer (2008). Transplantation under field conditions are needed to confirm whether populations were locally adapted or not.

Our hypotheses linked to the local rarity of plant species were not confirmed, neither with the indicator value for dominance *in-situ* (Landolt et al., 2010) nor with the population size. This means that locally rare species did not respond differently to breeding treatments compared with locally common species in our study. This contrasts with Angeloni et al. (2011), who found that inbreeding depression increases with population size (as categorical variable). Several causes may have precluded our ability to confirm patterns linked to local rarity. Firstly the indicator value for dominance *in-situ* may reflect a species' ability to form dominating stands rather than its typical population size. Secondly, except two populations, the estimated size of all populations in our models ranged within 10–100 individuals, whereas larger populations would probably have been needed to find a reliable pattern. Finally, it is possible that recent population-size reduction (due to fragmentation) may have a stronger impact on breeding effects on plant fitness than population size *per se*. Hence, future studies should include precise count of (reproductive) individuals as well as population history, such as recent bottle necks.

4.2. Plants across habitats benefit from outbreeding

In accordance with our hypothesis, we found that inbreeding and outbreeding effects on early life fitness varied with plant habitat resource-richness. However, with the exception of inbreeding effects on seedling performance and seed number varying strongly with nutrient richness and moisture level, respectively, breeding effects on fitness-related traits were rather constant within the nutrient richness and moisture range of the species' habitat. Inbreeding had generally negative effects, while outbreeding had beneficial effects on the species's early life fitness within the nutrient-richness and moisture range covered by their habitat (Fig. 3).

Negative inbreeding effects were predicted to be more pronounced under stress (Armbruster and Reed, 2005; Hayes et al., 2005; but see Nason and 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 lower than outbred seedling survival. Similar results have been reported in *Lychnis (Silene) flos-cuculi* by Hauser and Loeschcke (1996) and by Leimu et al. (2008). Yet, in our study we did not test responses of inbred and outbred plants to environmental stressors but rather, whether plants from different habitat conditions respond differently to inbreeding and outbreeding. Possibly, our findings would have been different if our experimental plants had been grown under more realistic settings, for instance in a field experiment. The relatively consistent effect of inbreeding and outbreeding on plant fitness across gradients of habitat conditions suggests that our results are unlikely to be systematically biased by the difference between the conditions encountered by species in the greenhouse and in their natural habitat. Furthermore, recent literature questions the triggering effect of stress on inbreeding depression (Sandner et al., 2021) and suggests that environmental variation increases phenotypic variation and subsequently increases the probability of detecting inbreeding depression.

4.3. Large variation of breeding effects on slow- and fast-growing plants

In accordance with our hypothesis, we found that inbreeding and outbreeding effects on early plant fitness varied with plant traits linked to resource-allocation strategy, such as SLA and mean plant size (Westoby, 1998; Wright et al., 2004; Moles and Leishman, 2008; Fig. 4). However, we observed a considerable variability of plant responses to inbreeding and outbreeding between low- and high-SLA and large and small species, precluding the confirmation of any pattern about the relationship between inbreeding, outbreeding and resource-allocation strategy of the plant. Finding this important variability conforms with earlier studies reporting variation in the level of negative inbreeding effects between traits and life-cycle stages (e.g. Husband and Schemske, 1996; Glaettli and Goudet, 2006; Sedlacek et al., 2012). Notably, Ågren and Schemske (1993) and Picó et al. (2004) found that inbreeding depression levels varied independently among different fitness-related traits. The origin of such a variation has been suggested to be a differential mutation rate and selection over time and between genes, as some of them are life-stage specific (Charlesworth and Charlesworth, 1987; Husband and Schemske, 1996; Angeloni et al., 2011). Thus, if inbreeding depression is caused by several genes, some of which acting at a specific life stage, selection against stage-specific deleterious and recessive mutations is expected to vary over time (Husband and Schemske, 1996), possibly explaining the variation in the level of negative inbreeding effects found in our study. However, we focused on the early stages of life and are therefore unable to assess whether the level of negative inbreeding effects evolves in a certain direction over the whole life course, as predicted by the evolutionary theory of aging (Hamilton, 1966; Husband and Schemske, 1996). Finally, it has been reported that the intraspecific phenotypic variability of plants grown in a greenhouse versus

in the field is generally lower than the interspecific variability, provided that the greenhouse plants are not fertilized (Mokany and Ash, 2008, who measured 17 traits on 14 plant species). Given that our plants were not fertilized, our measurement of mean plant size and SLA at species level under standardized conditions in a greenhouse most likely reflects consistent size and SLA differences between species and, therefore, differences in adaptive strategies between species. Despite this, further studies are needed to verify whether the inter-taxa pattern we found for the effect of SLA and mean plant size on plant response to inbreeding and outbreeding holds when these traits are measured in the field.

4.4. Conclusion, conservation implications and caveats

The first important result we found is that our regionally rare and non-competitive species did not generally suffer more from adverse effects of inbreeding than common and competitive species in their early life stages. The second is that outbreeding had most often beneficial effects on early fitness-related traits of plant species across the gradients of regional rarity, competitive ability, habitat nutrient-richness and moisture we tested. This suggests that outbreeding may be typically beneficial, rather than detrimental, for early life fitness of plant species, including rare and non-competitive ones. This is congruent with Leimu and Fischer (2008)'s conclusion that negative outbreeding effects (outbreeding depression) due to the disruption of local adaptations may be less common than often assumed. In contrast, although rare plant species did not strongly suffer from inbreeding in their early life stages, it appeared that common plant species did. Our study thus also supports Angeloni et al. (2011)'s call to consider negative effects of inbreeding on regionally common plant species, as their fitness may be more immediately threatened by inbreeding.

Based on our study, we suggest considering between-population mixing as an adequate management strategy to improve plants' early F1 fitness (Charlesworth and Willis, 2009), when propagating plant material and planning conservation actions. We recommend considering population mixing for common and competitive plant species, as well as for regionally rare and non-competitive species, as a measure to increase fitness and genetic diversity. High genetic diversity allows evolutionary resilience and genetic pre-adapation to future climate and disturbances (Gamfeldt and Källström, 2007; Jump et al., 2009; Weeks et al., 2011), which are essential for their survival under climate changes.

Our study presents three caveats that require attention when considering our recommendation. Firstly, positive outbreeding effects (heterosis) may fade and negative outbreeding effects (outbreeding depression) may appear in the second generation (Tallmon et al., 2004; Edmands, 2007). Future studies and conservation projects should thus test whether outbreeding increases plant fitness beyond the F1 generation and whether fitness traits in later life stages are similarly affected than early fitness traits. Secondly, our study is designed to identify pattern of inbreeding and outbreeding on plant fitness across species under a standardized common environment, thus bearing high inference. However, to be conservative, these inferences should be confined to perennial entomophilous species from Central Europe. Finally, the broad nature of the explanatory variables employed in our analyses allow to elucidate general trends regarding the effects of inbreeding and outbreeding on plant fitness among species and across a diverse spectrum of adaptive strategies. Consequently, our study cannot identify species-specific patterns (van Kleunen et al., 2014) and patterns observed in the field. Further studies should investigate how environmental conditions affect patterns of plant fitness in function of inbreeding and outbreeding directly in field settings or by applying environmental stressors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be available on Dryad as soon as the manuscript is accepted.

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Supporting information

Additional supporting information may be found in the online version of the article at the publisher's website.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2024.e03081.

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