Similar but not identical: cryptic speciation of *Saxifraga rosacea*

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Similar but not identical:
cryptic speciation of *Saxifraga rosacea*

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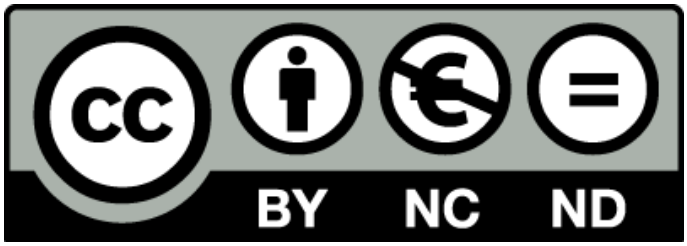

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CHAPTER 1

General Introduction

GENERAL INTRODUCTION

BACKGROUND

Since the *Origin of species* describing the Darwin‘s theory of phenotypic speciation, evolutionary biology has found evidence that not only selection drives speciation but also random processes like genetic drift and mutations within the framework of the neutral theory of molecular evolution (Kimura, 1983). The notion of speciation had been revised with numerous models explaining how new species arise (Dobzhansky, 1937; Mayr, 1942; Coyne & Orr, 2004; Schluter, 2009). The relative importance of selection and random genetic divergence for the speciation process has long been discussed (Orr and Smith, 1998; Rundle and Nosil, 2005; Matute and Cooper, 2021). However, most researchers agree that speciation events are more likely to occur in small and isolated populations when they are subject to new and strong selection pressures (Orr & Orr, 1996; Gavrilets, 2003; Khatri & Goldstein, 2015). Climate change is a potentially strong mechanism for speciation by introducing vicariant barriers with the shift of climate zones, by modifying the dispersal of populations through migration to more suitable climate space or by applying new selection pressures when populations are establishing in a new environment (Vrba & DeGusta, 2004; Pardi & Smith, 2012). The Quaternary period encompassing the last 1.8 million years is characterized by drastic climate changes with several glacial advances and retreats. Thus, at a geological scale, these rapid and extreme climatic cycles may have accelerated speciation rates because of their severe effects on the geographical ranges of plants and animals, by increasing the isolation of populations and by decreasing their size while they were submitted to new environmental pressures (Davis & Shaw, 2001; McCarty, 2001; Parmesan & Yohe, 2003, Liow & Stenseth, 2007).

In post glacial periods, recurrent geographical expansions and contractions of ranges and back-and-forth advances may have isolated then reconnected part or whole populations (Harris, 2013). Evolutionary forces as genetic drift or divergence selection induced by changes in population size or environmental conditions, may have led to fixed changes in allele frequencies like polyploidization, a well-known genome modification involved in the evolution of plant species (Grant, 1981; Masterson, 1994; Soltis et al. 2009; Rice et al, 2015). Genome duplication is a predominant mode of speciation of angiosperms (Coyne & Orr, 2004; Adam & Wendel, 2005; Meyers & Levin, 2006; Venditti & Pagel, 2010) that can result in physiological, morphological, demographic and/or ecological changes of attributes of the

different cyotypes (Levin, 2002; Parisod et al. 2010; Ramsey, 2011). The relative frequency of polyploids and the level of ploidy of flowering plants increase towards the poles (Stebbins, 1984; Brochmann et al., 2004; Arrigo et al. 2016). Polyploids appear to be more successful colonizers after deglaciation as their fixed heterozygous genomes may counter the loss of genetic variation in small founding populations typical for colonizers (Guldahl et al., 2005; Leitch & Leitch, 2008; Brochmann & Brysting, 2008). Frequent heterozygosity within polyploids may be a buffer against extinction risks during periods of climatic change (Chapman et al. 2006; Lovell et al, 2021) by increasing their plasticity and by facilitating cytotypes with higher ploidy level to occupy a wider range of environments, or by conferring them a higher potential for local adaptation due to more diverse sets of alleles contained in the higher number of genome copies (Mitton & Grant, 1984; Brochmann & Elven, 1992; Te Beest et al. 2012, Van de Peer et al. 2017).

Human activities are currently accelerating climate change and impact on plant populations through the degradation, destruction and fragmentation of habitats. Therefore, numerous studies have investigated recently fragmented and/or isolated populations of plant species (Newman & Tallmon, 2001; Aguilar et al., 2008; Frankham et al., 2017) to provide the basis for suitable conservation management programs for these newly threatened species. However, little is known about the consequences of fragmentation for ‘old’ rare species that occupy naturally rare habitats with restricted areas that have been fragmented for a long time. Understanding the life cycle, determining the dynamics of populations and estimating the extinction risks of rare plant species are essential to evaluate if management plans are required and to set up long-term management schemes (Oostermeijer et al., 2003; Heinken & Weber, 2013), but genetic diversity of fragmented and isolated populations is often considered as the main issue. Conservation strategies endeavor to enhance the genetic diversity of plant populations to enhance their chances to evolve, to increase the probability that they have preadapted alleles that may help them to cope with changing environmental conditions or to prevent genetic deterioration by inbreeding and genetic drift (Lindenmayer & Fischer, 2013; Frankham et al. 2019). Several decades of using genetic rescue to restore appropriate gene flow in populations of endangered plant species have shown the benefits of such management programs (Frankham, 2015, Whiteley et al. 2015), yet recent studies exposed limitations of these management techniques (Ralls et al., 2020; Robinson et al., 2021). With the improvement of molecular technics, it is now more common to investigate population genetics/gene flow (Fischer and Matthies, 1998; Ouborg et al., 1999; Ellstrand, 2014) prior to conservation decisions as it is crucial to establish accurate degrees of genetic

differentiation between populations. In the case of different cytotypes, significant genetic differences may lead to reproductive isolation (Frankham et al., 2002) raising the question of their taxonomic status and also of their legal status of protection (Ouborg et al., 2006, Bosch et al, 2019), and questioning the need to favor a specific gene flow between them.

OUTLINE OF THE THESIS

In an integrative ecological, demographic and taxonomic approach, I studied the consequences of different levels of ploidy on ecological niche differentiation, adaptation and plasticity, population dynamics and reproductive isolation of two closely related plant taxa. *Saxifraga rosacea* s.l. is an ice-age relict occupying naturally rare and long-term fragmented screes and rock-face habitats (Thorn, 1960; Walter and Straka, 1970). The two subspecies *Saxifraga rosacea* subsp. *rosacea* and *Saxifraga rosacea* subsp. *sponhemica* (hereafter referred to as *S. rosacea* and *S. sponhemica* for brevity) have been found to have different ploidy levels (2n = 64 (Philp, 1934; Webb, 1950) and 2n = 46 - 52 (Drábková, 2000; Oberdorfer, 2001), respectively). Both cytotypes are morphologically very similar and are found in similar habitats (Webb & Gornall, 1989). However, *S. sponhemica* has a scattered distribution from Belgium to the Czech Republic, whereas *S. rosacea* is more common and occurs from Iceland and Norway to Central Europe (Jalas, 1999; GBIF, 2019) and despite their spread and without know overlapping distribution areas, no mixed populations are known.

I assumed that a polyploidization event may have led to several differentiations, explaining the current geographical distribution of the two subspecies. The higher ploidy level of *S. rosacea* may have conferred advantages to occupy a wider range of ecological niches because of its larger genome with higher potential for adaptation. The two *Saxifraga* cytotypes may also differ in their sensitivity to current disturbances and thus have different extinction risks. Moreover, the differences in ploidy level may have caused reproductive isolation preventing the two subspecies to hybridize, questioning their current taxonomical status as subspecies and the conservation management plans for those naturally rare plant taxa.

This thesis consists of three studies:

In chapter 2 (‘Ecological niche differences between two polyploid cytotypes of *Saxifraga rosacea*’), the ecological differentiation between *Saxifraga rosacea* and *Saxifraga sponhemica* is investigated by analyzing the environmental conditions in 22 populations

spread over the distribution areas of both cytotypes and by testing their tolerance to boundary climatic conditions. Habitat characteristics were studied to define their ecological niches, mean plant trait values in populations were evaluated, and niche modeling was used to predict possible suitable habitats and occurrence probabilities in Europe. The tolerance to harsh climatic conditions was assessed through a cold resistance experiment and by recording the performance of plants at two transplant sites established beyond the range of the other cytotype to test for local adaptation of both *Saxifraga* cytotypes.

In Chapter 3 (‘Population dynamics and extinction risks of two closely related plant taxa’), population fate and demography were investigated for four years in five populations of *Saxifraga rosacea* and four populations of *Saxifraga sponhemica*. Plant size and reproductive traits were monitored to determine vital rates and population growth rates of the two cytotypes, to evaluate temporal and spatial variation of population dynamics, to establish their life cycle and to analyse differences between the two *Saxifraga* cytotypes. The most sensitive life stage transitions of both cytotypes were identified and correlated to recorded disturbances. Environmental stochasticity and local climatic data were correlated with population dynamics and life cycle to simulate the extinction risk probabilities for *Saxifraga rosacea* and *Saxifraga sponhemica*.

In Chapter 4 (‘Reproductive isolation of two cytotypes of *Saxifraga rosacea*: genetic rescue issues in rare plant conservation’), crossing experiments were carried out with plants from 15 populations of *Saxifraga rosacea* and 15 populations of *Saxifraga sponhemica* to study the effects of inbreeding and outbreeding, to clarify the taxonomic status of the morphologically defined subspecies and to determine if the two *Saxifraga* should be considered as different species. Two generation of offspring involving manual crosses within and between populations, hybridization and backcrosses, were produced to investigate reproductive fitness. Seed traits and offspring performance were assessed to estimate the degree of hybrid vigour, outbreeding depression and inbreeding depression. Moreover, morphological traits of the descendants from the crossing experiments were studied to evaluate the relevancy of the current taxonomical criteria used to distinguish the two cytotypes.

CHAPTER 2

Ecological niche differences between two polyploid cytotypes of *Saxifraga rosacea*

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Lucile Decanter carried out the practical work, contributed significantly to the planning of the experiments, and was responsible for the analysis of the data and writing the paper. G.C., D.M., N.E. and S.H. contributed to the planning of the study and reviewed the manuscript, D.M. and G.C. supported the analysis of the data.

ABSTRACT

Different cytotypes of a species may differ in their morphology, phenology, physiology and their tolerance of extreme environments. We studied the ecological niches of two subspecies of *Saxifraga rosacea* with different ploidy levels, the hexaploid Central European endemic ssp. *sponhemica* and the more widely distributed octoploid ssp. *rosacea*. For both cytotypes, we recorded local environmental conditions and mean plant trait values in populations across their areas of distribution, analyzed their distributions by niche modelling, studied their performance at two transplant sites with contrasting conditions, and tested their cold resistance experimentally.

Mean annual temperature was higher in hexaploid than in octoploid populations and experiments indicated that frost tolerance of the hexaploid is lower than that of the octoploid. Reproduction of octoploids from both Iceland and Central Europe was higher than that of hexaploids at a transplant site in subarctic Iceland whereas the opposite was true in temperate Luxembourg indicating adaptation of the octoploids to colder conditions. Temperature variables were also most important in niche models predicting the distribution of the two cytotypes. Genetic differences in survival among populations were larger for the octoploids than for the hexaploids in both field gardens suggesting that greater genetic variability may contribute to their larger distributional range.

Our results support the hypotheses that different cytotypes may have different niches leading to spatial segregation and that higher ploidy levels can result in a broader ecological niche and in particular greater tolerance of more extreme conditions.

KEY WORDS: Cytotype distribution; frost tolerance; niche modeling; polyploidy; population differences; reciprocal transplant experiment; spatial segregation.

INTRODUCTION

Polyploidization (genome duplication) is a well–known phenomenon in the evolution of angiosperms (Grant, 1981; Masterson, 1994; Otto and Whitton, 2000; Soltis et al., 2009; Soltis et al., 2014; Rice et al., 2015). Polyploid species contain more than two sets of chromosomes and can originate through genome duplication within a species (autopolyploidy) or hybridisation between different species and subsequent genome duplication (allopolyploidy, Soltis and Soltis 1993, 1999; Comai, 2005). Polyploidization often creates new lineages that contribute to biodiversity in plants (Glennon et al., 2014).

Whole genome duplication can result in changes in plant morphology, phenology, physiology and demography (Li et al., 1996; Levin, 2002; Raabová et al., 2008; Maherali et al., 2009) and generate individuals that exploit new niches, have a higher tolerance of extreme environments (Weiss-Schneeweiss et al., 2013), are more resistant against herbivores (Stutz et al., 2016) or may outcompete their parent species (Leitch and Leitch, 2008). Polyploids may also be more able to colonize new habitats and can be more invasive (Richardson et al., 2000; te Beest et al., 2012). Further polyploidization events and backcrosses can lead to cytotypes with an even higher number of chromosome sets and to species with multiple cytotypes that differ in their chromosome numbers. However, maintaining large genomes also present metabolic costs for polyploid cytotypes that can result in lower growth rates (Otto, 2007; Neiman et al., 2013; Guignard et al., 2016). Little is known about the ecological consequences of different levels of polyploidy (Brittingham et al., 2018).

A recent review concluded that co–occurrence of different cytotypes within populations of mixed–ploidy species is common, but spatial segregation at different scales can occur (Kolář et al., 2015). In the case of mosaic parapatry, mostly single-cytotype populations may be spatially intermingled, or dominant cytotypes may be spatially separated with limited contact zones (large–scale parapatry; Kolář et al., 2015). The pattern of the geographic distribution of cytotypes can provide important insights about the origin and maintenance of different ploidy levels (Baack, 2005; Rieseberg and Willis, 2007; Kolář et al., 2009; Muñoz–Pajares et al., 2018). A random distribution of cytotypes or the frequent occurrence of mixed–cytotype populations can indicate similar habitat requirements, whereas strong spatial segregation may indicate niche differentiation, reproductive isolation, limited dispersal or separation of cytotypes by historical factors (see Muñoz–Pajares et al., 2018 and references therein).

Climate is a major determinant of the distribution of plant species and is also thought to explain a large part of the spatial separation of lineages with different ploidy levels (Glennon et al., 2014; McAllister et al., 2015; Muñoz–Pajares et al., 2018). Ecological niche modelling (Mairal et al., 2018; Muñoz–Pajares et al., 2018) and multivariate analysis of niche variables allow a quantitative evaluation of the ecological divergences of plant lineages with different ploidy levels and also permit a statistical comparison of the niche overlap of the different taxa (Warren et al., 2008; Broennimann et al., 2012). However, the evidence for climatic or ecological niche differentiation between different cytotypes of a species is still inconclusive. While some studies have found correlations between cytotype distribution and climate variables and differences in habitat conditions between cytotypes (e.g. Raabová et al.,

2008; Kolář et al., 2009; Sonnleitner et al., 2010; Richardson and Hanks, 2011; Ramsey 2011; Mráz et al., 2012; McAllister et al., 2015; Visger et al., 2016; Muñoz–Pajares et al., 2018), others have found evidence for shared broad–scale climatic niches between cytotypes and no evidence for differences in climatic requirements (Godsoe et al., 2013; Glennon et al., 2014 and references therein). Ideally the study of niche differentiation would include experiments testing the effects of environmental factors on different cytotypes, but there are not many studies involving such experiments (Ramsey 2011; Kolář et al., 2015; McIntyre and Strauss, 2017). As polyploidization can also drive changes in phenotypic traits in natural populations (Comai, 2005; Chen and Sun, 2010), the study of phenotypic differences between different cytotypes may be important to understand their ecology (Segraves et al., 1999; Nuismer and Cunningham, 2005; Münzbergova, 2006).

It has also been proposed that historical factors may strongly influence the distribution of cytotypes (Stebbins, 1984; Brochmann, 2004). The current distribution of polyploids in regions affected by the ice ages may be linked to events of colonization and retreats in refugial zones of plant species, associated with the glaciation–deglaciation periods of the recent geological past. It has been shown that the frequency and level of ploidy of flowering plants increases towards the poles and circum-arctic area (Favarger, 1967; Löve and Löve, 1974; Brochmann et al., 2004) suggesting that because of their fixed-heterozygous genomes polyploids are buffered against inbreeding and genetic drift and were more successful at colonizing deglaciated areas than their relatives with lower ploidy levels (Brochmann et al., 2004).

In the family *Saxifragaceae* the diversity of cytotypes within a species can be complex because of multiple euploid and aneuploid polyploidization events (Soltis et al., 2007). Within the genus *Saxifraga*, the section *rosacea* has many closely related taxa that differ in their ploidy level (Webb and Gornall, 1989). In this study, we combined climate niche modelling, measurements of temperature in natural populations, analyzes of substrate composition and vegetation, and experimental tests of cold resistance to study the niches of two subspecies with different morphology and cytotype, the octoploid *Saxifraga rosacea* ssp. *rosacea* and the hexaploid *S. rosacea* ssp. *sponhemica* (C.C. Gmel.) D.A. Webb. In addition, we tested local adaptation by studying survival, growth and reproduction of plants from 20 populations across the range of the two cytotypes transplanted into two transplant sites, each of them situated within the range of one of the cytotypes and beyond the range of the other. We also investigated mean trait values in a large number of populations of both cytotypes of *S. rosacea*. Despite occurring in similar habitat types (scree, cliffs, rock walls), the two

cytotypes have distinct distribution areas without known overlapping zones (Jalas et al., 1999). Both taxa are considered to be ice age relics (Thorn, 1960; Walter and Straka, 1970; Walisch et al., 2015).

We address the following specific questions: (1) Do the two subspecies differ consistently in their chromosome number? (2) Do the cytotypes differ in their ecological niches, and in particular is there evidence for a wider niche and a greater tolerance of extreme conditions of the taxon with the higher ploidy level? (3) Do plants in populations of the two cytotypes differ in their size and reproduction?

MATERIALS AND METHODS

Study species—

Saxifraga rosacea Moench is a perennial plant with a fragmented distribution in Europe. Three subspecies have been distinguished (Webb and Gornall, 1989), but we studied only *S. rosacea* subsp. *rosacea* Moench and *S. rosacea* subsp. *sponhemica* (C.C.Gmel.) D.A. Webb. The third subspecies *Saxifraga rosacea* subsp. *hartii* (D. A. Webb) D. A. Webb is only known from Arranmore Island in Ireland (Chater, 1987; Webb and Gornall, 1989).

The phylogenetic relationsships of *S. rosacea* s.l. are not clear. In a phylogenetic study of *Saxifraga* sec. *Saxifraga*, Vargas (2000) identified a polytomic subclade consisting of *S. rosacea*, *S. hartii*, *S. graeca*, *S. granulata* and *S. cespitosa*. More recently, Tkach et al. (2015) found *S. terektensis*, *S. cespitosa* ssp. *monticola* and *S. hypnoides* to be closely related to *S. rosacea*. The two taxa we studied were originally described as different species and in the interest of brevity, we will refer to them as *S. rosacea* and *S. sponhemica*. Chromosome numbers for *S. sponhemica* have been reported to be 46-52 in Central and Eastern Europe (Drábková 2000; Oberdorfer et al. 2001), whereas plants of *S. rosacea* from Clare Island and Blackhead in Western Ireland had 64 chromosomes (Philp 1934; Webb 1950). Preliminary results from crossing experiments indicate that reproduction of hybrids between *S. sponhemica* and *S. rosacea* is very low (Decanter unpubl.).

Both *S. sponhemica* and *S. rosacea* have a disjunct distribution (Fig. 1). *S. sponhemica* occurs in an area extending from the Belgian Ardennes to the German Hunsrück, in the French Jura, in the Czech Republic, and in the extreme south of Poland. *S. rosacea* occurs in Central and Eastern Germany, the Faroe Islands, Western Ireland, Norway, Sweden, Finland and in Iceland (Webb and Gornall, 1989; GBIF.org, 2019).

Both subspecies grow as compact cushions ranging in size from one to 600 rosettes and occur in stable environments where competition is low, like rocky substrates, screes,

stony slopes and stone walls (Webb and Gornall, 1989). The lobes of the leaves of *S. rosacea* are obtuse, acute or shortly mucronate with hairs that are predominantly glandular, whereas the segments of the leaves of *S. sponhemica* are apiculate, narrow and have hairs that are mostly non–glandular (Webb and Gornall, 1989). In continental Europe, both taxa flower from April to July, whereas in Iceland *Saxifraga rosacea* flowers from June to August. The white protandrous flowers are entomophiles and flowers not visited by insects produce hardly any seeds (Webb and Gornall, 1989).

Study sites—

We selected nine populations of *S. sponhemica* spread over its area of distribution: three populations in the Czech Republic, three in the Ardennes (Belgium and Luxembourg), two in the Hunsrück region of Germany and one in the French Jura. For the subspecies *S. rosacea*, 13 populations were selected: four in Eastern Germany, three in Southern Germany, four in Iceland, one in the French Jura and one in the Vosges mountains of France (Table 1).

Chromosome counts—

Chromosomes were counted for plants from all the study populations (excluding HERM and WENW) using the protocol of Inceer and Hayirlioglu-Ayaz (2007). In summer 2012, one rosette each from at least 20 cushions was collected in each of the populations, placed on wet cotton and brought to the laboratory. The rosettes were placed onto soaked peat pellets (Jiffy®) to grow roots and in spring 2013 planted into pots. The plants were kept in the garden of the National Museum of Natural History of Luxembourg. In autumn 2014, roots of three individuals per population were sampled in the morning, gently cleaned with ddH_2O taking care not to damage the rootlets and placed for 3 h in a 0.05% solution of colchicine. Once wiped, the roots were placed in a 3:1 mix of ethanol / acetic acid for 2 h at room temperature. After rinsing with ddH_2O, the rootlets were placed together with drop of a 1% aceto–orcein solution between a slide and a cover glass and softly squashed. Chromosomes were counted under a microscope with a magnification of 1000X (Motic serie BA210 Digital, Motic®, Hong Kong).

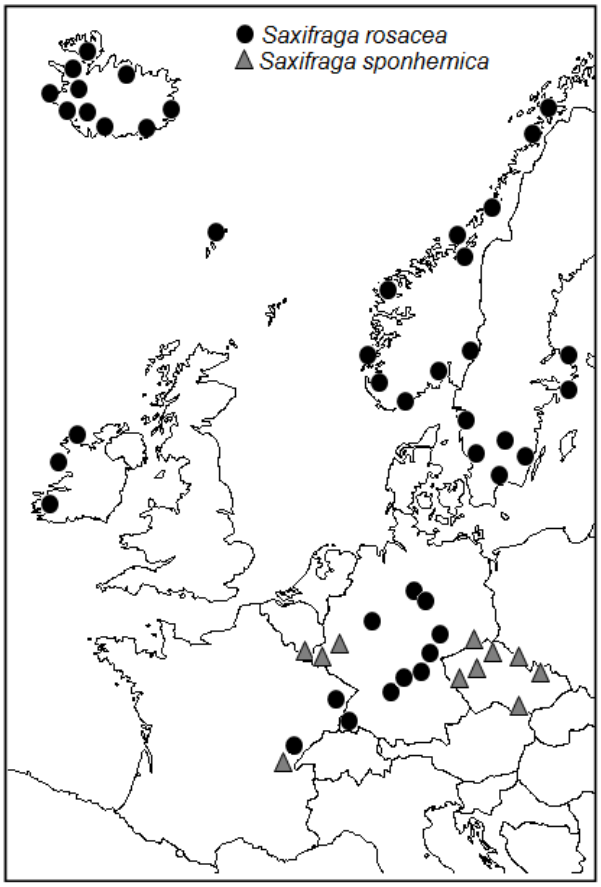

FIGURE 1. Map showing the distribution of *Saxifaga rosacea* and *Saxifaga sponhemica* (modified from GBIF 2019 and Atlas Flora Europeae; Jalas et al.,1999).

Habitat characterization—

In June 2012, we randomly selected plots of 1 x 1 m in which *Saxifraga* was present in the study populations. In most populations, five plots were selected, but in the populations Arbois, Hermetingen, Aðaldalshraun and Skogafoss only two to four plots could be established because parts of the populations were difficult to access. In each plot we recorded the cover of each vascular species and the total cover of bryophytes, litter and bare soil. At each site, we took a rock sample, and recorded the habitat type (rock wall, scree), slope and altitude. The content of SiO_2, Al_2O_3, Fe_2O_3, and CaO in the rock samples was analyzed by X–ray fluorescence spectrometry (ARL PERFORM–X 9400 Sequential XRF XP, Thermo Scientific®, USA). Loss on ignition was determined by preparing samples (0.5 g) on glass discs with lithium metaborate, finely grounding them and incinerating them for 2 h at 1000°C.

In July 2013, we recorded canopy openness in the study populations by taking a hemispherical photograph at a height of 1 m above one randomly selected *Saxifraga* cushion near the centre of each population with a camera (Canon EOS 5D, © Canon Inc., JP) equipped with a Fisheye lens (EF 8–15mm f / 4L). The pictures were processed with the GapLight Analyzer software (Version 2.0, Frazer et al. 1999). From 2012 to 2015, we

recorded local temperature four times a day (6 a.m., 12 a.m., 6 p.m. and 12 p.m.) in all populations except ROB and HERM with data loggers (Tiny tag Transit 2 TG–4080 and Tiny tag Plus 2 TGP–4500, Gemini Data Loggers®, U.K) placed at the soil/rock level avoiding direct exposition to the sun (Table 1). Growing degree days (GDDs), a measure of heat accumulation, were assessed using this equation for each day of the year:

$$GDD = \frac{Tmax + Tmin}{2} - Tbase$$

We chose 5 °C as Tbase, as both studied *Saxifraga* occur in cold areas. To obtain the GDDs per population, we took the sum of all the GDD values for the days and summed them per year (McMaster and Wilhelm, 1997).

We also obtained the following four fundamental bioclimatic variables for each study site at a grid size of 0.86 km² (30 arc s) from the WorldClim v2.0 database (Fick and Hijmans, 2017): mean annual temperature, maximum temperature of the warmest month, minimum temperature in coldest month, and precipitation.

Population size structure—

In July 2013, we randomly selected five plots of 1 x 1 m in each population, except for Arbois, Hermetingen and Skogafoss where only 2 – 3 plots could be placed due to problems in accessing the plants. We recorded in each plot the maximum length and width of all *Saxifraga* cushions and the number of inflorescences per cushion and calculated the mean number of inflorescences per flowering plant. To estimate the mean number of flowers per inflorescence, we counted the number of flowers on five randomly selected inflorescences per cushion. We calculated the area of each cushion by multiplying the maximum length and width of the rosette and then calculated the density of inflorescences per cm^2 of cushion.

We distinguished three classes of plants based on their status and size (cushion area): (1) single vegetative rosettes, (2) medium sized cushions (one flowering rosette or more than one rosette and cushion area < 46 cm^2), and (3) large cushions (> 46 cm^2). The value of 46 cm^2 is the geometric mean of the cushion area of 1476 observed plants (excluding single vegetative rosettes).

TABLE 1. Location of the 22 populations of *Saxifraga rosacea* and *Saxifraga sponhemica* in which the environmental conditions, the vegetational composition and the population structure were studied. In a number of populations additional studies were carried out.

Region	Population	Abbreviation	Altitude (m a.s.l)	Lat.	Long.	Studies
Saxifraga rosacea* spp. *sponhemica						
Ardennes	Bouillon	BOU	230	49.79	5.06	T, F, Ts, Cc
	Kautenbach	KAU	277	49.95	6.01	T, F, Ts, Cc
	Robertville	ROB	474	50.45	6.10	F, Ts, Cc
Hunsrück	Frauenberg	FRA	313	49.66	7.28	T, F, Ts, Cc
	Gerolstein	GER	417	50.19	6.62	T, F, Ts, Cc
Czech Republic	Blesno	BLE	466	50.45	14.14	T, F, Ts, Cc
	Tetínské skály	TET	249	49.95	14.10	T, F, Ts, Cc
	Voskov	VOS	237	49.91	14.19	T, F, Ts, Cc
Jura	Arbois	ARB	531	46.87	5.81	T, F, Ts, Cc
Saxifraga rosacea* spp. *rosacea						
East Germany	Hatzfeld	HAT	359	50.99	8.54	T, F, Ts, Cc
	Hersbruck	HERS	454	49.51	11.48	T, F, Ts, Cc
	Moschwitz	MOS	345	50.53	12.16	T, F, Ts, Cc
	Rubeland	RUB	413	51.75	10.84	T, F, Ts, Cc
South Germany	Hermetingen	HERM	659	48.19	9.21	
	Wental	WEN	622	48.73	10.01	T, F, Ts, Cc
	Wentalwieble	WENW	615	48.71	10.01	T
Iceland	Aðaldalshraun	ADA	4	65.93	-17.49	T, Ts, Cc
	Road 427	R427	39	63.85	-22.20	F, Ts, Cc
	Skogafoss	SKO	79	63.53	-19.51	T, F, Ts, Cc
	Svart	SVA	52	63.88	-22.41	T, F, Ts, Cc
Vosges	Hartmannswillerkopf	HAR	909	47.85	7.16	T, F, Ts, Cc
Jura	Baume–les–messieurs	BAU	416	46.69	5.64	T, F, Ts, Cc

Note: F = samples selected for the frost tolerance experiment, T = temperature data logger placed at site, Ts = rosettes from population planted into transplant sites, Cc = number of chromosomes counted.

Frost tolerance experiment—

We tested the frost tolerance of *S. rosacea* and *S. sponhemica* by measuring leaf electrolyte leakage using a protocol adapted from Lindén (2002). Experimental plants had been grown clonally from rosettes sampled from different mother plants in 19 populations (10 of *S. rosacea*, 9 of *S. sponhemica*) in 2012 (s. Table 1). The experiment was conducted in

February 2014 to take advantage of the acclimatization of the plants to winter temperatures in the botanical garden of the Natural History Museum in Luxembourg. We collected three pairs of leaves from each of five mother plants per population. Each pair of leaves was cleaned and placed for 24 hours pairwise into a small plastic bag filled with ddH_2O to maximize leaf turgor. The three leaf pairs from each maternal plant were then briefly placed on absorbing paper to remove any liquid water, and each pair was placed in sealed plastic bags and subjected to one of the three temperatures in a dark climatic chamber: –10 °C, –20 °C or + 23 °C as control. After 24 hours we collected twenty small circular samples (0.5 mm diameter) from each leaf pair with a leaf puncher (Harris Uni–Core 0.5, Sigma-Aldrich®, USA), transferred them into Eppendorf tubes with 5 mL of ddH_2O and stirred them (250 rpm) at room temperature for 24 h. Conductivity was then measured with a conductivity meter (SensION+ EC71, Hach®, USA). The leaf samples in the Eppendorf tubes were then placed for 20 min in a water bath at 92 °C, stirred again (250 rpm) at room temperature for 24 h, and conductivity was measured again for each leaf pair. The electrolyte leakage ratio was calculated as the ratio between the first and the second conductivity measure and expressed as a percentage.

Test of local adaptation—

We grew plants of each cytotype at two common transplant sites, one in Luxembourg (49°57’54”N, 5°58’09”E, 297 m a.s.l) and a second one in northern Iceland (65°44’27”N, 17°59’02”W, 508 m a.s.l). The two transplant sites were located near extant populations of *S. sponhemica* and *S. rosacea,* respectively, but beyond the range of the other cytotype. The transplant site in Luxembourg was established in an old slate quarry near Merkholtz. All existing plant cover was removed, and the area was covered with a TYPAR® geotextile to avoid competition by local vegetation. The geotextile was covered with a 10 cm layer of a local schist and soil mixture. The transplant site in northern Iceland was set up on top of a flat mountain ridge (540 m) near Akureyri that was covered by a very sparse and eroded heathland vegetation. The site was fenced in order to avoid grazing by sheep and horses. Temperature data were recorded for 24 months at each site with data–loggers (Tiny tag Transit 2 TG–4080) placed at a soil depth of 5 cm starting in August 2013 (Iceland) and October 2013 (Luxembourg).

In June 2013 four newly formed rosettes were sampled from five randomly selected mother plants from each of the populations used for the frost tolerance experiment (Table 1).

Each sampled rosette was planted into a peat pellet (38mm) (Jiffy®, NL) and grown in the botanical garden of the Natural History Museum in Luxembourg.

We planted two daughter rosettes from each mother plant (genotype) into each of the two transplant sites at the end of July 2013 (Iceland) and mid–September 2013 (Luxembourg). The daughter rosettes were planted with their peat pellets into the local soil. In total 200 plants originating from eleven *S. rosacea* and nine *S. sponhemica* populations were planted per transplant site. To avoid intra–specific competition, we randomly planted 20 plants separated by a distance of 50 cm on ten transects. The distance between transects was 1 m. In July (Iceland) and August (Luxembourg) 2014 and 2015 we recorded survival, size and flowering of the plants. Plant size was recorded as the largest diameter of a cushion. Reproductive characteristics were recorded by counting the total number of inflorescences per cushion.

Environmental niche modelling and niche overlap—

Environmental niche modelling was performed with MaxEnt software (Phillips et al., 2006) with default parameters, except for number of replicates (10), and maximum number of iterations (5000). We used the area under the curve statistic (AUC) to evaluate model accuracy. We used all 19 bioclimatic variables available from the WorldClim v2.0 database (Fick and Hijmans, 2017) in a grid size of 0.86 km² (30 arc s) for tests run separately for both taxa. Based on the relative contribution of each bioclimatic variable to the final model, we selected the following variables for the niche modelling: mean diurnal range, temperature seasonality, minimum temperature of the coldest month, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of the coldest quarter, precipitation of wettest month, and precipitation of the warmest quarter.

Overlap between niches of *S. sponhemica* and *S. rosacea* was evaluated with Schoener's D using ENMTools (Warren et al., 2008; Warren et al., 2010). Identity of the two niches was also tested with ENMTools using 100 replicates. We used an extended dataset of 39 populations of *S. sponhemica* and 193 populations of *S. rosacea* for the niche modelling (see Appendix 1). Occurrence data were extracted from GBIF (GBIF.org, 10.8.2018 & 31.8.2018/ GBIF occurrence downloads doi: 10.15468/dl.kpjvnr; 10.15468/dl.ifynrf; 10.15468/dl.nediim; 10.15468/dl.nhrrzt) and herbarium specimens (Herbaria LUX and ICEL). Final niche model maps were made with QGIS3 based on the average niche models of ten replicate Maxent runs calculated separately for the two taxa.

Statistical analysis—

Differences in climatic variables (data from local data–loggers and the WorldClim database), environmental characteristics, rock composition, population characteristics among Iceland and Central Europe populations of *Saxifraga rosacea* and *Saxifraga sponhemica* populations were assessed by analysis of variance and Tukey tests.

Differences in community composition among the populations of the two taxa were studied by conducting a nested canonical correspondence analysis (CCA) of log–transformed values of the cover of co–occurring vascular plant species and bryophytes using the identity of the *Saxifraga* taxon present and population identity as explanatory variables. The effects of taxon identity on community composition were tested against the variation among populations while those of population identity were tested against the variation among plots. We also compared the vegetation of Iceland and continental sites with *S. rosacea* with a CCA.

We distinguished three types of populations for the analyzes of frost tolerance and transplant sites: Populations of *S. sponhemica*, Central European populations of *S. rosacea*, and Iceland populations of *S. rosacea*. The effects of population type, population as a random factor nested within population type and frost treatment were investigated by nested analysis of variance. We partitioned the effect of population type into two orthogonal contrasts between (1) populations of *S. rosacea* in Iceland vs. Central European populations of *S. rosacea* and *S. sponhemica* and (2) Central European populations of *S. rosacea* vs. those of *S. sponhemica* in the same region to disentangle the effects of differences between the two cytotypes in the same region from regional adaptation to the conditions in Iceland. Of interest were the interactions between the two contrasts and frost treatment, which according to the rules for analyzing mixed models were tested against the frost treatment by population interaction (Zar 2010).

The effects of transplant site, population type, population of origin as a random factor, and genotype (random) on growth and reproductive characteristics were studied by nested analyzes of variance or deviance (binomial variables). The effect of population type was partitioned into the same two orthogonal contrasts as in the analysis of frost tolerance. The effects of transplant site were tested against the population by site interaction, that of the contrasts against the variation among populations, the effect of population against the variation among genotypes, and that of the interactions of the site with the two contrasts against the site by population interaction.

Variables were log–transformed if necessary to obtain normally distributed residuals and homoscedasticity. Vegetation analyzes were carried out with R for Windows (Version

3.5.1), and the Vegan package v2.5–4 (Oksanen, 2018). All other statistical analyzes were performed using IBM–SPSS 25.0 (IBM Corp. 2017).

RESULTS

Ploidy differences and habitat characteristics—

All studied *S. rosacea* plants were octoploid (8x = 64) while nearly all *S. sponhemica* plants were hexaploid (6x = 48). One *S. sponhemica* plant from KAU and one from ROB had 52 chromosomes.

The mean monthly temperatures at the Iceland sites of *S. rosacea* measured at the sites by the data loggers were several degrees lower than that at Central European sites of *S. rosacea* and *S. sponhemica* in nearly all months (Fig. 2) resulting in a significantly lower mean annual temperature at the Iceland sites (Table 2). Moreover, mean monthly temperatures and mean annual temperatures in the Central European populations of *S. rosacea* were lower than in those of *S. sponhemica*. This suggests a requirement for higher temperatures for *S. sponhemica*. Most other climatic variables differed only significantly between the Iceland populations of *S. rosacea* and the central European populations of both subspecies (Table 2). The values indicate that at least the Iceland populations of *S. rosacea* can grow at much lower temperatures and need less warmth than those of *S. sponhemica*. Iceland populations of *S. rosacea* occurred at lower elevations and on slopes that were on average less steep than those of the Central European populations of both subspecies (Table 2). The composition of the rocks on which the *S. rosacea* plants were growing in Iceland differed from that of the Central European ones. In contrast, there were no clear differences between the rocks on which *S. sponhemica* and *S. rosacea* grow in Central Europe and no clear differences in canopy openness among the three population types.

The CCA provided no evidence for differences between the composition of the plant communities at sites with *S. sponhemica* and *S. rosacea* ($F_{1,20}$ = 1.05, P = 0.41). However, both the individual populations of *S. sponhemica* ($F_{8,32}$ = 1.79, P < 0.001) and those of *S. rosacea* ($F_{12,45}$ = 2.05, P < 0.001) varied strongly in their plant communities. The vegetation of sites with *S. rosacea* in Iceland differed from that of the continental sites ($F_{1,11}$ = 1.81, P < 0.002). The cover of vascular plant species was generally low. *Geranium robertianum* was the only plant species common to most sites of both cytotypes (present in all sites except at TET, WEN and in Iceland). The cover of bryophytes was generally also low except in one Icelandic population (R427) where *Racomitrium lanuginosum* was a dominant species. The median

number of species including bryophytes was 23.5 species per 5 m². In total 228 species of vascular plants and mosses were observed co–occurring with the two cytotypes (164 species with *S. rosacea* sites and 124 species with *S. sponhemica*).

TABLE2. Environmental conditions in Iceland and Central Europe populations of *Saxifraga rosacea* and in *Saxifraga sponhemica* populations.

	Saxifraga rosacea		*Saxifraga sponhemica*	*F*	
	Iceland	C–Europe	C–Europe		
Data from WorldClim					
Annual temperature (°C)	4.45 ^{A}	7.86 ^{B}	8.50 ^{B}	44.75	***
Max. temperature of warmest month (°C)	12.93 ^{A}	21.92 ^{B}	22.27 ^{B}	68.27	***
Min. temperature of coldest month (°C)	–2.35 ^{A}	–3.69 ^{A}	–2.97 ^{A}	2.19	
Precipitation (mm)	1048.00^{A}	846.11 ^{A}	792.78 ^{A}	1.74	
Data from local data loggers					
Annual temperature (°C)	4.35 ^{A}	8.43 ^{B}	10.28 ^{C}	44.89	***
Summer temperature (°C)	9.42 ^{A}	15.98 ^{B}	18.01 ^{B}	19.07	***
Maximum temperature (°C)	18.70 ^{A}	36.46 ^{B}	38.32 ^{B}	3.72	*
Minimum temperature (°C)	–4.91 ^{A}	–2.96 ^{A}	–4.77 ^{A}	0.82	
Growing degree days	514.87 ^{A}	1762.19 ^{B}	2272.74 ^{B}	28.84	***
Other characteristics					
Slope (°)	22.50 ^{A}	53.56 ^{B}	54.67 ^{B}	3.71	*
Altitude (m)	43.50 ^{A}	532.44 ^{B}	354.89 ^{B}	16.45	***
Canopy openness (%)	53.67 ^{A}	37.50 ^{A}	37.83 ^{A}	1.24	
SiO_2 (%)	52.53 ^{A}	17.92 ^{B}	40.22 ^{AB}	3.61	*
Al_2O_3 (%)	15.17 ^{A}	4.45 ^{B}	10.42 ^{AB}	4.69	*
Fe_2O_3 (%)	11.81 ^{A}	3.36 ^{B}	7.65 ^{AB}	3.85	*
CaO (%)	8.89 ^{A}	32.41 ^{A}	17.82 ^{A}	2.29	
Loss on ignition (%)	2.02 ^{A}	30.35 ^{B}	14.02 ^{AB}	4.28	*

Notes: F–test for differences between the three regions: *, $P < 0.05$; ***, $P < 0.001$. Mean values with different letters in the exponent are significantly different (Tukey test, $P < 0.05$).

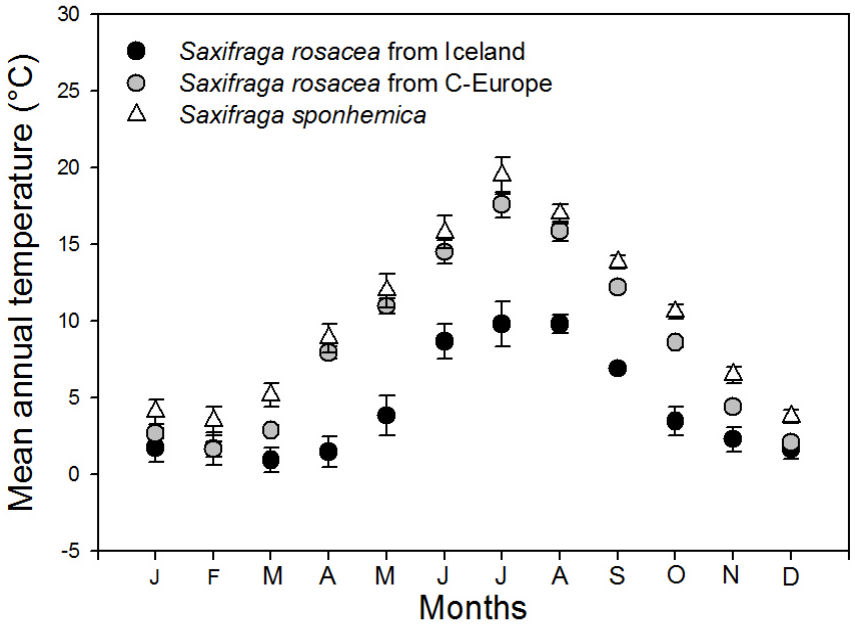

FIGURE 2. Variation in mean temperature per month among sites with *Saxifraga rosacea* in Iceland (*n* = 3), sites with *S. rosacea* in Central Europe (*n* = 8) and sites with *Saxifraga sponhemica* (*n* = 8) as measure by data loggers at the sites. Means ± 1SE.

Population structure—

Cushions in the Icelandic *S. rosacea* populations were very small (Table 3). The mean cushion area did not differ much between cytotypes of both subspecies in Central Europe, but was more than six times greater than in the Icelandic *S. rosacea* populations. In contrast, inflorescences of *S. rosacea* produced on average more flowers than those of *S. sponhemica* in the central European populations and also more than those of *S. rosacea* in Iceland. The population structure of the Icelandic populations did strongly differ from that of the Central European populations, because Icelandic populations had hardly any large cushions and a much higher proportion of medium–sized plants and more vegetative rosettes (Table 3).

Frost tolerance and response to environment at the transplant sites—

In the frost tolerance experiment, electrolyte leakage of the leaves of *S. rosacea* from Iceland was lower than that of the leaves from Central European populations of *S. rosacea* and *S. sponhemic*a ($F_{1,16}$ = 5.17, P = 0.037; Fig. 3), also at frost temperatures indicating higher frost tolerance. Electrolyte leakage of leaves of *S. sponhemica* from Central European populations was much higher at -20 ^{o}C than that of leaves of *S. rosacea* from the same region

($F_{2,32}$ = 3.19, P = 0.055; Fig. 3), indicating lower frost tolerance of the hexaploid *S. sponhemica*.

TABLE 3. Characteristics of Iceland and Central European populations of *Saxifraga rosacea* and populations of *Saxifraga sponhemica*.

	Saxifraga rosacea		*Saxifraga sponhemica*		
Population characteristic	Iceland	C–Europe	C–Europe	*F*	*P*–value
Cushion area (cm²)	5.9^{A}	46.2^{B}	39.6^{B}	17.2	<0.001
Mean number of inflorescences per cm^2 of cushion	0.14^{A}	0.04^{B}	0.06^{B}	11.4	<0.001
Mean number of flowers per inflorescence	1.80^{A}	4.48^{B}	3.11^{A}	12.5	<0.001
Proportion of single vegetative rosettes (%)	17.1^{A}	4.0^{B}	8.6^{A,B}	4.0	0.035
Proportion of medium cushions (%)	81.7^{A}	43.4^{B}	38.1^{B}	18.7	<0.001
Proportion of large cushions (%)	1.3^{A}	52.6^{B}	53.2^{B}	26.6	<0.001

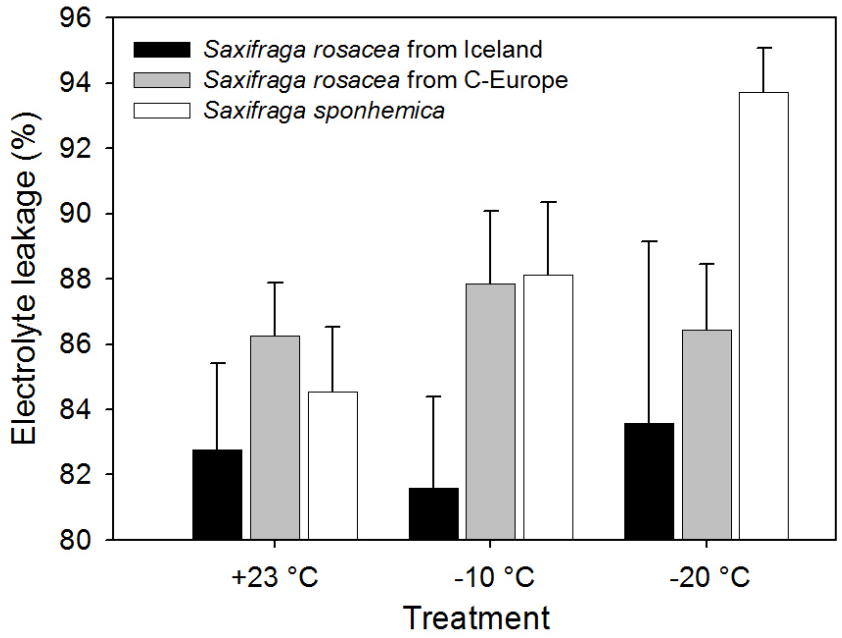

FIGURE 3. Mean (± 1SE) electrolyte leakage ratioat three temperatures for leaves of *Saxifraga rosacea* from Iceland and Central Europe and leaves of *Saxifraga sponhemica*.

Mean annual temperatures at the transplant sites were 2.5 °C in Iceland and 11.9 °C in Luxembourg. The sites strongly influenced all growth and reproductive characteristics. In 2015, 86% of the surviving plants flowered in Luxembourg but only 15% survived in Iceland. However, the cytotypes reacted differently to the conditions at the two transplant sites. Survival until 2015 in Luxembourg was much lower (zero) for populations of *S. rosacea* from Iceland than for Central European populations of both cytotypes, whereas in Iceland survival of the Iceland *S. rosacea* populations was higher than that of populations of both Central European cytotypes (Fig. 4a, Table 4), indicating local adaptation of the Iceland and Central European populations. However, octoploid and hexaploid populations from Central Europe did not differ in their response to the two environments.

Cushion diameter was much larger in Luxembourg (mean 2014: 8.3 cm) than in Iceland (mean 2014: 1.4 cm) after one year of growth (Table 5). Mean plant size did not differ between Central European populations of the two cytotypes and the two cytotypes did not react differently to the conditions at the sites. However, plants from Iceland populations were smaller than those from both cytotypes from Central Europe and this difference was particularly pronounced at the transplant site in Luxembourg (4.2 vs. 8.3 cm diameter), indicating local adaptation.

The Central European populations of the two cytotypes responded differently in their reproduction to the contrasting environments (Table 4). A higher proportion of the plants of the octoploid *S. rosacea* than of the hexaploid *S. sponhemica* flowered in Iceland in 2014, whereas more *S. sponhemica* plants flowered in Luxembourg (Fig. 4b). Reproduction as measured by the number of inflorescences produced by the flowering plants in 2014 was higher for the few surviving plants from the Iceland populations at the site in Luxembourg than for the Central European populations (Table 5, Fig. 4c). However, all the plants from Iceland in Luxembourg died until the next year. Central European plants of the octoploid *S. rosacea* produced more inflorescences under the cold condition in Iceland than plants of the hexaploid *S. sponhemica*, while the reproductive success of *S. sponhemica* was higher than that of the *S. rosacea* plants from the same region at the warmer site in Luxembourg, indicating contrasting responses of the two cytotypes to the two environments.

TABLE 4. Analyzes of deviance for the effects of the two transplant sites, the contrast between the Iceland populations of *S. rosacea* and the Central European populations of *S. rosacea* and *S. sponhemica*, the contrast between the Central European populations of *S. rosacea* and *S. sponhemica*, the population of origin, and genotype on survival from 2013 to 2014 and from 2013 to 2015, and flowering of the *Saxifraga* plants that survived until 2014.

	Survival					Flowering		
	2013 – 2014			2013 – 2015		2014		
	df	*F*		*F*		*df*	*F*	
Site	1	5.5	*	0.4		1	1.6	
Iceland vs. C-Europe populations	1	4.7	*	3.8	+	1	0.5	
C-Europe *S. rosacea* vs. *S. sponhemica*	1	0.5		2.1		1	1.9	
Population	17	2.0	*	1.2		17	1.5	
Genotype	80	1.3	+	1.2		71	1.1	
Site x (Iceland vs. C-Europe populations)	1	23.3	***	19.0	***	1	1.5	
Site x (C-Europe *S. rosacea* vs. *S. sponhemica*)	1	0.8		0.9		1	22.1	***
Site x population	17	1.1		2.5	**	15	1.8	+
Site x genotype	80	1.1		1.1		38	1.0	

Note: Quasi–*F* values are given. +, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

The various populations of the two cytotypes varied in their reaction to the two transplant sites (Fig. 5). Plants from all *S. rosacea* populations from Iceland survived better in Iceland than in Luxembourg, indicating local adaptation (Fig. 5a). Similarly, the five populations of *S. sponhemica* with high survival (> 60%) in Luxembourg had particularly low survival in Iceland. Genetic differences in survival were larger among populations (Icelandic and Central European populations together) of the octoploid *S. rosacea* than for the hexaploid *S. sponhemica* at both transplant sites. In summer 2018, when all plants had to be removed from the Iceland site to avoid the potential spread of non–native genotypes, only 9.5% of all plants had survived. Out of the 19 surviving plants, 16 plants belonged to the cytotype *S. rosacea* (14 Central European and two plants from Iceland). The three surviving *S. sponhemica* plants all originated from one Czech population (Voskov).

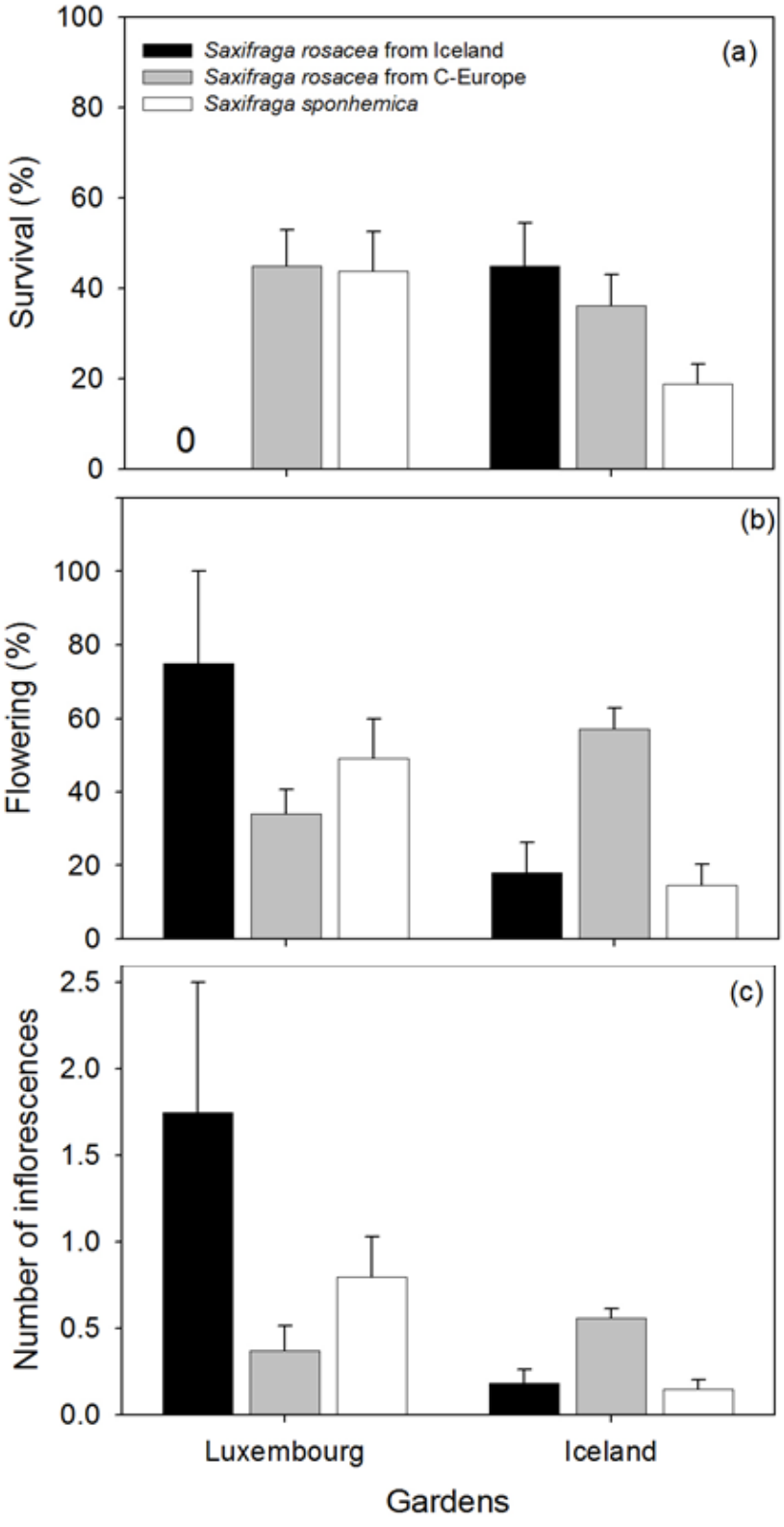

FIGURE 4. Fitness measures (mean ± 1SE) for *Saxifraga rosacea* from Iceland and Central Europe, and *S. sponhemica* at the transplant sites in Luxembourg and Iceland: (A) the probability of survival from 2013-2015, (B) the probability of flowering in 2014, and (C) the number of inflorescences per cushion in 2014.

In terms of flowering probability, nearly all populations of *S. sponhemica* flowered less in Iceland than in Luxembourg whereas both the Icelandic and Central European populations of *S. rosacea* strongly varied in their response to the two sites (Fig. 5b).

At the site in Luxembourg, variation in the growth of plants among the individual populations of both cytotypes from Central Europe was very large, whereas plants from Icelandic populations of *S. rosacea* were all very small (Fig. 5c), indicating low plasticity and an inability to profit from the warmer conditions at the Luxembourg site. In contrast, in Iceland, variation in the growth of the various populations of both cytotypes was rather low.

TABLE 5. Analyzes of variance for the effects of the two transplant sites, the contrast between the Iceland populations of *S. rosacea* and the Central European populations of *S. rosacea* and *S. sponhemica*, the contrast between the Central European populations of *S. rosacea* and *S. sponhemica*, the population of origin, and genotype on plant size and the number of inflorescences per cushion of the two *Saxifraga* subspecies in 2014.

Source of variation		Cushion diameter		Number of inflorescences	
	df	*F*		*F*	
Site	1	182.4	***	7.3	*
Iceland vs. C-Europe populations	1	3.0	+	0.1	
C-Europe *S. rosacea* vs. *S. sponhemica*	1	<0.1		<0.1	
Population	17	2.0	*	0.9	
Genotype	71	1.3		2.9	***
Site x (Iceland vs. C-Europe populations)	1	3.3	+	5.1	*
Site x (C-Europe *S. rosacea* vs. *S. sponhemica*)	1	0.6		18.6	***
Site x population	15	1.6		0.5	
Site x genotype	38	1.5	+	4.7	***

Note: +, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

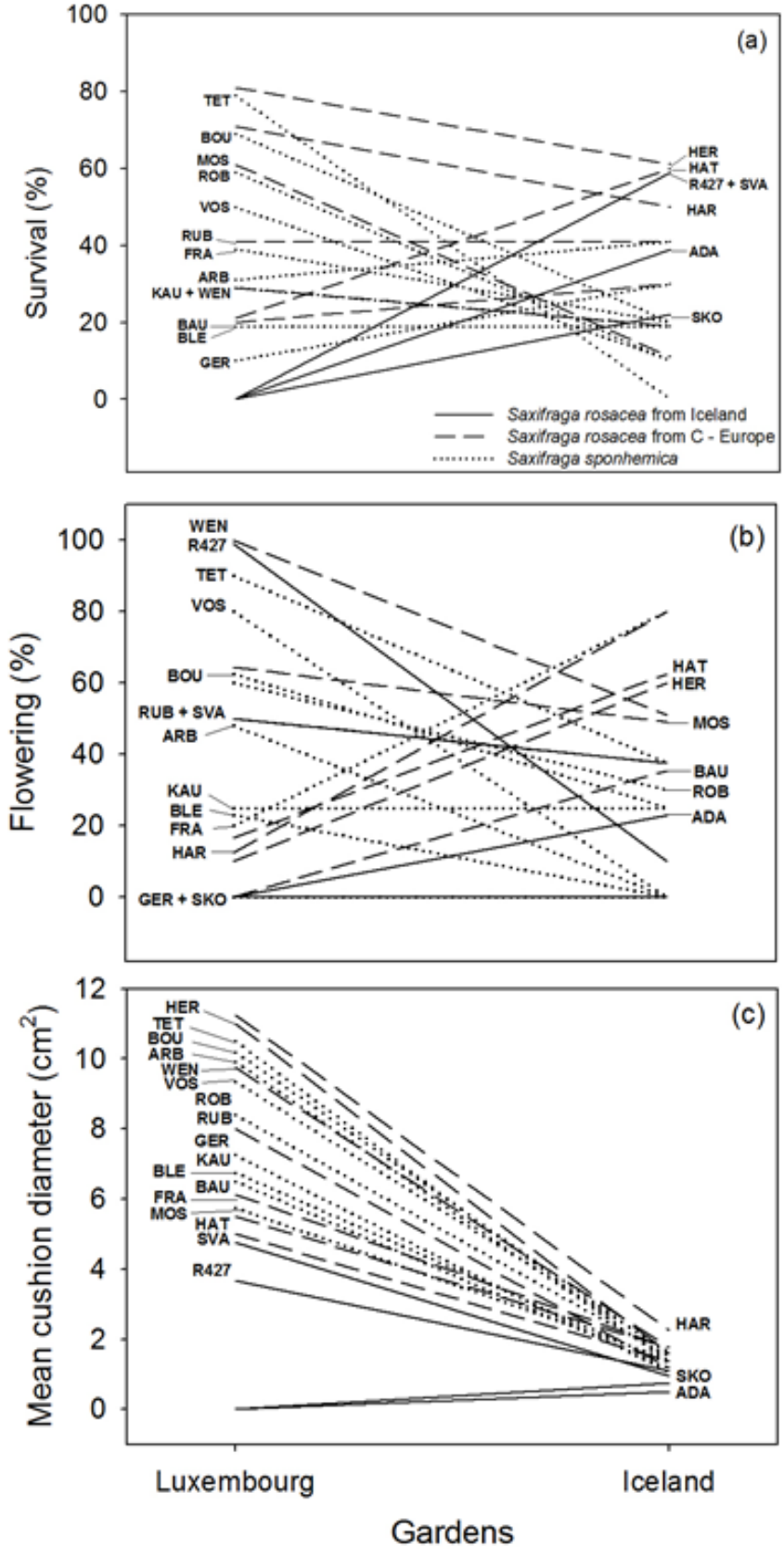

FIGURE 5. Reaction norm of four Icelanic and seven Central European *S. rosacea* populations, and nine *S. sponhemica* population for (A) the probability of survival from 2013-2015, (B) the proportion of flowering individuals in 2014, and (C) the diameter of the cushions in 2014 at two transplant sites.

Environmental niche modelling and niche overlap—

The estimated niche models using ten climatic variables had very high AUC scores for *S. sponhemica* (0.977 ± 0.013) and *S. rosacea* (mean 0.959 ± 0.013) populations, indicating negligible rates of false negative and false positive suitability predictions. Nearly all populations of *S. sponhemica* and *S. rosacea* were situated in areas with high predicted probabilities (Fig. 6a,b). Niche overlap between the two taxa was 56.6% (Schoener's D), which was significantly lower than expected by chance in the niche identity test (83.7% ± 1.9%, $P < 0.001$). In the final model for *S. sponhemica* mean temperature of the coldest month had the highest importance value (56.5), whereas temperature annual range had the highest contribution (49.4%) to the model for populations of *S. rosacea* (Appendix 2).

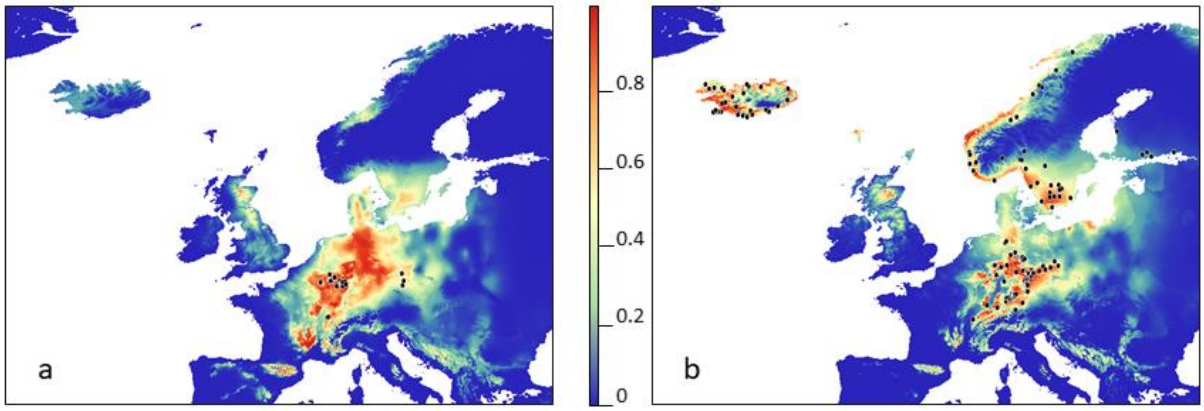

FIGURE 6. Probability of occurrence of (A) *Saxifraga sponhemica* and (B) *Saxifraga rosacea* according to Maxent niche models based on 10 climatic variables (see text details). Black dots represent the populations used for the niche models (see Appendix S1). Blue indicates unsuitable habitats, and red indicaes highly suitable habitats.

DISCUSSION

The results of our study confirmed that both morphologically defined taxa have different ploidy levels (all *S. sponhemica:* 6x = 48; all *S. rosacea:* 8x = 64). Our chromosome numbers for *S. rosacea* are in agreement with those reported previously for the taxon by Philps (1934), and Catcheside and Heslop–Harrison (reported in Webb, 1950) for material from Ireland. The 48 chromosomes found for the subspecies *sponhemica* are within the range (46 – 52) reported in a study by Drábková (2000) using material from Czechia. In contrast to most other studies of species with different ploidy levels (Kolář et al., 2015) we found no

mixed cytotype populations but complete spatial segregation of the two cytotypes.

There are at least three non–exclusive explanations for the complete spatial segregation of the cytotypes of *Saxifraga*. Minority cytotypes may become quickly excluded in mixed populations (Levin, 1975), cytotypes may have different ecological requirements (Ramsey 2011; Kolář et al., 2015; McAllister et al., 2015; Muñoz–Pajares et al., 2018), and historical factors like differences in recolonisation history and stochastic events may have shaped the distribution of the cytotypes (Sexton et al., 2009).

The minority cytotype exclusion hypothesis (Levin, 1975) predicts that if a different cytotype invades an established population of plants of another cytotype the reproductive success will be frequency–dependent and the minority cytotype will be rapidly excluded from the population. In *Chamerion angustifolium*, Husband and Schemske (2000) found clear evidence that minority cytotype exclusion can operate in natural populations and the reproductive costs facing the minority cytotype plants may also explain the spatial segregation in *Ranunculus adoneus* (Baack, 2005). In the case of our 6x and 8x *Saxifraga* cytotypes exclusion of the minority cytotype due to infertility of hybrid heptaploid progeny resulting from crosses between hexaploid *S. sponhemica* and octoploid *S. rosacea* might also have contributed to the spatial segregation of the two cytotypes in Central Europe.

However, several lines of evidence suggest that niche differentiation may be the main factor responsible for the spatial segregation. The WorldClim data as well as the data from our local data loggers and the performance of the two cytotypes at the transplant site in Iceland indicate that populations of *S. rosacea* can exist at much lower mean annual temperatures than those of *S. sponhemica*. The lower frost tolerance of *S. sponhemica* was corroborated by the results of the freezing experiment, in which the tolerance to severe frost of the hexaploid *S. sponhemica* from Central Europe was much lower that that of the octoploid *S. rosacea* from the same region. Conversely, reproductive performance of the populations of *S. sponhemica* in the warmer climate of the transplant site in Luxembourg was much higher than that of *S. rosacea* from the same region and mean annual temperatures measured by data loggers placed in the Central European populations were higher in populations of *S. sponhemica* than in those of *S. rosacea.* There was some evidence of adaptation to regional conditions in *S. rosacea.* The subarctic populations of *S. rosacea* survived slightly better in Iceland than the plants from Central Europe, but were clearly maladapted to the warmer conditions in Luxembourg, where no plants survived over two years.

Other studies that investigated differences in environmental characteristics between populations of different cytotypes also found differences in climate (Nakagawa, 2006; Li et al., 2010; Manzaneda et al., 2012; Thompson et al., 2014; Muñoz–Pajares et al., 2018), but this is not a general pattern (Godsoe et al., 2013; Glennon et al., 2014). Habitats of cytotypes may also differ in soil characteristics (Černá and Münzbergová, 2015) or be segregated by altitude (Husband and Schemske, 2000; Šafářová, 2011).

In contrast to the many studies that have investigated correlations between the distribution of different cytotypes and environmental conditions, few studies have used experiments to investigate ecological differences between cytotypes although it has been stressed that niche differentiation between cytotypes should be ideally studied using reciprocal transplant experiments (Ramsey 2011; Kolář et al., 2017). Reciprocal transplant experiments with diploid and tetraploid *Anthoxanthum* species revealed local adaptation of the different cytotypes (Flegrová and Krahulec, 1999). Similarly, a study of *Chamerion angustifolium* cytotypes found that diploids and tetraploids survived best at their native elevations (Martin and Husband, 2013) and McIntyre and Strauss (2017) found evidence for local adaptation of cytotypes in *Claytonia perfoliata*. In contrast, other reciprocal transplant experiments failed to find clear evidence of local adaptation of cytotypes. Diploid and polyploid populations of *Mercurialis annua* were ecologically differentiated, but did not show local adaptation (Buggs and Pannell, 2007) and there was only limited support for the existence of local adaptation within cytotypes of tetraploid, pentaploid and hexaploid *Alllium oleraceum* (Duchoslav et al., 2017). For the *Senecio carniolicus* complex it has been suggested that there may be no clear–cut answer to the question if the resident or the higher ploidy species shows superior performance, but rather that relative performance may depend on life–history stage (Hülber et al., 2018; see also Raabová et al., 2008). Our experimental planting of rosettes from many populations of the two cytotypes of *Saxifraga* into two transplant sites with contrasting climates was not a reciprocal transplant experiment in the strict sense because the plants did not occur naturally at exactly those sites, but the results nevertheless suggest greater adaptation of *S. rosacea* to cold conditions and of *S. sponhemica* to warmer conditions.

In spite of the overall clear geographical separation, niche modelling suggested that both cytotypes of *Saxifraga* could co–occur in many parts of Central Europe and indeed there is partial geographic sympatry (s. Fig. 1). Moreover, we found no differentiation in terms of accompanying vegetation or substrate composition between the two taxa in Central Europe. Both are rare species restricted to rock habitats where there is hardly any competition from

other species. However, there are no contact zones between the cytotypes in central Europe. A possible reason are subtle differences in the climatic niches and the microhabitats which are not reflected in the larger scale climatic data (WordClim and niche modelling), but are reflected in the higher mean annual temperatures measured locally with the data loggers in the Central European populations of *S. sponhemica* than in those of *S. rosacea*. Furthermore, historical factors may have affected the distributions. Both taxa are considered to be ice–age relicts which today are rare and restricted to isolated rock habitats, but may have been much more common during the ice–age (Thorn, 1960; Walter and Straka, 1970). There could have been a large random component in the extinction of populations due to the invasion of trees after the ice age contributing to the current pattern of distribution in central Europe. Its greater frost tolerance may have allowed *S. rosacea* to colonize habitats in Scandinavia once they became available, while this appears not to have been possible for *S. sponhemica*.

Several authors have advanced the hypothesis that higher ploidy levels may be associated with a greater tolerance of extreme conditions and a greater niche breadth (e.g. Grant, 1981; Soltis et al., 2014), but results of studies of the relationship between niche breadth and ploidy level have been conflicting. In the genus *Clarkia* polyploid species have significantly larger ranges compared with diploid species (Lowry and Lester, 2006) and in *Claytonia perfoliata* polyploids occupy distinct and broader niches relative to diploids (McIntyre, 2012). In contrast, no differences in mean range breadth were observed between diploid and polyploid congeners in a sample from diploid and polyploid species of North American angiosperms (Martin and Husband, 2009). In a study of the genus *Phalaris* there was no general support for broader niche breadths of polyploids (Visser and Molofsky, 2015), and in *Primula* climatic niches of polyploid species were narrower than those of diploid species (Theodoridis et al., 2013). In the Potentilleae tribe of the Rosaceae transitions to higher ploidy are actually associated with reduced range size and abiotic breadth (Brittingham et al., 2018).

Our results support the hypothesis of increasing niche breadth with ploidy level as they suggest that the greater hardiness of the octoploid *S. rosacea* may explain why its range extends much further to the north than that of the hexaploid *S. sponhemica*. The distribution of the closely related decaploid *S. cespitosa* (10 x = 80) extends even further to the north (up to 80 °N). The three taxa may thus be an example of increasingly greater genetic flexibility to cope with the harsher conditions in the subarctic and arctic environments with increasing ploidy level (Brochmann et al., 2004; Rice et al., 2019). Tolerance of a large range of environmental conditions and a greater distributional range may be due to genetic

differentiation among populations or due to phenotypic plasticity. Genetic differences in survival among populations were larger for the octoploid *S. rosacea* than for the hexaploid *S. sponhemica* at both transplant sites suggesting that greater genetic variability contributes to the larger distributional range of *S. rosacea*. The considerable variation found in the response of the different populations of the two cytotypes of *Saxifraga* to environmental conditions suggests that studies should not only consider the overall niche differentiation among cytotypes, but also genetic variation among populations within cytotypes.

In Central Europe where both cytotypes of *Saxifraga* occur under broadly similar conditions there were no differences in mean population characteristics except for a higher number of flowers per inflorescence in *S. rosacea*, which was, however, compensated by a lower number of inflorescences per cushion area. In contrast, plants in the Iceland populations were much smaller and there were hardly any large plants. The very small size of adult *S. rosacea* plants in Iceland may indicate that this taxon is at its northern range limit in the subarctic. However, plants from Iceland also grew much slower than those from other populations when grown in Central Europe, were less plastic and did not survive until the end of the experiment, suggesting adaptation to the subarctic conditions.

CONCLUSIONS

Our results suggest that the different geographical distribution of the octoploid *S. rosacea* and the hexaploid *S. sponhemica* can at least be partially explained by the greater cold hardiness of *S. rosacea*. In the absence of strong habitat differences in Central Europe, reproductive isolation could explain why both taxa are not sympatric in Central Europe and why there are no known mixed populations where both taxa occur. In order to clarify the taxonomic status of the two *Saxifraga rosacea* subspecies further molecular genetic studies and crossing experiments will be necessary. Our results support the hypothesis that different cytotypes may have different niches which lead to spatial segregation, and that higher ploidy levels may result in a broader ecological niche and in particular greater tolerance of more extreme conditions.

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CHAPTER 3

Population dynamics and extinction risks of two closely related plant taxa of *Saxifraga rosacea*

ABSTRACT

The study of population dynamics of closely related plant taxa under different environmental conditions may identify important life history traits and provide the basis for appropriate management schemes. We studied the dynamics and the viability of five populations of *Saxifraga rosacea* subsp. *rosacea* and of four of *Saxifraga rosacea* subsp. *sponhemica* occurring in similar habitats over four years at the rosette (ramet) and cushion (genet) level. Finite rate of increase based on mean transition matrices indicated that most populations will decline ($\lambda < 1$), if current conditions continue, but this trend was more pronounced for *S. sponhemica*. Life-table response experiment analysis revealed that the low rate of increase of *S. sponhemica* populations was mainly due to low growth transitions, low fecundity and the stasis of small and medium-size plants. Elasticity analyses indicated that the survival of adults contributed to a large part to population growth rate of both long-lived *Saxifraga* but this phenomenon was more important in populations of *S. sponhemica*. The contribution of adults to sexual reproduction was low for both taxa, but higher in *S. rosacea* populations.

Our results indicated also a possible trade-off between survival and reproduction. In the case of *S. sponhemica*, simulations suggest that management measures should favor the recruitment of young plants by creating new microsites through small disturbances. Population growth rates of both *Saxifraga* strongly decreased with increasing maximum temperatures at the sites. Projections of the quasi-extinction probability indicated an unfavorable future for populations of the two *Saxifraga* taxa under current environmental conditions. This negative trend was stronger for *S. sponhemica*, suggesting differences in the future prospects of *S. rosacea* and *S. sponhemica* populations. The two taxa will therefore require specific conservation and management approaches under the scenario of climate change.

KEY WORDS: *Saxifraga rosacea* subspecies, demography, population growth rate, LTRE analysis, population viability.

INTRODUCTION

Understanding the population dynamics of rare and endangered plant species can be very helpful for planning effective management programs for them (Beissinger & Westphal 1998, Reed et al. 2001, Menges 2000). Knowing the demography of a species enhances the

understanding of which factors may be important for the success of conservation plans. This statement is particularly relevant in the context of increasing habitat fragmentation, as small and isolated populations of rare and endangered plant species are more sensitive to stochastic factors, changing environmental conditions and are more subject to genetic drift (Lienert 2004, Aguilar et al 2008, Frankham et al. 2017). Demographic studies may allow to assess the viability of populations in space and time (Lande 1988, Beissinger et Westphal 1998, Oostermeijer et al. 2003), and an increasing number of demographic studies shows the importance of this approach for plant population management, using different structured and unstructured demographic modeling techniques (Jongejans et al. 2008a, Crone et al. 2011). Demographic studies often use analyses based on stage-structured matrix models as can be seen by the development of the plant demography database COMPADRE (Salguero-Gómez et al. 2015) initiated by Jonathan Silvertown and Miguel Franco (Silvertown et al. 1992, 1993, 1996, Franco and Silvertown 1996, 2004). The widespread use of matrix models in plant demography is also reflected by the extensive literature about demographic models (Cochran & Ellner 1992, Tuljapurkar & Caswell 1997, Groom & Pascual 1998, Caswell 2001) and the development of analytical tools for carrying out comparative demographic studies based on stage-structured matrix models, in which elasticities of demographic rates are analyzed in *G-L-F* triangles (Silvertown et al. 1992). The importance of external factors that affect specific transitions of the life cycle can be studied by Life Table Response Experiment analysis (LTRE, Caswell 1989). These techniques evaluate similarities and differences in the demography of different species in the same habitat (Münzbergová 2013, Černá & Münzbergová 2013) or populations of the same species in different habitats (Colling & Matthies 2006, Kabiel et al., 2016).

By integrating stochasticity into the modelling of plant demography it becomes possible to estimate extinction risks, to determine important stages in the life cycle and to evaluate the robustness level of population dynamics (Menges 1998, 2000, Higgins et al. 2000). The long-term population viability of a population is assessed by simulating the extinction probability taking into account stochastic demographic and environmental variation (Beissinger et al. 1998, Dinnétz et al. 2002, Kaye et al. 2003, Colling et al. 2006, Schleuning et al. 2007). As environmental stochasticity is considered to be the main stochastic factor of influence (Menges 1992, 1998), including environmental variation in the simulations allows also to determine which transitions are most sensitive to disturbances (Henle et al. 2004, Lehtilä et al. 2006). Demographic characteristics like the longevity of a species, its dispersal ability, the form of seed bank may have important consequences on the ability of a species to

adapt to changing climatic conditions in a time of rapid climate change (García & Zamora 2003, Morris et al. 2008, Erhlén & Morris 2015).

Few studies have investigated the population dynamics of two closely related taxa by a comparative demographic study and concerned mostly comparisons between diploids and tetraploids (Bucharová et al. 2010, de Groot et al. 2012, Černá & Münzbergová 2013). We studied the demography of two closely related endangered plant taxa, the hexaploid Central European endemic *Saxifraga rosacea* ssp. *sponhemica* and the more widely distributed octoploid *S. rosacea* ssp. *rosacea* to provide a base for assessing the risk of extinction of populations and possible conservation measures. The two cytotypes have similar ecological niches but the octoploid was found to be more frost tolerant (Decanter et al., 2020). We determined the fate of plants in several populations of both taxa over several years and analysed their demography using matrix models. The aims of the study were: (1) to compare the demography of the two closely related taxa, (2) to determine which transitions in their life cycle have the greatest influence on their population growth rate and may be critical for population persistence, and (3) to carry out a demography-based population viability analysis for several populations of each taxon.

MATERIALS & METHODS

Study species and selected sites—

Saxifraga rosacea Moench is a long-lived perennial plant with a fragmented distribution in Europe extending from France to Norway and from Iceland to S-Poland with some populations on the west coast of Great Britain and Ireland and in the Faroe Islands. Throughout its distribution three subspecies have been described (Webb and Gornall, 1989), but we studied only the two closely related *Saxifraga rosacea* subsp. *rosacea* and *Saxifraga rosacea* subsp. *sponhemica*, as the third taxon *Saxifraga rosacea* subsp. *hartii* (D. A. Webb) D. A. Webb is only known from scarce populations on Arranmore Island in Ireland (Chater, 1987; Webb and Gornall, 1989). The two studied taxa are referred to in the following as *S. rosacea* and *S. sponhemica*.

Both *S. rosacea* and *S. sponhemica* occur on rocky substrates such as stony slopes, screes and stone walls (Webb and Gornall, 1989). These types of habitat are naturally isolated and fragmented and considered to be in decline in Europe (Rocky Habitat, Habitats Directive - Article 17). The subspecies *sponhemica* is considered to be extremely rare or critically endangered (Korneck *et al*., 1996; Holub and Procházka, 2000; Colling, 2005; Mirek *et al*.,

2006) whereas *S. rosacea* is more widespread (Jalas et al., 1999). Both taxa grow as cushions (genets) consisting of 1 - 600 rosettes (ramets), interconnected by stolons. Plants reproduce sexually by developing cymose inflorescences mainly from the apex of rosettes, rarely from lateral buds at the rosette base (pers. obs.) from April to June in Central Europe, and until August in northern populations. A wide range of insects pollinate the white protandrous flowers and the very small seeds of the capsule-type fruits are easily dispersed (Webb and Gornall, 1989). *S. sponhemica* can also propagate itself clonally via detached rosettes (Walisch et al. 2015) and can expand further on the substrate via stolons (Tutin et al. 1968).

We selected four populations of *S. sponhemica* for the study, one in the Czech Republic, two in the Ardennes region (Belgium and Luxembourg) and one in the Hunsrück region of Germany. We selected five populations of *S. rosacea,* one in the French Jura, one in the Vosges mountains of France, one in E-Germany and two in S-Germany (Table 1).

TABLE 1. Location of the nine studied populations of *Saxifraga rosacea* and *Saxifraga sponhemica*.

Species	Region	Population	Abbrev.	Lat.	Long.
Saxifraga rosacea* spp. *sponhemica					
	České Středohoří	Blešno	BLE	50.46	14.15
	Ardennes	Robertville	ROB	50.45	6.10
	Oesling	Kautenbach	KAU	49.95	6.02
	Hunsrück	Gerolstein	GER	50.20	6.63
Saxifraga rosacea* spp. *rosacea					
	Jura	Baume-les-messieurs	BAU	46.69	5.64
	Vosges	Hartmannswillerkopf	HAR	47.86	7.17
	East Germany	Moschwitz	MOS	51.76	10.85
	South Germany	Rübeland	RUB	48.71	10.02
		Wentalwieble	WENW	50.54	12.17

Field methods—

In early summer 2012 we established in each study population one permanent transect 10 m long and 4 m wide and monitored 30 to 80 randomly selected cushions per population, from 2012 to 2015. The selected plants were permanently marked with a thin metal wire sealed by a numbered metallic stamp and the local spatial coordinates of each plant were recorded. When a genet was too small (< 0.5 cm) to support the wire, a paint dot was sprayed on the rock at the base of the cushion. In three populations we had to establish 2-4 smaller sub-transects (maximum distance 10 m) instead of one large transect for reasons of accessibility to the steep rock slopes. At the genet level, we recorded each year the size of each marked plant (number of rosettes) and the number of flowering stems.

If a plant disappeared, we selected a new genet of the same size category and marked it with a new number. We randomly selected one rosette within each of the marked genets for the demographic study at the rosette (ramet) level. The selected rosettes were marked with small differently coloured plastic strings. Each year we recorded the size of the main rosette (diameter), the number of secondary rosettes connected to the main rosette, the total number of stems and flowers of each rosette.

Due to the crumbling rocky substrate at many sites, new plants appearing in the study transects could also have been detached rosettes from disturbed adult individuals. Therefore from 2013 to 2015, only plants with a diameter of less than 0.5 cm were considered as yearlings originating from seeds and recorded as such.

From 2012 to 2015, we recorded local temperature four times a day (at 6 a.m., 12 a.m., 6 p.m. and 12 p.m.) in all populations except ROB with data loggers (Tiny tag Transit 2 TG–4080, Gemini Data Loggers®, U.K) placed at the soil/rock level avoiding direct exposition to the sun.

Analytical methods—

Defining demographic stages, estimation of fecundities and construction of transition matrices

We constructed transition matrices based on the size of the plants to investigate the pattern of recruitment, growth and mortality in each population. We defined mortality as the disappearance of marked plants during the study.

At the genet level, we distinguished four size classes based on the number of rosettes: yearling (diameter < 0.5 cm, one rosette, not flowering), small (diameter > 0.5 cm, 1 - 10 rosettes), medium (11-50 rosettes) and large (> 50 rosettes) (Fig. 1). Plants of all size classes except yearlings flowered. No seed stage was included in this study in order not to introduce a delay of one year in the process of reproduction (Caswell 2001).

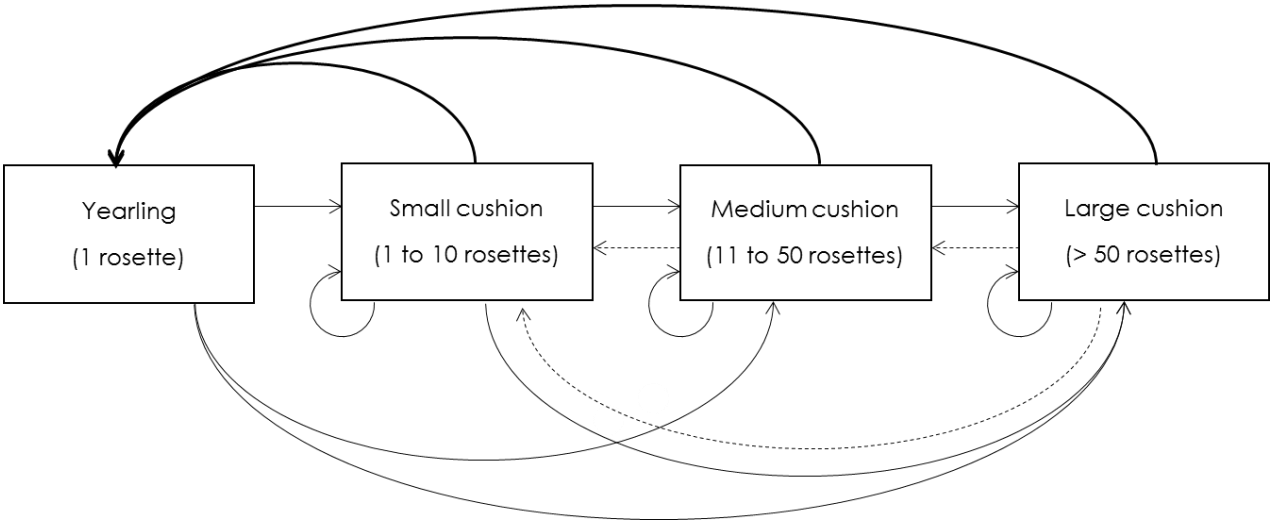


FIGURE 1. Life-cycle diagram for both *Saxifraga rosacea* at the genet level

To estimate fecundity transitions, we calculated the average number of flowers per plant in each size class and calculated the contribution of each stage to the production of flowers. We then partitioned the number of yearlings (newly developed plants) among the genet size classes according to its contribution to the total number of flowers. Because it was not possible to distinguish yearlings in the first year of study from older plants, all non-flowering plants of less than 0.5 cm diameter were considered as yearlings. In the following years, only newly recruited plants were recorded as yearlings.

We constructed separate transition matrices for each population for each of the three transitions resulting in 27 matrices. To compare the demography of the two taxa we calculated a mean transition matrix per taxon over all populations for each transition by calculating the arithmetic means of the corresponding elements in the matrices. We also calculated standard deviations. Due to severe anthropogenic disturbances at two *S. rosacea* sites (Baume-les-Messieurs [BAU] and Hartmannswillerkopf [HAR]) during the study period, a third mean transition matrix was calculated for *S. rosacea* excluding the disturbed populations for the transitions that were affected by disturbance.

Population growth rate and LTRE

To evaluate the overall performance of the studied populations, we calculated their finite rates of increase λ for each transition and their coefficients of variation through time. To evaluate the contribution of each matrix element to λ, we calculated elasticities for the mean transition matrices per taxon of the pooled data for all study years (De Kroon et al., 2000; Caswell 2001). Following Silvertown *et al.* (1993), composite elasticities representing growth

(*G*, advance in stage), survival (*L*, stasis or regression in stage) and fertility (*F*, sexual reproduction) were calculated between 2012 and 2015, to evaluate the relative contribution of each stage to the finite rate of increase, λ. The effects of recorded climatic conditions on geometric means of the finite rate of increase were studied by regression.

Life-table response experiment (LTRE) were used to analyze which transitions contributed most to differences in growth rates between the two taxa (Caswell 2001). We first calculated a mean matrix per taxon per transition and then calculated overall mean matrices over the three transitions; one for *S. sponhemica*, one for *S. rosacea*, and one for *S. rosacea* excluding the transitions of populations that were subject to disturbance. We chose the stage matrix of *S. sponhemica* as reference to which we compared the matrix of *S. rosacea.*

Rosette survival and flowering

We studied the effects of plant taxon, genet and rosette size, and sexual reproduction on the survival and flowering of rosettes between 2012 and 2013 by analyses of deviance with a logit link and binomial errors (Table 2).

TABLE 2. Skeleton of the binary logistic linear model for the survival and flowering probabilities of a rosette of the two *Saxifraga rosacea*.

Source of variation	d.f.	Error term
Taxon	1	Population
Population	7	Residuals
Genet size	1	Residuals
Ramet size	1	Residuals
No. of flowers	1	Residuals
Taxon x genet size	1	Population x genet size
Taxon x ramet size	1	Population x ramet size
Taxon x no. of flowers	1	Population x no. of flowers
Population x genet size	7	Residuals
Population x ramet size	7	Residuals
Population x no. of flowers	7	Residuals
Residuals	304-518	

Stochastic simulation of population growth and extinction risks.

To integrate environmental stochasticity, we calculated stochastic growth rates by matrix sampling (Morris & Doak 2002). For the matrix-sampling method, population growth for each *Saxifraga* taxon was projected over 50 000 time intervals using at each time step one of the pooled matrices (pooled over populations) selected at random with equal probability.

The stochastic log growth rate was calculated as the arithmetic mean of all pairs of $\log(N_{(t+1)}/N_{(t)})$.

To determine the extinction risk of the two *Saxifraga* taxa over the pooled populations per taxon, we investigated the probability of quasi-extinction in a stochastic environment. We used the same matrices as for the log stochastic growth rate calculation and projected the model over 120 years for the two *Saxifraga* subspecies, with 1000 iterations. The threshold value for quasi-extinction was set to 10% of the average number of individuals in the nine populations studied, i.e. 10. As initial population vector we used the pooled number of individuals per size category over all populations per taxon in the first study year. All simulations were calculated with the R Popbio package (Stubben & Milligan 2007, version 2.7) using R version 4.1.0 (R Core Team, 2021).

RESULTS

Temporal and spatial variation—

Both taxa showed similar demographic patterns over time. The finite rate of increase of the *S. rosacea* and *S. sponhemica* populations varied over time and space for the three transitions (Table 3). However, for most transitions of the populations λ was smaller than 1, and only in two populations was the mean population growth rate larger than 1 (BAU and KAU) over the whole study period.

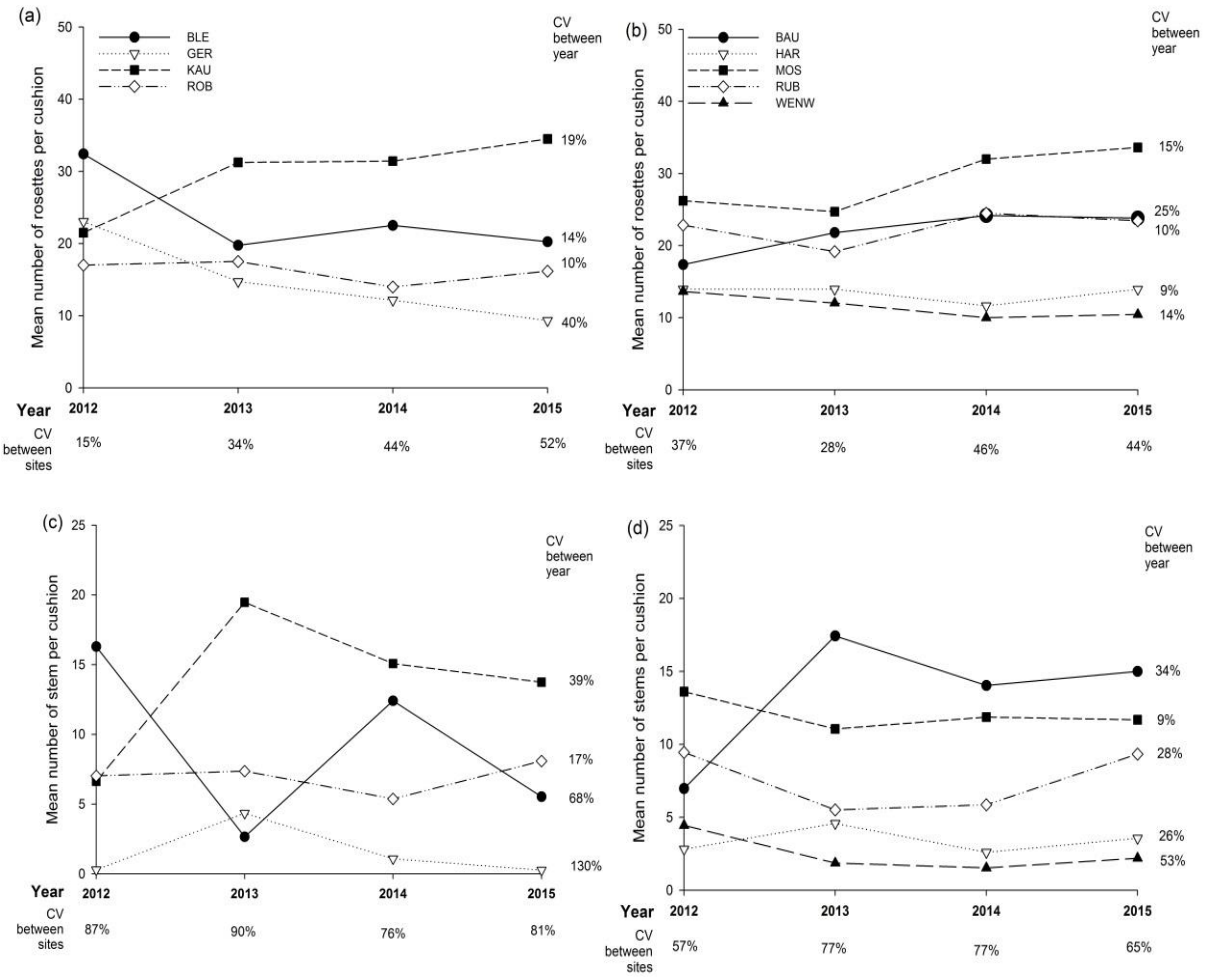
Two populations of *Saxifraga rosacea* were strongly affected by direct habitat destruction during the study period due to the construction of safety installations permitting access to the sites for the general public (Table 3). Consequently, the HAR site showed a sharp decrease in the finite rate of increase for transition 2013-2014 ($\lambda = 0.375$), whereas the BAU site suffered a strong decrease in transition 2014-2015 ($\lambda = 0.693$). Among the populations of *S. sponhemica*, strong decreases of the growth rate appeared also in GER and ROB populations (Table 3).

Both taxa showed similar patterns in their spatial and temporal variation of plant size expressed as mean number of rosettes per plant per population and reproduction (mean number of flowering stems per cushion per population) (Fig. 2). Plant size fluctuated slightly over the years except for one population of *S. sponhemica* (GER), where genet size decreased continuously (Fig. 2 a, b). The variation in the mean number of stems per cushion over time was much higher suggesting that plant reproduction might be more influenced by environmental stochasticity than plant growth (Fig.2 c, d). Plant size and the mean number of stems per cushion varied considerably among populations and were asynchronous.

TABLE 3. Finite rate of increase of *Saxifraga rosacea* and *Saxifraga sponhemica* populations in different years. λ and CV are based on the matrices of transitions. The transitions that were disturbed are marked with an asterisk. All transitions were included in the mean matrix calculation.

	Saxifraga rosacea populations					*Saxifraga sponhemica* populations			
Transition	BAU	HAR	MOS	RUB	WENW	BLE	GER	KAU	ROB
2012 → 2013	1.108	0.922	1.039	0.956	1.000	0.849	0.859	1.174	1.067
2013 → 2014	1.035	0.375*	1.063	0.896	0.861	0.895	0.915	1.011	0.799
2014 → 2015	0.693*	0.591	0.888	0.930	1.139	0.896	0.656	1.048	0.810
CV (%)	23.43	43.73	9.49	3.25	13.90	3.01	16.83	7.96	17.03
Mean matrix 2012 → 2015	1.001	0.690	0.990	0.936	0.952	0.864	0.816	1.092	0.889

FIGURE 2. Temporal (among years) and spatial (among populations) variation in the mean number of rosettes per cushion and in the mean number of stems per cushion in *Saxifraga sponhemica* (a, c) and *Saxifraga rosacea* (b, d).



Influence of rosette size and reproduction on survival and flowering—

There were no significant differences in rosette survival between the two taxa (Table 4). Flowering in the previous year reduced rosette survival indicating a trade-off between rosette survival and reproduction (Table 4). This effect differed slightly between taxa (species*flowering interaction). Flowering probability increased significantly with rosette size in the previous year (Table 4), while genet size had only a marginally significant effect. This suggests that in these cushion plants, resources for flowering are allocated at the individual rosette level independent of genet size.

TABLE 4. Analyses of deviance of the effects of species, population, genet size, ramet size and number of flowers the previous year on the survival and the flowering probabilities of a rosette of the two *Saxifraga* taxa studied in 2013.

Source of variation	Deviance change	d.f.	Mean deviance	Quasi-*F*	
Survival probability					
Taxon	0.11	1	0.11	0.03	
Population	30.37	7	4.34	3.40	***
Genet size	0.95	1	0.95	0.74	
Ramet size	0.94	1	0.94	0.74	
No. of flowers $_{(t-1)}$	9.33	1	9.33	7.30	***
Taxon*Genet size	0.06	1	0.06	0.02	
Taxon*Ramet size	0.59	1	0.59	0.62	
Taxon* No. of flowers $_{(t-1)}$	3.95	1	3.95	5.20	+
Population*Genet size	18.76	7	2.68	2.10	*
Population*Ramet size	6.71	7	0.96	0.75	
Population* No. of flowers $_{(t-1)}$	5.32	7	0.76	0.59	
Residual	662.02	**518**	1.28		
Flowering probability					
Taxon	0.03	1	0.025	0.010	
Population	17.46	7	2.494	2.050	*
Genet size	3.33	1	3.325	2.734	+
Ramet size	21.75	1	21.748	17.880	***
No. of flowers $_{(t-1)}$	3.44	1	3.435	2.824	+
Taxon*Genet size	0.06	1	0.061	0.060	
Taxon*Ramet size	2.93	1	2.932	0.856	
Taxon* No. of flowers $_{(t-1)}$	6.15	1	6.153	3.878	+
Population*Genet size	7.10	7	1.014	0.834	
Population*Ramet size	23.97	7	3.425	2.815	***
Population* No. of flowers $_{(t-1)}$	11.11	7	1.587	1.304	
Residual	369.77	**304**	1.216	0.010	

Note: +, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Comparative demography of S. rosacea and S. sponhemica—

The two *Saxifraga* taxa had very similar size class distributions with a dominance of small and medium-sized cushions (78.7% in *S. rosacea* populations and 77.1% in *S. sponhemica* populations) and low proportions of yearlings and large cushions (Fig. 3). The populations may thus be classified as 'stable' in contrast to 'dynamic' or 'aged' populations where either young or old plants dominate.

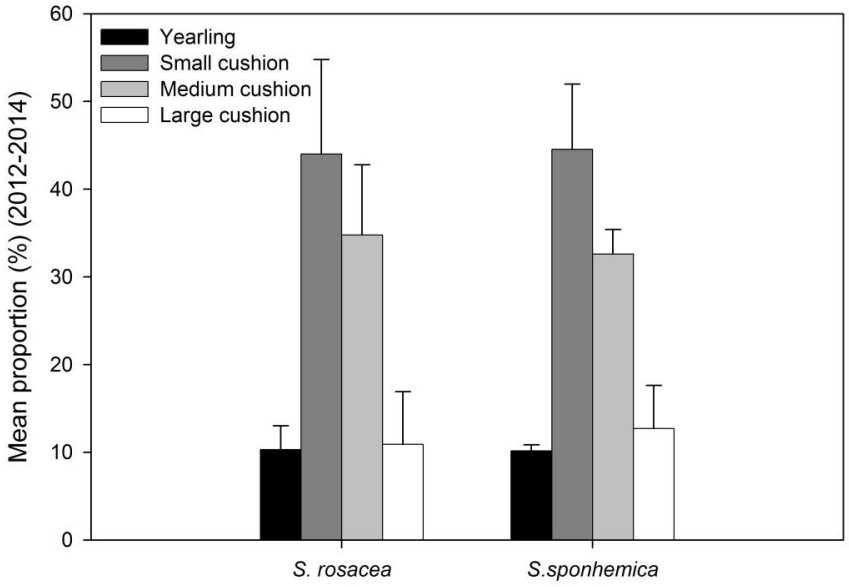


FIGURE 3. Mean proportion of plants 2012 - 2014 in the different size classes for populations of *S. rosacea* and *S. sponhemica*. Vertical bars indicate 1 standard error of mean.

For all years combined, *S. sponhemica* had a slightly lower finite rate of increase (0.9181) than *S. rosacea* (0.9462). When the transitions of the populations HAR and BAU that were affected by disturbance were excluded from the analysis, the difference in the mean finite rate of increase between the two taxa was even more pronounced (0.9181 vs 1.0137) (Table 5).

TABLE 5. Finite rate of increase of the populations of *Saxifraga rosacea* (with and without disturbed populations) and *Saxifraga sponhemica* for the study period (2012-2015).

	S.rosacea (all populations)	*S. rosacea* (without disturbed populations)	*S. sponhemica*
2012 → 2013	1.0134	1.0134	1.0034
2013 → 2014	0.9185	1.0013	0.8933
2014 → 2015	0.9029	0.9998	0.8659
Mean 2012 → 2015	0.9462	1.0137	0.9181

Over the study period, mean λ decreased for both taxa and was consistently lower than 1 after 2013 indicating a continuous decline of the studied populations (Table 5). However, when the transitions affected by disturbance in the *S. rosacea* populations were excluded from the analysis, *S. rosacea* performed much better than *S. sponhemica* (Table 5). Including environmental stochasticity reduced the growth rates of both taxa only slightly (Table 6).

Table 6. Finite rate of increase and stochastic growth rates of populations of *Saxifraga rosacea* (with and without disturbed populations) and *Saxifraga sponhemica* populations. In parentheses 95% confidence intervals are given.

	Deterministic growth rate	Stochastic growth rate (CI)
S. sponhemica	0.9181	0.9161 (0.9154-0.9169)
S. rosacea	0.9462	0.9448 (0.9438-0.9457)
S. rosacea without disturbed populations	1.0137	1.0137 (1.0128-1.0146)

The geometric mean finite rate of increase of both taxa without disturbed transitions decreased with mean maximum temperature at the study sites (r = -0.82, P = 0.013; Fig. 4) indicating that high summer temperatures are not favorable for the survival of both cytotypes; this trend remained when disturbed transitions were included in the analysis (r = -0.69, P = 0.05).

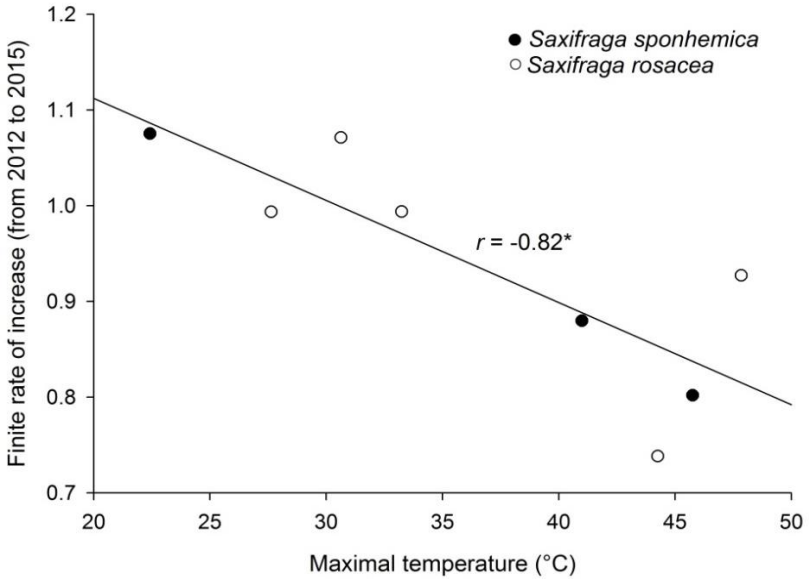


FIGURE 4. The relationship between the finite rate of increase of *Saxifraga rosacea* and *Saxifraga sponhemica* populations and the mean maximal temperature at the study sites measured for the period 2012 – 2015 without disturbed transitions.

Elasticity analysis indicated that the survival of adult plants contributed more to population growth for *S. sponhemica* (67.2%) than for *S. rosacea* (57.4% and 61% excluding disturbed transitions of populations HAR and BAU) (Table 7). In contrast, the contribution of sexual reproduction was very low, but higher for *S. rosacea* (7.7% with and 7.2% without disturbed transitions of populations HAR and BAU) than for *S. sponhemica* (3.9%) (Table 7). In *G-L-F* ordination triangles the populations of both taxa were positioned in the bottom right part of the diagrams, corresponding to a demographic pattern dominated by high longevity (*eL*) and low fecundity (*eF*) (Fig. 5). In populations of *S. rosacea* the contribution of survival (*eL*) to λ was lower that of fecundity (*eF*) in comparison to populations of *S. sponhemica*.

Mortality of yearlings and small cushions was important in both taxa (Table 7). The mortality of yearlings was slightly lower in *S. rosacea* (34.9%) than in *S. sponhemica* (38.6%), but substantially lower without disturbed populations (24.8%).

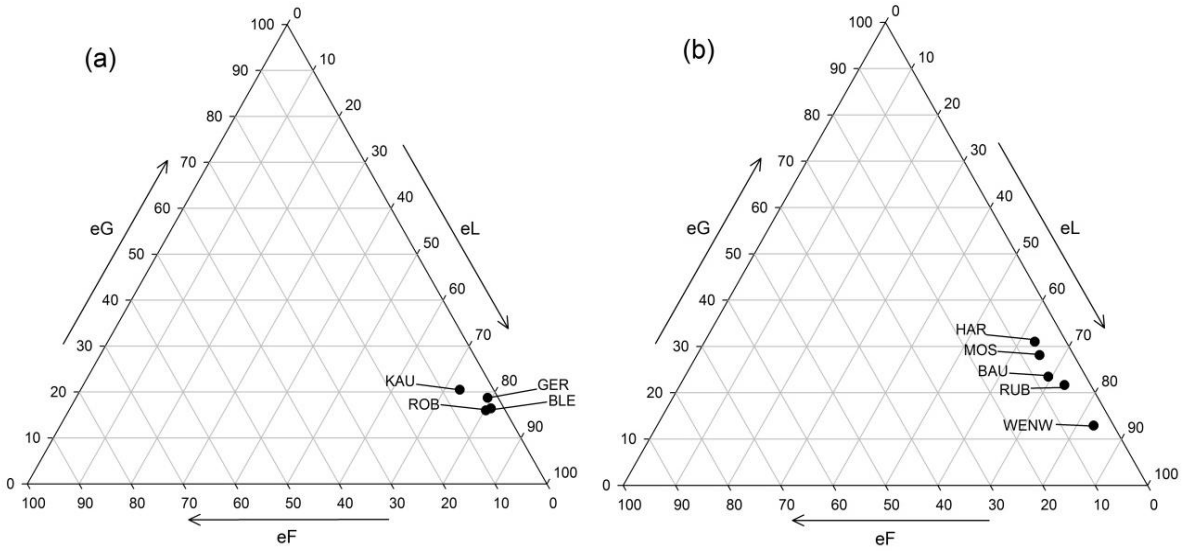


FIGURE 5. Triangular G-L-F ordination of the elasticities of the mean matrices for the studied populations of (a) *Saxifraga sponhemica* and (b) *Saxifraga rosacea*.

Conditional life spans of *Saxifraga* plants were estimated for both taxa from the mean transition matrices pooled over all years. The conditional life spans of *S. sponhemica* and *S. rosacea* plants were similar when the disturbed transitions of *S. rosacea* populations were excluded (9.6 years vs. 9.1 years). Life expectancy did not significantly differ between the four studied size stages of the two taxa. The estimated longevity for large genets for *S. sponhemica* was 10.4 years and 8.2 years for *S. rosacea*. Life expectancy of large genets of *S. rosacea* increased to 13.7 years when transitions from partially destroyed populations were excluded.

The two taxa differed in their vegetative growth patterns. *S. rosacea* produced much fewer secondary rosettes per mother rosette than *S. sponhemica* (4.27 ± 0.16 vs. 6.03 ± 0.20; $F_{1,7} = 8.171$, $P = 0.024$).

LTRE analysis—

LTRE analyses were conducted to analyse differences in demographic patterns between populations of *S. sponhemica* and all populations of *S. rosacea*, and between populations of *S. sponhemica* and only the undisturbed populations of *S. rosacea*. In both LTRE analyses, the largest differences between the two taxa were found for the fecundity of large cushions (Fig. 6a, b). However, the LTRE analysis revealed that these contrasts did not solely contribute to the differences between population growth rates. The low rate of increase of *S. sponhemica* populations was mainly due to low growth transitions (yearling to medium size plant, small plant to medium-size plant and medium-size plant to large plant), low fecundity and the stasis of small and medium-size plants in their stages (Fig. 6c). When only undisturbed *S. rosacea* populations where included in the LTRE analysis, the lower rate of increase of *S. sponhemica* populations was mainly due to a lower growth of small to medium plants and medium to large plants and to a lower survival of medium-sized and large plants (Fig. 6d).

TABLE 7. Mean transitions (2012-2015) (±1 SD) and elasticities for populations of *Saxifraga sponhemica*, *Saxifraga rosacea* and undisturbed populations of *Saxifraga rosacea*. Abbreviations are used for the transitions: Y = yearling, S = small plant, M = medium-sized plant and L = large plant.

	Transitions				Elasticities				
S. sponhemica ($\lambda = 0.9181$)									
	Y	S	M	L		Y	S	M	L
Y	0	0.006 ± 0.006	0.083 ± 0.087	0.625 ± 0.211	Y	0	0.001	0.015	0.023
S	0.614 ± 0.072	0.640 ± 0.007	0.191 ± 0.033	0.043 ± 0.041	S	0.039	0.218	0.053	0.002
M	0	0.144 ± 0.074	0.670 ± 0.018	0.367 ± 0.097	M	0	0.093	0.354	0.038
L	0	0	0.069 ± 0.027	0.566 ± 0.112	L	0	0	0.063	0.100
S. rosacea ($\lambda = 0.9462$)									
Y	0	0.07 ± 0.004	0.102 ± 0.043	1.059 ± 0.557	Y	0	0.001	0.019	0.057
S	0.524 ± 0.103	0.559 ± 0.103	0.158 ± 0.048	0.057 ± 0.067	S	0.051	0.131	0.035	0.004
M	0.127 ± 0.098	0.175 ± 0.069	0.614 ± 0.101	0.320 ± 0.135	M	0.026	0.085	0.284	0.043
L	0	0.004 ± 0.004	0.105 ± 0.059	0.573 ± 0.060	L	0	0.004	0.100	0.159
S. rosacea without disturbed populations ($\lambda = 1.0137$)									
Y	0	0.007 ± 0.004	0.125 ± 0.074	0.891 ± 0.691	Y	0	0.001	0.022	0.049
S	0.677 ± 0.190	0.613 ± 0.127	0.149 ± 0.058	0.044 ± 0.075	S	0.060	0.145	0.032	0.003
M	0.075 ± 0.116	0.195 ± 0.075	0.690 ± 0.121	0.252 ± 0.088	M	0.013	0.090	0.290	0.033
L	0	0.004 ± 0.004	0.100 ± 0.093	0.681 ± 0.103	L	0	0.004	0.082	0.176

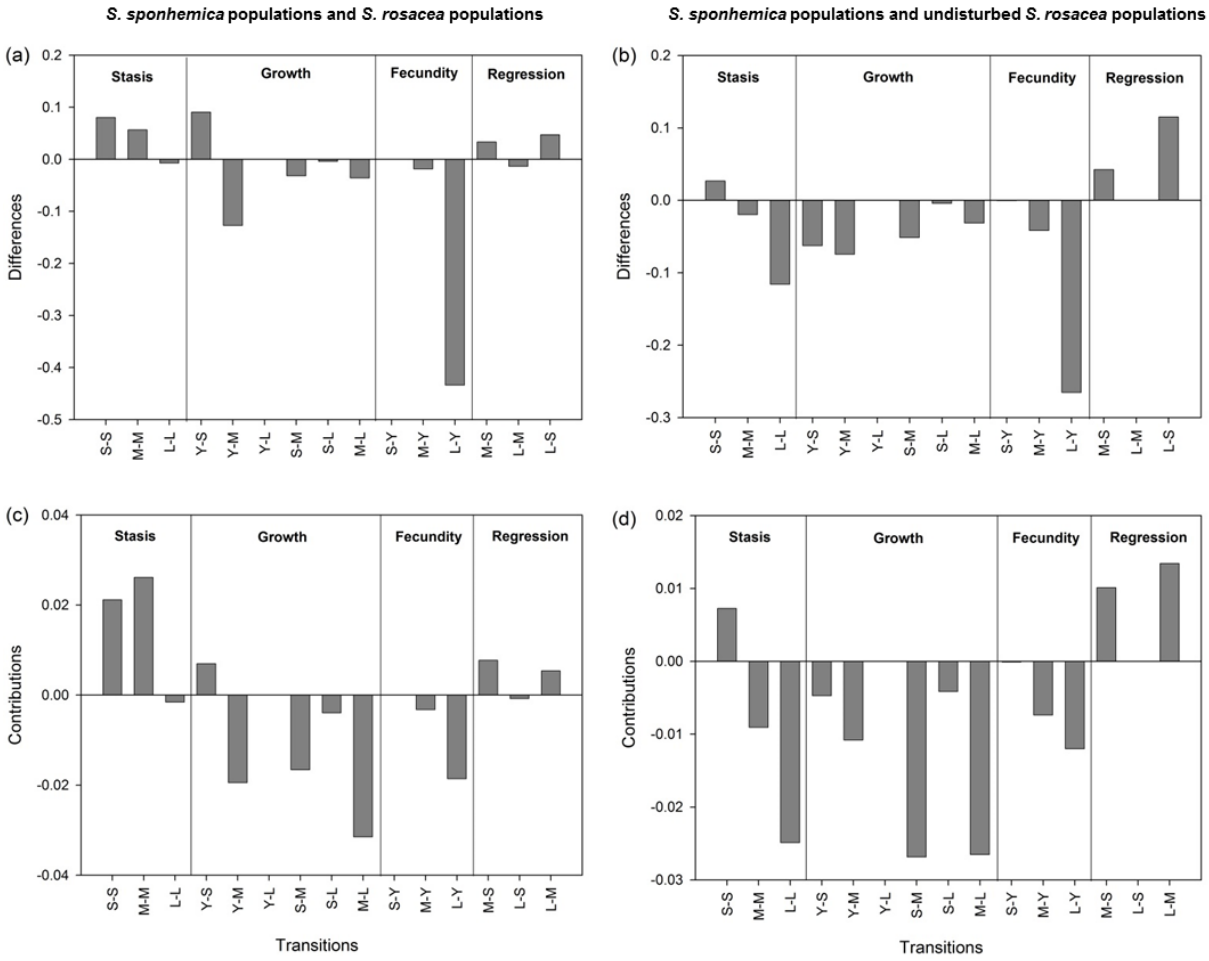


FIGURE 6. Differences (a) between elements in the mean matrix for populations of *Saxifraga sponhemica* and populations of *Saxifraga rosacea* and (b) between elements in the mean matrix for populations of *Saxifraga sponhemica* and undisturbed populations of *Saxifraga rosacea*, and the contributions of the differences in population growth rate (c) between populations of *Saxifraga sponhemica* and populations of *Saxifraga rosacea* and (d) between populations of *Saxifraga sponhemica* and undisturbed populations of *Saxifraga rosacea*. Abbreviations: Y = yearling, S = small plant, M = medium-size plant and L = large plant.

Stochastic simulations and extinction risks—

Stochastic simulations including environmental stochasticity were used to calculate extinction risks for the two taxa. Extinction risks were modelled for populations of *S. sponhemica*, populations of *S. rosacea* including the disturbed populations and populations of *S. rosacea*; operated models performed without disturbed *S. rosacea* populations did not show probabilities of quasi-extinction. The probability of quasi-extinction differed between the two taxa. For *S. sponhemica*, projections showed that quasi-extinction probabilities of 50% would be reached within 50 years. For *S. rosacea*, the same quasi extinction probability would be

reached in 77 years. The risk of extinction quickly increased with time and reached 90% after 59 years for *S. sponhemia* and 88 years for *S. rosacea*. (Fig. 7)

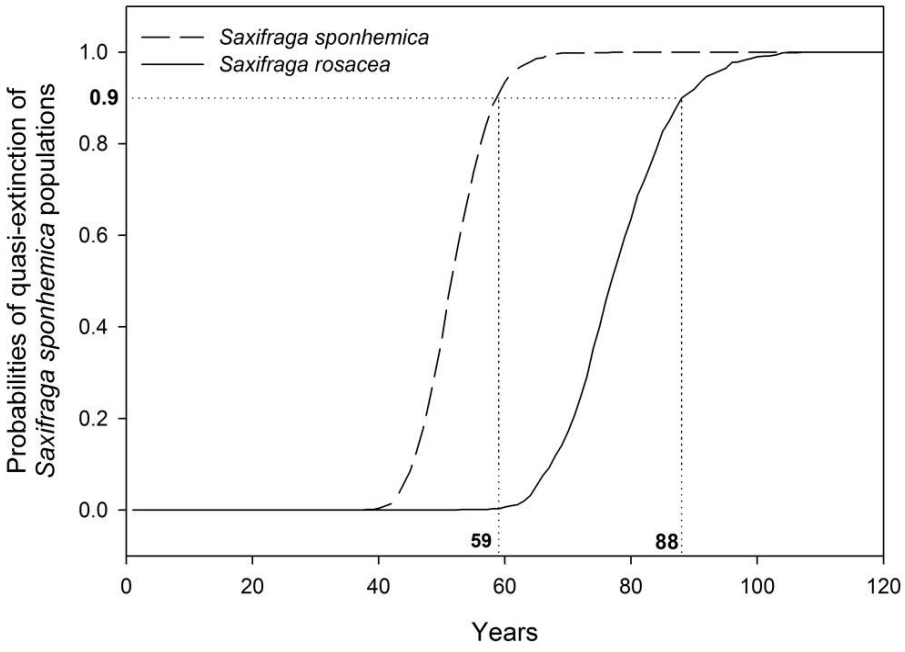


FIGURE 7. Projected probability of quasi-extinction for the populations of *Saxifraga rosacea* and *Saxifraga sponhemica* over 120 years.

To evaluate the effects of different fecundities on the finite rate of increase in both *Saxifraga*-taxa, we studied the effect of different fecundity values (yearlings per plant). We used the mean matrices of *S. sponhemica* and *S. rosacea* and excluded for *S. rosacea* the disturbed transitions. The finite rates of increase strongly increased for both taxa with higher fecundity. With current fecundity values, a growth rate > 1 was obtained when the disturbed populations of *S. rosacea* were excluded. The simulation with all populations of *S. rosacea* reached a positive growth rate when doubling current fecundity values. However, in the case of *S. sponhemica* current fecundity values would have to be at least quadrupled to obtain a growth of the studied populations (Fig. 8).

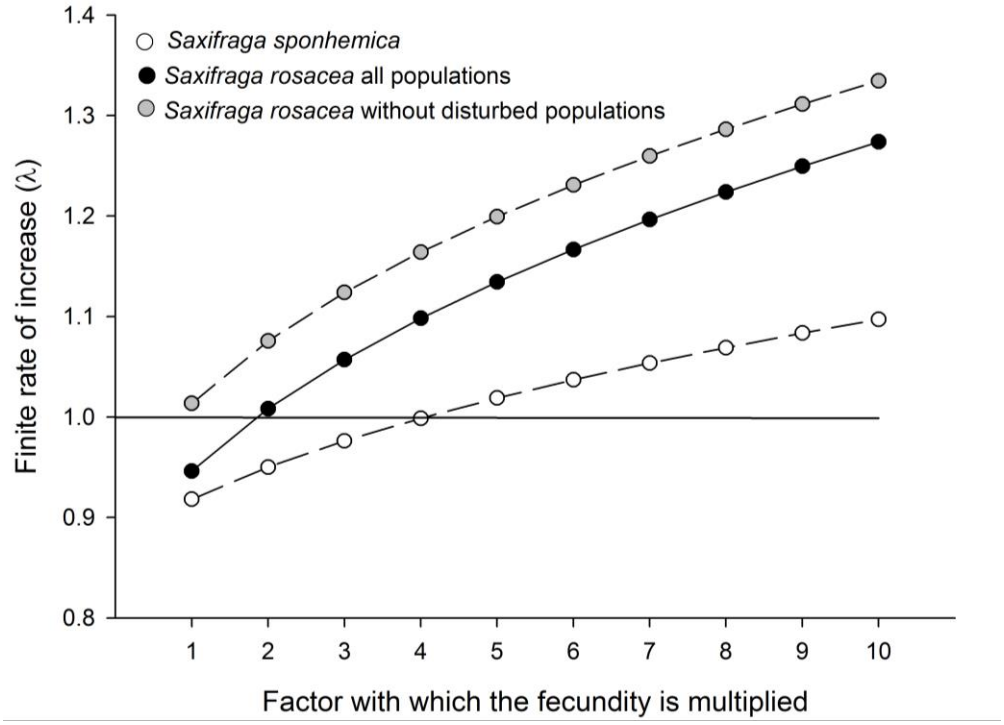


FIGURE 8. Stochastic simulation of finite rate of increase of the two studied *Saxifraga* taxa as influenced by different fecundities (increasing the number of yearlings per flowering plant by a factor of 2 to 10).

DISCUSSION

Few differences were found between the population dynamics of the two closely related *Saxifraga*-taxa. Their life spans were similar, and like other *Saxifraga*-species (Molau 1993; Erschbamer et al. 2004; Reisch 2004) they are long-lived plants.

Populations of the two taxa had very similar size distributions and they consisted mainly of cushions of intermediate size and few juveniles. Oostermeijer et al. (1994) defined this type of population structure, based on the analysis of *Gentiana pneumonanthe* populations under different management regimes, as 'stable'. This population type has a finite rate of increase near one with a high proportion of intermediate sized plants (small and medium-size plants), few large plants and few yearlings. In *S. rosacea* and *S. sponhemica*, the low proportion of young plants could be due to the limited number of suitable microsites quickly occupied in limited space habitat such as rock walls, slopes and screes. With few other plant species co-occurring in populations of both *Saxifraga rosacea* (Decanter et al. 2020); yearlings may more often encounter competition with other individuals from the same species (Schmida & Ellner 1984).

The proportion of large cushions may be restricted by the size of the cracks and crevices in which the plants root. Root growth would be limited to microspaces within rocks

to access water and nutrients, restraining the expansion of cushions. Although *S. sponhemica* develops stolons (Webb & Gornall 1989), the new ramets do not produce roots and thus do not incease the supply of water and nutrients although they enable cushions to spread further on the substrate.

In spite of the low proportion of large cushions in *S. rosacea* s.l. populations, large plants contributed most to the production of young plants, presumably because larger individuals produced most of the seeds as they had the largest number of flowers. Other demographic studies also found that larger plants contributed most to seed production (Colling et al. 2006, Ellner and Rees 2006, Tenhumberg et al. 2008, 2015, Kienberg & Becker 2017). The two taxa produce a large amount (120 per capsule; Decanter, unpublished) of small seeds (0.8 x 0.55 mm; Webb & Gornall, 1989; c. 30 μg, Decanter, unpublished), but the recruitment of juveniles is not necessarily correlated to the number of seeds. Dispersal of the seeds by wind on the rock substrates (Geiger, 1966) is limited, especially given the limited and decreasing number of available rocky habitats due to reforestation or habitat destruction. Yet for juveniles to establish, a microsite must provide adequate environmental conditions (Fowler, 1988; Callaway & Walker, 1997; Leck et al., 2008).

Large scale perturbations and disturbances like direct habitat destruction may have strong impacts on the survival probability of plant populations. However, small scale disturbances are known to favor seedling establishment in many endangered plant species (Anderson et al. 2007; Albrecht et al. 2016). In sensitive environments such as low nutrient wet grasslands, small scale perturbations may decrease the establishment of yearlings (Colling et al. 2002, Jongejans et al. 2008b). However, in rocky habitats the absence of small-scale habitat disturbances in *S. rosacea* s.l populations could lead to their extinction in the long term. Occasional small-scale perturbations could create safe microsites for new individuals to establish and rejuvenate the populations. In rocky habitats, perturbations such as crumbling or cryoturbation events are known to increase the population dynamics (García & Zamora 2003; Tuitele-Lewis 2004, Csergö et al. 2009).

Few differences between the two *Saxifraga* were found in the analysis of the regional elasticities in the *G-L-F* triangle. The patterns found for the two *Saxifraga* were similar to those found for woody plants and other long-lived forbs (Silvertown and Nillson 1993; Pico & Riba 2002; García 2003; Colling and Matthies 2006). The population dynamics of both taxa relied essentially on plant longevity (*L*) and survival of older plants with a life expectancy in the range of a decade. However, *S. rosacea* populations had a slightly higher growth (*G*) and a lower mortality of juveniles, thus populations of *S. rosacea* appeared to be

more viable than those of *S. sponhemica*. The LTRE analyses showed that largest contrasts between the two taxa were due to their fecundities. In *S. rosacea* populations large individuals produced more juveniles than in *S. sponhemica* populations, but these differences did not contribute most to the difference in the rate of increase. The largest contributions to the differences were due to the stasis of small and medium cushions of *S. sponhemica* individuals in their own size class. As these categories mainly contributed to the population structure, an important mortality rate in these categories may be jeopardize their long-term survival. In both LTRE analyses, the lower growth rates of *Saxifraga sponhemica* were mainly due to a higher plant growth of *Saxifraga rosacea* population. Furthermore, the LTRE analysis of the partially destroyed populations of *S. rosacea* showed that were the growth of intermediate size class cushions into next size class and the survival of large individuals contributed the most to the differences in rate of increase. Survival, replacement and growth of the oldest individuals strongly impacted the viability of populations as this size class contributes the most to seed production. In *S. rosacea* populations the current proportion of juveniles generated by the oldest size class is sufficient to have a finite rate of increase close to 1, but in *S. sponhemica* populations, their proportion is largely insufficient. Other studies comparing vital rates of closely related species with different cytotypes also found only subtle differences (Hahn et al. 2012, Černá & Münzbergová 2013, Rokaya et al. 2017).

As in other long-lived species, the fate of older plants was the most important factor for the population dynamics. *Saxifraga rosacea* s.l. is sensitive to the loss of large plants, because, as in many other perennial plant species (Franco & Silvertown 1996, Ehrlén & Lehtilä 2002, Goave & Ticktin 2010) individuals spend most of their life span in this stage. The strategy to rely on the survival of large individuals and a trade-off between rosette survival and reproduction may enable populations to endure unfavorable years (García and Zamora 2003, Maurice et al. 2012, Münzbergová 2013).

Several studies have investigated the response of the population dynamics of plants to a changing climate. Dirnböck and Dullinger (2004) simulated the expansion of *Pinus mugo* populations and found that pine shrubs will invade and displace current alpine vegetation under future climate change scenarios due to changes in recruitment and dispersal limitation. Life-history traits such as survival and fecundity may be particularly affected by changing climate conditions especially for long-lived species (García, 2008) or arctic plants (Callaghan & Carlsson, 1997). Even if *S. rosacea* has been shown to have a higher tolerance to frost and been more able to cope with harsher climatic conditions than *S. sponhemica* (Decanter et al., 2020), an increase of maximum temperature due to climate change would have negative

effects on the population growth of both *Saxifraga* taxa which are considered to be ice age relicts (Walisch et al. 2015). Higher temperatures will reduce the relative humidity of north-exposed rock faces and lead to drought in micro-cracks and crevices. The weakly developed roots of yearlings and small cushions may not allow them to survive the predicted higher temperatures due to climate change. Climate projections estimate an increase in the mean annual temperature between 2 to 4.5 °C until the end of the century (Jacob et al, 2013) for the continental regions where *S.rosacea* and *S. sponhemica* populations occur, and up to 5.5 ° C for the northern distribution areas of *S. rosacea* as well as an increase of heavy rain events (Jacob et al., 2013). The steep slopes of the substrate on which *S. rosacea* s.l grows causes heavy rainfall to run off and the small amount of soil available does not hold much water (Sanchez & Peco, 2007; Kienberg & Becker 2017).

We found that the demography of the two closely related *Saxifraga* taxa was similar, although the subtle differences appeared to affect the long-term viability of the populations. The simulation of quasi-extinction showed that the extinction risks are substantially higher for *S. sponhemica* populations. Without direct habitat destruction, the studied populations of *S. rosacea* are viable over the long term and may slowly expand, whereas the *S. sponhemica* populations face a high risk of extinction within the next century. Münzbergová (2007) also found similarities between population growth rates of two cytoypes of *Aster amellus* but differences in the risk of extinction. Because of its much larger distribution area (Jalas et al., 1999) and much higher number of extant populations (GBIF database, 2019), *S. rosacea* faces a lower risk of extinction than *S. sponhemica*, as rare species are more sensitive to strong external disturbances (McIntyre & Lavorel 1994, Pavlovic 1994), notably when populations are fragmented (Henle et al. 2004). Moreover, the strong genetic fragmentation of the populations of *S. sponhemica* could restrict their evolution, and thus prevent necessary adaptation to climate change (Walisch et al. 2015). Despite the close similarities between the demography of *S. rosacea* and *S. sponhemica*, the two taxa may need different conservation and management approaches as the future of their populations may diverge with current environmental conditions. It could be necessary to create new populations for both *Saxifraga*-taxa in cool valleys on rocky habitats orientated north with little direct sunlight, and to keep the habitat of existing populations open to avoid competition with shrubs and trees that may lead to the disappearance of large adult plants (Walisch T., 2009). Furthermore in populations of *S. sponhemica* it will be crucial to create new microsites through small perturbations to increase the recruitment of young plants.

CONCLUSIONS

Our results suggest that although populations of *S. rosacea* and *S. sponhemica* have comparable population dynamics, their populations may face different fates. Populations of both *S. rosacea* and *S. sponhemica* consisted mostly of plants of intermediate size, while young and old individuals were rare. Differences in the contribution of each size-class to the population dynamics led to a certain stability of *Saxifraga rosacea* populations whereas most *Saxifraga sponhemica* populations declined. However, stochastic simulations showed that populations of the two *Saxifraga* taxa have a significant extinction risk within the next century without suitable management and conservation measures. The results of our study suggest that populations of closely related species growing in similar habitats and with similar demographic characteristics may nevertheless have different extinction risks in the face of climate change, especially rising temperatures.

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CHAPTER 4

Reproductive isolation of two cytotypes of *Saxifraga rosacea*: genetic rescue issues in rare plant conservation

ABSTRACT

Habitat fragmentation may strongly impact populations of endangered plant species. The increased isolation reduces the genetic diversity within populations through genetic drift and inbreeding and increases extinction risks. Establishing artificial gene flow is often considered as a management plan for populations of rare and threatened species. However unclarified taxonomic statuses can lead to unappropriated conservation measures especially in closely related taxa.

To clarify the taxonomic status of two closely related cytotypes of *Saxifraga rosacea* we used crossing experiments involving within and between population crosses, hybridization and backcrosses and studied fitness and taxonomically important traits of the descendants over two generations. Hybrids between the two cytotypes showed reduced fitness in the first generation, indicating outbreeding depression. The reproductive isolation between both cytotypes was confirmed by the drastic fitness loss of cytotype-hybrids in the second generation. Between-population crosses within the same cytotype showed hybrid vigor compared to within population crosses, indicating inbreeding depression in the fragmented and isolated populations of both taxa. The study of the morphological traits of the descendants from the crossing experiments indicated that the taxonomical criteria to distinguish both cytotypes should be revised. Our results indicate that the two *Saxifraga rosacea* taxa are reproductively isolated and that they should be considered as two different species. Our study highlights the importance of clarifying the taxonomic status of closely related taxa by means of integrative taxonomy before genetic management measures are to be considered.

KEY WORDS: *Saxifraga rosacea*, crossing experiment, leaf morphology, integrative taxonomy, reproductive fitness.

INTRODUCTION

Habitat fragmentation is known to apply important pressures on populations and the increased isolation strongly impacts genetic diversity through genetic drift and a reduction in gene flow (Young et al., 1996; Willi et al., 2007). The loss of genetic diversity reduces the ability to respond to stochastic changes in environmental conditions and may increase the extinction risk of isolated populations in the long term. The conservation of genetic diversity is thus an important issue in plant conservation (Matthies et al., 2004; Frankham, 2005; Lande, 2018).

The genetic management of populations is often invoked when plant species are critically endangered due to increased isolation and fragmentation (Heinken & Weber, 2013; Frankham et al, 2017). Small populations of vulnerable plant species often suffer from the loss of genetic diversity due to increased inbreeding and may have a lower potential to adapt to a changing environment (Lande & Shannon, 1996; Rieseberg & Willis, 2007). However, some naturally rare plant species occur since many generations in small and/or isolated populations due to migration history, geological events or niche restrictions (Huenneke, 1991; Leimu et al., 2006; Finger et al., 2011). The necessity of implementing genetic rescue plans aiming to increase genetic diversity in isolated populations should be carefully considered in such 'old rare' species, especially if a genotypic balance between reproductive fitness and adaptation to local conditions is known to contribute to the long term survival of their populations (Ellstrand & Elam, 1993; Honnay & Jacquemyn, 2007). The artificial increase of gene flow among isolated plant populations to counter inbreeding depression can positively affect their conservation (Lesica & Allendor, 1999; Frankham, 2015; Ellstrand & Rieseberg, 2016), but this measure should be applied with caution (Hufford et al. 2012; Waller, 2015). Investigating the risks of genetic management can avoid causing outbreeding depression through the introduction of deleterious genes, and the overdominance of specific genetic material (Edmands & Timmerman, 2003; Marsden et al. 2013). Several mechanisms are known to generate outbreeding depression including local adaptation to different environments or due to chromosomal differences like different ploidy levels (Frankham et al., 2017). Such process could also result from loss of variation through genetic drift because of bottleneck or founder effect affecting allelic structure or the breakup of coadapted gene complexes by recombination (Hufford and Mazer 2003). The inadequate mixing of different genotypes may be very damaging in plant conservation (Schneller, 1996; Goto et al., 2011), especially when mixing different cytotypes (Murray & Young, 2001; Severns & Liston, 2008; Schmidt-Lebuhn et al., 2018). The study of ploidy level in plant populations should thus be an important part of genetic management plans (Frankham et al., 2002, 2015).

An important step in plant species conservation before applying management measures is the clarification of taxonomic uncertainties (Frankham et al., 2002). However, plant taxonomy is essentially based on morphological descriptions of herbarium sheets, assorting specimens into groups with a large variability of characteristics within and among populations (Henderson, 2005; Efimov, 2011; Jakubska-Busse et al., 2017; McAlister et al. 2019) or based on incomplete data (Botes, et al. 2020; Borges et al. 2020). Linnæus' work, based on the morphological studies on collection specimens, established the 'morphospecies concept'. In

the biological species concept (BSC), a species was defined by Dobzhansky (1937) and Mayr (1942) as a "group of interbreeding natural populations which are reproductively isolated from other such groups". It has been subsequently recognized that the reproductive isolation could be incomplete but, if the isolation was rather established, entities could be considered as evolutionary separated. However, this concept is not suitable for allopatric groups or organism without sexual reproduction (Paterson, 1985). As a consequence numerous alternative concepts emerged (Coyne & Orr, 2004) which are based on miscellaneous characteristics such as biochemistry, cytology or morphology. Aside from the philosophical prospect of assorting entities into categories (Ghiselin, 1974; 1997; Richards, 2010), classification of groups into species is fundamental for several biology fields and especially for species conservation. The accurate taxonomic status of endangered species should be first established (Frankham et al., 2002; Ouborg et al., 2006) prior to any conservation decisions. However, because of the current problems of habitat destruction, climate change and the direct and indirect human impacts on ecosystems, management plans are often quickly enacted (Forest et al., 2015).

Unsettled taxonomy issues can result in the underestimation or overestimation of the number of taxonomic entities (Hey et al., 2003), population sizes or distribution areas and may lead to the reevaluation of the legal protection status of these species (see Daco et al. 2019; Zhao, 2020). The application of integrative taxonomy (Ouborg et al., 2006; Padial et al, 2010; Pante et al. 2015) which makes use of information on genetics, ecological niches and morphological characteristics can help to accurately allocate resources to well identified threatened species (Shafer et al, 2015, Fitzpatrick et al., 2015, Whiteley et al., 2015).

We investigated the reproductive isolation of two closely related rare subspecies of *Saxifraga rosacea* Moench which is broadly distributed in Europe (GBIF database, 2019). The two subspecies *S. rosacea* ssp. *rosacea* (D.A.Webb) and *S. rosacea* ssp. *sponhemica* (C.C.Gmel.) have few morphological differences but are known to have different cytotypes (Webb & Gornall, 1989; Oberdorfer et al., 2001; Decanter et al. 2020). *S. rosacea* subsp. *rosacea* (octoploid) and *S. rosacea* subsp. *sponhemica* (hexaploid) have distinct distribution areas in Continental Europe without known mixed populations. *S. rosacea* subsp. *sponhemica* has a smaller and disjunct distribution area (Jalas et al., 1999) in comparison to *S. rosacea* subsp. *rosacea* which occurs from Central Europe to the subarctic (Decanter et al. 2020). The aim of this study was to investigate the reproductive isolation of the two *Saxifraga* subspecies using crossing experiments within and among populations and to study offspring performance over two generations. We recorded seed production, seed mass, germination rate, survival and

multiplicative fitness, and the main distinctive morphological traits described in the literature (Webb, 1951; Webb & Gornall, 1989).

We addressed the following questions: (1) Is there evidence for high genetic load in the isolated populations of these rare taxa? (2) Will artificially increasing gene flow between populations result in heterosis or outbreeding depression? (3) Are the two closely related taxa S. *rosacea* subsp. *rosacea* and S. *rosacea* subsp. *sponhemica* reproductively isolated?

MATERIALS AND METHODS

Studied species—

Saxifraga rosacea MOENCH is a perennial plant species with a large distribution area in Europe (Jalas et al. 1999). Webb and Gornall (1989) described three subspecies: *S. rosacea* subsp. *rosacea*, *S. rosacea* subsp. *sponhemica* and *S. rosacea* subsp. *hartii* which is only known from Arranmore Island in Ireland. In the present study we focused on the first two subspecies, called *Saxifraga rosacea* and *Saxifraga sponhemica* in the following. Although the two *Saxifraga* taxa grow in similar habitats such as screes, stony slopes or walls, they differ in their ecological niche (Decanter et al., 2020) and no mixed populations are known. *S. sponhemica* has a disjunct area of distribution and occurs in three regions: the Czech Republic, the Ardennes - Hunsrück region and the Jura region (Jalas et al., 1999). *S. rosacea* is distributed in central and eastern Germany, the Faroe Islands, Norway, eastern Ireland and Iceland (Webb and Gornall, 1989; GBIF database, 2019).

The two *Saxifraga* subspecies have a similar general morphology and reproductive system. The plants form cushions consisting of 1 to 600 rosettes. Floral stems grow from the rosette apex – sometimes from the rosette basis (personal obs.) – and develop small white protandrous flowers grouped into simple cymes. Plants bloom from April to July in continental Europe and from June to August in Iceland, where only *Saxifraga rosacea* occurs. Flowers are self-compatible and pollinated by a wide range of insect species such as Dipterae (Muscidae and Syrpidae), Apidae and Coleoptera (Webb & Gornall, 1989; Walisch et al., 2015) and produce small capsules (3.8 x 4.7 mm) consisting of two locules and may contain over 200 seeds (0.8 x 0.5 mm). Covered with papillae, the dark brown seeds have a mass between 5 and 80 μg (personal obs.). The two subspecies are taxonomically distinguished by their leaf morphology. The lobes of the leaves of *S. rosacea* are obtuse, acute, or shortly mucronate with trichomes that are predominantly glandular, whereas the segments of the

leaves of *S. sponhemica* are apiculate, narrow, and have trichomes that are mostly non-glandular (Webb and Gornall, 1989).

The two subspecies are also genetically differentiated (Philp, 1934; Webb & Gornall, 1989; Weber, 1995; Decanter et al. 2020), as they have different cytotypes: *S. rosacea* plants are octoploid (8x = 64), while *S. sponhemica* plants are hexaploid (6x = 48).

Sampling of plants and crossing experiment – F1 generation—

For the crossing experiments, we collected seeds or rosettes in 15 populations of *S. rosacea* and 15 populations of *S. sponhemica* (Table 1) from 3 to 14 different plants per population depending on population size and available ripe seeds. Seeds were stored at 6 °C in paper bags with silica gel until they were placed for germination (spring 2007) on moist filter paper in Petri dishes in a growth chamber at 20 °C under a 12 h day/12 h night light regime with lighting by fluorescent tubes (Sylvania®, Gro-lux F58W/T8, Erlangen, Germany). When plantlets had reached a size of about 1 cm they were transplanted to soaked peat pellets (38 mm) (Jiffy, Jiffy Group, Kristiansand, Norway) kept at the same light conditions at room temperature. Rosettes collected in the field were immediately placed on wet cotton in individual sampling containers and then transplanted to soaked peat pellets (38 mm) (Jiffy, Jiffy Group, Kristiansand, Norway) kept at room temperature under a 12 h day/12 h night light regime provided by fluorescent tubes. In September 2009, all plants were transferred to pots of 11 cm diameter filled with low-nutrient soil (138 mg/L N, 108 mg/L P_2O_2, 158 mg/L K_2O) and kept in the garden of the National Museum of Natural History of Luxembourg until the start of the crossing experiment.

For the first generation offspring experiment (F1) in spring 2010, three plants (individuals) of each population were used to realize three different pollination treatments (Fig 1.): (1) Within Population Crosses (WPC) were carried out by pollinating a flower of an individual plant with pollen from another individual plant from the same population (2) Between Population Crosses (BPC) were carried out by pollinating a flower of an individual plant with pollen from another plant from another population of the same cytotype, (3) Between Cytotype Crosses (BCC) were carried out by pollinating a flower with pollen from another plant of the other cytotype. All crossings were carried out in a glasshouse sealed with a mosquito net to keep natural pollinators out. The position of all plants was randomized every week.

TABLE 1. Location of the 30 populations of *Saxifraga rosacea* and *Saxifraga sponhemica* used for the pollination experiment.

Subspecies	Subregion	Population	Abbr.	Lat.	Long.	Sampling year	Raised from seeds (S) or rosettes (R)
S. rosacea	Jura	Baume-les-messieurs	BAU	46.69	5.64	2008	R
	Vosges	Hartmannswillerkopf	HAR	47.86	7.17	2008	R
	Central Germany	Bronn	BR	49.73	11.46	2008	R
		Gräfenwarth	GR	50.53	11.71	2008	R
		Harzer-Hexen-Stieg	HS	51.76	10.84	2008	R
		Lochbauer	LOCH	50.54	12.17	2008	R
		Neuahus	BVV	49.63	11.55	2008	R
		Neutal	NEUT	49.06	11.57	2008	R
		Pegnitz	PEG	49.69	11.55	2008	R
		Pfaffenhafen	PFAFF	49.64	11.53	2008	R
		Pönitzal	POR	50.50	11.73	2008	R
		Reschwer	RENZ	50.54	12.17	2008	R
		Seew	SEEW	49.69	11.54	2008	R
		Velden	V	49.61	11.52	2008	R
		Wied	W	49.51	11.50	2008	R
S. sponhemica	Jura	Arbois	ARB	46.88	5.81	2003	S
		Salin	S	46.93	5.92	2008	R
	Ardennes	Bouillon	BOU	49.80	5.07	2003	S
	Eifel	Gerolstein	GER	50.20	6.63	2008	R
		Hammerstein	HAMM	49.69	7.30	2003	S
	Nahetal	Idar-Oberstein	N	49.68	7.29	2003	S
	Czech Republic	Blesno	BLE	50.48	13.90	2003	S
		Borec	BOR	50.52	13.99	2003	S
		Ostry	O	50.53	13.95	2003	S
		Tétinské	TET	49.57	14.06	2003	S
		Voskov	VOS	49.92	14.18	2003	S
	Oesling	Bettel	BET	49.92	6.22	2003	S
		Kautenbach	KAU	49.95	6.02	2003	S
		Michelau	M	49.89	6.12	2003	S
		Unterschlinder	U	49.92	6.07	2003	S

In order to avoid self-pollination, receiver flowers were emasculated before maturation of their anthers. Hand-pollination was performed by gently rubbing two anthers from the donor flower on each ripe stigma of the receiver flower. Each pollinated flower was marked by a colored wool thread tied to the base of the sepals. Ripe fruits (capsules) were collected and separately stored in small paper bags and dried at room temperature. Developed and undeveloped seeds were counted, weighed and stored at 6 °C. Because the seeds of the

Saxifraga taxa have a very low seed mass (< 0.1 mg), we weighed all developed seeds from each capsule together and calculated mean seed weight per capsule by dividing by the number of seeds.

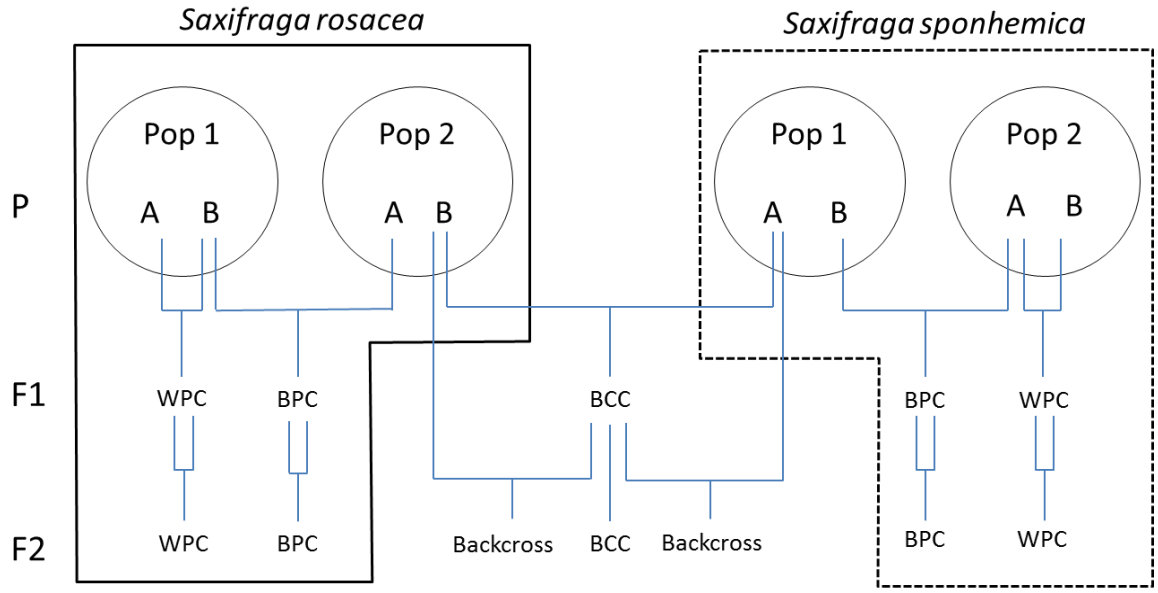

FIGURE 1. Schematic diagram showing the various pollination treatments to obtain the F1 and F2 generations.

In autumn 2012, 20 to 40 developed seeds from each capsule of each F1 generation crossing were put for germination on moist filter paper in two Petri dishes placed into a cooled incubator (IPP260plus – Memmert GmbH + Co.KG, Büchenbach, Germany) at 20 °C with a 16 h day and 8 h night regime. The number of seeds put to germinate varied because some treatments generated less than 40 seeds per capsule. The position of each Petri dish was randomized in the incubator every three days and the number of germinated seeds was recorded. Plantlets were transferred into peat pellets (38 mm) (Jiffy, Jiffy Group, Kristiansand, Norway) when they had a size of about 1 cm and they were placed under a 12 h day/12 h night light regime provided by fluorescent tubes at room temperature. After 16 weeks of growth, in spring 2013 the plants were transferred into pots (11 cm diameter) filled with low nutrient soil (138 mg/L N; 108 mg/L P_2O_2; 158 mg/L K_2O) and placed in the experimental garden of the Luxembourg National Museum of Natural History. In summer 2014 the number of surviving plants was recorded for each treatment.

Crossing experiment – F2 generation—

For the F2 generation experiment, descendants from the different crosses were selected in spring 2014 to carry out four pollination treatments (Fig. 1): (1) Within Population Crosses (WPC) were obtained by crossing two individuals descending from the WPC treatment (2) Between Population Crosses (BPC) were obtained by crossing descendants from each BPC treatment among themselves (3) Between Cytotype Crosses (BCC) were carried out by crossing individuals descending from the BCC treatment among themselves (4) Backcrosses were carried out by crossing descendants from WPC with a descendant of BCC, one of whose parents was from the same population as the WPC individual.

Pollination, seed storage and measures on seeds of the F2 generation were performed in the same manner as for the F1 generation. In autumn 2014, seeds produced by the F1-generation were germinated following the protocol for the previous generation. When plantlets reached a diameter of c. 1 cm, they were transferred into peat pellets (38 mm) (Jiffy, Jiffy Group, Kristiansand, Norway) and grown under a 12 h day/12 h night light regime provided by fluorescent tubes at room temperature. Survival of the plants was recorded in summer 2015.

Leaf morphology measures—

The leaf morphology of 221 plants of the F1 generation and 206 plants of the F2 generation was investigated by recording the number of lobes, the presence/absence of apiculated leaves, the presence/absence of glandular/non-glandular trichomes, and the trichome density in three classes (low, moderate, abundant). For the F1 generation, we recorded morphological data for the offspring of *S. rosacea* and *S. sponhemica* (WPC) and the hybrid plants (BCC). For the F2 generation, we recorded the same morphological data for the within population crossings of *S. rosacea* and *S. sponhemica* (WPC), and for the F2 hybrids (BCC) and backcrossed individuals (see Fig. 1).

For the F1 and F2 generation, we sampled two fresh leaves at the base of two different rosettes from each plant and weighed them immediately to determine the fresh mass per leaf. The leaves were then dried at room temperature for two weeks, weighed again, mounted on herbarium sheets and scanned (Konica-Minolta Bizhub 423, resolution 400 x 400 dpi). The digital images were processed using Image J v.1.5 (Schneider 2012) to determine leaf area. The data were used to calculate specific lef area (SLA) and leaf dry matter content (LDMC).

Data analysis—

For each pollinated flower in the two pollination experiments, the number of developed seeds per fruit, the mean mass of developed seeds, the proportion of seeds germinating and the survival of the descendants planted until summer 2014 (F1) and spring 2015 (F2) were recorded. Multiplicative fitness measures were calculated for each generation as follows:

$$F1\ Multiplicative\ fitness = Mean\ number\ of\ developed\ seeds\ per\ F1\ fruit \times F1\ germination\ rate \times F1\ survival\ rate\ after\ 2\ years$$

$$F2\ Multiplicative\ fitness = Mean\ number\ of\ developed\ seeds\ per\ F2\ fruit \times F2\ germination\ rate \times F2\ survival\ rate\ after\ 5\ months$$

Prior to statistical analyses, the mean number of developed seeds per capsule of the parental generation was log-transformed and the F1 multiplicative fitness was square-root transformed. Statistical analyses on plant fitness measures were performed using linear mixed-effects models or generalized linear mixed-effects models using the R packages lme4 (Bates et al., 2015) and glmmTMB (Brooks et al., 2017) with cytotype of the mother and pollination treatment as fixed factors and population of the mother as random effect (Table 2). Pairwise comparisons between pollination treatments were performed with Tukey tests using the R package emmeans (Searle et al., 1980). To compare the effects of the different pollination treatments on multiplicative fitness, the results of the WPC treatment were set to a relative reference value of 1 for both the F1 and F2 generation. The statistical analyses of the crossing experiments were conducted using R version 3.6.3 (R Core Team, 2020).

To investigate the effects of the different pollination treatments on the leaf morphology of the F1 and F2 generations, ANOVA and post-hoc Tukey tests were performed on continuous variables (number of lobes, SLA and LDMC), and Pearson Chi^2 analyses were performed on categorical variables (trichome density, apiculated leaves, glandular trichomes). SLA and LDMC were log-transformed prior to analyses. The statistical analyses of leaf morphology were conducted using IBM–SPSS 23.0 (IBM Corp. 2015).

TABLE 2. Fixed factors, random effects and models of analyses selected to investigate the mean number of developed seeds, the mass of one developed seed (mg), the germination rate, the survival rate and the multiplicative fitness (number of developed seeds per fruit*germination rate*survival rate) of the F1 and the F2 generation.

	Parents	**F1 generation**
Mean number of developed seeds	*Model:* lm *Fixed factors:* Taxon of the mother*Pollination treatments	*Model:* zero inflated glmmTMB with negative binomial errors *Fixed factor:* Pollination treatment
Mass of one developed seed (in mg)	*Model:* glmmTMB *Fixed factors:* Taxon of the mother*Pollination treatment *Random effect:* Population of the mother	
	F1 generation	**F2 generation**
Germination	*Model:* glmmTMB with betabinomial error *Fixed factors:* Taxon of the mother*Pollination treatment *Random effect:* Population of the mother	*Model:* glmmTMB with betabinomial errors *Fixed factor:* Pollination treatment
Survival	*Model:* glmmTMB with binomial error *Fixed factors:* Taxon of the mother*Pollination treatment *Random effect:* Population of the mother	*Model:* glmmTMB with betabinomial error *Fixed factor:* Pollination treatment
Multiplicative fitness	*Model:* glmmTMB *Fixed factors:* Taxon of the mother*Pollination treatment *Random effect:* Population of the mother	*Model:* zero inflated glmmTMB with truncated negative binomial error *Fixed factor:* Pollination treatment

RESULTS

F1 and F2 crossing experiment—

The number of seeds developed by the parent generation and their mass were significantly affected by the pollination treatments ($F_{2,67}$ = 4.79, P = 0.01 and χ^2 = 7.02, P = 0.033, respectively). In contrast, neither mother taxon ($F_{1,67}$ = 1.85, P = 0.18) nor its interaction with pollination treatment ($F_{2,67}$ = 0.35, P = 0.70) had a significant effect on the number of developed seeds. Capsules from between population crosses (BPC) contained more developed seeds (138.04 +19.91/-16.79) than capsules from within population crosses (107.15 +23.44/-20.03) and capsules from between cytotype crosses (BCC; 75.86 +9.47/-8.42), and

seed mass was higher for BPC (32.2 ± 2.5 μg) than for WPC (25.8 ± 2.7 μg) and BCC crosses (24.6 ± 1.9 μg; Fig. 2a). The proportion of undeveloped seeds per capsule was high for all crossing types (WPC: 50%, BPC: 39%, and BCC: 59%). Fully developed seeds from all pollination treatments germinated similarly well (WPC: 74%, BPC: 66%, and BCC: 64%) and the differences between treatments were not significant ($P = 0.30$). No significant differences were found between the two mother cytotypes *S. rosacea* and *S. sponhemica* for the number of developed seeds ($F_{1,67} = 1.85$, $P = 0.18$), mean seed mass ($\chi^2 = 0.03$, $P = 0.86$) and the proportion of seeds germinating ($\chi^2 = 2.76$, $P = 0.10$).

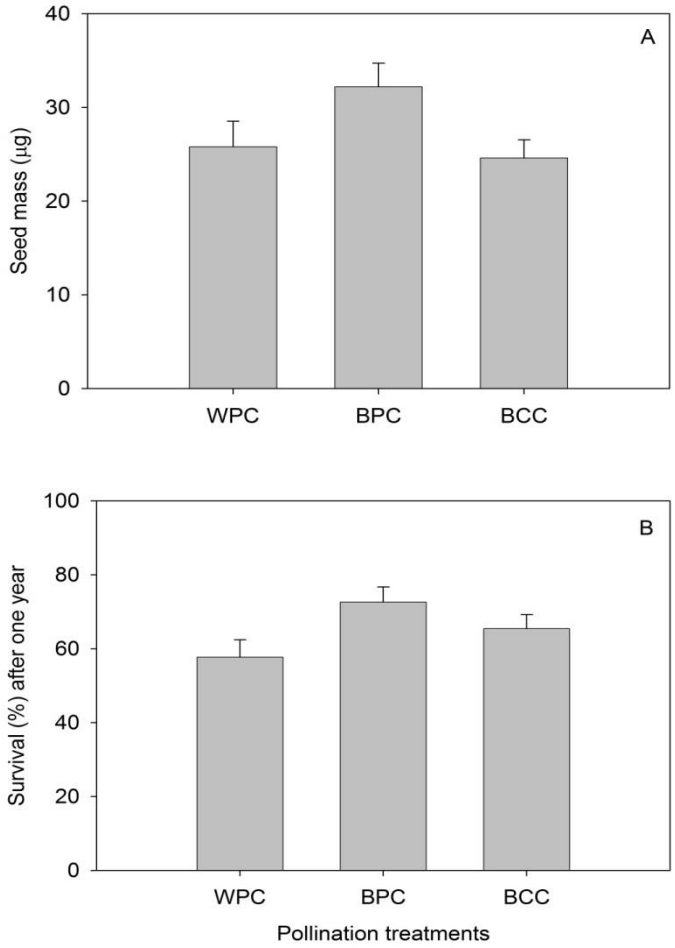

FIGURE 2. Effects of pollination treatments (WPC: within population crosses, BPC: between population crosses, BCC: between taxa crosses) on (A) the mean mass per developed seed, and (B) the proportion of survival after one year of the F1 generation. Vertical bars indicate 1 SE.

Pollination treatments significantly affected the survival ($\chi^2 = 6.66$, $P = 0.036$) and the multiplicative fitness ($\chi^2 = 5.92$, $P = 0.052$) of the parental generation. Individuals resulting from BPC had a significant higher survival than individuals resulting from within population crosses (WPC) after one year ($P = 0.0372$; Fig. 2b) indicating inbreeding in the studied

Saxifraga populations. Individuals from BPC also had a higher multiplicative fitness than individuals resulting from BCC indicating outbreeding depression for crossings between cytotypes (Fig 2b, Tukey contrast, $P = 0.047$). The cytotype of the mother taxon also had significant effects, individuals with a *S. rosacea* mother had a higher multiplicative fitness ($\chi^2 = 6.45$, $P = 0.011$) with more individuals (70.5%) surviving after one year than individuals with a *S. sponhemica* mother (60.2% survival) ($\chi^2 = 4.39$, $P = 0.036$; Fig. 3). There were no significant effects of the cytotype of the father plants on seed production, seed mass, germination rate, survival and multiplicative fitness of the F1 individuals.

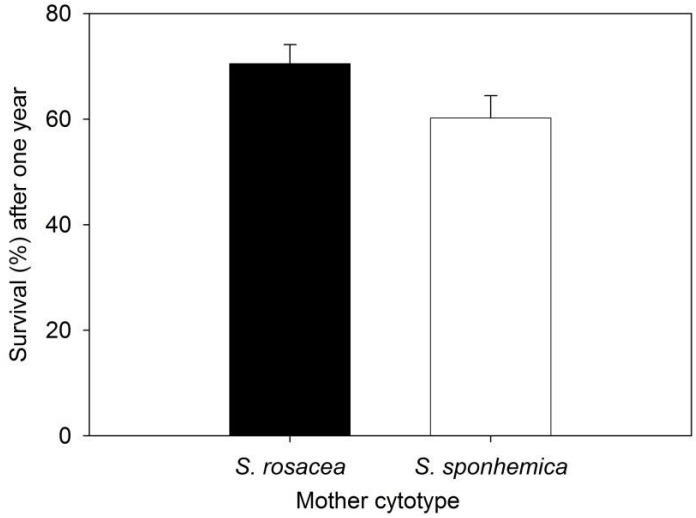

FIGURE 3. Effect of the cytotype of the mother plant on the survival of the F1 generation after one year. Vertical bars indicate 1 SE.

The F1 parents produced a very high proportion of undeveloped seeds with all pollination treatments (WPC: 72%, BPC: 93% and BCC: 97%). The germination of offspring from all second pollination treatments was high (WPC: 78%, BPC: 82% and BCC: 69%), and there was no significant effect of crossing type ($P = 0.15$).

Seed mass did not differ among the pollination treatments of the F1 parents ($P = 0.91$), while significant differences were found for the number of seeds produced by plants that produced seeds at all ($\chi^2 = 4.48$, $P < 0.001$). Within population crosses (WPC) produced more developed seeds (70.6) than BPC (27.0; $P = 0.004$), BCC (20.1; $P = 0.079$) and backcrosses (11.3; $P < 0.001$), and BCC produced more developed seeds than backcrosses (Tukey contrasts, $P = 0.060$). Germination of the seeds was not influenced by pollination treatment ($\chi^2 = 5.25$, $P = 0.15$). Survival after 5 months was significantly affected by pollination treatment ($\chi^2 = 8.42$, $P = 0.038$). Descendants from BPC crosses had a higher survival after 5

months (77.6%) than descendants from WPC crosses (55.6%; Tukey contrast, $P = 0.03$) indicating inbreeding depression.

Offspring from within population crosses (WPC) had a higher multiplicative fitness ($\chi^2 = 41.94$, $P < 0.001$) than BPC, BCC and backcrosses (Fig. 4), and BPC had a higher multiplicative fitness than backcrosses (Tukey contrasts, $P < 0.001$) indicating increasing outbreeding depression with increasing crossing distance. At the first generation of the descendants, the relative multiplicative fitness of BPC was considerably higher (1.64) in comparison to the multiplicative fitness of WPC set as reference treatment with a relative value of 1 indicating genetic load in extant populations. In contrast, the lower relative multiplicative fitness of the BCC treatment (0.80) indicated outbreeding depression. At the second generation, the relative multiplicative fitness of BPC was notably lower (0.22) in comparison to the reference treatment WPC with a relative value of 1 suggesting loss of hybrid vigor through segregation. The relative multiplicative fitness of BCC was also much lower (0.06) suggesting severe outbreeding depression through reproductive isolation between the two cytotypes. Similarly, the relative multiplicative fitness of backcrosses was extremely low (0.03) compared to WPC, indicating that introgression of genes from the other taxon has negative effects in the long-term.

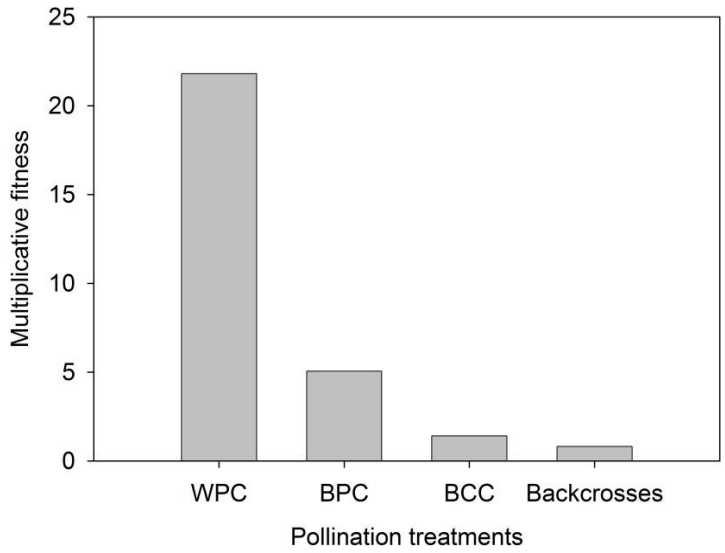

FIGURE 4. Effects of pollination treatments (WPC: within population crosses, BPC: between population crosses, BCC: between cytotype crosses, Backcrosses) on the multiplicative fitness (mean number of developed seeds per fruit × germination × survival after 5 months) of the F2 generation.

F1 and F2 leaf morphology—

The analysis of leaf morphology of the F1 generation revealed significant differences among crossing types for trichome density (χ^2 = 41.57, P < 0.001), glandular trichomes presence (χ^2 = 9.28, P = 0.01) and the proportion of apiculated leaves (χ^2 = 36.45, P < 0.001). F1 individuals resulting from crosses between *S. rosacea* parents (SRR) had a higher trichome density with trichomes being mostly glandular and the leaves were largely not apiculated in comparison to individuals resulting from crosses between *S. sponhemica* parents (SRS) whereas hybrid individuals had intermediate values (Fig. 5a, b, c). Specific leaf area (SLA) also differed significantly among cross types ($F_{2,219}$ = 3.96, P = 0.02), with SRR descendants having the lowest SLA (144.54 cm^2/g, +3.37/-3.29), hybrid BCC_{F1} individuals having intermediate values (154.88 cm^2/g, +3.61/-3.53) and SRS_{F1} individuals having the highest SLA values (173.78 cm^2/g, +12.43/-11.6).

In the F2 generation there were also significant differences among crossing types for trichome density (χ^2 = 39.95, P < 0.001), glandular trichome presence (χ^2 = 11.86, P = 0.008) and the proportion of apiculated leaves (χ^2 = 27.08, P < 0.001). Descendants from crosses between *S. rosacea* individuals (SRR) and BCC descendants produced more trichomes on mostly non apiculated leaves in comparison to descendants resulting from crosses between *S. sponhemica* individuals (SRS; Fig. 6a, b, c) confirming that the proportion of apiculated leaves is an important morphological character to distinguish *S. rosacea* and *S. sponhemica*. Descendants from backcrosses had less trichomes and had a higher proportion of apiculated leaves than SRR and BCC descendants, but had more trichomes and had a lower proportion of apiculated leaves than SRS descendants (Fig. 6a, b). SRR and backcrosses produced leaves with a lower proportion of glandular trichomes in comparison to SRS_{F2} descendants (Fig. 6c).

Significant differences between cross types were also found for specific leaf area (SLA; $F_{3,102}$ = 2.99, P = 0.02) and leaf dry mater content (LDMC; $F_{3,101}$ = 16.56, P < 0.001). Descendants of backcrosses had a lower SLA (426.58 cm^2/g +20.10/-19.20) and a higher LDMC (97.72 $\mu g/\mu g^{-1}$ +6.99/-6.52) than SRR (SLA 630.96 cm^2/g +60.87/-5.52 and LDMC 75.86 $\mu g/\mu g^{-1}$ +5.43/-5.06), SRS (602.56 cm^2/g +73.52/-65.53 and 74.13 $\mu g/\mu g^{-1}$ +7.15/-6.52) and BCC (616.60 cm^2/g +59.49/-54.25 and 67.61 $\mu g/\mu g^{-1}$ +3.19/-3.04) descendants.

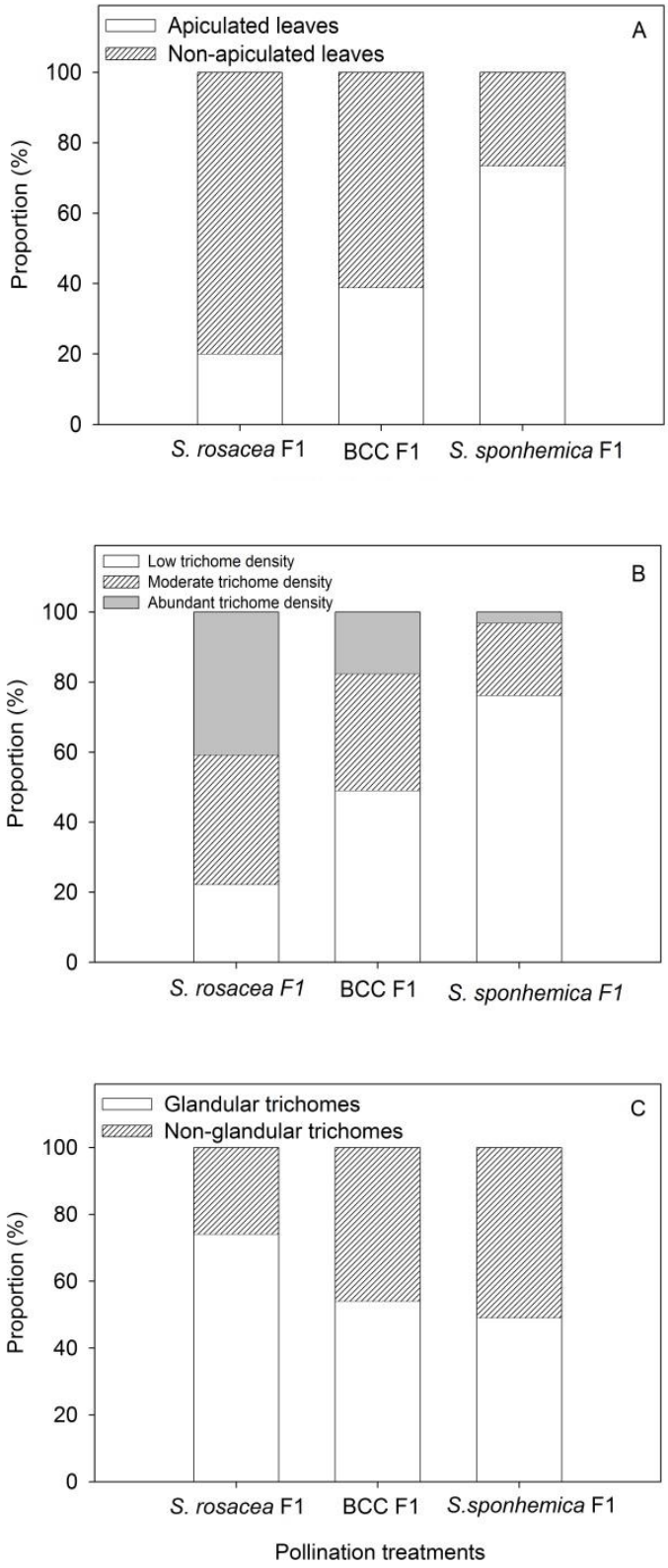

FIGURE 5. Effects of pollination treatments (Between *S. rosacea* individuals, between *S. sponhemica* individuals, and between the two cytotypes [BCC]) on the leaf morphology of the F1 generation. (A) Proportion of apiculated and non-apiculated leaves, (B) trichome density, and (C) glandular and non-glandular trichomes.

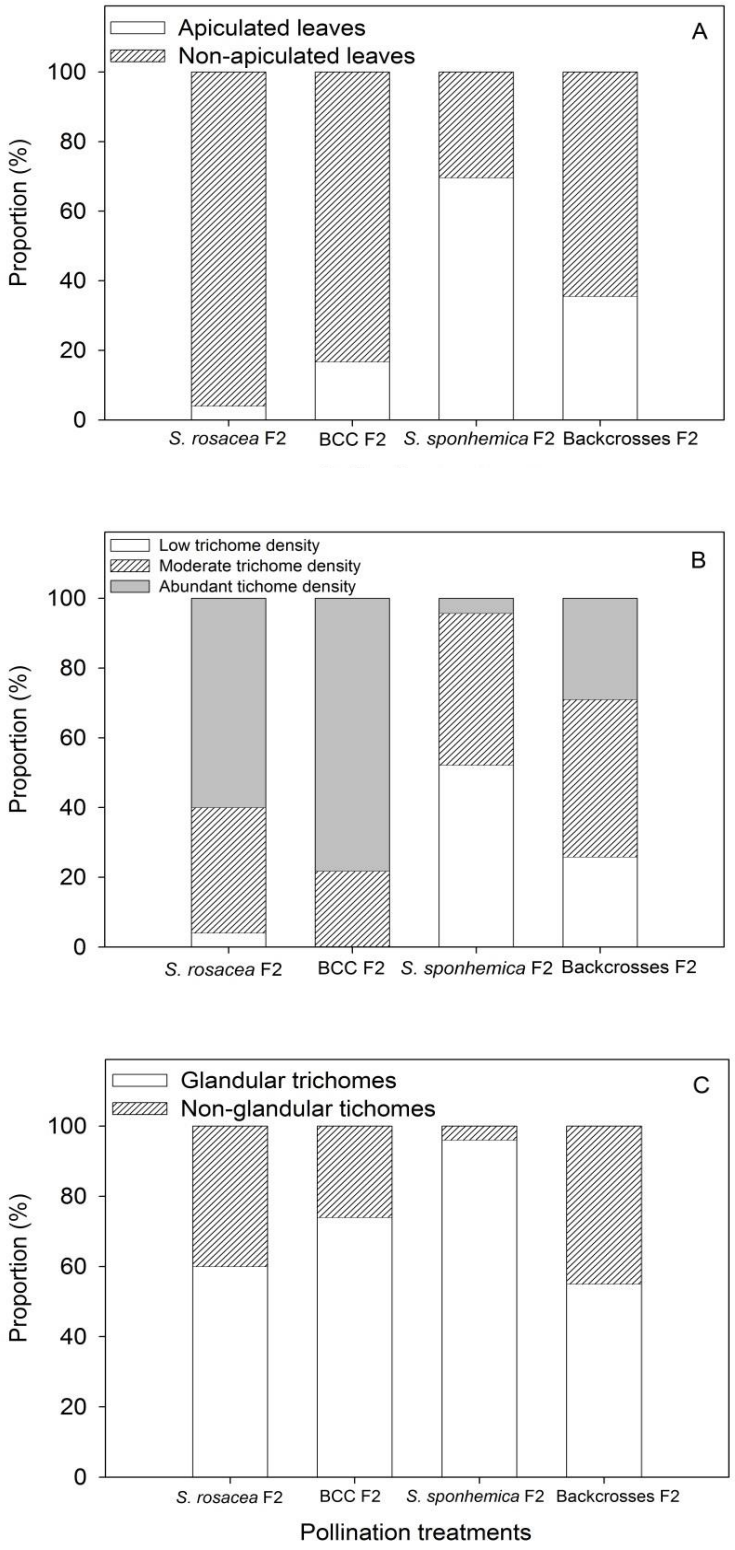

FIGURE 6. Effects of pollination treatments (Between *S. rosacea* individuals, between *S. sponhemica* individuals, and between the two cytotypes [BCC]) on the leaf morphology of the F2 generation. (A) Proportion of apiculated and non-apiculated leaves, (B) trichome density, and (C) glandular and non-glandular trichomes.

DISCUSSION

Several lines of evidence indicate that *S. rosacea* and *S. sponhemica* are reproductively isolated and should be considered as two different species. Previous studies reported 64 chromosomes for *S. rosacea* (Philp, 1934; Webb, 1950) and 46 to 52 chromosomes for *S. sponhemica* with (Drábková, 2000; Oberdorfer et al., 2001). A recent study (Decanter et al., 2020) confirmed that *S. rosacea* is octoploid (2n = 64) and that *S. sponhemica* is hexaploid (2n = 48). Our results show that genetically unbalanced hybrid progeny from crosses between 6x and 8x *Saxifraga* cytotypes displayed signs of infertility with a high proportion of undeveloped seeds and severe loss fitness. The reduced fitness in the first generation of *Saxifraga* hybrids (BCC) indicated outbreeding depression, often considered to be a step towards reproductive isolation (Frankham et al. 2011) especially if genetic differentiation is high (Montalvo & Ellstand, 2001; Becker et al., 2008).

Moreover, the higher performances of between population (BPC) individuals – seed production, seed mass, germination rate, survival and multiplicative fitness – showed heterosis within each *Saxifraga* cytotype. This hybrid vigor observed in the first generation offspring indicates inbreeding depression between parents from both taxa. Due to segregation (Rieseberg et al., 1999), heterosis may have mask the breakup of coadapted gene complexes (Hufford and Mazer, 2003) and strong negative effects of severe outbreeding depression were revealed in the second generation with the drastic fitness loss of BCC and backcrosses individual.

Reproductive isolation have been found in crossings between other closely related taxa (Ramsey et al, 2003; Kay, 2006) and crossings between different cytotypes (Husband & Sabara, 2004; Suda et al., 2007; Dobeš et al. 2013). With higher number of genome copies, genetic diversity of individuals may arise due to chromosomal rearrangements or epigenetic remodeling (Osborn et al., 2003; Gu et al. 2004; Chen & Ni, 2006) and thus increase genetic distances between cytotypes. The genetic dissimilarities between the *S. rosacea* and *S. sponhemica* cytotypes strongly reduced the survival probabilities of hybrids. Thus, only crosses between genetically similar individuals may lead to successful following generations contributing to the isolation of populations of the different taxa (Higgs & Derrida, 1992).

Reproductive isolation between cytotypes has been investigated mainly between diploids and tetraploid and has been attributed to the 'triploid block' (Burton & Husband, 2000; Köhler et al., 2009), considered as a deadlock hybridization. But hybridization followed by polyploidization may generate genetic stability for triploids to get established (Greig et al., 2002; Buggs et al. 2009). However, triploid individuals face reduced viability and fertility

resulting from the genomic imbalance in the endosperm of uneven-ploidy cytotypes, a mechanism that may also be expected with higher uneven-ploidy cytotypes such as resulting from between cytotype crosses in our study. However, fertile triploids have been found to be produced through the production of unreduced gametes (Burton & Husband, 2001; Brownfield & Köhler, 2011) and generating offspring by selfing or by backcrossing of triploids with diploids or tetraploids (Bretagnolle et al., 1995; Ramsey & Schemske, 1998; Henry et al., 2005). Even though backcrossed offspring were produced between the two *Saxifraga* cytotypes in the second generation, their number was very low or non-viable as noted in other studies (Müntzing, 1936; Ramsey & Schemske, 1998; Zhou, 2007).

The decline of fitness in the offspring from two genetically distant groups or populations is often observed in botanic garden breeding programs (Havens et al. 2004; Ensslin et al. 2015; Volis, 2017). Artificial crosses often discard pre-zygotic factors, such as differences in the morphology, phenology, ecological niches or local adaptation. Combining numerous factors such as biological characteristics, genetics, ecology or morphology in the species concept was proposed by Frankham et al. (2002) because the consideration of only one aspect may lead to unappropriated classification. *S. rosacea* and *S. sponhemica* have very similar morphological characteristics, and only a few criteria based on leaf morphology described by Webb (1951) and Webb & Gornall (1989) allow to distinguish the two taxa, although even these characters show some variability within taxa. In our study, offspring from crosses within *S. rosacea* had mainly non-apiculated leaves and the offspring from crosses within *S. sponhemica* had apiculated leaves confirming Webb & Gornall's (1989) description. However, our results do not support the second taxonomic criterion that *S. rosacea* has "leaf-hair predominantly non glandular" "with few or none of hairs gland-tipped" (see Webb, 1951). More than 50% of the offspring of *S. rosacea* and *S. sponhemica* WPC and BPC crosses had glandular trichomes. Moreover, this criterion showed large variation within populations. Trichome density and the proportion of glandular trichomes is often variable within the same species (Werker, 2000). However, little is known about *Saxifraga* trichomes although they are considered for taxonomic classification (Webb, 1951; Gornall, 1986; Webb & Gornall, 1989; Rawat et al. 2019). Even less is known about the gland-tips, their function, their metabolites or their chemical compounds. Some studies found important roles of trichomes for plants, such as protection from UV light or herbivory (Ehleringer, 1984; Tattini et al. 2007, Dalin et al., 2008), or in frost tolerance (Agrawal et al., 2004; Purwanto et al., 2021). Dhawan (2016) showed that a higher level of chemical content (proline) in glandular trichomes of *Ocimum kilimandscharicum* leaves is important for this species to survive cold

stress. The hexaploid *S. sponhemica* was found to have a different ecological niche and to be less frost tolerant than the octoploid *S. rosacea* (Decanter et al., 2020). The higher trichome density and the lower SLA of *S. rosacea* may contribute to the higher frost tolerance of *S. rosacea* in comparison to *S. sponhemica*. In contrast, the higher SLA of *S. sponhemica* may indicate a higher potential for rapid growth. This type of growth strategy is often observed in species occurring in less stressed environments (Galmes et al., 2005). In contrast to *S. sponhemica,* whose distribution is restricted to Central Europe, *S. rosacea* has a much larger distribution including Scandinavia and sub-arctic Iceland (Decanter et al. 2020).

With the higher ploidy level in *S. rosacea*, genetic interactions like epistasis (Turelli & Orr, 2000; Galloway & Etterson, 2005) are more likely to occur. Interactions between genes could have influenced the phenotypic traits of *S. rosacea* with adaptive differences in leaf morphology in comparison to the lower ploidy cytotype *S. sponhemica*, thus enhancing plant performance such as survival and fitness. Interactions between nuclear and cytoplasmic genes, suggested by the transitional leaf characteristics of the *S. rosacea* x *S. sponhemica* hybrids (BCC_{F1} and BCC_{F2} crosses) may also have enforced the reproductive isolation between the two cytotypes (Rawson & Burton, 2002). The severe loss of fitness of *S. rosacea* x *S. sponhemica* hybrids in the second generation (BCC_{F2}) is in line with the Bateson-Dobzhansky-Muller theory of speciation, where genetic incompatibilities can occur when populations are hybridizing due to genetic changes accumulated in each of the two closely related *Saxifraga* taxa.

Genetic issues are important for management and conservation plans (Ellstrand, 1992; Fahrig & Merriam, 1994; Gittleman et al., 2000; Frankham et al, 2017; Ralls et al., 2018). Although it is essential to maintain an adequate gene flow among populations in order to avoid inbreeding depression, establishing artificial gene flow among isolated populations may lead to outbreeding depression (Fischer & Matthies, 1997; Frankham et al. 2011), or moderate hybrid vigor as over-performant hybrids could quickly dominate a population (Charlesworth & Willis, 2009; Liberatore et al., 2013). The different crosses between and within the two taxa *S. rosacea* and *S. sponhemica* resulted in both heterosis and outbreeding depression in the first generation, illustrating the importance of taking into account genetic aspects in conservation projects for *Saxifraga rosacea* Moench. In the second generation our results indicate a clear reproductive isolation between both taxa which may explain why no mixed populations have been recorded. The two taxa have no protection status at a global (IUCN) or European scale (CITES, European Environmental Agency). However, many countries distinguish the two cytotypes as subspecies in their national red lists (Colling, 2005; Grulich et al., 2012; UICN

France, 2018; Metzing et al., 2018). A clear taxonomical classification is essential and should be the first aspect to be considered when planning the conservation of species (Franckham et al., 2002; Daco et al., 2019).

Within the current taxonomic classification, *S. sponhemica* is considered to be a subspecies of *Saxifraga rosacea* Moench. Based on their reproductive isolation and their different ecological niches (Decanter et al. 2020), we suggest that both taxa should be considered as different species. Two *Saxifraga* taxa might then benefit from a higher state of protection. Moreover, suitable management plans in order to maintain, reinforce or establish new populations could be developed, which avoid a genetic mix between the two cytotypes that would have a high risk of leading to outbreeding depression and maladapted genotypes.

CONCLUSIONS

Our results suggest that *S. rosacea* and *S. sponhemica* are reproductively isolated and that the very low performance of F2-generation hybrids and of backcrosses have led to spatial segregation and could explain the absence of mixed populations in Central Europe. The two taxa should be considered as two different species and morphological leaf characteristics described previously should be partially reevaluated. Mixing populations of the two cytotypes could undermine the conservation of wild populations of *S. rosacea* and *S. sponhemica.* However, establishing artificial gene flow among the isolated populations of each cytotype could be considered as a genetic rescue measure to counteract inbreeding depression, although the possible local adaptation of populations has to be considered (see Walisch et al. 2015). Our study strongly supports the use of integrative taxonomy in the establishment of conservation measures for closely related taxa.

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APPENDIX

Appendix 1 Extended dataset of 39 populations of *S. sponhemica* and 193 populations of *S. rosacea* for the niche modelling

Locations of *Saxifraga rosacea*

Country	Locality	latwgs84	longws84	Collector
France	Beaumes-les-Messieurs, Reculée de Beaumes	46.68957	5.63775	Tania Walisch
France	Beaumes-les-Messieurs, Bois de Saint-Aldegrin	46.69697	5.65441	Tania Walisch
France	Hartmannswillerkopf	47.86003	7.16583	Nora Elvinger
Germany	MTB 4814: Lennestadt (North Rhine-Westphalia)	51.174912	8.041437	GBIF - FlorKart BfN
Germany	Bodensteiner Ley near Runkel (North Rhine-Westphalia)	50.39192	8.17723	Nora Elvinger
Germany	MTB 5615: Villmar (Hesse)	50.374899	8.20811	GBIF - FlorKart BfN
Germany	MTB 8315: Waldshut-Tiengen (Baden-Württemberg)	47.67488	8.29145	GBIF - FlorKart BfN
Germany	Bad Laasphe (Hesse)	50.9278306	8.425361	Nora Elvinger
Germany	MTB 5016: Bad Laasphe (North Rhine-Westphalia)	50.924909	8.458118	GBIF - FlorKart BfN
Germany	MTB 5017: Biedenkopf (Hesse)	50.974918	8.541454	GBIF - FlorKart BfN
Germany	MTB 4917: Battenberg (Eder) (Hesse)	51.024918	8.624786	GBIF - FlorKart BfN
Germany	MTB 4918: Frankenberg (Eder) (Hesse)	51.024918	8.708124	GBIF - FlorKart BfN
Germany	MTB 6120: Obernburg am Main (Bavaria)	49.824909	9.041475	GBIF - FlorKart BfN
Ireland	Caher Connel (County Clare)	53.04072	9.13926	Nora Elvinger
Germany	MTB 7821: Veringenstadt (Baden-Württemberg)	48.174896	9.208142	GBIF - FlorKart BfN
Germany	MTB 7721: Gammertingen (Baden-Württemberg)	48.224893	9.208143	GBIF - FlorKart BfN
Germany	Hermentingen (Baden-Württemberg)	48.19618	9.22099	Nora Elvinger
Ireland	Black Head (County Clare)	53.15351	9.26426	Nora Elvinger
Germany	MTB 7521: Reutlingen (Baden-Württemberg)	48.474896	9.291479	GBIF - FlorKart BfN
Germany	MTB 4822: Gudensberg (Hesse)	51.174922	9.37481	GBIF - FlorKart BfN
Germany	MTB 7522: Bad Urach (Baden-Württemberg)	48.474899	9.458154	GBIF - FlorKart BfN
Germany	MTB 7623: Mehrstetten (Baden-Württemberg)	48.324899	9.624825	GBIF - FlorKart BfN
Germany	MTB 7624: Schelklingen (Baden-Württemberg)	48.324902	9.708157	GBIF - FlorKart BfN
Germany	MTB 7524: Blaubeuren (Baden-Württemberg)	48.424902	9.70816	GBIF - FlorKart BfN
Ireland	Macgillycuddy Reeks (County Kerry)	52.00291	9.75764	Nora Elvinger

Germany	MTB 7324: Geislingen an der Steige-West (Baden-Württemberg)	48.674899	9.791494	GBIF - FlorKart BfN
Germany	MTB 7225: Heubach (Baden-Württemberg)	48.774909	9.874834	GBIF - FlorKart BfN
Germany	MTB 7525: Ulm-Nordwest (Baden-Württemberg)	48.424902	9.958165	GBIF - FlorKart BfN
Germany	Wentalweible (Baden-Württemberg)	48.71369	10.016	Nora Elvinger
Germany	Wental (Baden-Württemberg)	48.73406	10.01634	Nora Elvinger
Germany	Bernstadt (Baden-Württemberg)	48.51801	10.03513	Nora Elvinger
Germany	MTB 7526: Ulm Nordost (Baden-Württemberg)	48.474906	10.041501	GBIF - FlorKart BfN
Germany	MTB 7226: Oberkochen (Baden-Württemberg)	48.724915	10.041502	GBIF - FlorKart BfN
Germany	MTB 7426: Langenau (Baden-Württemberg)	48.524909	10.041504	GBIF - FlorKart BfN
Germany	Königsbronn (Baden-Württemberg)	48.73524	10.11465	Nora Elvinger
Germany	MTB 7326: Heidenheim an der Brenz (Bavaria)	48.624915	10.124836	GBIF - FlorKart BfN
Germany	Eselsburgertal (Baden-Württemberg)	48.6082077	10.1784619	Nora Elvinger
Germany	MTB 7327: Giengen an der Brenz (Baden-Württemberg)	48.624909	10.20817	GBIF - FlorKart BfN
Germany	MTB 7227: Neresheim-West (Baden-Württemberg)	48.774915	10.208174	GBIF - FlorKart BfN
Ireland	Mount Brandon (County Kerry)	52.23679	10.25107	Nora Elvinger
Germany	MTB 8527: Oberstdorf (Bavaria)	47.474896	10.291515	GBIF - FlorKart BfN
Germany	MTB 5328: Wasungen (Thuringia)	50.624931	10.374845	GBIF - FlorKart BfN
Germany	MTB 7228: Neresheim-Ost (Baden-Württemberg)	48.774918	10.374847	GBIF - FlorKart BfN
Germany	MTB 4229: Braunlage (Lower Saxony)	51.774947	10.541514	GBIF - FlorKart BfN
Germany	MTB 4129: Bad Harzburg (Lower Saxony)	51.824944	10.624846	GBIF - FlorKart BfN
Germany	MTB 4230: Elbingerode im Harz (Saxony-Anhalt)	51.72495	10.708186	GBIF - FlorKart BfN
Germany	MTB 4330: Benneckenstein im Harz (Saxony-Anhalt)	51.674947	10.791521	GBIF - FlorKart BfN
Germany	MTB 4130: Wernigerode (Saxony-Anhalt)	51.82495	10.791523	GBIF - FlorKart BfN
Germany	Rübeland, Hermannshöhlen (Saxony-Anhalt)	51.75403	10.8366	Nora Elvinger
Germany	Rübeland (Saxony-Anhalt)	51.75315	10.84278	Nora Elvinger
Germany	Neuwerk (Saxony-Anhalt)	51.75759	10.86583	Nora Elvinger
Germany	MTB 4231: Blankenburg (Saxony-Anhalt)	51.77495	10.874858	GBIF - FlorKart BfN
Germany	MTB 4331: Hasselfelde (Saxony-Anhalt)	51.674947	10.958192	GBIF - FlorKart BfN
Germany	MTB 4232: Quedlinburg (Saxony-Anhalt)	51.724954	11.041526	GBIF - FlorKart BfN
Germany	MTB 4132: Halberstadt (Saxony-Anhalt)	51.82495	11.041526	GBIF - FlorKart BfN
Germany	MTB 7932: Utting am Ammersee (Bavaria)	48.074912	11.041536	GBIF - FlorKart BfN
Germany	MTB 4332: Harzgerode (Saxony-Anhalt)	51.62495	11.124862	GBIF - FlorKart BfN
Germany	MTB 5232: Stadtilm (Thuringia)	50.724941	11.124866	GBIF - FlorKart BfN
Germany	MTB 6133: Muggendorf (Bavaria)	49.874934	11.208208	GBIF - FlorKart BfN
Germany	MTB 4333: Pansfelde (Saxony-Anhalt)	51.674947	11.291538	GBIF - FlorKart BfN

Germany	MTB 6233: Ebermannstadt (Bavaria)	49.774928	11.291539	GBIF - FlorKart BfN
Germany	MTB 6033: Hollfeld (Bavaria)	49.974938	11.29154	GBIF - FlorKart BfN
Germany	MTB 6133: Muggendorf (Bavaria)	49.824931	11.291542	GBIF - FlorKart BfN
Germany	MTB 7034: Kipfenberg (Bavaria)	48.974922	11.374874	GBIF - FlorKart BfN
Germany	MTB 6334: Betzenstein (Bavaria)	49.624931	11.374874	GBIF - FlorKart BfN
Germany	Kinding (Bavaria)	48.98774	11.43491	Nora Elvinger
Germany	MTB 5434: Leutenberg (Thuringia)	50.574944	11.458207	GBIF - FlorKart BfN
Germany	MTB 6434: Hersbruck (Bavaria)	49.524928	11.458209	GBIF - FlorKart BfN
Germany	MTB 6234: Pottenstein (Bavaria)	49.724934	11.45821	GBIF - FlorKart BfN
Germany	MTB 5334: Saalfeld a.d. Saale (Thuringia)	50.624938	11.45821	GBIF - FlorKart BfN
Germany	MTB 6934: Beilngries (Bavaria)	49.024925	11.458213	GBIF - FlorKart BfN
Germany	Bronn (Bavaria)	49.72732	11.46012	Nora Elvinger
Germany	Hersbrück (Bavaria)	49.50737	11.49935	Nora Elvinger
Germany	Velden (Bavaria)	49.6097	11.51539	Nora Elvinger
Germany	Pfaffenhofen (Bavaria)	49.63659	11.53114	Nora Elvinger
Germany	MTB 5335: Ziegenrück (Thuringia)	50.624944	11.541543	GBIF - FlorKart BfN
Germany	MTB 6235: Pegnitz (Bavaria)	49.724938	11.541544	GBIF - FlorKart BfN
Germany	MTB 6135: Creußen (Bavaria)	49.874938	11.541547	GBIF - FlorKart BfN
Germany	MTB 5435: Liebengrün (Thuringia)	50.574947	11.541547	GBIF - FlorKart BfN
Germany	MTB 6335: Auerbach in der Oberpfalz (Bavaria)	49.624934	11.541547	GBIF - FlorKart BfN
Germany	MTB 6935: Dietfurt an der Altmühl (Bavaria)	49.074928	11.54155	GBIF - FlorKart BfN
Germany	Seeweiher (Bavaria)	49.69057	11.54637	Nora Elvinger
Germany	Auerbach-Michelfeld (Bavaria)	49.696114	11.546667	observation
Germany	Michelfeld (Bavaria)	49.62971	11.54772	Nora Elvinger
Germany	Dietfurt (Bavaria)	49.05828	11.57027	Nora Elvinger
Germany	Drognitz (Thuringia)	50.59522	11.58729	Nora Elvinger
Germany	MTB 7035: Schamhaupten (Bavaria)	48.974931	11.624885	GBIF - FlorKart BfN
Germany	MTB 6435: Pommelsbrunn (Bavaria)	49.524934	11.624886	GBIF - FlorKart BfN
Germany	MTB 5536: Hirschberg an der Saale (Thuringia)	50.474941	11.708214	GBIF - FlorKart BfN
Germany	MTB 5436: Schleiz (Thuringia)	50.524941	11.708218	GBIF - FlorKart BfN
Germany	Gräfenwarth (Thuringia)	50.52518	11.71307	Nora Elvinger
Germany	Pöritzsch (Thuringia)	50.49714	11.72978	Nora Elvinger
Germany	MTB 5737: Schwarzenbach an der Saale (Bavaria)	50.274947	11.958223	GBIF - FlorKart BfN
Germany	Fichtelgebirge nature parc (Bavaria)	50.063309	11.962051	observation
Germany	Wojaleite national protection zone (Bavaria)	50.253891	11.972593	observation

Germany	MTB 5138: Gera (Thuringia)	50.82495	12.041558	GBIF - FlorKart BfN
Germany	MTB 5238: Weida (Thuringia)	50.774947	12.041558	GBIF - FlorKart BfN
Germany	MTB 5638: Bobenneukirchen (Saxony)	50.324947	12.04156	GBIF - FlorKart BfN
Germany	MTB 5438: Plauen (Saxony)	50.57495	12.124901	GBIF - FlorKart BfN
Germany	Meschwitz (Saxony)	50.54024	12.16931	Nora Elvinger
Germany	Reschwitzmühle (Saxony)	50.551675	12.1754167	Nora Elvinger
Germany	MTB 5439: Treuen (Saxony)	50.524947	12.208238	GBIF - FlorKart BfN
Germany	MTB 5343: Geyer (Saxony)	50.67496	12.87492	GBIF - FlorKart BfN
Germany	MTB 4945: Roβwein (Saxony)	51.07497	13.2916	GBIF - FlorKart BfN
Germany	MTB 5147: Frauenstein (Saxony)	50.82497	13.541606	GBIF - FlorKart BfN
Germany	MTB 5050: Königstein (Saxony)	50.974973	14.124958	GBIF - FlorKart BfN
Germany	MTB 4553: Nochten (Saxony)	51.474986	14.541635	GBIF - FlorKart BfN
Germany	MTB 4855: Gorlitz (Saxony)	51.124986	14.958315	GBIF - FlorKart BfN
Iceland	Adaldalsrhaun	65.93488	-17.49859	Nora Elvinger
Iceland	after Ólafsfjördur	65.95189	-18.83559	Nora Elvinger
Iceland	Austuràrgil Aðalbòlsheiði	65.09406	-20.61188	Hörður Kristinsson
Iceland	Böðvarsdalur Vopnafjörður	65.754	-14.537	Eypor Einarsson
Iceland	Eldgjà	63.946	-18.646	Eypor Einarsson
Iceland	Fagurhòlsmyri Öraefum	63.877	-16.648	Bergpor Johannsson 2005
Iceland	Fljòtshlíð	63.723	-19.95	Bergpor Johannsson 2005
Iceland	Geirmundarstadir	65.78751	-21.75386	Nora Elvinger
Iceland	Grindavík	63.841	-22.429	Bergpor Johannsson 2005
Iceland	Herdísarvík Reykjanesskaga	63.869	-21.814	Eypor Einarsson
Iceland	Heydalsà Steingrímsfjörður	65.636	-21.535	Eypor Einarsson
Iceland	Hörgshlíð Mjòafjörður	65.844	-22.604	Eypor Einarsson
Iceland	Hrunamannahreppi	64.258	-20.235	Eypor Einarsson
Iceland	Keilir Reykjanesi	63.941	-22.161	Bergpor Johannsson 2005
Iceland	Kròkamyri Reykjanesskaga	63.901	-22.104	Kristbjörn Egilsson
Iceland	Maelifellsdalur Skagafjörður	65.37991	-19.3974	Hörður Kristinsson
Iceland	Mjòlkà, Arnarfjörður	65.774	-23.159	Bergpor Johannsson 2005
Iceland	near Egilsstadir	65.25416	-14.42315	Nora Elvinger
Iceland	near road 515	64.6206	-21.36601	Nora Elvinger
Iceland	near Svartifoss	64.02471	-16.94057	Nora Elvinger
Iceland	on road 1 close to Laufskálavarða (Kir)	63.62068	-18.44808	Nora Elvinger
Iceland	on road 427	63.85175	-22.20126	Nora Elvinger

Iceland	on road 43 to Grindavik	63.88741	-22.40578	Nora Elvinger
Iceland	on road 43 to Grindavik	63.88518	-22.41832	Nora Elvinger
Iceland	Öxi	64.81394	-14.62265	Nora Elvinger
Iceland	Raudaberg	64.3499	-15.67652	Nora Elvinger
Iceland	Reykjanes Reykjanesi	63.817	-22.698	Bergpor Johannsson 2005
Iceland	Seljafoss	63.61587	-19.98787	Nora Elvinger
Iceland	Seljalandsfoss	63.614	-19.998	Bergpor Johannsson 2005
Iceland	Skaftafell Vatnajökull National Park	64.01318	-16.96764	Nora Elvinger
Iceland	Skeljabrekka Borgarfjörður	64.538	-21.749	Eypor Einarsson
Iceland	Skógafoss	63.53151	-19.5122	Nora Elvinger
Iceland	Skógafoss	63.531	-19.531	Bergpor Johannsson 2005
Iceland	Skògar undir Eyjafjallajökull	63.525	-19.493	Bergpor Johannsson 2005
Iceland	Spillir Sùgandafjörður	66.127	-23.546	Bergpor Johannsson 2005
Iceland	Torfnafjall Fljótum	66.12657	-19.03662	Hörður Kristinsson
Iceland	Vìk	63.42259	-19.01556	Nora Elvinger
Iceland	Vík Myrdal	63.42	-19.026	Bergpor Johannsson 2005
Iceland	Vogastapi Reykjanesi	63.97	-22.443	Eypor Einarsson
Sweden	Sm. Jönköping. Fäng tr. Jönköpings Simsällskap	57.776933	14.15806	Eriksson, Erik
Sweden	Sm. Jönköping.	57.781999	14.159527	Eriksson, Eskil
Sweden	Ekenhaga	57.184036	14.048284	Petersson, Bernhard
Sweden	Småland, Berget.	56.805334	14.037221	Christoffersson, Judith
Sweden	Hools prästgård.	57.974568	12.664744	Åberg, Sven
Sweden	Göteborg Slottsskogen	57.685899	11.938741	Johansson, Yngve
Sweden	Halland. Ränneslöv s:n, Ålstorp. Förvildad utanför en trädgård.	56.460745	13.152915	Johansson, Yngve
Sweden	Lindsdal, Lidhemsväg, ödehus vid IP. Förvildad trädgård	56.727111	16.295299	Davidsson, Sven
Norway	Salhus i havets nivaa	60.50349	5.26739	Ivar Jørstad, Finn Wischmann
Norway	Indplantet i Tøien bot. have fra Hovlandsfjeld paa Modum";" Petrus.	59.91708	10.77061	N. Moe, Johannes Lid scr.
Norway	Lørenskog Låsby, langs veien	59.89159	10.98375	Halfdan Rui
Norway	Stavanger: Vølstadveien, N-siden av Lt. Stokkavatn.	58.97285	5.68682	John Inge Johnsen
Norway	Espa stasjon, vegkant langs vegen opp til nåvaerende E6	60.58123	11.27083	Anders Often, Tore Berg, Reidar Haugan
Norway	Gimle	58.16303	8.00435	Torleiv Hannaas, Finn Wischmann
Norway	Ved Malms stue, Vik	65.31047	12.17789	Knut Strompdal
Norway	Vandeskog, Sveio. Hage.	59.53806	5.28189	Olav Vandeskog
Norway	Salhus ved Bergen	60.50349	5.26739	Trygve Slettebakken
Norway	Etne Kambo Have	59.67501	5.95618	Chr. Sommerfelt, Finn Wischmann

Norway	Fra fru Strømsøs have [paa] Aasvei - Trondhjem	63.41893	10.37213	Einar Fondal
Norway	Byaasen,	63.41005	10.36169	Einar Fondal
Norway	Salhus ved Bergen	60.50349	5.26739	Trygve Slettebakken
Norway	Udsigten, Aasveien	63.42341	10.37234	Einar Fondal
Norway	Hjartland, ytre Strandberg	66.05357	12.85024	Tommy Prestø
Norway	Pl. i steinbed hos frk. Sellaeg. Jonsv.v.	63.42302	10.4124	Einar Fondal
Finland	Svisskärsfjärden, Kristinestad	62.248368	21.363466	Finnish Museum of Natural History Collections
Finland	Villinki, Helsingfors	60.155933	25.125525	Finnish Museum of Natural History Collections
Finland	Hiidenvesi	60.331021	24.145236	Finnish Museum of Natural History Collections
Finland	Nurmijärvi	60.502349	24.639979	Finnish Museum of Natural History Collections
Finland	Virolahti	60.531059	27.55244	Finnish Museum of Natural History Collections
Norway	Hinnøya: Kanbeogen, mellom Damveien og Kanebogelva ved trafostasjonen.	68.77104	16.54431	Torbjørn Alm, Unni Bjerke Gamst
Norway	Vefsn: Knausen i Halsøy. Steinbedd.	65.86467	13.20262	Peter Benum
Norway	Tverlandet naturreservat	67.270391	14.720531	Grete Nytrøen Kvavik, Mats Nettelbladt, Ingvild Gabrielsen, Gunnar Rofstad
Norway	Fana, Paradis.	60.3366	5.346	G. F. Heiberg, R. Elven
Norway	Fredrikshald,	59.13014	11.39395	E. Ryan scr., Sigmund Sivertsen
Sweden	Rinkaby	55.984255	14.269795	Knut Andersson
Sweden	Karlstad	59.381584	13.505989	Oskar Wer
Norway	Svorkmo, 'Austli' v. Aspøl	63.18386	9.74338	Kjetil Bevanger
Norway	Byåsveien	63.41902	10.36212	Arne Lie
Norway	Pl. i steinbed hos fru dommer Eriksen	63.42322	10.39237	Einar Fondal
Norway	Austbygda kirke	60.02576	8.82806	Elisabeth Bodvin, Jan-Henrik Bodvin, Reidar Elven
Norway	Ved Eg	58.16276	7.97037	John Nuland
Sweden	Vetlanda N	57.48913	15.3375	T. Merkert
Sweden	Växjö Ö	56.86252	15.08008	Curt Mossberg
Sweden	Bäckseda, Vetlanda	57.400563	15.076892	Alf Lindström
Sweden	Aneby Ö	57.80614	14.99194	Jörgen Josefsson
Sweden	Alvesta C	56.84864	14.531	Åke Widgren

Locations of *Saxifraga sponhemica*

Country	Locality	latwgs84	longws84	Collector
Belgium	Bouillon castle lower part	49.79275	5.063833	Tania Walisch
Belgium	Bouillon, Bastion de Bretagne	49.796944	5.069527	Tania Walisch
Belgium	Robertville	50.452566	6.1025777	Nora Elvinger
Czech Republic	Blešno	50.48203	13.90565	Tania Walisch
Czech Republic	Děkovka	50.49008	13.92368	Tania Walisch
Czech Republic	Ostry	50.5318	13.95135	Tania Walisch
Czech Republic	Boreč 1	50.5151	13.98975	Tania Walisch
Czech Republic	Tetinske Skaty	49.57007	14.06241	Tania Walisch
Czech Republic	Voškov 2	49.920555	14.183027	Tania Walisch
Czech Republic	Voškov 1	49.917944	14.197166	Tania Walisch
France	Planches/Arbois	46.879066	5.812994	Tania Walisch
France	Salins	46.926122	5.918511	Tania Walisch
Germany	Mettendorf (Rhineland-Palatinate)	49.924877	6.20805	GBIF - FlorKart BfN
Germany	Mürlenbach (Rhineland-Palatinate)	50.174893	6.624731	GBIF - FlorKart BfN
Germany	Gerolstein (Rhineland-Palatinate)	50.19539	6.62792	Nora Elvinger
Germany	Lebach (Saarland)	49.474883	6.874738	GBIF - FlorKart BfN
Germany	Sohren (Rhineland-Palatinate)	49.92489	7.208082	GBIF - FlorKart BfN
Germany	Frauenburg	49.666912	7.282493	Tania Walisch
Germany	Naheloreleifels	49.679854	7.288151	Tania Walisch
Germany	Hammerstein crossing	49.690271	7.289085	Tania Walisch
Germany	Birkenfeld (Rhineland-Palatinate)	49.674896	7.291417	GBIF - FlorKart BfN
Germany	Hammerstein road	49.687337	7.29939	Tania Walisch
Germany	Baumholder (Rhineland-Palatinate)	49.674896	7.374754	GBIF - FlorKart BfN
Germany	Gemünden (Rhineland-Palatinate)	49.82489	7.374755	GBIF - FlorKart BfN
Germany	Bockenau (Rhineland-Palatinate)	49.49656	7.40896	Nora Elvinger
Germany	Kirn (Rhineland-Palatinate)	49.774896	7.45809	GBIF - FlorKart BfN
Germany	Norheim (Rhineland-Palatinate)	49.4818	7.48537	Nora Elvinger
Germany	Hellberg im Nahebergland (Rhineland-Palatinate)	49.79314	7.495806	GBIF - naturgucker
Germany	Waldböckelheim (Rhineland-Palatinate)	49.824893	7.791434	GBIF - FlorKart BfN
Germany	Dannenfels (Rhineland-Palatinate)	49.624893	7.874766	GBIF - FlorKart BfN
Luxembourg	Kautenbach, railroad crossing	49.95164	6.016199	Tania Walisch

Luxembourg	Kautenbach, Hockslay	49.945055	6.026922	Tania Walisch
Luxembourg	Unterschlinder (rock)	49.921747	6.071765	Tania Walisch
Luxembourg	Erpeldange 1 (scree)	49.894458	6.115189	Tania Walisch
Luxembourg	Vianden (parking)	49.934721	6.198395	Tania Walisch
Luxembourg	Vianden (castle wall)	49.933399	6.207622	Tania Walisch
Luxembourg	Bettel, Bicycle road	49.922687	6.218112	Tania Walisch
Luxembourg	between Vianden and Bettel	49.922849	6.219157	Tania Walisch
Luxembourg	Vianden, Roth	49.928734	6.225124	Tania Walisch

Appendix 2 Environmental niche modelling with MaxEnt

All 19 bioclimatic variables available from the WorldClim v2.0 database (Fick and Hijmans, 2017) in a grid size of 0.86 km² (30 arc s) were used for tests run separately for *Saxifraga sponhemica* and *S. rosacea*. Based on the relative contribution of each bioclimatic variable to the final model the following variables were selected for the niche modelling: mean diurnal range, temperature seasonality, minimum temperature of the coldest month, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of the coldest quarter, precipitation of wettest month, and precipitation of the warmest quarter.

Analysis of variable contributions

a) *Saxifraga sponhemica*
b)

Variable	Percent contribution	Permutation importance
BIO7	41.5	0
BIO6	14.8	56.6
BIO11	8.9	9.6
BIO18	7.9	2.9
BIO9	7.9	2.2
BIO4	7.7	3.2
BIO2	4.9	3.7
BIO10	3.2	17.9
BIO13	2.6	3.1
BIO8	0.6	0.9

b) *Saxifraga rosacea*

Variable	Percent contribution	Permutation importance
BIO7	49.4	13.1
BIO11	19.1	25.2
BIO6	9.2	47
BIO9	8.4	3.1
BIO2	5.6	0.7
BIO13	3	2
BIO10	2.9	3
BIO8	1.8	0.6
BIO18	0.4	1.1
BIO4	0.3	4.1

The tables a) and b) give estimates of relative contributions of the WorldClim variables to the Maxent models. To determine the first estimate, in each iteration of the training algorithm, the increase in regularized gain is added to the contribution of the corresponding variable, or subtracted from it if the change to the absolute value of lambda is negative. For the second estimate, for each environmental variable in turn, the values of that variable on training presence and background data are randomly permuted. The model is reevaluated on the permuted data, and the resulting drop in training AUC is shown in the table, normalized to percentages. Values shown are averages over replicate runs.

Of all climatic variables in the Maxent model, for *S. sponhemica* the estimate of relative importance was highest for BIO6 (56.6), and for S. rosacea BIO7 contributed most (49.4%) to the model.

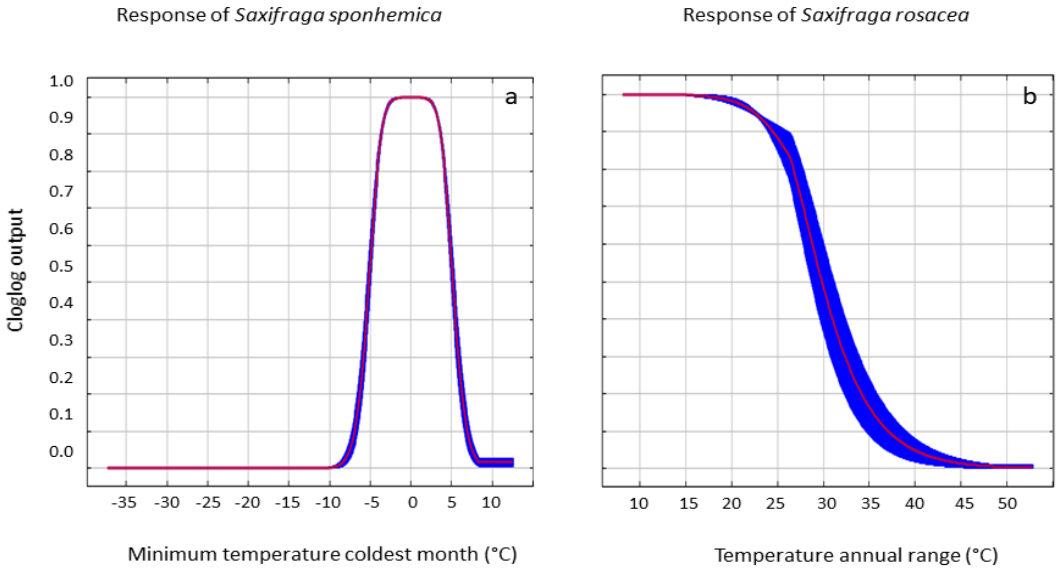

FIGURE S1. Predicted probability of (a) the presence of *Saxifraga sponhemica* in relation to the minimum temperature of the coldest month (BIO6), and (b) the presence of *Saxifraga rosacea* in relation to temperature annual range (BIO7). The curves show the mean response of the 10 replicate Maxent runs (red) +/– 1 standard deviation (blue shade).

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SUMMARY

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Numerous models have been proposed to explain how new species arise. Climate change is one of the mechanism known to apply strong selection pressures and increase the chance of speciation. During the Quaternary period, glacial advances and retreats redrafted landscapes by modifying the geographical ranges of plants and animals. Their distribution area were subjected to dramatic envionmental changes that could have accelerate the speciation rate, due to divergences selection, genetic drift or population isolation. In plant species, inability to quickly migrate with drastic climate space movements may have favor genome modifications like polyploidization, a well-known abrupt mode of speciation in angiosperm species by genome duplication. A higher number of genome copies thus a more divers set of alleles, may provide advantages to cope with harsher climatic conditions, contributing to species differenciation of plants with different cytotypes. This thesis investigates the ecological, demographical and taxonomical differences between two glacial plants relicts of *Saxifraga rosacea* with distinct distribution areas and different ploidy levels, the hexaploid *Saxifraga rosacea* subsp. *sponhemica* and the octoploid *Saxifraga rosacea* subsp. *rosacea*.

The investigation of 22 populations of the two closely related *Saxifraga* cytotypes showed that plants of the octoploid *Saxifraga* occured in sites with lower mean annual temperature and were more cold tolerant than plants of the hexaploid *Saxifraga*. Also, plants performed better in their region of origin in a two transplant sites experiment with contasting conditions indicating local adaptation. But among populations of the octoploid, genetic differences in survival were larger than among populations of the hexaploids suggesting a greater genetic variability that may enable the *Saxifraga* taxa with a higher ploidy level to occupy a larger distribution range, as revealed by probabilities of occurence according to our Maxent niche modelling. Our results suggest that cytotypes differences may lead to ditribution segregation and a higher ploidy level may enable to occupy broader ecological niches. Differences in geographical distribution and habitat fragmentation may have consequences on population dynamics, as growth rate or population sturcture, and extinction risks of the two cytotypes thus require different conservation plans.

Monitoring, for several years, the variability and demography dynamics of *Saxifraga rosacea* subsp. *sponhemica* populations and *Saxifraga rosacea* subsp. *rosacea* populations enlightened strong decline of population growth rates of both *Saxifraga* and this trend was stronger in *sponhemica* ssp. populations than in *rosacea* ssp. populations. Despite decade longevity of both *Saxifraga* plants and comparable population dynamics with few young and

few old individuals, the two cytotypes may encounter different fates. Analyses of populations size-class structures showed that with current climatic conditions, populations of octoploids could remain stable but populations of hexaploids will decline due to low fecundities. Still, extinction risks models project negative futures for both *Saxifraga* and need management and conservation programs. This finding support the hypothesis that with similar habitat and demography characteristic, two closely related cytotypes may still have different fates with ongoing climate changes. Demographic studies are important to establishing suitable conservation actions for fragmented populations.

Management of isolated populations of endangered species often includes genetic diversity and gene flow studies however taxonomy clarification should also be investigated, especially with closely related taxa. We studied the effects of within and between population crosses, hybridization and backcrosses of the two *Saxifraga* cytotypes on reproductive fitness, performances and taxonomical traits of two generations of offspring. Evidences of outbreeding depression were found at F_1 generation on hybrids between the two cytotypes and drastic loss of fitness at F_2 generation confirmed a reproductive isolation between the hexaploid *Saxifraga rosacea* subsp. *sponhemica* and the octoploid *Saxifraga rosacea* subsp. *rosacea*. Taxonomy of the two *Saxifraga* as subspecies should be revised, the two cytotypes should be established as two different species and their respective area of distribution should be reviewed. Taxonomical traits should also be reevaluated as two of three leaves morphological traits used in previous literature showed too important variations to remain reliable taxonomical criterion. It would be essential to reexamine the legal protection status of the two *Saxifraga* and to set up appropriate management plans. Artificial gene flow among the isolated populations of each cytotype could be considered to counter signs of inbreeding depression revealed by the hybrid vigor of between population crosses compared to within population crosses. Also the low performances of hybrids in the F_2 generation could explain the absence of known mixed population thus mixing populations of the two cytotypes could subvert the conservation of wild populations of the two *Saxifraga*.

Overall, the results of this thesis explore the performances of plants with different ploidy levels and provide new insights on the role of polyploidization in increasing the evolutionary potential of plant species to adapt to changing environmental conditions. It highlights the importance of using integrative approaches by combining ecological, demographical and taxonomical studies to establish appropriate management plans for the conservation of rare and endangered closely related plant species.

ZUSAMMENFASSUNG

ZUSAMMENFASSUNG

Es wurden zahlreiche Modelle vorgeschlagen, um zu erklären, wie neue Arten entstehen. Der Klimawandel ist einer der Mechanismen, von dem bekannt ist, dass er einen starken Selektionsdruck ausübt und die Wahrscheinlichkeit der Artbildung erhöht. Während des Quartärs haben die Gletschervorstöße und -rückzüge die Landschaften neu gestaltet, indem sie die geografischen Verbreitungsgebiete von Pflanzen und Tieren veränderten. Die Verbreitungsgebiete waren dramatischen Umweltveränderungen ausgesetzt, die die Speziationsrate aufgrund von divergenter Selektion, genetischer Drift oder Isolation der Populationen beschleunigt haben könnten. Bei Pflanzenarten könnte die Unfähigkeit, schnell mit drastischen klimatischen Raumbewegungen zu wandern, Genommodifikationen wie Polyploidisierung, ein bekannter abrupter Modus der Speziation bei Angiospermenarten durch Genomduplikation, begünstigt haben. Eine höhere Anzahl von Genomkopien und damit ein vielfältigerer Satz von Allelen könnte Vorteile bieten, um mit härteren klimatischen Bedingungen zurechtzukommen, und so zur Artdifferenzierung von Pflanzen mit unterschiedlichen Zytotypen beitragen. In dieser Arbeit werden die ökologischen, demographischen und taxonomischen Unterschiede zwischen den beiden Zytotypen von *Saxifraga rosacea* mit unterschiedlichen Verbreitungsgebieten und unterschiedlichen Ploidiegraden untersucht: der hexaploiden *Saxifraga rosacea* subsp. *sponhemica* und der oktoploiden *Saxifraga rosacea* subsp. *rosacea*.

Die Untersuchung von 22 Populationen der beiden eng verwandten *Saxifraga*-Zytotypen zeigte, dass Pflanzen des oktoploiden Zytotyps an Standorten mit niedrigerer Jahresmitteltemperatur vorkamen und kältetoleranter waren als Pflanzen des hexaploiden Zytotyps. Außerdem schnitten die Pflanzen in einem Transplantationsexperiment mit kontrastierenden Umweltbedingungen in ihrer Herkunftsregion besser ab, was auf eine lokale Anpassung hinweist. Die genetischen Unterschiede in der Überlebensrate waren zwischen den Populationen des oktoploiden Zytotyps größer als zwischen den Populationen des hexaploiden Zytotyps, was darauf hindeutet, dass eine höhere genetische Variabilität es S. rosacea ermöglicht, ein größeres Verbreitungsgebiet zu besetzen. Dies stimmte mit den Vorkommenswahrscheinlichkeiten gemäß unserer Maxent-Nischenmodellierung überein. Unsere Ergebnisse deuten darauf hin, dass Unterschiede im Zytotyp zu einer Segregation der Verbreitung führen können, und dass ein höherer Ploidiegrad bei *S. rosacea* dieses Taxon in die Lage versetzen kann, eine breitere ökologische Nische zu besetzen.

Unterschiede in der geografischen Verteilung und der Habitatfragmentierung können Auswirkungen auf die Populationsdynamik, wie z. B. die Populationswachstumsrate oder die

Populationsstruktur, und damit auf das Aussterberisiko der beiden Zytotypen haben, was dann möglicherweise spezielle Erhaltungspläne erfordert. Die Analyse der Variabilität und der demographischen Dynamik mehrerer *Saxifraga sponhemica*- und *Saxifraga rosacea*-Populationen über mehrere Jahre hinweg zeigte einen starken Rückgang der Populationswachstumsraten beider *Saxifraga*-Taxa. Allerdings war dieser negative Trend bei den Populationen von *Saxifraga sponhemica* stärker ausgeprägt als bei den Populationen von *Saxifraga rosacea*. Trotz der jahrzehntelangen Langlebigkeit beider *Saxifraga*-Taxa und ihrer vergleichbaren Populationsstruktur mit wenigen jungen und wenigen alten Individuen könnten die beiden Zytotypen unterschiedliche Schicksale erfahren. Analysen ihrer Populationsstrukturen zeigten, dass unter den gegenwärtigen klimatischen Bedingungen die oktoploiden Populationen stabil bleiben, die hexaploiden Populationen jedoch aufgrund der geringen Fekundität abnehmen werden. Die Analyse der Lebensfähigkeit der Populationen zeigte, dass das Aussterberisiko für die Populationen beider *Saxifraga*-Zytotypen hoch ist, und dass ein Bedarf an spezifischen Management- und Erhaltungsprogrammen besteht. Unsere Ergebnisse unterstützen die Hypothese, dass zwei eng verwandte Zytotypen mit ähnlichen Habitat- und Demographie-Charakteristika bei anhaltenden Klimaveränderungen ein unterschiedliches Schicksal haben können. Detaillierte demographische Studien sind wichtig, um geeignete Erhaltungsmaßnahmen für fragmentierte Populationen von eng verwandten seltenen und gefährdeten Pflanzenarten festzulegen.

Das Management von isolierten Populationen gefährdeter Arten beinhaltet oft Studien zur genetischen Vielfalt und zum Genfluss. Aber auch die Klärung der Taxonomie sollte in Betracht gezogen werden, insbesondere bei eng verwandten Taxa. Wir untersuchten die Auswirkungen von Kreuzungen innerhalb und zwischen Populationen, Hybridisierung und Rückkreuzungen der beiden Saxifraga-Zytotypen auf die reproduktive Fitness, die Leistungen und die taxonomischen Merkmale von zwei Generationen von Nachkommen. Bei Hybriden zwischen den beiden Zytotypen wurden in der F1-Generation Anzeichen für eine Auszuchtdepression gefunden, und ein drastischer Verlust der Fitness in der F2-Generation bei Hybriden bestätigte eine reproduktive Isolation zwischen der hexaploiden *Saxifraga rosacea* subsp. *sponhemica* und der oktoploiden *Saxifraga rosacea* subsp. *rosacea*.

Der aktuelle taxonomische Status der beiden *Saxifraga*-Taxa als Unterarten sollte überarbeitet werden. Wir schlagen vor, die beiden Zytotypen als zwei verschiedene Arten zu betrachten und ihre jeweiligen Verbreitungsgebiete entsprechend zu überprüfen. Taxonomische Merkmale, die eine Unterscheidung der beiden Taxa erlauben, sollten ebenfalls neu bewertet werden, da zwei von drei in der Literatur verwendeten

blattmorphologischen Merkmalen eine zu große Variation aufweisen, um als zuverlässige taxonomische Kriterien zu gelten. Infolgedessen wird es unerlässlich sein, den rechtlichen Schutzstatus der beiden Saxifraga-Taxa erneut zu überprüfen und entsprechende Managementpläne aufzustellen. Künstlicher Genfluss zwischen den isolierten Populationen jedes Zytotyps könnte in Betracht gezogen werden, um Anzeichen von Inzuchtdepression entgegenzuwirken, die sich in der Hybridstärke von Kreuzungen zwischen Populationen im Vergleich zu Kreuzungen innerhalb von Populationen zeigt. Die geringe Leistung der Hybriden in der F2-Generation könnte das Fehlen bekannter Mischpopulationen erklären, so dass das Mischen von Populationen der beiden Zytotypen die Erhaltung der Wildpopulationen beider Saxifraga-Taxa untergraben könnte.

Insgesamt zeigen die Ergebnisse dieser Doktorarbeit die Leistungen zweier eng verwandter Pflanzentaxa mit unterschiedlichem Ploidiegrad und lieferten neue Erkenntnisse über die Rolle der Polyploidisierung bei der Erhöhung des evolutionären Potenzials von Pflanzenarten zur Anpassung an sich ändernde Umweltbedingungen. Außerdem wurde die Bedeutung der Verwendung integrativer Ansätze durch die Kombination ökologischer, demographischer und taxonomischer Studien hervorgehoben, um geeignete Managementpläne für die Erhaltung seltener und gefährdeter eng verwandter Pflanzenarten zu erstellen.

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ERKLÄRUNG

Erklärung

Ich versichere, dass ich meine Dissertation

“Similar but not identical:cryptic speciation of Saxifraga rosacea”

selbständig, ohne unerlaubte Hilfe angefertigt und mich dabei keiner anderen als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe.

Die Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.