






RESEARCH ARTICLE

Gene flow effects on populations inhabiting marginal areas: Origin matters

Javier Morente-López¹  | Carlos Lara-Romero^{1,2}  | Alfredo García-Fernández¹  |
María Luisa Rubio Teso¹  | Samuel Prieto-Benítez^{1,3} | José María Iriondo¹ 

¹Área de Biodiversidad y Conservación, Dpto. de Biología, Geología, Física y Química Inorgánica, ESCET, Universidad Rey Juan Carlos, Móstoles, Spain

²Ecology Area. Life Science Department, University of Alcalá (UAH), Alcalá de Henares, Spain

³Ecotoxicology of Air Pollution Group, Environmental Department, CIEMAT, Madrid, Spain

Correspondence

Javier Morente-López
Email: javimorente@gmail.com

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Abstract

1. The evolutionary potential of populations inhabiting marginal areas has been extensively debated and directly affects their conservation value. Gene flow is one of the main factors influencing selection, adaptive potential and thus, local adaptation processes in marginal areas. The effects of differential gene flow provenance are still not well understood, since studies on gene flow between marginal populations have been underrepresented in the literature. This kind of gene flow can be especially beneficial because it can provide both adaptive allelic combinations originated under similar environmental conditions and genetic variation on which selection can act.
2. We conducted a study on the effects of different gene flow provenance on marginal populations of Mediterranean alpine *Silene ciliata* Pourret (Caryophyllaceae) replicated in three mountain ranges of Central Spain. The delineation of optimal and marginal areas of the species distribution was based on environmental differentiation and relied on the ecological definition of centrality and marginality. We experimentally tested the effect of three different types of pollen-mediated gene flow on germination rate, seedling size and survival rate in marginal populations and assessed their effects by establishing in situ common gardens. To further assess the evolutionary potential of marginal populations, we performed a reciprocal sowing experiment and measured the same fitness components to determine the extent of local adaptation.
3. We found that gene flow between marginal populations improved germination rate and seedling survival with regard to gene flow from optimal to marginal populations and within marginal populations. In reciprocal sowing experiments, seedling survival rate was higher when the seed source was from marginal areas than when it was from optimal areas in both marginal and optimal sowing sites.
4. *Synthesis.* Our results suggest that gene flow between marginal populations from similar environmental conditions increases the fitness of the recipient population by increasing genetic diversity and simultaneously providing adaptive alleles generated under similar selective pressures. Results also highlight the adaptive potential of marginal populations as genetic diversity from marginal areas may provide a fitness advantage to the populations in optimal areas. In this context, the adaptive

value of marginal populations increases their relevance and potential use in conservation management.

KEYWORDS

adaptation, conservation value, ecological gradients, gene flow, in situ common garden, marginal populations, reciprocal plantings

1 | INTRODUCTION

Climate change is causing species to experience new environmental pressures, leading to changes in species distributions and affecting species ecological niches (Davis & Shaw, 2001; Lenoir, Gégout, Marquet, De Ruffray, & Brisse, 2008; Walther et al., 2002). At the heart of theoretical relationships between niche and distribution, it is well known that populations inhabiting different locations inside the species range may experience environmental variability that causes fundamental demographic, genetic and phenotypic differences among them (Hardie & Hutchings, 2010; Pironon et al., 2017; Soule, 1973). Several authors have defined central and marginal areas inside species distributions based on environmental variability (e.g. Hargrove & Rotenberry, 2011; Pironon et al., 2017; Pouget et al., 2013), although their delimitation and characteristics are still a topic of great interest and debate in the literature (see review by Pironon et al., 2017). Central areas can be assigned to sites with the environmental conditions where the species populations are most frequently found, as environmental conditions in these areas are presumably optimal for the species. Similarly, marginal areas can be assigned to those that correspond to the environmental conditions where the species is less frequently found and are probably harsher for the species (Brown, 1984; Soule, 1973). These populations could be expected to be smaller, less abundant and more fragmented than central populations (Kawecki, 2008; Pironon et al., 2017). Effects of climate change on natural populations may vary depending on whether they occur in optimal or marginal areas of the species distribution and cause stronger selective pressures under marginal environmental conditions (Kawecki, 2008; Sexton, McIntyre, Angert, & Rice, 2009).

The conservation value of populations inhabiting marginal areas at distribution limits has been extensively debated (Abeli & Orsenigo, 2018; Hunter & Hutchinson, 1994; Lesica & Allendorf, 1995; Millar & Libby, 1991; Papuga, Gauthier, Pons, Farris, & Thompson, 2018) and, to a large extent, depends on their evolutionary potential (Vucetich & Waite, 2003). Historically, populations inhabiting marginal areas have been considered genetically impoverished, and thus maladapted, due to genetic drift and inbreeding processes, becoming of little significance in terms of evolutionary potential (Eckert, Samis, & Loughheed, 2008; Lande, 1994; Lynch & Gabriel, 1987; Whitlock, 2003; Whitlock, Ingvarsson, & Hatfield, 2000). However, these populations can also have adaptive value and be especially valuable in a climate change context. If marginal populations maintain a substantial standing genetic variation, they can adaptively diverge from populations

inhabiting optimal areas (Barrett & Schluter, 2007; Hoffmann & Sgró, 2011), playing an important role in the generation and maintenance of biological diversity (Channell & Lomolino, 2000). In this context, the origin and distribution of adaptive alleles inside the species range and, thus, its probability of emergence in marginal populations, directly affect the conservation value of these populations (Rolland, Lavergne, & Manel, 2015).

Divergent selection strength, genetic variation availability and gene flow are the three factors that have the greatest influence on adaptation potential in marginal populations (Blanquart, Kaltz, & Gandon, 2013; Hoffmann & Sgró, 2011; Kawecki & Ebert, 2004). When selection is spatially heterogeneous, local adaptation patterns are expected to emerge (Blanquart et al., 2013). In this context, the stronger divergent selection is, the higher the intensity of local adaptation will be. The availability of standing genetic variation and the ability of new alleles to arise and remain within populations directly influence successful local adaptation (Hancock et al., 2012). Gene flow is one of the main factors influencing divergent selection and local adaptation processes (Eckert et al., 2008; Hoffmann & Sgró, 2011; Holt & Gomulkiewicz, 1997; Sexton, Strauss, & Rice, 2011), although its effects on marginal population performance and adaptation are still under discussion and underrepresented in the literature (Sexton et al., 2011). Theory on the evolution of distribution limits predicts that gene flow from large optimal populations to marginal populations can provide marginal populations with a large number of maladaptive genes that hinder adaptation and, thus, niche expansion or preservation (Bridle & Vines, 2006; Garcia-Ramos & Kirkpatrick, 1997; Lenormand, 2002; Sexton et al., 2009). Alternatively, gene flow from optimal to marginal populations may increase effective population size and reduce genetic diversity loss in marginal populations, enhancing biological efficacy (Eckert et al., 2008; Hardie & Hutchings, 2010; Lesica & Allendorf, 1995; Ohsawa & Ide, 2008). In this way, gene flow can even contribute to the expansion or preservation of the population range and the emergence of local adaptation processes (Alleaume-Benharira, Pen, & Ronce, 2006; Barton, 2001; Hämälä & Savolainen, 2019; Holt, Gomulkiewicz, & Barfield, 2003; Sexton et al., 2011). As a result of these contrasting processes, mediated by the environmental and genetic context, metapopulations can simultaneously exhibit significant variation in inbreeding depression, heterosis and outbreeding depression among their populations (Escobar, Nicot, & David, 2008; Ronce, Shaw, Rousset, & Shaw, 2009).

An aspect still poorly studied is the effect of gene flow between marginal populations, which can be especially beneficial because it can provide both adaptive allelic combinations originated under similar environmental conditions and genetic variation on which selection can act (Sexton et al., 2011). It is also worthy to note that the origin and distribution of adaptive alleles inside species ranges remain largely unclear (Feldman, Brodie, Brodie, & Pfrender, 2009; Rolland et al., 2015). This aspect is a crucial point, as the location of the emergence of novel adaptive alleles (i.e. in optimal or marginal areas) directly affects adaptive alleles distribution within the species range (Rolland et al., 2015) and thus, gene flow effects and local adaptation patterns (Fedorka, Winterhalter, Shaw, Brogan, & Mousseau, 2012).

To fill this gap and provide further insight into this subject, we experimentally tested the effects of three different types of pollen-mediated gene flow (within marginal populations, and between marginal–marginal and optimal–marginal populations) on marginal populations of *Silene ciliata* Pourr. (Caryophyllaceae), a Mediterranean alpine plant species. To complement this study, we also carried out a local adaptation test by implementing reciprocal sowing experiments. This research is essential because experimental studies jointly testing gene flow provenance effects and local adaptation of marginal populations are scarce despite the importance and close relatedness of these two processes (but see Sexton et al., 2011).

Alpine ecosystems have highly variable environmental conditions at the local scale (i.e. small changes in elevation can cause great changes in temperature, humidity, exposure and other variables; Hovenden & Vander Schoor, 2003); consequently, divergent selection pressures can also greatly vary at short distances (Herrera & Bazaga, 2008). In geographically close but environmentally distant areas, gene flow can play a key role in the evolutionary potential of marginal populations. Declining growth rates and local extinction risk have been found in *S. ciliata* populations inhabiting low elevation range limit areas, in contrast with the stable growth rate trend found in populations at higher elevations (Giménez-Benavides, Albert, Iriondo, & Escudero, 2011; Lara-Romero, Robledo-Arnuncio, García-Fernández, & Iriondo, 2014). Potential adaptive value of populations at the low elevation range limit has been also found (García-Fernández, Iriondo, & Escudero, 2012; Giménez-Benavides, Escudero, & Iriondo, 2007a). Taken together, *S. ciliata* is a suitable case study that allows us to test the effects of gene flow on marginal populations. We hypothesized that the heterogeneous environmental conditions found in Mediterranean alpine environments promote divergent selection that generates adaptation patterns. We predicted that: (a) populations inhabiting marginal areas will be better adapted to face the environmental conditions that occur in such habitats, (b) gene flow from other populations will reduce inbreeding and the genetic load of target populations and, thus, cause an improvement in fitness and (c) gene flow from populations inhabiting marginal habitats and thus, experiencing the same environmental conditions, will provide greater fitness to marginal populations than gene flow from populations inhabiting optimal environmental conditions.

2 | MATERIALS AND METHODS

2.1 | Study species

Silene ciliata Pourr. (Caryophyllaceae) is a dwarf cushion perennial plant, which inhabits mountain ranges of the northern Mediterranean area from Portugal to Bulgaria (see Kyrkou et al., 2015; Tutin et al., 1964), reaching its southernmost limit in the Sistema Central of the Iberian Peninsula. It is pollinated by diurnal insects and nocturnal moths (Giménez-Benavides, Escudero, & Iriondo, 2007b). Seeds are dormant and need cold stratification to germinate (García-Fernández, Escudero, Lara-Romero, & Iriondo, 2014). Seed germination occurs just before snowmelt mainly at the end of April and May (Lara-Romero et al., 2014). In addition, the seedling stage in this species has been documented to be strongly affected by great selective pressures (García-Fernández, Iriondo, et al., 2012; Giménez-Benavides et al., 2007a; Lara-Romero, García-Fernández, et al., 2016). *Silene ciliata* has been well studied with regard to physiology, phylogeography, demographic history, phenotypic genetic differentiation and landscape genetics (García-Fernández, Segarra-Moragues, et al., 2012; Giménez-Benavides, Albert, et al., 2011; Giménez-Benavides, García-Camacho, et al., 2011; Giménez-Benavides et al., 2007b; Kyrkou et al., 2015; Lara-Romero, de la Cruz, et al., 2016; Lara-Romero, García-Fernández, et al., 2016; Morente-López et al., 2018).

2.2 | Study region and source populations

The Sistema Central is an approximately 500 km long West–East oriented mountain range located in the centre of the Iberian Peninsula. It is composed by three main southwest–northeast oriented mountain ranges: Béjar, Gredos and Guadarrama. *Silene ciliata* populations from the Sistema Central have the same phylogenetic origin (Kyrkou et al., 2015). In these areas, the species grows in dry cryophilic pastures above the tree line (Rivas-Martínez, Fernández-González, Sánchez-Mata, & Pizarro, 1990) from 1,850 m a.s.l. to the highest mountain peaks (c. 2,600 m a.s.l.). This Mediterranean Alpine ecosystem presents marked and sharp environmental gradients characterized by strong daily and seasonal temperature fluctuations, a long period of snow cover and a pronounced summer drought combined with high solar radiation which induces typical xerophilic characteristics in the inhabiting species (Rivas-Martínez et al., 1990).

Environmental variation within the distribution area of *S. ciliata* in the Sistema Central was previously studied by modelling potential habitat suitability (hereafter, HS) using the MAXENT algorithm (Phillips, Anderson, & Schapire, 2006). Potential HS was based on the occurrence probability of this species in each mountain range (Morente-Lopez et al., 2020). We defined optimal and marginal environments based on the environmental differentiation summarized by HS and relying on the ecological definition of centrality and marginality (Pironon et al., 2017; Soule, 1973). The 'optimal' category was assigned to the areas with HS values in the highest 33rd percentile of the distribution, whereas the 'marginal' category was

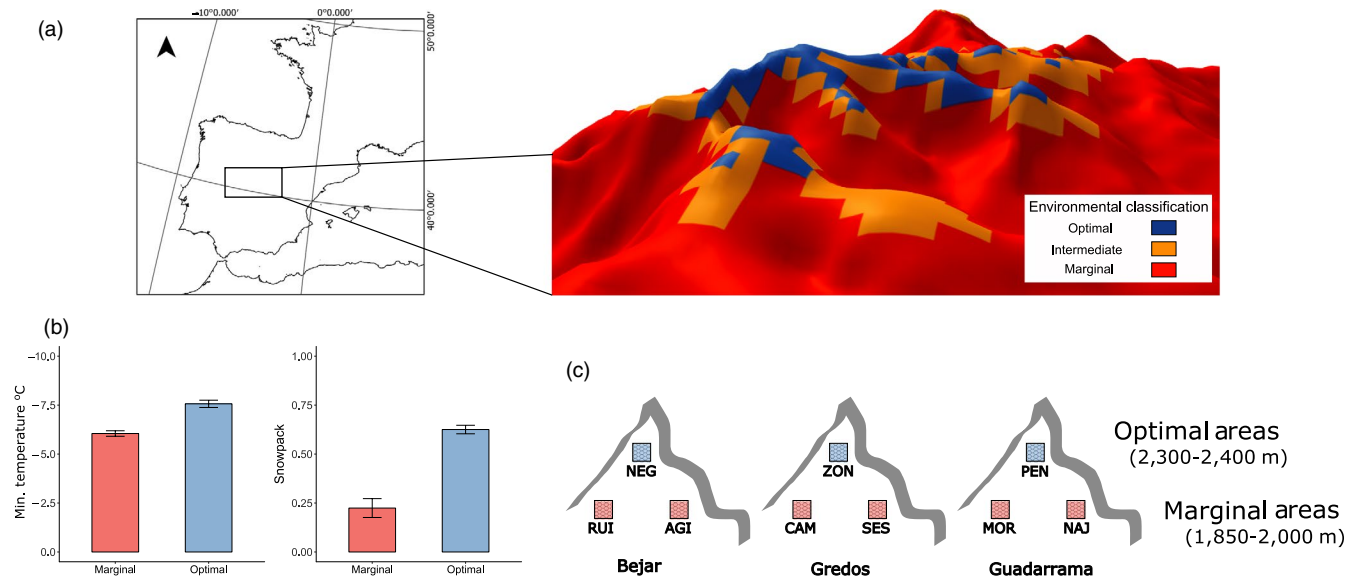


FIGURE 1 (a) *Silene ciliata* ecological classification in optimal, intermediate and marginal areas in Guadarrama mountain range (Sistema Central of the Iberian Peninsula). The classification was made by modelling the potential habitat suitability of the territory using the MAXENT algorithm, categorizing habitat suitability values into optimal, intermediate and marginal classes and projecting them in the space (see Appendix 1; Morente-Lopez et al., 2020). (b) Environmental differences between optimal and marginal areas regarding the main environmental variables (minimum annual temperature and snowpack accumulation in thaw months; February, March and April). (c) Representation of the three mountain ranges and the six populations used in the experiments located in optimal and marginal areas. Abbreviations and additional information about populations used for the experiments are specified in Table 1

composed of the areas with HS values in the lowest 33rd percentile of the distribution. The distribution was generated from 120 *S. ciliata* occurrence records in the Sistema Central obtained from the Global Biodiversity Information Facility (www.GBIF.org, accessed up to October 2013). Optimal and marginal populations essentially correspond to those that occur at the high and low edge of the elevation range, respectively, and large climatic differences were found between those populations (Figure 1a,b and see Appendix 1). This classification is congruent with demographic trends obtained by previous studies in populations in these two areas (Giménez-Benavides, Albert, et al., 2011; Lara-Romero, de la Cruz, et al., 2016; Lara-Romero et al., 2014). Ground-truthing of the model was made by prospecting populations along the HS gradient in the three mountain ranges of study. We selected nine populations of *S. ciliata*, one population located in an optimal area and two populations in marginal areas for each of the three mountain ranges of the Sistema Central (Table 1; Figure 1c).

2.3 | Effective population size and phenotypic diversity of studied populations

We estimated effective population size (N_e) as a measure closely related to the evolutionary potential of each population using the VarEff method (Variation of Effective size) implemented in the R package 'VAREFF' (Cornuet & Luikart, 1996; Nikolic & Chevalet, 2014). The method estimates present and historical effective population size from microsatellite markers by resolving coalescence theory and using

approximate likelihoods in a Monte Carlo Markov Chain approach (Nikolic & Chevalet, 2014). Genetic characterization of 20 individuals of each of the nine studied populations with eight microsatellite markers was obtained from Morente-López et al. (2018; see Appendix 2 for more details). We also estimated the phenotypic diversity of each population as a measure of available adaptive genetic diversity using the multivariate metrics Rao's quadratic entropy (RaoQ; Botta-Dukát, 2005) and data of reproductive and phenology traits from Morente-López et al. (2019; see Table 1). In Morente-López et al. (2019) plants were grown under the same common garden conditions, and therefore the observed phenotypic diversity can be related to genetic diversity. RaoQ is a classical multi-trait diversity index that can be used to quantify phenotypic diversity at different ecological units from populations to taxa and communities (Carmona, de Bello, Mason, & Lepš, 2016). RaoQ was estimated using FD R package (Laliberté & Legendre, 2010).

2.4 | Gene flow experiment

2.4.1 | Ex situ common garden plant collection and gene flow experiment

At the end of the summer of 2013, we collected a rosette from at least 30 adult plants from each of the nine selected populations. We made cuttings of each rosette to generate a variable number of clones of each genet. Cuttings were treated with an organic root activator (Neudofix rooting, Neudorff) and planted in 88 ml plastic pots filled with commercial potting substrate enriched

TABLE 1 *Silene ciliata* populations of the Sistema Central (Spain) used in the gene flow and reciprocal sowing studies. For each population several geographical, environmental and genetic data are provided as well as sample sizes for each of the experiments developed in this study

Population	ID	Mountain range	Environment	Snow-pack	MinT (°C)	Latitude	Longitude	Elevation (m)	HS values	N _e	H _e	Phenotypic diversity	Gene flow experiment		Reciprocal sowing experiment	
													No. mothers	No. seeds sown	No. mothers	No. seeds sown
Canchal Negro	NEG	Béjar	Optimal	0.59	-7.2	40°20'19.97"N	5°41'22.27"W	2,360	0.80	166 (64,265)	0.72	0.039	—	—	32	994
Las Cimeras (ruinas)	RUI	Béjar	Marginal	0.27	-6.7	40°21'7.03"N	5°40'59.71"W	2,000	0.60	142 (102,193)	0.72	0.047	30	2,310	32	679
Pico El Aguila	AGI	Béjar	Marginal	0.19	-6.1	40°21'12.36"N	5°41'46.52"W	1,950	0.51	141 (104,188)	0.76	0.045	33	2,001	30	920
Altos del Morezón	ZON	Gredos	Optimal	0.62	-7.7	40°14'57.5"N	5°16'8.3"W	2,380	0.85	154 (73,229)	0.67	0.044	—	—	34	1,238
El Sestil	SES	Gredos	Marginal	0.18	-5.9	40°16'24.45"N	5°14'54.93"W	1,900	0.43	141 (89,193)	0.70	0.048	32	1,167	30	918
Los Campanarios	CAM	Gredos	Marginal	0.34	-6.0	40°15'42.63"N	5°12'55.74"W	2,000	0.61	137 (95,189)	0.68	0.048	25	1,183	33	921
Pico de Peñalara	PEÑ	Guadarrama	Optimal	0.67	-7.8	40°51'2.11"N	3°57'24.02"W	2,400	0.87	201 (73,599)	0.54	0.047	—	—	42	1,488
Najarra baja	NAJ	Guadarrama	Marginal	0.03	-5.9	40°49'23.46"N	3°49'52.53"W	1,850	0.30	205 (98,325)	0.65	0.036	32	1,941	40	968
Morrena Peñalara	MOR	Guadarrama	Marginal	0.33	-5.7	40°50'11.82"N	3°57'0.91"W	1,980	0.63	137 (93,187)	0.68	0.049	28	2,188	43	956
Total											180	10,790	316	9,082		

Abbreviations: Elevation, metres above sea level; Environment, environmental classification; H_e, expected heterozygosity (Morente-López et al., 2018); HS values, habitat suitability values; ID, identification of each population; MinT, minimum annual temperature. Latitude and Longitude, geographical coordinates; Mountain Range, mountain range of origin; N_e, effective population size and confidence interval in brackets; No. mothers and No. seeds sown, sample size expressed as the number of mother plants and seeds used for data analysis in both experiments; Phenotypic Diversity, phenotypic diversity estimated by the multivariate metrics Rao's quadratic entropy; Snowpack, snowpack accumulation values in thaw months.

with NPK (Klasmann®). The whole cutting collection was kept for 3 months in the Universidad Rey Juan Carlos CULTIVE laboratory greenhouse (690 m a.s.l.; <https://www.urjc.es/actualidad/noticias/948-laboratorio-de-cultivo-vegetal-cultive>) to let the cuttings root and grow. In January 2014, the resulting plants were taken outside of the greenhouse to the CULTIVE experimental field, so the plants could experience freezing temperatures to promote flowering and a more natural development. In March 2014, 2,200 plants of 330 different genets were transplanted into 2.5 L pots. In April 2014, we grouped plants of each population separately and isolated them from wild pollinators with net structures (white 10 × 16 thread/cm² net). Overall, plants were grown in common garden conditions for 7 months before the gene flow experiments started to minimize carry-over effects from the original environment related with the environmental conditions found at the natural populations (Bischoff & Müller-Schärer, 2010). The experiment provided homogeneous environmental conditions for all plants in terms of water availability and light exposure to minimize differential plastic responses. When plants started flowering in mid-April, we simulated gene flow in the six marginal populations by pollinating the stigmata of plants with mature anthers from different population origins. We performed three types of crosses summarized in Figure 2a: (a) within-population gene flow with pollen from the same marginal population (MM_{WP}), (b) between-population gene flow with pollen from the other marginal population of the same mountain range (MM_{BP}) and (c) between-population gene flow with pollen from the optimal population of the same mountain range (OM_{BP}).

2.4.2 | In situ common garden experiment and data collection

From each of the three gene flow treatments in each of the six marginal populations from the three different mountain ranges, we randomly selected 40 seeds from at least 25 different mothers. Therefore, the experimental design consisted of 40 seeds/mother × (a minimum of) 25 mothers/gene flow treatment × 3 gene flow treatments/population × 6 marginal populations amounting to over 14,400 seeds or over 2,400 seeds per population. In early autumn 2014, we sowed the seeds in plots located near the original marginal populations (Table 1). At each planting site, we removed the existing vegetation and the first 5 cm of soil, containing the roots and the potential *S. ciliata* seed bank, and added some soil from nearby areas. Each common garden experiment had a randomized block design with four blocks. Each block contained 10 seeds/mother × (a minimum of) 25 mothers/gene flow treatment × 3 gene flow treatments/mother involving over 600 seeds. Each lot of 10 seeds from the same mother was taken as a replicate unit for data analysis. Each block was a randomized copy, with the same number of seeds from the same mothers. During autumn, winter and early spring of 2014–2015, seeds experienced natural cold stratification in the field. In late spring of 2015, right after the snowmelt, seedling emergence was monitored. Six blocks were discarded in the first visit because they were damaged by soil cryoturbation and wild ungulate activities. We used a total of 10,790 seeds for data analysis (Table 1). We recorded seedling emergence, size and survival of the plantlets once a month from the end of May (after snowmelt) until the end of August. We stopped the surveys when <5% of the overall seedlings emerged were alive.

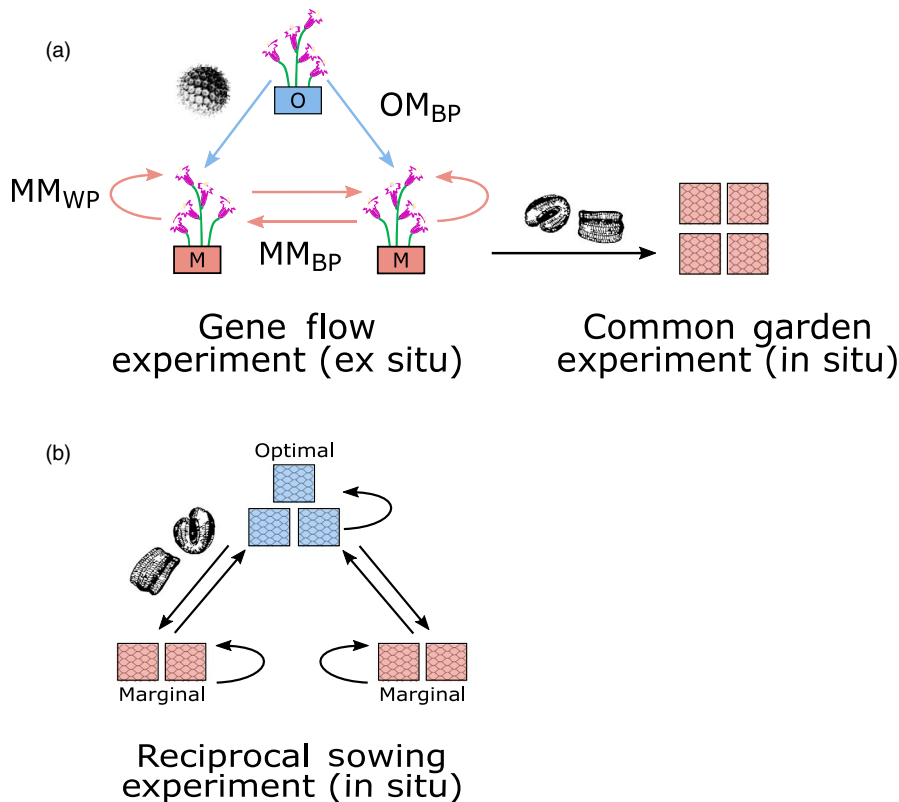


FIGURE 2 (a) Schematic representation of the gene flow experiment with the three different pollen treatments and the subsequent in situ common garden experiment. Coloured arrows represent the pollen-mediated gene flow treatments. MM_{BP} gene flow between two different marginal populations; MM_{WP} gene flow within marginal populations; OM_{BP} gene flow from optimal to marginal populations. The gridded squares represent the four blocks established in each marginal population where plantings took place. (b) Schematic representation of the reciprocal sowing experiment. Each arrow represents a different seeds translocation treatment. Gridded squares represent the experimental blocks established in each marginal and optimal population where sowings took place. As in Figure 1, red squares represent marginal areas and blue squares optimal areas

2.5 | Reciprocal sowing experiment

2.5.1 | Seed collecting, in situ sowings and data gathering

Seeds from a minimum of 30 plants were haphazardly collected in each of the six marginal and three optimal populations selected for this study at the end of summer 2016 (Table 1). Seeds were grouped by mother plant, cleaned and named accordingly. The seeds of each mother plant were reciprocally sowed in the population of origin and in the population with opposite environmental classification (optimal vs. marginal) from the same mountain range (see Figure 2b). For each sowing site, we prepared the soil as indicated in the gene flow experiment. A small grid for soil stabilization (DuPont™ Plantex® Groundgrid®, DuPont) was established in each sowing site to avoid substrate movement and loss. Moreover, a fence was established around the perimeter of the experiment to avoid wild herbivore disturbance. Two blocks were established in marginal sowing sites and three blocks in optimal sowing sites since the number of seeds sown in optimal areas was greater because seeds from three populations were sown. Each block contained 16 seeds/mother × (a minimum of) 25 mothers/population × 3 populations sown in optimal sowing sites or 2 populations sown in marginal sowing sites, involving over 800 and 1,200 seeds in marginal and optimal sowing sites, respectively. Thus, a total of over 9,000 seeds were sown. Two seeds were sown in each of the grid cells after they were filled with a 1:4 mix of sand and peat. Each set of seeds from the same mother was sown in the same row of eight cells of the grid and labelled. Cell rows with seeds from marginal populations were interposed with cell rows with seeds from optimal populations. Each cell row was used as a replicate unit for data analysis. Seeds experienced natural cold stratification during autumn, winter and early spring of 2016–2017. Seedling emergence was monitored right after the snowmelt. We used a total number of 9,082 seeds for data analysis (Table 1). We recorded seedling emergence, size and survival every 15 days from the end of April (after snowmelt) until the beginning of September when <5% of overall seedling emergence was alive at any of the sowing sites.

2.6 | Data analysis

We used the data collected in the two experiments to calculate the following variables: (a) germination rate as the proportion of seeds that germinated and emerged above ground; (b) seedling size as the maximum diameter of the rosette; (c) survival rate as the proportion of emerged seedlings that were alive on the date of the survey. For the gene flow experiment, these variables were analysed at the census before the one in which seedling survival was <5% for at least one gene flow treatment, population or mountain. Similarly, for the reciprocal sowing experiment, the variables were analysed at the census prior to the one in which seedling survival was <5% for at least one seed source, seed sowing site or mountain.

We used generalized and linear mixed models to analyse the data. We considered germination rate, seedling size and survival rate as response variables for the gene flow and reciprocal sowing experiments. For both experiments and each dependent variable, we followed a sequential approach. First, we optimized error distributions using the same full fixed effects structure (see Section 2.6.1). Then, once error distributions were optimized, we performed model selection for fixed effects. In all cases, model evaluation was made based on the Akaike information criterion (AIC) approach (Burnham & Anderson, 2002). Models with $\Delta\text{AIC} > 2$ relative to the best model were discarded, as they have less statistical support (Burnham & Anderson, 2002).

2.6.1 | Error distribution selection and random effect specification

Recent research recommends testing the relative fit of models made under different error distributions, such as binomial and beta-binomial, to deal with possible overdispersion and zero inflated error distributions concomitant to proportion data (Harrison et al., 2018; Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Following this approach, we used the methodology proposed by Brooks and collaborators and implemented in `GLMMTMB` R package (Brooks et al., 2017) to deal with possible overdispersion and zero inflated error distributions on germination rate and survival rate data. We fitted four models considering all combinations of binomial/beta-binomial and with/without zero inflated error distributions and maintaining the same full fixed effect structure explained below. Response variable transformation for all models was logit-link function. For seedling size, we selected between Gaussian error with and without log-link function following the same methodology. All models were fitted computing the full form of log-likelihood without dropping constant terms, which allows comparison of information criteria between different models with different error distributions (Brooks et al., 2017; Burnham & Anderson, 2002).

Mother plant and block within population and mountain were considered the complete random effect structure in all fitted models. However, models of germination rate and seedling survival rate often had convergence problems related to the inclusion of a random-effect variance that is estimated as zero (see Brooks et al., 2017). When convergence failed, random effects terms with intra-class correlation coefficient (ICC) equal to zero were removed. ICC was calculated by dividing between-group-variance (random intercept variance) by total variance (i.e. sum of between-group-variance and within-group residual variance; Lüdtcke, 2018). ICC can be interpreted as the proportion of the variance explained by the grouping random structure (Hox, 2002). Then, an ICC value of zero indicates that there is no variation in the response variable across levels of the random effect. ICCs were estimated using `SJSTATS` R package (Lüdtcke, 2018). Final model structures are specified in the results tables. Random effects structures were always consistent between the different error structures tested.

2.6.2 | Fixed-effect model selection

Maintaining the optimized error distribution and random structure, we fitted models with fixed effects combinations and their interactions based on a meaningful representation of the biological hypothesis of interest (Burnham & Anderson, 2002; Harrison et al., 2018).

For the gene flow experiment, we fitted models containing gene flow treatment (three levels: MM_{WP} , MM_{BP} and OM_{BP} ; see Figure 2a) and the mountain where the sowing experiments were done (three levels: Béjar, Gredos, Guadarrama) as fixed factors. We also included their interaction to test if the effect of gene flow origin was consistent among mountains. The resulting fitted models are summarized in Table A 3.1 in Appendix 3.

For the reciprocal sowing experiment, we fitted models containing combinations of seed source environment (two levels: Optimal and Marginal), sowing site environment (two levels: Optimal and Marginal) and mountain as fixed effects to analyse their effect on the response variables. To test if the effect of seed provenance was consistent between sowing sites and mountains, we included seed source interaction with each factor and the triple interaction. Final models designed for both experiments are summarized in Table A 3.2. in Appendix 3.

2.6.3 | Assessment of model performance

Model residuals were checked graphically for normality and homogeneity of variances using diagnostic plots (Zuur et al., 2009). Models were assessed for goodness-of-fit to the data using the marginal (R_m^2) and conditional (R_c^2) R^2 described by (Nakagawa & Schielzeth, 2013). R_m^2 represents the variance explained by fixed factors, while R_c^2 is interpreted as variance explained by both fixed and random factors (i.e. the entire model). We also calculated the Akaike weight (w_i) of each model as a proxy of model quality of adjustment (Burnham & Anderson, 2002).

3 | RESULTS

3.1 | Effective population size and phenotypic diversity of source populations

We found similar levels of effective population size (N_e) across populations ($M \pm SD N_e$: marginal = 150.5 ± 24.42 , optimal = 173.67 ± 24.42 ; non-parametric Wilcoxon rank sum test: $W = 11$, $p = 0.69$; Table 1). Moreover, the modelling of N_e found no evidence of significant changes over the last 200 generations (Table A 5.1 in Appendix 5), indicating the absence of historical events such as bottlenecks and expansions. We also found similar levels of within population phenotypic diversity (RaoQ) across populations (Table 1) and environments ($M \pm SD$ RaoQ: marginal = 0.041 ± 0.012 , optimal = 0.043 ± 0.04 ; non-parametric Wilcoxon rank sum test: $W = 3$, $p = 0.121$).

3.2 | Gene flow experiment

Around 18% of the sown seeds germinated under field conditions and only 19% of emerged seedlings had survived at the end of August (Table 2). The block within population and mountain was selected in the random structure for germination, survival rates and seedling size, while the mother plant was only selected for seedling size (Table 3). Germination rate presented a Beta-binomial structure with zero inflation, while survival rate adjusted better to a binomial model without zero-inflation structure. For germination and survival rates, only the model considering gene flow treatment and mountain as fixed factors, without any interactions, was selected (Table 3; Appendix 4 for model estimators). Germination rate of seeds from gene flow between marginal populations (MM_{BP}) was 7.2% higher than that obtained in seeds from gene flow within marginal populations (MM_{WP}) and 1.1% higher than that found in seeds from gene flow from optimal to marginal populations (OM_{BP} ; Figure 3a). Similarly, the survival rate of seeds from gene flow between marginal populations was 8.2% higher than that found in seeds from gene flow within marginal populations and

	Sowed seeds	Germinated seeds	Germination rate	Seedling size (cm)	Survival rate
Mountain					
Guadarrama	4,129	1,506	0.36 ± 0.01	4.94 ± 0.16	0.19 ± 0.01
Gredos	2,350	83	0.04 ± 0.01	5.57 ± 0.24	0.33 ± 0.06
Bejar	4,311	304	0.07 ± 0.01	5.63 ± 0.04	0.05 ± 0.01
GF treatment					
MM_{WP}	3,598	605	0.16 ± 0.01	5.57 ± 0.09	0.18 ± 0.02
MM_{BP}	3,629	654	0.18 ± 0.01	5.60 ± 0.07	0.19 ± 0.02
OM_{BP}	3,563	634	0.17 ± 0.01	5.30 ± 0.06	0.14 ± 0.02
Total	10,790	1,893			

TABLE 2 *Silene ciliata* seeds sowed and germinated in each mountain and for each gene flow treatment. Germination rate, seedling size and survival rate ($M \pm SE$)

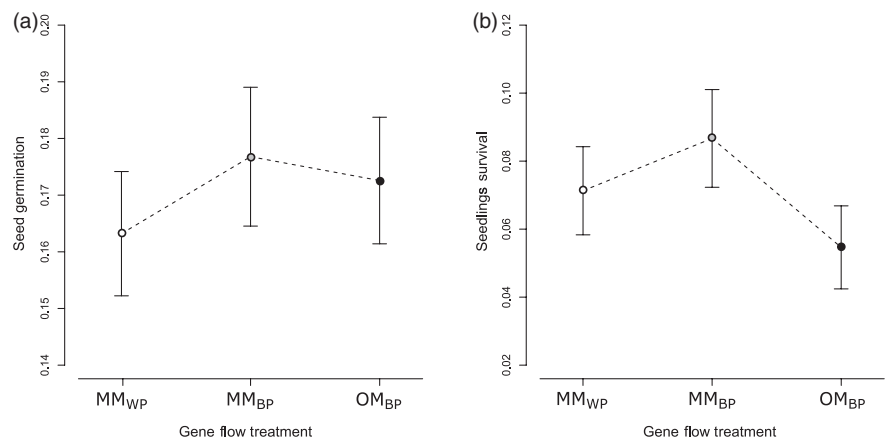
Abbreviations: GF treatment, gene flow treatment; MM_{BP} , gene flow between two different marginal populations; MM_{WP} , gene flow within marginal populations; OM_{BP} , gene flow from optimal to marginal populations.

TABLE 3 Set of best-ranked mixed models of the gene flow experiment (i.e. models with $\Delta\text{AICc} < 2$) fitted for germination rate, seedling size and survival rate

Model	df	log(L)	AICc	ΔAICc	Akaike weight (w_i)	R_m^2	R_c^2
1. Germination rate							
Germination rate ~ intercept + GF treatment + Mt	8	-1,498.35	3,012.7	0	0.81	0.28	0.37
Random effects structure selected: (1 Mt:Pop:Block). Error distribution selected: Betainomial with zero inflation logit-link function							
2. Seedling size							
Size ~ intercept	4	-564.99	1,138.0	0	0.87	0	0.29
Random effects structure selected: (1 Mother Plant) + (1 Mt:Pop:Block). Error distribution selected: Gaussian log-link function							
3. Survival rate							
Survival rate ~ intercept + GF treatment + Mt	6	-248.37	508.7	0	0.89	0.20	0.38
Random effects structure selected: (1 Mt:Pop:Block). Error distribution selected: Binomial logit-link function							

Abbreviations: ΔAICc , AICc differences; AIC, Akaike information criterion; GF treatment, gene flow treatment; Log(L), maximized log-likelihood function; Mt, mountain; Pop, population; w_i , Akaike weights.

FIGURE 3 Gene flow treatment differences in (a) seed germination rate and (b) seedling survival rate. MM_{BP} , gene flow between two different marginal populations; MM_{WP} , gene flow within marginal populations; OM_{BP} , gene flow between optimal and marginal populations. Error bars represent standard errors



35.8% higher than seeds from gene flow from optimal to marginal populations (Figure 3b). Germination and survival rates were substantially different in each mountain. These differences were particularly influenced by the high germination rate observed in Guadarrama and the low survival rate found in Béjar (Table 2). All selected models reached values of w_i above 0.8. R_c^2 were 0.37 and 0.38 for germination and survival rates, respectively, and the variance explained by fixed factors (R_m^2) represented a large fraction in both cases (76% and 63%, respectively). The null model considering only the intercept was selected when seedling size was analysed (Table 3).

3.3 | Reciprocal sowing experiment

Around 10% of the sown seeds germinated under field conditions and only 26% of the emerged seedlings had survived at the beginning of September (Table 4). Two models were selected for germination rate. The first model included seed source and mountain as fixed effects and the second also included sowing site without interactions (Table 5; Appendix 5 for model estimators). Seeds from optimal areas

had higher germination rates than seeds from marginal areas when they were sowed in both marginal and optimal sowing sites (Figure 4a). Nevertheless, differences in germination between seed source were greater in the marginal sowing site than in the optimal sowing site (19% vs. 2%, respectively, Figure 4a). R_m^2 values for these models reached values close to 0.1 (Table 5). Germination rate was again substantially different among mountains and was specially influenced by the lower germination values found in Gredos (Table 4). Regarding sowing sites, germination rates in optimal areas were lower than those found in marginal areas (Table 4).

The only model selected for survival rate was affected by seed source, sowing site, their interaction and a triple interaction of these two with mountain (Table 5). Seedlings originating from marginal areas had higher survival rates than seedlings from optimal areas when they were sown in both marginal and optimal sowing sites (32.4% and 66.8% higher, respectively, Figure 4b). With regard to sowing site, survival rate was higher in marginal areas than in optimal areas (Table 4; Figure 4b). Nevertheless, the pattern found was not always consistent in some of the sowing sites within mountains since the triple interaction was selected (Table 5). This inconsistency

	Seeds sown	Seeds germinated	Germination rate	Seedling size (cm)	Survival rate
Mountain					
Guadarrama	3,412	421	0.13 ± 0.01	8.81 ± 0.38	0.18 ± 0.02
Gredos	3,077	152	0.05 ± 0.01	7.35 ± 0.35	0.39 ± 0.05
Bejar	2,593	341	0.13 ± 0.01	6.30 ± 0.21	0.20 ± 0.03
Total	9,082	914			
Sowing site					
Optimal	3,720	285	0.08 ± 0.01	4.74 ± 0.32	0.08 ± 0.02
Marginal	5,362	629	0.12 ± 0.01	8.37 ± 0.22	0.30 ± 0.02
Total	9,082	914			

TABLE 4 Seeds sown and germinated, germination rate, seedling size and survival rate of *Silene ciliata* in the reciprocal sowing experiment measured in each mountain and in each sowing site ($M \pm SE$)

TABLE 5 Set of best-ranked mixed models of the reciprocal sowing experiment (i.e. models with $\Delta AICc < 2$) fitted for germination rate, seedling size and seedling survival rate

Model	df	log (L)	AICc	$\Delta AICc$	Akaike weight (w_i)	R_m^2	R_c^2
1. Germination rate							
Germination rate ~ intercept + SdSr + Mt	5	-252.83	515.7	0	0.30	0.08	0.14
Germination rate ~ intercept + SdSr + SwSt + Mt	6	-251.99	516.0	0.33	0.25	0.09	0.14
Random effects structure selected: (1 Mt:Pop:Block). Error distribution selected: Binomial logit-link function							
2. Seedling size							
Size ~ intercept + SdSr + SwSt + SdSr:SwSt + SdSr:SwSt:Mt	15	-2,329.90	4,689.8	0	0.99	0.006	0.009
Random effects structure selected: (1 Mother Plant) + (1 Mt:Pop:Block). Error distribution selected: Gaussian log-link function							
3. Survival rate							
Survival rate ~ intercept + SdSr + SwSt + SdSr:SwSt + SdSr:SwSt:Mt	13	-213.00	452.0	0	1.00	0.77	0.77
Random effects structure selected: (1 Mt:Pop:Block). Error distribution selected: Binomial logit-link function							

Abbreviations: $\Delta AICc$, AICc differences; AIC, Akaike information criterion; Log (L), maximized log-likelihood function; Mt: mountain; SdSr, seed source; SwSt, sowing site; w_i , Akaike weights.

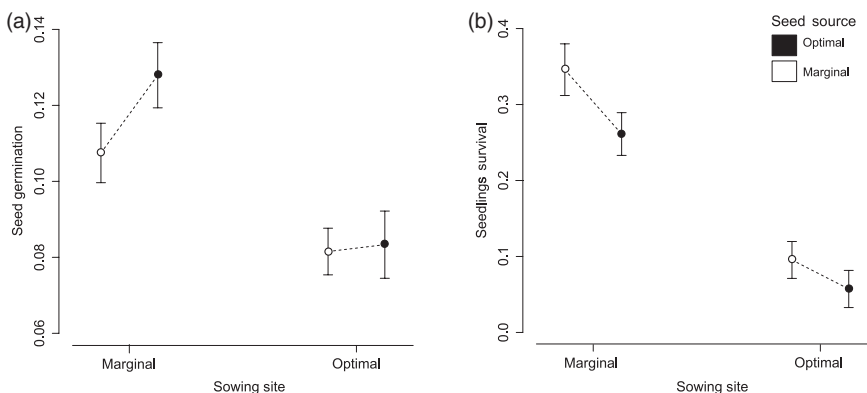


FIGURE 4 Seed source and sowing site differences in (a) seed germination rate and (b) seedling survival rate. Error bars represent standard errors

was explained by the lack of differences in survival rates between marginal and optimal seed sources in Gredos optimal sowing sites and in Béjar marginal sowing sites (Table A 6.1 in Appendix 6). When

seedling size was analysed, a model considering seed source, sowing size, their interaction and the triple interaction including mountain was selected, but its R_m^2 was close to zero (Table 5).

4 | DISCUSSION

4.1 | Gene flow effects on marginal populations

Although the effects of gene flow on adaptation have been extensively discussed from a theoretical perspective (Kawecki, 2008; Kawecki & Ebert, 2004; Sexton et al., 2009), the differential effect of gene flow depending on the environmental conditions existing at the source has been overlooked in the literature (Bontrager & Angert, 2018; Sexton et al., 2011; Yeaman & Jarvis, 2006). In our study, the commonly unconsidered gene flow between marginal populations (MM_{BP}) improved seedling survival compared to gene flow from optimal to marginal populations (OM_{BP}) and within marginal populations (MM_{WP}). This pattern was consistent with our third hypothesis, supporting the idea that gene flow between marginal populations provides greater fitness to marginal populations by increasing genetic variation and providing favourable alleles and/or genetic combinations that are potentially adaptive in such areas. This result is congruent with the few studies carried out so far on gene flow between marginal populations (Bontrager & Angert, 2018; Sexton et al., 2011), which reported similar findings.

The contrasting results between germination rate and survival rate obtained from the effect of gene flow from optimal to marginal populations (increase in germination vs. decrease in survival) compared to gene flow within marginal populations also support the theoretical grounds, suggesting that gene flow from large optimal populations to marginal populations can have two contrasting effects. The first one increases the fitness of marginal populations due to the increase of genetic diversity and the decrease of inbreeding depression (Alleaume-Benharira et al., 2006; Barton, 2001; Bridle & Vines, 2006; Sexton et al., 2009). The latter is especially important in the case of outbreeding species, such as *S. ciliata*, and in marginal populations with a small population size. The second one hinders adaptation to selective pressures occurring in marginal populations due to the contribution of maladaptive alleles or gene combinations (Kawecki, 2008; Kirkpatrick & Barton, 1997; Lenormand, 2002). In addition, progeny resulting from crosses between genetically distant individuals (outcrossing) can exhibit poor performance.

The different effects on germination and survival rates could be related to the different time of exposure to selective pressures and sensitivities to habitat stress experienced by seeds and seedlings (Halbritter, Billeter, Edwards, & Alexander, 2015; Körner, 2007). While seeds are exposed a shorter time to the environmental filter, seedlings are more exposed to existing environmental selective pressures. These selective pressures are even more remarkable if we consider the high mortality rates of this species in early life stages found in previous works (García-Fernández, Iriondo, et al., 2012; Giménez-Benavides et al., 2007a; Lara-Romero, de la Cruz, et al., 2016). This finding highlights the importance of discerning the provenance of gene flow in a context of divergent selection and adaptation (Sexton et al., 2011). It also highlights the importance

of measuring adaptive responses throughout the ontogeny of the species since selection forces may act with different strengths at different life stages and fitness components of organisms (Bontrager & Angert, 2018; Halbritter et al., 2015; Hoffmann & Sgró, 2011; Lara-Romero, de la Cruz, et al., 2016; Lara-Romero et al., 2017). Similarly, environmental stochasticity among years is an additional factor to consider that can condition adaptive processes and thus gene flow effects (Kawecki, 2008). In a previous study with the same species carried out in the Guadarrama mountain, gene flow from optimal to marginal populations also increased germination rates, but non-significant effects were found with regard to survival rate (García-Fernández, Iriondo, et al., 2012). However, in our gene flow experiment, precipitation was 100 mm lower during the May–September 2015 interval than the mean of the period between 1981 and 2010 (191 mm vs. 297 mm; data from Peñalara National Park and Spanish Meteorological Agency, AEMET). The higher-than-average drought experienced this year probably intensified selective pressures during our experiment, and, thus, it may have triggered the better performance of seedlings that received gene flow from marginal populations (i.e. populations that have evolved under drier and warmer conditions; Bontrager & Angert, 2018). It is difficult to single out the traits that are driving these plant survival and growth patterns, but phenological traits, root growth and morphological and physiological traits associated with drought tolerance may play a relevant role (Monneveux, Jing, & Misra, 2012; Savi, Love, Dal Borgo, Martellos, & Nardini, 2017).

Adaptive alleles capacity to emerge and remain in marginal populations is influenced by the strength of selective gradients between optimal and marginal populations and by population genetic characteristics (Barrett & Schluter, 2007). In this context, genetic diversity is one of the main factors defining population adaptive potential since it is closely linked with the standing genetic variation of populations to deal with selective pressures (Barrett & Schluter, 2007; Hoffmann & Sgró, 2011). Previous molecular characterization of the studied populations and the effective population size and phenotypic diversity values of this study indicate that marginal populations have similar genetic diversity levels than those found in optimal populations (Morente-López et al., 2018). Thus, in our Mediterranean alpine ecosystem, marginal populations may maintain enough standing genetic variation to develop adaptive processes. Furthermore, the presence of significant gene flow between marginal populations and between optimal and marginal populations across the elevation range within mountains has been documented (García-Fernández, Segarra-Moragues, et al., 2012; Morente-López et al., 2018). Nevertheless, the latter is constrained by differences in flowering phenology between optimal and marginal populations that reduce the overlap of the flowering period (Giménez-Benavides et al., 2007b; Morente-López et al., 2019). Hence, the beneficial admixture among marginal populations found in this study is feasible in nature and even favoured in the field when compared to gene flow from optimal to marginal populations. Previous studies in other species have also shown substantial levels of genetic diversity within local populations and

low levels of genetic differentiation along elevation gradients, probably related to the high occurrence of gene flow among populations (Davis & Shaw, 2001; Halbritter et al., 2015; Jump, Mátyás, & Peñuelas, 2009). Moreover, gene flow between populations could maintain genetic variance without hampering local adaptations (Yeaman & Jarvis, 2006).

4.2 | Adaptation processes in marginal populations

In the reciprocal sowing experiment, seedling survival rate was higher when the seed source (SdSr) was from marginal areas than when it was from optimal areas at both marginal and optimal sowing sites (SwSt). However, we should consider that the model selected for seedling survival rate also included the triple interaction between seed source, sowing site and mountain. Thus, our findings were context dependent since no differences in seedling survival rate were found between seed sources in some sowing sites in some mountains (Gredos mountain optimal SwSt and Béjar mountain marginal SwSt). On the other hand, the very low variance explained by the fixed factors of the models fitted for germination rates and seedling size (i.e. <10% in all models) denoted that seed source and sowing site had a very low effect on these variables.

As in the gene flow experiment, the stronger signal for seedling survival rate compared to the other traits can be related to the fact that adaptation responses are the most intense when they concern plant survival (Halbritter et al., 2018). In agreement with our first hypothesis, our seedling survival rate results suggest greater adaptive capacity to existing environmental conditions of marginal populations since seedlings from marginal seed sources responded better in both sowing sites. This finding supports previous research showing that differentiation and adaptation patterns can occur in elevation gradients between close populations, even in the presence of significant gene flow (Byars, Papst, & Hoffmann, 2007; Gonzalo-Turpin & Hazard, 2009). However, they do not conform to the concept of local adaptation sensu Kawecki and Ebert (2004). Under their local versus foreign definition of local adaptation, populations should perform better in their own habitat than translocated populations from other habitats, regardless of whether populations are in optimal or marginal areas (Blanquart et al., 2013; Kawecki & Ebert, 2004). Local adaptation experiments, such as the one carried out in this study, rely on the ability of the standing genetic variation of each population to respond to the environmental conditions occurring at the precise time and place where the experiment takes place (Galliart et al., 2019; Nadeau & Urban, 2019). The high mortality observed at the end of our study was also found in previous *in situ* seed sowing experiments conducted with the same species and in the same ecosystems (García-Fernández, Iriondo, et al., 2012; Giménez-Benavides et al., 2007a). These results denote the severity of environmental conditions that seeds and seedlings of Mediterranean alpine habitats have to confront.

5 | CONCLUSIONS

Our results support the idea that populations inhabiting marginal areas can generate valuable adaptive genetic combinations for the species (Sexton et al., 2011). In the southernmost populations of *Silene ciliata* in Central Spain, the optimal and marginal areas found along the elevation gradients are different enough in environmental terms to generate divergent selection and thus, differential adaptive processes. In our case study, the environmental conditions of the marginal areas can be considered to mimic environmental conditions in the optimal areas in future decades as a result of global warming. Thus, the adaptive processes that have already taken place in populations in the marginal areas may provide a fitness advantage to the populations at the optimal areas, as observed in our experiment in a particularly dry year.

The evolutionary potential and conservation value of marginal populations, although controversial (Abeli & Orsenigo, 2018; Lesica & Allendorf, 1995; Papuga et al., 2018), needs to be considered on a case by case basis, since the ecological originality of such areas can give populations the capacity to generate unique gene combinations (Papuga et al., 2018). Thus, environmentally marginal populations must be considered in conservation planning efforts. In those cases where marginal populations contain genetic combinations that provide an adaptive advantage under climate change conditions, assisted gene flow from these populations to other populations lacking these features may help increase the chances of a species to successfully adapt to climate change (Aitken & Whitlock, 2013).

Finally, our results highlight the importance of studying the genetically based phenotypic variation inside gradients in addition to molecular genetic diversity patterns in order to properly assess the evolutionary potential of marginal populations (Holderegger, Kamm, & Gugerli, 2006; Ohsawa & Ide, 2008).

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AUTHORS' CONTRIBUTIONS

J.M.-L., C.L.-R. and J.M.I. designed this study; J.M.-L. and C.L.-R. analysed the data; J.M.-L. wrote the paper with the help of C.L.-R. and J.M.I. All authors performed the field work, reviewed the paper and approved the final manuscript for publication.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/1365-2745.13455>.

DATA AVAILABILITY STATEMENT

The databases used in this study are freely available in figshare repository: <https://doi.org/10.6084/m9.figshare.11861886.v1> (Morente-López et al., 2020).

ORCID

Javier Morente-López  <https://orcid.org/0000-0001-9141-8581>

Carlos Lara-Romero  <https://orcid.org/0000-0003-0962-0567>

Alfredo García-Fernández  <https://orcid.org/0000-0002-7672-9872>

María Luisa Rubio Teso  <https://orcid.org/0000-0002-1019-2101>

José María Iriondo  <https://orcid.org/0000-0003-2710-3889>

REFERENCES

- Abeli, T., Vamosi, J. C., & Orsenigo, S. (2018). The importance of marginal population hotspots of cold-adapted species for research on climate change and conservation. *Journal of Biogeography*, *45*(5), 977–985. <https://doi.org/10.1111/jbi.13196>
- Aitken, S. N., & Whitlock, M. C. (2013). Assisted gene flow to facilitate local adaptation to climate change. *Annual Review of Ecology, Evolution, and Systematics*, *44*, 367–388. <https://doi.org/10.1146/annurev-ecolsys-110512-135747>
- Alleaume-Benharira, M., Pen, I. R., & Ronce, O. (2006). Geographical patterns of adaptation within a species' range: Interactions between drift and gene flow. *Journal of Evolutionary Biology*, *19*(1997), 203–215. <https://doi.org/10.1111/j.1420-9101.2005.00976.x>
- Barrett, R. D. H., & Schluter, D. (2007). Adaptation from standing genetic variation. *Trends in Ecology & Evolution*, *23*, 38–44. <https://doi.org/10.1016/j.tree.2007.09.008>
- Barton, N. H. (2001). Adaptation at the edge of a species' range. *Special Publication-British Ecological Society*, *14*, 365–392.
- Bischoff, A., & Müller-Schärer, H. (2010). Testing population differentiation in plant species – How important are environmental maternal effects. *Oikos*, *119*(3), 445–454. <https://doi.org/10.1111/j.1600-0706.2009.17776.x>
- Blanquart, F., Kaltz, O., Nuismer, S. L., & Gandon, S. (2013). A practical guide to measuring local adaptation. *Ecology Letters*, *16*, 1195–1205. <https://doi.org/10.1111/ele.12150>
- Bontrager, M., & Angert, A. L. (2018). Gene flow improves fitness at a range edge under climate change. *Evolution Letters*, *3*(1), 55–68. <https://doi.org/10.1002/evl3.91>
- Botta-Dukát, Z. (2005). Rao's quadratic entropy as a measure of functional diversity based on multiple traits. *Journal of Vegetation Science*, *16*(5), 533–540. <https://doi.org/10.1111/j.1654-1103.2005.tb02393.x>
- Bridle, J. R., & Vines, T. H. (2006). Limits to evolution at range margins: When and why does adaptation fail? *Trends in Ecology & Evolution*, *22*(3), 140–147. <https://doi.org/10.1016/j.tree.2006.11.002>
- Brooks, M. E., Kristensen, K., Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., ... Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*, 378–400. <https://doi.org/10.32614/RJ-2017-066>
- Brown, J. H. (1984). On the relationship between abundance and distribution of species. *The American Naturalist*, *124*(2), 255–279. <https://doi.org/10.1086/284267>
- Burnham, K., & Anderson, D. (2002). *Model selection and multi-model inference* (2nd ed.). New York, NY: Springer Verlag. Retrieved from https://cds.cern.ch/record/1608735/files/9780387953649_TOC.pdf
- Byars, S. G., Papst, W., & Hoffmann, A. A. (2007). Local adaptation and cogradient selection in the alpine plant, *Poa hiemata*, along a narrow altitudinal gradient. *Evolution*, *61*(12), 2925–2941. <https://doi.org/10.1111/j.1558-5646.2007.00248.x>
- Carmona, C. P., de Bello, F., Mason, N. W. H., & Lepš, J. (2016). Traits without borders: Integrating functional diversity across scales. *Trends in Ecology & Evolution*, *31*(5), 382–394. <https://doi.org/10.1016/j.tree.2016.02.003>
- Channell, R., & Lomolino, M. V. (2000). Dynamic biogeography and conservation of endangered species. *Letters to Nature*, *403*, 84–86. <https://doi.org/10.1038/47487>
- Cornuet, J. M., & Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, *144*(4), 2001–2014. <https://doi.org/10.1109/TAC.1974.1100705>
- Davis, M. B., & Shaw, R. G. (2001). Range shifts and adaptive responses to quaternary climate change. *Science*, *292*(5517), 673–679. <https://doi.org/10.1126/science.292.5517.673>
- Eckert, C. G., Samis, K. E., & Loughheed, S. C. (2008). Genetic variation across species' geographical ranges: The central-marginal hypothesis and beyond. *Molecular Ecology*, *17*(5), 1170–1188. <https://doi.org/10.1111/j.1365-294X.2007.03659.x>
- Escobar, J. S., Nicot, A., & David, P. (2008). The different sources of variation in inbreeding depression, heterosis and outbreeding depression in a metapopulation of *Physa acuta*. *Genetics*, *180*(3), 1593–1608. <https://doi.org/10.1534/genetics.108.092718>
- Fedorka, K. M., Winterhalter, W. E., Shaw, K. L., Brogan, W. R., & Mousseau, T. A. (2012). The role of gene flow asymmetry along an environmental gradient in constraining local adaptation and range expansion. *Journal of Evolutionary Biology*, *25*(8), 1676–1685. <https://doi.org/10.1111/j.1420-9101.2012.02552.x>
- Feldman, C. R., Brodie, E. D., Brodie, E. D., & Pfrender, M. E. (2009). The evolutionary origins of beneficial alleles during the repeated adaptation of garter snakes to deadly prey. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(32), 13415–13420. <https://doi.org/10.1073/pnas.0901224106>
- Gallart, M., Bello, N., Knapp, M., Poland, J., St Amand, P., Baer, S., ... Johnson, L. (2019). Local adaptation, genetic divergence, and experimental selection in a foundation grass across the US Great Plains' climate gradient. *Global Change Biology*, *25*(3), 850–868. <https://doi.org/10.1111/gcb.14534>
- García-Fernández, A., Escudero, A., Lara-Romero, C., & Iriondo, J. M. (2014). Effects of the duration of cold stratification on early life stages of the Mediterranean alpine plant *Silene ciliata*. *Plant Biology*, *17*(2), 344–350. <https://doi.org/10.1111/plb.12226>
- García-Fernández, A., Iriondo, J. M., & Escudero, A. (2012). Inbreeding at the edge: Does inbreeding depression increase under more stressful conditions? *Oikos*, *121*(9), 1435–1445. <https://doi.org/10.1111/j.1600-0706.2011.20219.x>
- García-Fernández, A., Segarra-Moragues, J. G., Widmer, A., Escudero, A., & Iriondo, J. M. (2012). Unravelling genetics at the top: Mountain islands or isolated belts? *Annals of Botany*, *110*(6), 1221–1232. <https://doi.org/10.1093/aob/mcs195>
- García-Ramos, G., & Kirkpatrick, M. (1997). Genetic models of adaptation and gene flow in peripheral populations. *Evolution*, *51*(1), 21. <https://doi.org/10.2307/2410956>
- Giménez-Benavides, L., Albert, M. J., Iriondo, J. M., & Escudero, A. (2011). Demographic processes of upward range contraction in a long-lived Mediterranean high mountain plant. *Ecography*, *34*(1), 85–93. <https://doi.org/10.1111/j.1600-0587.2010.06250.x>
- Giménez-Benavides, L., Escudero, A., & Iriondo, J. M. (2007a). Local adaptation enhances seedling recruitment along an altitudinal gradient in a high mountain mediterranean plant. *Annals of Botany*, *99*(4), 723–734. <https://doi.org/10.1093/aob/mcm007>
- Giménez-Benavides, L., Escudero, A., & Iriondo, J. M. (2007b). Reproductive limits of a late-flowering high-mountain Mediterranean

- plant along an elevational climate gradient. *New Phytologist*, 173(2), 367–382. <https://doi.org/10.1111/j.1469-8137.2006.01932.x>
- Giménez-Benavides, L., García-Camacho, R., Iriondo, J. M., & Escudero, A. (2011). Selection on flowering time in Mediterranean high-mountain plants under global warming. *Evolutionary Ecology*, 25(4), 777–794. <https://doi.org/10.1007/s10682-010-9440-z>
- Gonzalo-Turpin, H., & Hazard, L. (2009). Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *Journal of Ecology*, 97(4), 742–751. <https://doi.org/10.1111/j.1365-2745.2009.01509.x>
- Halbritter, A. H., Billeter, R., Edwards, P. J., & Alexander, J. M. (2015). Local adaptation at range edges: Comparing elevation and latitudinal gradients. *Journal of Evolutionary Biology*, 28(10), 1849–1860. <https://doi.org/10.1111/jeb.12701>
- Halbritter, A. H., Fior, S., Keller, I., Billeter, R., Edwards, P. J., Holderegger, R., ... Alexander, J. M. (2018). Trait differentiation and adaptation of plants along elevation gradients. *Journal of Evolutionary Biology*, 31, 784–800. <https://doi.org/10.1111/jeb.13262>
- Hämälä, T., & Savolainen, O. (2019). Genomic patterns of local adaptation under gene flow in *Arabidopsis lyrata*. *Molecular Biology and Evolution*, 36(11), 2557–2571. <https://doi.org/10.1093/molbev/msz149>
- Hancock, A. M., Brachi, B., Faure, N., Horton, M. W., Jarymowycz, L. B., Sperone, F. G., ... Bergelson, J. (2012). Adaptation to climate across the *Arabidopsis thaliana* genome. *Science*, 334(6052), 83–86. <https://doi.org/10.1126/science.1209244>
- Hardie, D. C., & Hutchings, J. A. (2010). Evolutionary ecology at the extremes of species' ranges. *Environmental Reviews*, 18, 1–20. <https://doi.org/10.1139/A09-014>
- Hargrove, L., & Rotenberry, J. T. (2011). Breeding success at the range margin of a desert species: Implications for a climate-induced elevational shift. *Oikos*, 120, 1568–1576. <https://doi.org/10.1111/j.1600-0706.2011.19284.x>
- Harrison, X. A., Donaldson, L., Correa-Cano, M. E., Evans, J., Fisher, D. N., Goodwin, C. E. D., ... Inger, R. (2018). A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ*, 6, 1–32. <https://doi.org/10.7717/peerj.4794>
- Herrera, C. M., & Bazaga, P. (2008). Population-genomic approach reveals adaptive floral divergence in discrete populations of a hawk moth-pollinated violet. *Molecular Ecology*, 17, 5378–5390. <https://doi.org/10.1111/j.1365-294X.2008.04004.x>
- Hoffmann, A. A., & Sgró, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, 470(7335), 479–485. <https://doi.org/10.1038/nature09670>
- Holderegger, R., Kamm, U., & Gugerli, F. (2006). Adaptive vs. neutral genetic diversity: Implications for landscape genetics. *Landscape Ecology*, 21, 797–807. <https://doi.org/10.1007/s10980-005-5245-9>
- Holt, R. D., & Gomulkiewicz, R. (1997). How does immigration influence local adaptation? A reexamination of a familiar paradigm. *The American Naturalist*, 149, 563–572. <https://doi.org/10.1093/past/69.1.132-a>
- Holt, R. D., Gomulkiewicz, R., & Barfield, M. (2003). The phenomenology of niche evolution via quantitative traits in a 'black-hole' sink. *Proceedings of the Royal Society B: Biological Sciences*, 270(1511), 215–224. <https://doi.org/10.1098/rspb.2002.2219>
- Hovenden, M. J., & Vander Schoor, J. K. (2003). Nature vs nurture in the leaf morphology of Southern beech, *Nothofagus cunninghamii* (Nothofagaceae). *New Phytologist*, 161, 585–594. <https://doi.org/10.1046/j.1469-8137.2003.00931.x>
- Hox, J. (2002). *Quantitative methodology series. Multilevel analysis techniques and applications*. Mahwah, NJ: Lawrence Erlbaum Associates Publishers.
- Hunter, M. L., & Hutchinson, A. (1994). The virtues and shortcomings of parochialism: Conserving species that are locally rare, but globally common. *Conservation Biology*, 8(4), 1163–1165. <https://doi.org/10.1046/j.1523-1739.1994.08041163.x>
- Jump, A. S., Mátyás, C., & Peñuelas, J. (2009). The altitude-for-latitude disparity in the range retractions of woody species. *Trends in Ecology & Evolution*, 24(12), 694–701. <https://doi.org/10.1016/j.tree.2009.06.007>
- Kawecki, T. J. (2008). Adaptation to marginal habitats. *Annual Review of Ecology, Evolution, and Systematics*, 39(1), 321–342. <https://doi.org/10.1146/annurev.ecolsys.38.091206.095622>
- Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, 7(12), 1225–1241. <https://doi.org/10.1111/j.1461-0248.2004.00684.x>
- Kirkpatrick, M., & Barton, N. H. (1997). Evolution of a species' range. *The American Naturalist*, 150(1), 1–23. <https://doi.org/10.1086/286054>
- Körner, C. (2007). The use of 'altitude' in ecological research. *Trends in Ecology & Evolution*, 22(11), 569–574. <https://doi.org/10.1016/j.tree.2007.09.006>
- Kyrkou, I., Iriondo, J. M., & García-Fernández, A. (2015). A glacial survivor of the alpine Mediterranean region: Phylogenetic and phylogeographic insights into *Silene ciliata* Pourr. (Caryophyllaceae). *PeerJ*, 3, 1–19. <https://doi.org/10.7717/peerj.1193>
- Laliberte, E., & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, 91(1), 299–305. <https://doi.org/10.1890/08-2244.1>
- Lande, R. (1994). Risk of populations extinction from fixation of new deleterious mutations. *Evolution*, 48(5), 1460–1469.
- Lara-Romero, C., de la Cruz, M., Escribano-Ávila, G., García-Fernández, A., & Iriondo, J. M. (2016). What causes conspecific plant aggregation? Disentangling the role of dispersal, habitat heterogeneity and plant-plant interactions. *Oikos*, 125(9), 1304–1313. <https://doi.org/10.1111/oik.03099>
- Lara-Romero, C., García-Fernández, A., Robledo-Arnuncio, J. J., Roumet, M., Morente-López, J., López-Gil, A., & Iriondo, J. M. (2016). Individual spatial aggregation correlates with between-population variation in fine-scale genetic structure of *Silene ciliata* (Caryophyllaceae). *Heredity*, 116(5), 417–423. <https://doi.org/10.1038/hdy.2015.102>
- Lara-Romero, C., Guzmán-M, E., Ramón, P., Vélez-Mora, D., & Espinosa, C. I. (2017). Does size matter? Ontogenetic responses of an Andean shrub to conspecific density-dependence. *Perspectives in Plant Ecology, Evolution and Systematics*, 25, 59–67. <https://doi.org/10.1016/j.ppees.2017.02.002>
- Lara-Romero, C., Robledo-Arnuncio, J. J., García-Fernández, A., & Iriondo, J. M. (2014). Assessing intraspecific variation in effective dispersal along an altitudinal gradient: A test in two Mediterranean high-mountain plants. *PLoS ONE*, 9(1), <https://doi.org/10.1371/journal.pone.0087189>
- Lenoir, J., Gégout, J. C., Marquet, P. A., De Ruffray, P., & Brisse, H. (2008). A significant upward shift in plant species optimum elevation during the 20th century. *Science*, 320(5884), 1768–1771. <https://doi.org/10.1126/science.1156831>
- Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends in Ecology & Evolution*, 17(4), 183–189. [https://doi.org/10.1016/S0169-5347\(02\)02497-7](https://doi.org/10.1016/S0169-5347(02)02497-7)
- Lesica, P., & Allendorf, F. W. (1995). When are peripheral populations valuable for conservation? *Conservation Biology*, 9(4), 753–760. <https://doi.org/10.1046/j.1523-1739.1995.09040753.x>
- Lüdtke, D. (2018). *Sjstats: Statistical functions for regression models. R Package Version 0.14, 3*. Retrieved from <http://cran.r-project.org/package=sjstats/>
- Lynch, M., & Gabriel, W. (1987). Environmental tolerance. *The American Naturalist*, 129, 283–303. <https://doi.org/10.1086/284635>
- Millar, C. I., & Libby, W. J. (1991). Strategies for conserving clinal, ecotypic, and disjunct population diversity in widespread species. *Genetics and Conservation of Rare Plants*, 149, 170.
- Monneveux, P., Jing, R., & Misra, S. C. (2012). Phenotyping for drought adaptation in wheat using physiological traits. *Frontiers in Physiology*, 3, 1–12. <https://doi.org/10.3389/fphys.2012.00429>

- Morente-López, J., García, C., Lara-Romero, C., García-Fernández, A., Draper, D., & Iriondo, J. M. (2018). Geography and environment shape landscape genetics of mediterranean alpine species *Silene ciliata* Poiret. (Caryophyllaceae). *Frontiers in Plant Science*, 9, 1698. <https://doi.org/10.3389/fpls.2018.01698>
- Morente-Lopez, J., Kass, J. M., Lara-Romero, C., Serra-Diaz, J. M., Soto-Correa, J. C., Anderson, R. P., & Iriondo, J. M. (2020). Ecological niche models as hypothesis generators of functional genetic differentiation and potential local adaptation in a Mediterranean alpine ecosystem. *BioRxiv*. <https://doi.org/10.1101/2020.02.18.954867>
- Morente-López, J., Lara-Romero, C., García-Fernández, A., Rubio Teso, M. L., Prieto-Benítez, S., & Iriondo, J. M. (2020). Data from the study of adaptive value and gene flow effects on populations inhabiting marginal using in situ common gardens. Dataset. *figshare*. <https://doi.org/10.6084/m9.figshare.11861886.v1>
- Morente-López, J., Scheepens, J. F., Lara-Romero, C., Ruiz-Checa, R., Tabarés, P., & Iriondo, J. M. (2019). Past selection shaped phenological differentiation among populations at contrasting elevations in a Mediterranean alpine plant. *Environmental and Experimental Botany*, 170, 103894. <https://doi.org/10.1016/j.envexpbot.2019.103894>
- Nadeau, C. P., & Urban, M. C. (2019). Eco-evolution on the edge during climate change. *Ecography*, 42(7), 1280–1297. <https://doi.org/10.1111/ecog.04404>
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 2, 133–142. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Nikolic, N., & Chevalet, C. (2014). Detecting past changes of effective population size. *Evolutionary Applications*, 7(6), 663–681. <https://doi.org/10.1111/eva.12170>
- Ohsawa, T., & Ide, Y. (2008). Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. *Global Ecology and Biogeography*, 17(2), 152–163. <https://doi.org/10.1111/j.1466-8238.2007.00357.x>
- Papuga, G., Gauthier, P., Pons, V., Farris, E., & Thompson, J. D. (2018). Ecological niche differentiation in peripheral populations: A comparative analysis of eleven Mediterranean plant species. *Ecography*, 41(10), 1650–1664. <https://doi.org/10.1111/ecog.03331>
- Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, 190(3–4), 231–259. <https://doi.org/10.1016/j.ecolmodel.2005.03.026>
- Pironon, S., Papuga, G., Vilellas, J., Angert, A. L., García, M. B., & Thompson, J. D. (2017). Geographic variation in genetic and demographic performance: New insights from an old biogeographical paradigm. *Biological Reviews*, 92(4), 1877–1909. <https://doi.org/10.1111/brv.12313>
- Pouget, M., Youssef, S., Migliore, J., Juin, M., Médail, F., & Baumel, A. (2013). Phylogeography sheds light on the central – Marginal hypothesis in a Mediterranean narrow endemic plant. *Annals of Botany*, 112, 1409–1420. <https://doi.org/10.1093/aob/mct183>
- Rivas-Martínez, S., Fernández-González, F., Sánchez-Mata, D., & Pizarro, J. M. (1990). Vegetación de la Sierra de Guadarrama. *Itinera Geobotanica*, 4, 3–132.
- Rolland, J., Lavergne, S., & Manel, S. (2015). Combining niche modeling and landscape genetics to study local adaptation: A novel approach illustrated using alpine plants. *Perspectives in Plant Ecology, Evolution and Systematics*, 17(6), 491–499. <https://doi.org/10.1016/j.ppees.2015.07.005>
- Ronce, O., Shaw, F. H., Rousset, F., & Shaw, R. G. (2009). Is inbreeding depression lower in maladapted populations? A quantitative genetics model. *Evolution*, 63(7), 1807–1819. <https://doi.org/10.1111/j.1558-5646.2009.00678.x>
- Savi, T., Love, V. L., Dal Borgo, A., Martellos, S., & Nardini, A. (2017). Morpho-anatomical and physiological traits in saplings of drought-tolerant Mediterranean woody species. *Trees*, 31(4), 1137–1148. <https://doi.org/10.1007/s00468-017-1533-7>
- Sexton, J. P., McIntyre, P. J., Angert, A. L., & Rice, K. J. (2009). Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics*, 40(1), 415–436. <https://doi.org/10.1146/annurev.ecolsys.110308.120317>
- Sexton, J. P., Strauss, S. Y., & Rice, K. J. (2011). Gene flow increases fitness at the warm edge of a species' range. *Proceedings of the National Academy of Sciences of the United States of America*, 108(28), 11704–11709. <https://doi.org/10.1073/pnas.1100404108>
- Soule, M. (1973). The epistasis cycle: A theory of marginal populations. *Annual Review of Ecology and Systematics*, 4(1), 165–187. <https://doi.org/10.1146/annurev.es.04.110173.001121>
- Tutin, T. G., Heywood, V. H., Burges, N. A., Valentine, D. H., Walters, S. M., & Webb, D. A. (1964). *Flora Europaea*. New York, NY: Cambridge University Press.
- Vucetich, J. A., & Waite, T. A. (2003). Spatial patterns of demography and genetic processes across the species' range: Null hypotheses for landscape conservation genetics. *Conservation Genetics*, 4, 639–645.
- Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., ... Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, 416(6879), 389–395. <https://doi.org/10.1038/416389a>
- Whitlock, M. C. (2003). Fixation probability and time in subdivided populations. *Genetics*, 164, 767–779.
- Whitlock, M. C., Ingvarsson, P. K., & Hatfield, T. (2000). Local drift load and the heterosis of interconnected populations. *Heredity*, 84, 452–457. <https://doi.org/10.1046/j.1365-2540.2000.00693.x>
- Yeaman, S., & Jarvis, A. (2006). Regional heterogeneity and gene flow maintain variance in a quantitative trait within populations of lodgepole pine. *Proceedings of the Royal Society B: Biological Sciences*, 273(1594), 1587–1593. <https://doi.org/10.1098/rspb.2006.3498>
- Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. Springer Science & Business Media. <https://doi.org/10.1007/978-0-387-87458-6>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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