Looking for a successful translocation: the case of *Astragalus edulis*

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Abstract. *Astragalus edulis* (Fabaceae) is an endangered annual plant species from the western Mediterranean region. It occurs in the Iberian Peninsula (SE), Morocco (two main locations in the NE and SW), and the easternmost Canary Islands (Lanzarote and Fuerteventura). After a thorough study assessing genetic diversity and phylogeographic patterns, and a proposal of a management plan to preserve the genetic diversity across the entire area of the species, it is about time to go further in the species conservation. It is necessary to recognize genetically impoverished populations to be able to successfully reinforce populations and find realistic places for translocations. For this, Nei's genetic diversity values were calculated for the three geographic areas and for their populations. Diversity values were further evaluated to calculate the optimal contribution for the populations within each area to maximize the genetic pool. To evaluate whether the optimum habitat for the species is expected to grow or decrease under a climate change scenario, the optimal model of current climatic suitability of *A. edulis* was projected into the IPCC (2070_RCP8.5) future scenario. The different geographical areas showed similar genetic diversity values. The populations that contribute to maximize the genetic pool in each geographic area were similar, but not equal, to those proposed in previous studies. The future potential habitat for the species shows a significant range decline, which will affect translocation efforts.

Keywords: Conservation; genetic diversity; species distribution models; western Mediterranean-Macaronesian area.

Introduction

Acquiring knowledge on species potential habitat, genetic diversity, and intraspecific phylogenetic relationships is essential to appropriately address species conservation (Commander *et al.*, 2018). The genetic patterns of biodiversity must be preserved as they hold the survival ability of the species (Pérez-Collazos *et al.*, 2008). Human-mediated changes to ecosystems and the climate change threaten species survival (Leadley *et al.*, 2010). These perturbations often promote habitat fragmentation leading to isolation and limitation of gene flow among populations. As a consequence, species genetic diversity will be negatively affected (Young & Clarke, 2000). This isolation additionally implies a decrease in the resilience of populations when facing environmental changes. Urgent conservation measures and restoration plans are required given the current scenario of biodiversity loss due to rapid environmental change (Christmas *et al.*, 2016; IUCN, 1998).

Climate change forces species to adapt, move (migrate), or become extinct (Christmas *et al.*, 2016; Nogués-Bravo *et al.*, 2018). Species can respond plastically to environmental variation being phenotypic plasticity considered a major evolutionary force (Noel

et al., 2007). Phenotypic plasticity can be adaptive (Thompson, 1991; Valladares *et al.*, 2006) being this essential for conservation biology. Despite this, the ability to adapt is often limited when the rate of environmental change is high, and in this context the dispersal abilities of species can be vital in preventing extinction (Tremlová & Münzbergová, 2007). These displacements occur naturally through the dispersive abilities of the species (dispersal of seeds and propagules). However, dispersive processes are generally stochastic, so the probability that the species will colonize habitats with climatic and edaphic suitable characteristics is also unlikely (Nathan, 2006). Species distribution models (SDMs) provide essential information about the ecological requirements of the species. This can help to find the optimal areas for the species in the future (Elith & Leathwick, 2009; Guisan *et al.*, 2013) and consequently it could be vital to ensure the success of conservation proposals (Dawson *et al.*, 2011; IUCN/SSC, 2013).

Regarding *in situ* conservation, several estimators have been proposed to answer how many and which populations should be conserved; all of which are based on the preservation of the higher amount of genetic diversity in the least number of populations and/or areas (Ryder, 1986; Moritz, 1994; Riddle &

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Hafner, 1999; Maes *et al*., 2004; Pérez-Collazos *et al*., 2008; among others). The selection of relevant genetic units for conservation (RGUCs) has been successfully used to propose sampling strategies for plant species, such as *Boleum asperum* Desv. (Pérez-Collazos *et al*., 2008), *Borderea pyrenaica* Miégev. (Segarra-Moragues & Catalán, 2010), and *Astragalus edulis* Bunge (Peñas *et al*., 2016). This method is based on the idea that rare alleles are essential in conservation as they represent unique evolutionary products that could provide the species with the ability to adapt to environmental changes (Pérez-Collazos *et al*., 2008). However, the conservation proposals are often focused on passive protection, which are often inappropriate for reducing accelerated losses of natural species and habitats (Fenu *et al*., 2019). The main objective of *in situ* conservation is to establish a method that combines the conservation of the entire genetic diversity with the socioeconomic viability of the conservation proposal. Thus, *ex situ* conservation is not feasible at large scales due to the costs, but it is feasible to apply to the most threatened species, which are precisely those that require greater *ex situ* conservation effort (Fay & Krauss, 2003). The gathering of all the genetic diversity of the species is essential to propose viable translocation measures (Caujapé-Castells & Pedrola-Monfort, 2004; Pearse & Crandall, 2004), and represents the basis of the creation and use of seed banks, the core of *ex situ* conservation strategy.

The reinforcement of existing populations and the creation of new populations (through the introduction of propagules or seeds to a new location within or outside the range of distribution of the species) are two types of translocation (Akeroyd & Wyse Jackson, 1995). The reinforcement of rare and threatened species populations has become essential for biodiversity conservation (Armstrong & Seddon, 2008). When reinforcing populations or creating new ones, it is common to use only a few individuals from one population or from several small populations, which inevitably leads to the failure of the conservation proposals (Godefroid *et al*., 2011). Small populations of endemic species and/or of fragmented habitat species hold limited genetic variation; therefore, increasing the size of the population may end up decreasing the number of individuals capable of reproducing within the populations, this is due to inbreeding depression that eventually reduces their genetic diversity (Frankham *et al*., 2002; Friar *et al*., 2000). Outbreeding depression must also be considered as it may influence the survival in species that inhabit ecologically or geographically different areas (Fenster & Galloway, 2000). The number of successful reintroductions of plant species is still small (Fenu *et al*., 2019). In order to reduce the failure rates, reintroductions require knowledge of the life history, demography, reproductive biology, ecology, and geography of the focal species (Armstrong &

Seddon, 2008; Bajomi *et al*., 2010). The failure of the translocations has probably diverse causes, but the reasons are partially unknown due to the fact that the studies are focused on monitoring the establishment and not on the explanation about the failure (Armstrong & Seddon, 2008).

Astragalus edulis (Fabaceae) is an endangered annual endemism from the western Mediterranean hotspot region. It occurs in South-Eastern (SE) Iberian Peninsula, Morocco (two main locations in the North East and South West), and the easternmost Canary Islands (Lanzarote and Fuerteventura). Previous studies have assessed intraspecific genetic diversity and phylogeographic patterns of *A. edulis*, which have led to propose an *in situ* management plan to preserve the genetic diversity across the entire distribution area of the species (Bobo-Pinilla *et al*., 2018; Peñas *et al*., 2016). As described by Peñas (2004), the dispersal ability of the species is very limited. The low values of diversity and rarity found have been interpreted as a consequence of recent colonization events or reduction of the effective population sizes (Bobo-Pinilla *et al*., 2018). Moreover, this species represents an ideal study system to test the utility of Species Distribution Modeling (SDMs) and diversity patterns as a tool to assess how climate change will affect the potential distribution of endangered species from semiarid ecosystems and a way to preserve taxa that have highly fragmented populations.

In the present study, we propose the reinforcement planning of the most degraded populations of *A. edulis* in order to preserve its genetic diversity. In addition, we will use SDMs to infer the areas that will present optimal climatic conditions for the survival of the species in the future and consequently propose realistic translocations.

Materials and Methods

Genetic diversity patterns

Amplified fragment length polymorphisms (AFLP) of *Astragalus edulis* obtained by Peñas *et al*. (2016) were used in this study as a source of genetic data. This data includes a total of 358 plants from 17 populations (6 from the Iberian Peninsula, 8 from Morocco, and 3 from the Canary Islands; Table 1 and Figure 1). AFLP-SURV 1.0 (Vekemans *et al*., 2002) was used to calculate the Nei (1987) gene diversity index for each population (H_{nei}). Nei's gene diversity was also calculated for the three main geographical areas separately (H_t; Table 1). The multilevel b-spline tool (Conrad *et al*., 2015) implemented in QGIS (QGIS-Development-Team, 2017) software was used to draw the genetic diversity patterns. This tool creates a diversity gradient map, interpolating the specific values of the populations.
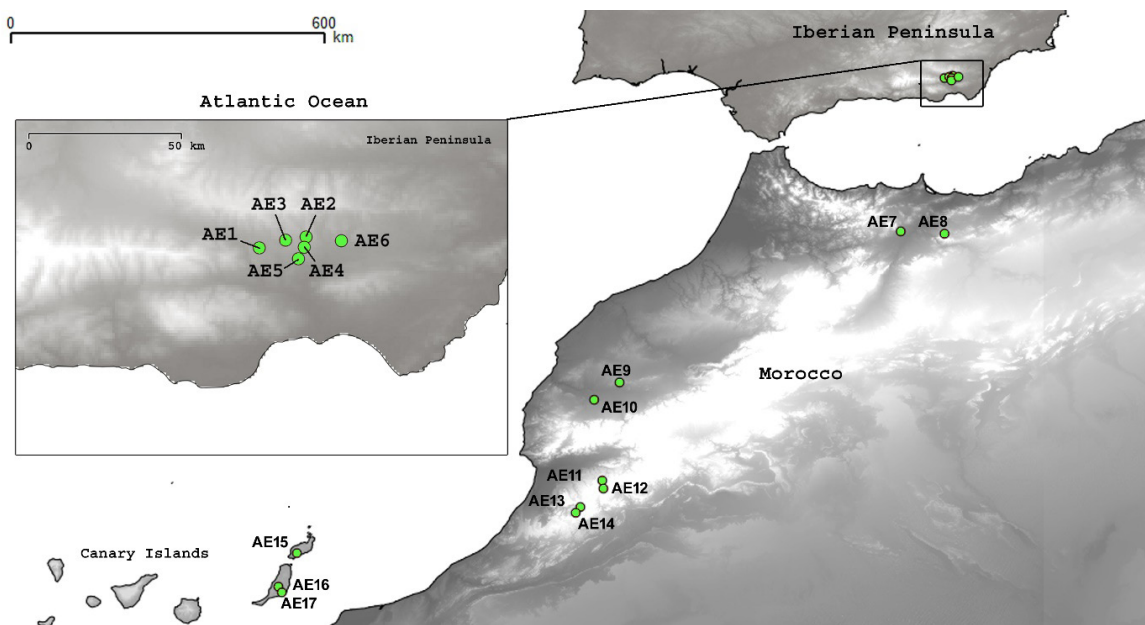
Figure 1. Location of the populations of *Astragalus edulis* studied.

Table 1. Geographic and genetic diversity features of the populations of *Astragalus edulis* (N, Number of individuals used for the AFLP analyses; H_{Nei}, Nei's gene diversity index; H_t, Nei's average gene diversity values per geographical area) and Metapop2 v2.2.1 results (ΔH_{nei}, proportional increment/decrement of the within-population gene diversity when the population data is removed in the analysis; ΔH_{dist}, proportional increment/decrement of Nei's average genetic distance between populations when the population data is removed in the analysis; C_x, expected proportion of seeds from the populations within the geographic areas in order to obtain the maximum diversity values in a synthetic population).

Area	Population	N	Longitude	Latitude	H_{nei}	H_t	ΔH_{nei}	ΔH_{dist}	C_x
Iberian Peninsula	*AE1*	16	-2.60	37.10	0.098	0.115	1.68	-2.97	8.5
	AE2	24	-2.46	37.13	0.096		2.00	0.77	0.0
	AE3	32	-2.53	37.12	0.109		-1.82	1.28	0.0
	AE4	24	-2.47	37.10	0.131		-4.96	0.28	30.9
	AE5	23	-2.49	37.07	0.133		-5.37	-0.54	60.6
	AE6	33	-2.36	37.12	0.076		7.78	-1.23	0.0
Morocco	*AE7*	17	-2.60	34.42	0.069	0.106	3.48	-2.42	0.0
	AE8	21	-3.35	34.47	0.107		-2.98	-0.54	44.1
	AE9	22	-8.19	31.87	0.107		-2.43	1.17	15.3
	AE10	14	-8.62	31.57	0.084		1.55	-0.26	0.0
	AE11	18	-8.48	30.18	0.097		-0.57	-0.26	17.5
	AE12	19	-8.45	30.05	0.095		0.16	0.10	0.0
	AE13	25	-8.86	29.74	0.096		-1.00	-1.15	23.1
	AE14	7	-8.94	29.64	0.084		1.19	-0.41	0.0
Canary Islands	*AE15*	29	-13.73	28.93	0.079	0.111	15.20	-1.61	0.0
	AE16	14	-14.03	28.36	0.134		-7.69	-2.68	82.5
	AE17	22	-13.99	28.27	0.111		-6.96	-0.68	17.5

Contribution of the populations to global genetic diversity

In order to calculate the relative contribution of the populations to a genetic pool of the species in the different areas, the proportional contribution of each population to Nei's gene diversity (ΔH_{nei}; Nei, 1987) and the proportional contribution of the average Nei's (ΔH_{dist}) minimum genetic distance between populations was calculated using the software Metapop2 v2.2.1 (López-Cortegano *et al.*, 2019). These contributions are obtained by disregarding each population from the analysis one by one in each area and calculating the proportional amount of diversity gained or lost after the removal of the population data. The software also calculates the proportion of gene diversity explained within and among populations in each area, giving an approximation of the contribution of each population to the total diversity of each area (Petit *et al.*, 1998). Moreover, Metapop2 v2.2.1 was also used to obtain the expected proportional contribution (C_x) of each population (within the areas) to a theoretical synthetic pool (e.g. germplasm bank) with maximum global gene diversity (D_{max}) (Table 1). This was done by maximizing the function $D_{max = 1 - \sum_{i=j}^{n} f_{ij} c_i c_j}$, where f_{ij} is the average coancestry between populations i and j, and c_i is the contribution of subpopulation i to the pool (Toro & Caballero, 2005).

Species distribution modeling

The optimal model of current climatic-suitability of *A. edulis* (Bobo-Pinilla *et al.*, 2018) was projected into the future scenario (2070_RCP8.5) climatic layers (available at www.worldclim.com) using the package 'raster' (Hijmans *et al.*, 2005); the most restrictive future scenario was chosen to ensure that the areas found will have the climatic characteristics matching the habitat suitability of the species. The climatic features found to have an influence on the ecology and range limits of *A. edulis* are: temperature seasonality (bio4), precipitation of the wettest quarter (bio16), and precipitation of the driest quarter (bio17) (Bobo-Pinilla *et al.*, 2018). QGIS (QGIS-Development-Team, 2017) software was used to search for matching areas of optimal habitat in current and future times; the values of habitat suitability over 0.5 at the present were compared with those over 0.5 in the future scenario (2070_RCP8.5).

Results

Genetic diversity patterns

Genetic Diversity values (H_{Nei}) ranged from 0.069 in the *Astragalus edulis* population AE7 (Morocco) to 0.134 in the population AE16 (Canary Islands). The average genetic diversity values (H_t) for the three areas considered were similar (0.115 for the Iberian Peninsula, 0.106 for Morocco, and 0.111 for the Canary Islands; Table 1).

Regarding the genetic diversity patterns, the Iberian populations showed the highest values of diversity in the central distribution area (populations AE3, AE4, and AE5, with 0.109, 0.131, and 0.133 respectively), whereas the westernmost and easternmost areas showed the lowest values (0.098 in population AE1 and 0.076 in population AE6; Figure 2A and Table 1), being population AE6 the most impoverished one. The Moroccan populations showed low diversity values, with the exception of populations AE9 and AE8 (with both having a value of 0.107; Figure 2B and Table 1). Thus, all populations in the south of the Atlas Mountains showed a pattern of low genetic diversity. With respect to the Canary Islands, the population of Lanzarote was found to have a remarkably low genetic diversity (0.079; Figure 2C and Table 1).

Contribution of the populations to global genetic diversity

The intra- and inter-population contributions to the total genetic diversity were 90.98% and 9.02% respectively for the Iberian Peninsula, 81.93% and 18.07% respectively for Morocco, and 96.75% and 3.25% respectively for the Canary Islands. In the Iberian Peninsula, populations AE4 and AE5 showed the largest loss of within-population diversity (ΔH_{nei} of 4.96% and 5.37%, respectively) when they are disregarded in the analysis (Table 1), indicating that these are the populations with a more important contribution to the intra-population diversity in the area. Population AE1 showed the largest contribution to between-population diversity (a loss of 2.97% of variation when removed from the analysis, ΔH_{dist}). In Morocco, populations AE8 and AE9 showed the largest contribution to within-population diversity, whereas AE7 and AE13 showed the largest contribution to between-population diversity. Finally, in the Canary Islands, populations AE16 and AE17 showed a substantial contribution to both within- and between-population diversity. The net overall contribution of the populations to global genetic diversity is clearly illustrated by the expected contributions of the populations to a pool of maximum diversity (C_x, Table 1), with contributions of AE1, AE4, and AE5 in the Iberian Peninsula, AE8, AE9, AE11, and AE13 in the Moroccan area, and AE17 and, particularly, AE16 in the Canary Islands.

Species distribution models

The current distribution of *A. edulis* mostly coincided with that predicted by the potential distribution of the species (Figure 3A). In general, the suitable habitat decreased significantly (Figure 3B), especially in the SE of the Iberian Peninsula and in the NE of Morocco. Only small regions of Lanzarote and the northern area situated at the SW part of the Atlas Mountains remained suitable for the species in the future considering its current distribution (Figure 3C).
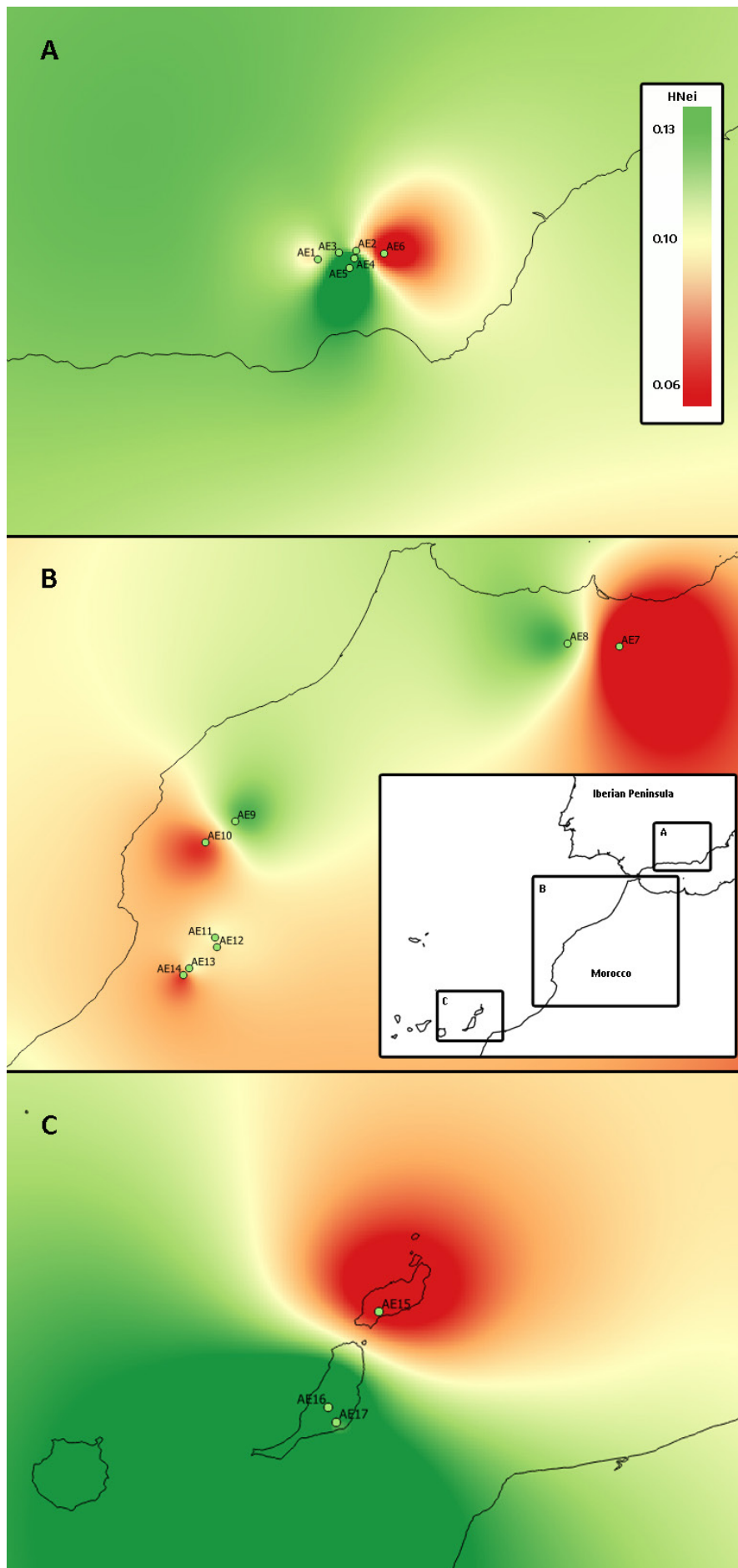
Figure 2. Nei's gene diversity pattern (red = low; yellow = medium; green = high) obtained with QGIS (QGIS-Development-Team, 2017); A, Iberian Peninsula; B, Morocco; C, Canary Islands.
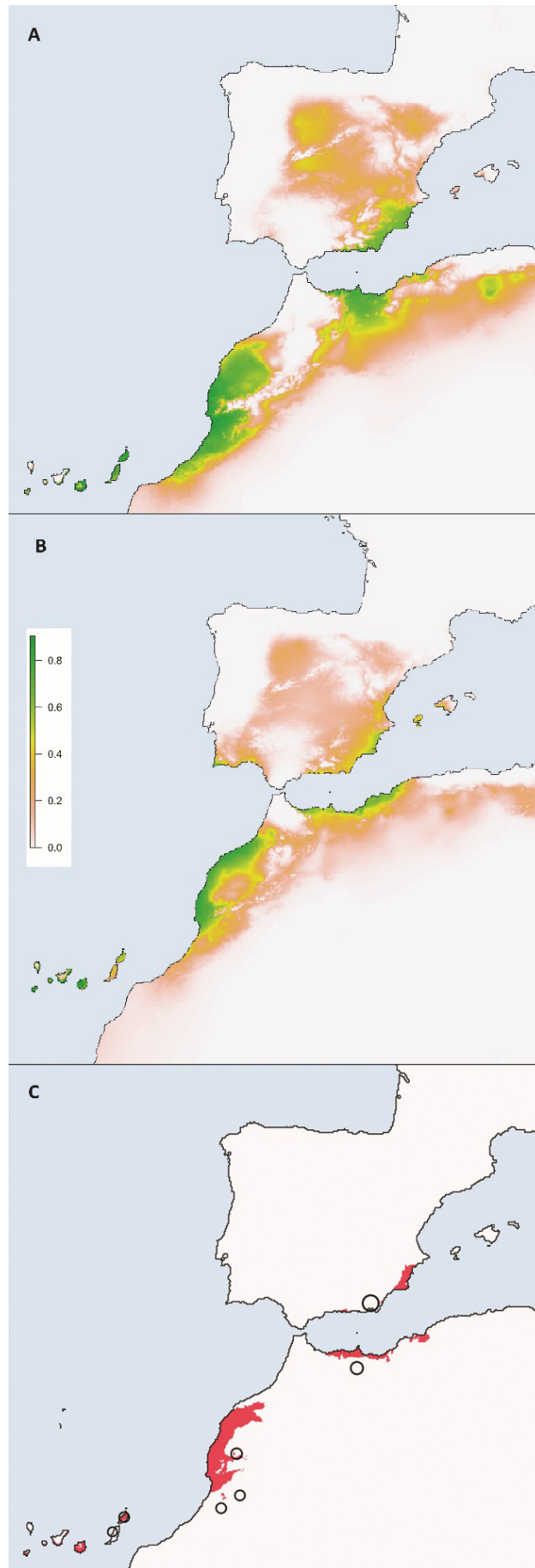
Figure 3. A, Distribution model representing the habitat suitability for the species in the present (red-yellow, medium, green, high; Bobo-Pinilla *et al.*, 2018); B, Projected habitat suitability values for the species in the future scenario 2070_RCP8.5 (red-yellow, medium, green, high); C, red areas represent suitable areas for the species in the present and future scenarios, circle represent present populations. (slightly modified from Bobo-Pinilla *et al.*, 2018).

Discussion

The mere designation of protected areas is not enough to protect biodiversity (Volis, 2019; and references within). A method that unifies the different aspects regarding the viability of populations as part of conservation proposals is thus necessary; such unification is especially deficient for *ex situ* conservation (Volis & Blecher, 2010). The aim of a successful proposal should be the combination of preserving the maximum variability and viability of the species as well as the efficient search for suitable locations for translocations of the species in a changing world.

Reinforcement of the genetically impoverished populations

The relevant genetic units for conservation (RGUCs) proposed for *Astragalus edulis* (Peñas *et al*., 2016) could be underestimating the importance of some populations with low genetic diversity. The proposal of RGUCs in Morocco was to preserve the populations AE8 and AE9; the results obtained here show that the optimal contributions to the genetic diversity of the Moroccan area would include these two populations with 44.1% and 15.3% respectively, but also populations AE11 and AE13 with 17.5% and 23.1% of the total Moroccan optimal seed proportion. The optimal proportions of the populations in the Iberian Peninsula to maximize the genetic pool corresponds with AE5 (60.6%), AE4 (30.9%), and AE1 (8.5%), coinciding with the proposal of RGUCs priority selection. In addition, the RGUC chosen for the Canary Islands was population AE16; the optimal contribution of this population to the maximization of the genetic diversity is 82.5%. Our results suggest population AE17 should also be considered as its optimal contribution is 17.5% of the seed optimal proportion. The selection of population AE17 to the optimal contribution seems to be the result of its main contribution to within-population diversity (a decrease of the genetic diversity by 6.96% when this population is removed from the analysis) but also to between-population diversity (a decrease of 0.68%). Regarding the Moroccan populations, the value of diversity explained among populations seems to be also underestimated by the RGUCs method. The genetic and geographic distance among populations make it difficult to encompass the majority of the genetic diversity with the preservation of the two populations proposed by the RGUCs method in the Moroccan area. It is imperative to consider the contribution to the genetic pool of the genetic distance among the populations within the genetically structured areas.

The aim of translocation proposals is to increase the survival of a given species (Commander *et al*., 2018; Volis & Blecher, 2010). Even considering that it is impossible to know the final implications of a translocation due to the multiple interacting variables (Friar *et al*., 2000), it is important to consider the possible risks of inbreeding and outbreeding depression (Barrett & Kohn, 1991). Inbreeding depression is common when only a few random individuals are used in translocations. The inevitable result is the decrease of fitness and genetic diversity of the populations (Young *et al*., 2005; Young & Clarke, 2000), and consequently, a loss in their ability to adapt to changing environmental conditions (Frankham, 2005). The proposal of obtaining optimal proportions of seeds from each population for translocation seems appropriate, not only because of the intended maximization of genetic diversity, but also because of the fact that collecting large quantities of seeds in natural populations may have a negative impact on local population dynamics (Broadhurst *et al*., 2008). Outbreeding depression may be an important risk in species that inhabit ecologically or geographically different areas (Fenster & Galloway, 2000). Exposure of individuals to new environments and the phenotypic changes that this generates can decrease the survival and reproductive success of individuals and, therefore, the success of translocation. (Lema & Nevitt, 2006). As shown by Bobo-Pinilla *et al.* (2018), this species exhibits a genetic pattern of three geographic areas (Iberian Peninsula, Morocco, and Canary Islands). As it is considered that the seeds from the same area will not affect the genetic pattern, the transference of plant material within these areas reduces the risk of outbreeding (Kaulfuß & Reisch, 2017). The phenotypic plasticity that can provide the adaptive traits of the species is generally unknown, and therefore conservation biologists still rely on quantitative genetic approaches to study phenotypic variation of endangered species and design efficient management programs (Noel *et al*., 2007). Following the predicted optimal contributions of the populations to global diversity in each area, a complete seed bank should be created and the populations most genetically impoverished (AE1, AE2, and AE6 from the Iberian Peninsula; AE7 and AE10–14 from Morocco; and AE15 from the Canary Islands) should be reinforced with the population proportions calculated for each area.

The future potential habitat of *Astragalus edulis*

Species distribution models are a recommended conservation tool that allow the proposal of areas where to create new populations of endangered species that are suffering under the climate change scenario (IUCN/SSC, 2013). Despite this, their use for such an inference has been limited to date (Keppel *et al*., 2012). The impact of climate change on biodiversity will depend on the ability of plant species to migrate to new sites with suitable habitat conditions (Normand *et al*., 2011). As the seeds of the species lack of evident adaptations for dispersal (Peñas, 2004), the geographic distance between present and future suitable areas for *A. edulis* (Figure 3) makes it unlikely that their seeds colonize the areas found to have optimal conditions for them in the future. This mismatch between the high rate of change in climatic conditions in the habitat of the species and the ability of species to follow these changes may affect species survival (Malcolm *et al*., 2002).

The model indicates that the desertification process favors the optimal conditions for the species in areas

with no frost influence, with optimal conditions decreasing in inland areas and shifting directionally to the North (Figure 3B). Notably, these areas are also highly affected by human pressure, which can become a major drawback when proposing specific areas for translocation. Moreover, the optimal areas in the future scenario are located mainly outside the actual areas for the species. This implies a significant challenge, as the translocations outside the actual range of the species could have unknown results (Seddon *et al.*, 2015). Shared suitable areas between present and future scenarios (Figure 3C) could be used to introduce plants corresponding with the closest genetic pattern (i.e., Iberian Peninsula, Morocco or Canary Islands). However, further research about fine scale distribution models is needed to find the specific sites, with adequate edaphic characteristics of the habitats, where to create the new populations.

Conclusion

We propose an approach that integrates genetic and geographic data to be used in *ex situ* and *in situ* complementary conservation measures as a tool to design efficient solutions for the conservation of endangered plant species. Applied to *Astragalus edulis*, we used genetic diversity data to propose an optimal proportion of seeds from the populations within the geographic areas in order to maximize the genetic pool contributing to storage the highest variability. This will strengthen the viability of the reinforcement planning of the most degraded populations of the species. Also, we detected the populations from the three geographic areas studied (Iberian Peninsula, Morocco and Canary Islands) that should be reinforced. Finally, we inferred, using the species distribution model, which areas will present optimal conditions for the survival of *A. edulis* in the future. A general pattern of range shifts towards coastal and northern areas have been detected, so further research is needed in order to find fine scale edaphic suitable sites to propose the creation of new populations given the decrease of suitable areas for the species in the current distribution range. Finally, in order to have a way to check the success of the translocation proposal, further analyses regarding the genetic diversity of the created and reinforced populations must be made and a monitoring of the population dynamics must be carried out. This study should help to propose genetically diverse and spatially explicit translocations.

Acknowledgements

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