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Genetic conservation strategies of endemic plants from edaphic habitat islands: The case of *Jacobaea auricula* (Asteraceae)

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ABSTRACT

Conservation genetics is a well-established and essential scientific field in the toolkit of conservation planning, management, and decision-making. Within its framework, phylogeography allows the definition of conservation strategies, especially in threatened endemic plants. Gypsum and salt-rich outcrops constitute a model example of an edaphic island-like habitat and contain rare and endemic species, many of them threatened. This is the case of *Jacobaea auricula*, an Iberian gypsohalophytic species with biological, ecological, and conservation interest. Genetic-based criteria were used to preserve the highest possible percentage of the species' genetic pool as well as to dispose of a set of genotypes for translocation and/or reinforcement planning of degraded populations. Relevant Genetics Units for Conservation (RGUCs) were selected as *in situ* conservation planning. As a complementary *ex situ* measure, the optimal contribution for the populations to maximize the genetic pool within each genetic cluster was calculated. To preserve the maximum genetic diversity and the highest percentage of rare AFLP bands possible, eight RGUCs were selected; the *ex situ* conservation design included twenty-one populations, gathering all haplotypes and ribotypes. Our genetic conservation proposal of *J. auricula* would improve the implementation of future genetic conservation measures, as a species model of endemic plants from edaphic habitat islands.

1. Introduction

It is necessary to develop and apply strategies and methods for the conservation of biodiversity due to historical losses of biodiversity (Margules & Pressey, 2000; Pärtel et al., 2005). Conservation biology aims to preserve current genetic diversity and the diversification processes that are taking place at species-level (Forest et al., 2007). Genetic diversity must be preserved as it holds the survival ability of the species (Hoban et al., 2020; Pérez-Collazos et al., 2008); to this effect, population genetics data are essential for both conceptual and applied biodiversity conservation programs. Moreover, conservation genetics is a well-established scientific field that will be essential (among other methods) in the toolkit of conservation planning, management, and decision-making (Frankham et al., 2004; Holderegger et al., 2019). Regarding diversification processes, phylogeny and phylogeography can enlighten how interactions between evolutionary and ecological processes influence diversity at multiple scales (Webb et al., 2002). For this reason, these disciplines could improve the proactive conservation planning (Avise, 2009; Médail & Baumel, 2018). Genetic patterns, species potential habitat and intraspecific phylogenetic relationships are essential to appropriately address species conservation (Commander et al., 2018). Therefore, the use of genetic diversity structure is necessary for defining conservation strategies, especially for threatened endemic plants within biodiversity hotspots.

Unfortunately, most of the phylogeographic studies have not placed much emphasis on establishing management and conservation proposals neither *in situ* units nor *ex situ*, *i.e.* maintaining genetic diversity in *ex situ* collections. According to Médail & Baumel (2018), who performed a review of the studies dealing with the genetic diversity structure of narrow endemic plants in the Mediterranean Basin hotspot, only 27 % of

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these studies used the information generated to establish priorities for the conservation of the species, and around 18 % inferred conservation units. Both *in situ* and *ex situ* conservation genetic strategies are essential for protecting rare and threatened plant species (Volis & Blecher, 2010). The success of conservation depends on whether the species are able to survive in the habitat and how they could be regenerated if it were necessary in the future.

Conservation genetics leads us to use genetic patterns in the conservation decision-making process (DeSalle & Amato, 2004), where an important objective is to search how many and which populations deserve conservation priority. In order to preserve the highest amount of genetic diversity possible in the least number of populations and/or areas, several estimators have been proposed over time: Evolutionary Significant Units (Ryder, 1986), Management Units (Moritz, 1994), Operational Conservation Units (Doadrio et al., 1996), Fundamental Geographic and Evolutionary Units (Riddle & Hafner, 1999), Functional Conservation Units (Maes et al., 2004), and Relevant Genetic Units for Conservation (RGUCs; Pérez-Collazos et al., 2008). The latter approach combines two methods that use genetic data (considering both common and rare alleles) to estimate the minimum number of conservation units that should be targeted for an adequate representation of the total genetic variability of a species. This method is based on the idea that rare alleles are essential in conservation because they represent unique evolutionary products that could provide the species with the ability to adapt to environmental changes (Bengtsson et al., 1995; Lopez et al., 2009; Pérez-Collazos et al., 2008; Shaw & Etterson, 2012). Moreover, this method allows the selection of those populations that hold the highest values of diversity and/or rarity within the geographical areas. The selection of RGUCs has been used to propose sampling strategies for 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).

The conservation proposals are often focused on passive protection which results inadequate for reducing accelerated losses of natural species and habitats (Fenu et al., 2019; Mace & Purvis, 2008). *In situ* conservation of all of the populations of threatened species is often not feasible at large scales due to the costs, but it is feasible to apply *ex situ* conservation to the most threatened species which require greater effort (Fay & Krauss, 2003). When creating a germplasm bank, the gathering of all the genetic diversity of the species is essential as this will allow the proposal of viable translocation measures in the future (Caujapé-Castells & Pedrola-Monfort, 2004; Pearse & Crandall, 2004) and represents the basis of the *ex situ* conservation strategy. *Ex situ* collections may contribute effectively to plant species conservation if their use is supported by a thorough understanding of the limiting factors, such as scarcity of source material, low viability, low genetic variation, and socioeconomic factors, among others (Abeli et al., 2020; Hyvärinen, 2020). The seed banks should contain an optimal number of haplotypes and allele copies (and the type of allele targeted) and thus, must contain populations for the maximization of genetic diversity.

The habitats with gypsum outcrops frequently associated with salt-rich deposits are interesting biodiversity hotspots (Gutiérrez et al., 2008). These soils present particular physical and chemical characteristics which are inhabited by numerous plant species that have significant adaptations to survive in them, such as the gypsohalophytic flora (Denaeyer–De Smet, 1970). Gypsum and salt-rich outcrops occupy disjunct areas in territories with arid or semiarid climate conditions. These habitats comprise a model example of an edaphic island-like habitat and are interesting for the study of plant distributions, gene flow, genetic diversity, and diversification (Escudero et al., 2015; Moore et al., 2014; Mota et al., 2011). The gypsohalophytic flora is rich in rare and endemic species, many of them threatened (Pérez–García et al., 2011), and characterize the Iberian gypsum steppes habitat, which is included within the UE "Priority habitat 1520" (Gypsophiletalia order) (Evans, 2006; Mota et al., 2011). To preserve this priority habitat in the Iberian Peninsula, the inclusion of fifty-one localities has been proposed (Mota et al., 2011). Other proposals have been presented to preserve specifically gypsohalophytic species, such as the establishment of micro-reserves (Eugenio et al., 2013; Salazar et al., 2011), or the inclusion within nature protection areas in Natura 2000 network (Salazar et al., 2011). Unfortunately, these *in situ* protection proposals were applied at local level, and do not take into account the levels of genetic diversity of the population nor how such gene diversity is distributed throughout the whole area of distribution of the species.

Jacobaea auricula (Bourg. Ex Coss) Pelter. (Asteraceae) is a characteristic species of gypsum and salt-rich habitats (Salmerón-Sánchez et al., 2017), with biological, ecological, and conservation interest. This is an herbaceous perennial species from the eastern part of the Iberian Peninsula that has a discontinuous distribution in scattered and small populations. This disjunct distribution is edaphically restricted to gypsiferous or marl soils, salt marshes and saltland pastures bordering lagoons or seasonal water courses (Ascaso & Pedrol, 1991; Pérez–García et al., 2011; Salazar & Peñas, 2011; Salazar et al., 2011). Several intraspecific morphological discontinuities associated with geographical areas have been described, which has traditionally led to the recognition of three different subspecies (Ascaso & Pedrol, 1991; De La Torre et al., 1997). All three subspecies are included in different Spanish regional lists of threatened species (Anthos, 2020; Mota et al., 2011), as well as in the Spanish Red List of Vascular Flora (VV.AA., 2000) where they are considered to be in the threat category VU (Vulnerable). Salmerón-Sánchez et al. (2017) studied the phylogeographical and evolutionary history of the species, and whether the classical taxonomic differentiation in subspecies is genetically supported. In this research, the authors concluded that it would be premature to recognize infraspecific taxa in *J. auricula* and that these molecular taxonomic results should be considered for conservation purposes of the species.

To assist the preservation and management plans of flora associated with gypsum and salt-rich outcrops, *J. auricula* is studied as a focal species for developing genetic conservation strategies. To achieve this, the specific objectives are: a) to select RGUCs, on the basis of the possession of both common and rare alleles developing an *in situ* conservation planning, and b) to select populations from which to collect seeds as an *ex situ* proposal, aiming to store the greatest genetic variability that would contribute to the future creation of new populations or to reinforce existing ones.

2. Materials and methods

2.1. Selection of relevant Genetic Units for Conservation (RGUCs)

Amplified fragment length polymorphisms (AFLP) dataset of *Jacobaea auricula* obtained by Salmerón-Sánchez et al. (2017) were used as a source of genetic data to select the Relevant Genetic Units for Conservation (RGUCs). Taking into account the availability of data for many of the endangered species and the methodologies regarding the conservation proposals, the use of existing AFLP datasets allows to carry out successful conservation approaches. The used dataset includes a total of 285 samples from 32 populations distributed along the full range of the species. Four selected AFLP primer combinations produced 1625 reproducible fragments which allowed for the determination of the population genetic structure and genetic diversity of this species. Among other genetic parameters obtained in this phylogeographical analysis, gene diversity indices, frequency and distribution of rare bands present in each population as well as the inference of distinct genetic clusters (Table1 and Fig. 1; data obtained from Salmerón-Sánchez et al., 2017) are useful to design conservation priorities (Médail & Baumel, 2018). In this study, our proposal is the use of this information to set conservation units, specifically, RGUCs (Pérez-Collazos et al., 2008).

RGUCs selection relies on two premises based on the population structure and on the probabilities of the loss of rare alleles (those with an overall frequency lower than 10 %, and present in less than 20 % of the populations; Table S1). In the method, the calculated values of

Table 1
Geographic and genetic diversity and rarity features of the populations of *J. auricula*. Assignment to the genetic clusters detected by Salmerón-Sánchez et al. (2017); Nei's GD, Nei's gene diversity index; DW, frequency down-weighted marker values; the last column refers to whether the populations are in a protected area; higher values of genetic diversity and rarity per cluster are indicated in bold.

Nº Pop.	Locality	Genetic cluster	Longitude/ latitude	Nei's GD	DW	Haplotypes	Ribotypes
1	Lo, Ribafrecha	B	−2.37°/42.35°	0,123	5,858	III(2)	I (2)
2	Na, Sesma	B	−2.08°/42.49°	0,121	5,940	VII(2)	I (2)
3	Na, Peralta, Barranco de Vallacuera	B	−1.85°/42.37°	0,105	5,278	VII(2)	I (2)
4	Na, Fitero	B	−1.88°/42.04°	0,098	4,699	III(2)	I (2)
5	Z, between Tudela and Ejea	B	−1.38°/42.12°	0,109	5,599	III(2)	I (2)
6	Z, Barranco Val de Vares, monte de la Mediana	B	−0.75°/41.52°	0,108	5,437	VII(2)	I (2)
7	Z, Bujaraloz, Laguna del Pez	B	−0.26°/41.38°	0,119	5,730	V(2)	I (2)
8	L, between La Sentiu de Sio and Balaguer	B	0.87°/41.80°	0,119	5,236	VI(3)	I (2)
9	L, La Noguera between Camarasa and Cubells	A	0.93°/41.85°	0,100	8,672	IX(3)	I (2)
10	L, Biosca-Sanahuja, Les Gesses	B	1.31°/41.84°	0,112	6,728	IV(2)	I (2)
11	T, La Albarca, Pla de la Devesa, Barranc de la Bova	B	0.90°/41.30°	0,112	5,983	I(1) & III(1)	I (2)
12	So, Monteagudo de las Vicarias	B	−2.14°/41.39°	0,114	5,935	I(2)	I (2)
13	So, Monteagudo de las Vicarias (b), Los Chorlitos	B	−2.18°/41.39°	0,120	5,949	I(2)	I (2)
14	Te, Las Cuerlas, Laguna de Gallocanta	B	−1.53°/40.97°	0,129	7,637	III(2)	I (2)
15	Te, Cubla-Villastar, Los Centenares	B	−1.13°/40.25°	0,120	4,882	III(2)	I(1) & III, IV(1)
16	M, Villaconejos	B	−3.51°/40.09°	0,115	5,579	I(1) & III(1)	I(1) & III, IV(1)
17	M, Aranjuez, El Salobral	B	−3.63°/39.99°	0,104	5,405	III(2)	III,IV(1) & I,V(1)
18	To, Villacañas, lagunas de Peña Hueca	B	−3.35°/39.51°	0,114	4,154	IX(2)	IV(2)
19	Cu, El Pedernoso, llanos de Montilla	B	−2.77°/39.49°	0,111	4,682	IX(3)	I(1) & III, IV(1)
20	Ab, Casas de Ves-Balsa de Ves, Corral del Caracol	B	−1.19°/39.29°	0,113	6,315	I(3)	I(1) & I, IV(1)
21	A, San Vicente de Raspeig	D	−0.57°/38.38°	0,092	5,989	II:2	I(1) & III, IV(1)
22	A, Saladar de Agua Amarga	D	−0.53°/38.28°	0,097	5,566	I:2	I(1) & I,III(1)
23	A, Laguna de la Mata	D	−0.68°/38.02°	0,091	7,146	II(3)	I(1) & III, IV(1)
24	Mu, Jumilla, Sierra Santa Ana, La Buitrera	D	−1.33°/38.42°	0,097	6,090	IX(3)	
25	Mu, El Rincón (Lorca)	C	−1.88°/37.87°	0,096	4,374	II(1) & VIII(2)	I(1) & II,III(1)
26	Al, Huercal Overa, Rambla de Santa Bárbara	C	−1.96°/37.37°	0,088	4,376	VIII(3)	II(2)
27	Gr, Galera, Barranco del Agua	C	−2.57°/37.73°	0,090	7,254	II(2)	II(2)
28	Gr, Cúllar, Rambla Amarguilla	C	−2.62°/37.56°	0,087	9,717	I(2)	II(2)
29	Gr, Baza, salar de Baza	C	−2.74°/37.55°	0,074	4,227	I(2)	II(2)
30	Al, Rambla de Tabernas	C	−2.45°/37.01°	0,071	3,902	II(2)	II(2)
31	Al, Rambla El Cautivo	C	−2.44°/37.01°	0,079	3,945	II(2)	II(2)
32	Al, La Sartenilla	C	−2.41°/37.02°	0,083	4,103	II(2)	II(2)

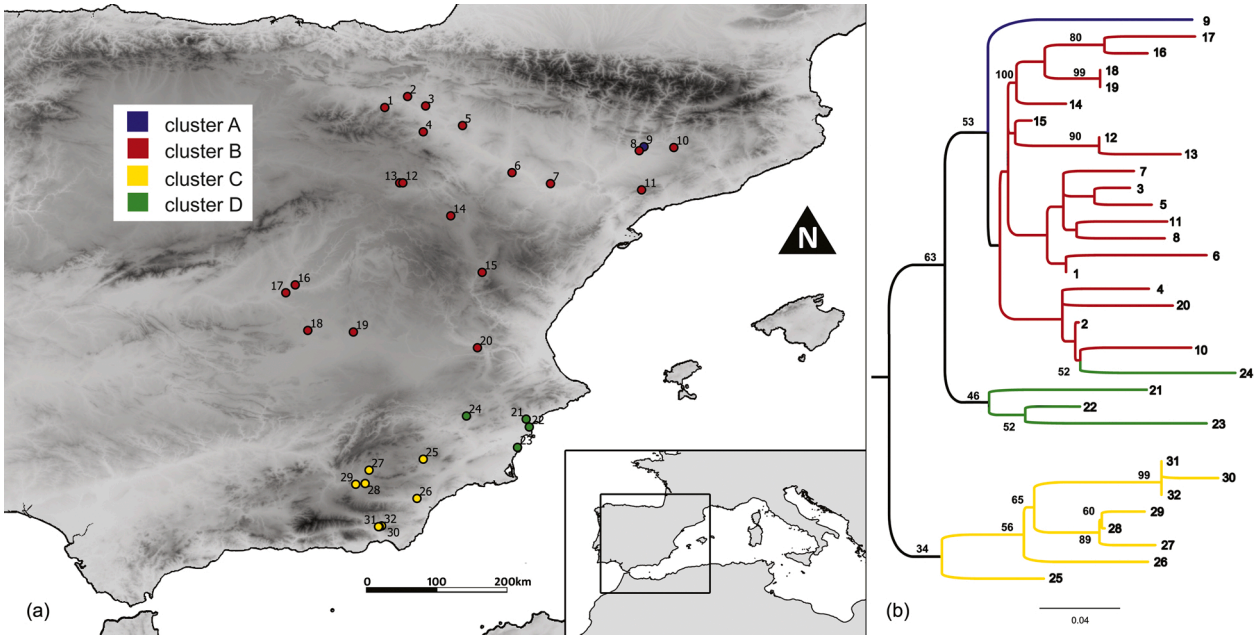

Fig. 1. (a) Location of the populations of *J. auricula* studied. Populations were assigned to the different genetic clusters following the results of STRUCTURE analysis over the AFLP dataset (Salmerón-Sánchez et al., 2017) (cluster A = blue; cluster B = red; cluster C = yellow; cluster D = green). (b) Neighbor-joining tree based on distance matrix of F_{ST} between every pair of populations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

probability of rare-allele loss are compared with the degree of inter-population subdivision (Caujapé-Castells & Pedrola-Monfort, 2004; Pérez-Collazos et al., 2008).

Before carrying out the analysis to determine the selection of populations to be preserved, it is necessary to establish the consistency of *a priori* potential subdivisions with genetic parameters. Plant genetic

diversity is spatially structured at different scales as a result of environmental influences, life-history traits, and the demographic past history of the species (Engelhardt et al., 2014). This could lead to the need for treatment of several subgroups. In the case of *J. auricula*, it is possible to adopt two different criteria; the application of Bayesian methods over the AFLP dataset (Salmerón-Sánchez et al., 2017) has allowed determining the number of genetic units on the basis of the detected polymorphism. STRUCTURE v. 2.3.4 (Pritchard et al., 2003) software showed the existence of four genetic clusters (A, B, C, and D; Fig. 1 & Table 1). When populations showed genetic admixture, they were assigned to the predominant cluster. The second criterion is focused on the use of plastid and ribosomal sequences for the establishment of phylogeographic patterns in the species (Salmerón-Sánchez et al., 2017). In both criteria, the groups are inferred exclusively from genetic data, although we finally selected the clustering generated in STRUCTURE, as they yielded a better split among groups of populations. These genetic clusters were considered as geographic units or sampling areas.

To support the geographic areas proposed, genetic relationships among populations were analyzed using a neighbor-joining tree (NJ; Saitou & Nei, 1987) based on distance matrix of F_{ST} between every pair of populations. For that, 1000 resampled F_{ST} distance matrices among populations were constructed by bootstrapping (Felsenstein, 1985) in AFLPSURV (Vekemans, 2002). F_{ST} values were calculated following Lynch and Milligan method (1994) after the estimation of allelic frequencies by means the method developed by Zhivotovsky (1999). Software package PHYLIP V3.6 (Felsenstein, 2005) was used to estimate the length of tree branches (FITCH, Fitch & Margoliash, 1967).

Following Ceska et al. (1997), the total number of populations that should be preserved (n) to represent a given proportion of the genetic diversity (P) was estimated with the modified equation $P = 1 - F_{ST}^{n}$ (Segarra-Moragues & Catalán, 2010). F_{ST} value was calculated using ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010). A proportion of 99 % and 99.9 % of the total genetic diversity was set for the populations of *J. auricula*.

To calculate the probabilities of loss of the rare alleles (in our case, rare bands as we work with dominant markers), the expression $L = (1 - p)^{2N}$ (Bengtsson et al., 1995) was used; here p represents the band frequency and N the number of populations in which a rare band is present. This expression is equally applicable for both codominant and dominant markers (Pérez-Collazos et al., 2008). For each rare AFLP band, the observed and expected probabilities of loss (L_o and L_e, respectively), and the representative value (R) were calculated, following the method described by (Pérez-Collazos et al., 2008). R-value indicates the proportion of rare alleles (bands in our case) captured by sampling only one population (Bengtsson et al., 1995; Caujapé-Castells & Pedrola-Monfort, 2004; Pérez-Collazos et al., 2008; Segarra-Moragues & Catalán, 2010). R-values were also calculated in each genetic cluster to obtain the proportion of rare bands captured by sampling one population within them.

The Preferred Sampling Area (PSA) for each rare AFLP band was chosen regarding the higher frequency within the areas (Table S1). Regarding the PSA percentages and the R-values of the genetic clusters, the optimal proportion of the populations to be sampled in each cluster was calculated. For each PSA, the populations were chosen by considering the higher value of Nei's gene diversity index (Salmerón-Sánchez et al., 2017) to manage the maximum amount of diversity.

The number of rare bands found in each population (Table 1) was also considered to test which percentage of them would be recovered after selecting the minimum number of populations of *J. auricula* following our managing scheme. Moreover, in order to verify the success of the proposal, the number of AFLP bands that would be captured if the population selection process had been random was calculated. Average values were calculated over 100 repetitions.

2.2. Contribution of the populations to global genetic diversity for ex situ conservation

In order to create a seed bank that maximizes genetic diversity, the software Metapop2 v2.2.1 (López-Cortegano et al., 2019) was used on the AFLP dataset. This software calculates the expected proportional contribution (C_x; Table 2) of each population (within the genetic clusters) to a theoretical synthetic pool with maximum global gene diversity (D_{max}). The software maximizes the function $D_{max} = 1 - \sum_{ij=1}^{n} f_{ij}\, c_i\, c_j$ where f_{ij} is the average coancestry between populations i and j, and c_i and cj is the contribution of subpopulation i and j to the pool (Toro & Caballero, 2005). Randomization process was applied over 1000 repetitions in order to check the success of the selection. Metapop2 v2.2.1 also calculates the proportional contribution of each population to Nei's gene diversity [ΔH_{nei}, (Nei, 1978)] and the proportional contribution of the average Nei's minimum genetic distance (ΔH_{dist}) between populations. These contributions (amount of genetic diversity and distance gained or lost) are calculated by disregarding each population one by one from the analysis in each genetic cluster; as a practical approach values under 2% were not considered. Moreover, as an estimation of the distribution of the genetic diversity, the software calculates the proportion of gene diversity explained within and among populations in each area (Petit et al., 1998).

2.3. Mapping genetic diversity and rarity patterns

To create a genetic diversity (Nei, 1978) and rarity (DW; Schönswetter & Tribsch, 2005) gradient map the Multilevel b-spline tool

Table 2
Metapop2 v2.2.1 results in each of the genetic groups considered (B, C and D): 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; ΔH_t, total variation; C_x, expected proportion of seeds from the populations in order to obtain the maximum diversity values in a synthetic population within each genetic cluster, values under 2% were not considered.

	Population	*ΔH_{nei}*	*ΔH_{dist}*	*ΔH_t*	*Cx (%)*
	1	−0.396	0.308	−0.088	7.30
	2	−0.237	0.102	−0.135	–
	3	0.377	−0.248	0.129	–
	4	0.535	−0.168	0.367	–
	5	0.140	0.104	0.244	–
	6	0.155	−0.311	−0.156	6.30
	7	−0.188	−0.157	−0.345	11.80
	8	−0.167	0.021	−0.145	2.80
	10	0.072	−0.870	−0.799	16.50
Cluster B	11	0.061	−0.503	−0.443	11.10
	12	0.045	0.072	0.117	–
	13	−0.056	0.067	0.011	0.70
	14	−0.404	−0.143	−0.546	19.20
	15	−0.122	0.202	0.080	–
	16	−0.088	0.228	0.140	0.20
	17	0.318	−0.305	0.013	5.80
	18	−0.081	0.334	0.253	–
	19	0.049	−0.161	−0.112	8.80
	20	−0.024	−0.373	−0.397	9.50
	25	−2.128	−2.968	−5.096	34.80
	26	−0.730	−1.798	−2.528	18.30
	27	−1.703	0.132	−1.571	18.30
	28	0.352	−0.809	−0.457	11.90
Cluster C	29	1.395	−0.442	0.953	–
	30	1.546	−0.327	1.220	2.00
	31	0.613	0.998	1.611	–
	32	0.196	−0.010	0.186	14.70
	21	0.510	−0.475	0.035	15.40
	22	−0.529	1.061	0.532	13.50
Cluster D	23	0.588	−7.669	−7.081	34.10
	24	−0.551	−7.993	−8.544	37.00

(Conrad et al., 2015) implemented in QGIS (QGIS-Development-Team, 2017) software was used. This tool interpolates the specific values of the populations drawing the genetic diversity and rarity patterns.

2.4. Plastid and ribosomal DNA patterns

Given the importance of including plastid DNA in conservation proposals as it represents the evolutionary history of plant species (Carvalho et al., 2019), the sequences of the three regions of the plastid DNA obtained for *J. auricula* in Salmerón-Sánchez et al. (2017) were downloaded from GenBank (psbA-3′trnKmatK (Shaw et al., 2005), rpl16 (Small et al., 1998) and trnQ-5′rps16 (Shaw et al., 2007); Table S2). The 75 sequences were assembled and edited using Geneious v 5.5.7 (Drummond et al., 2012) and aligned with Clustal W2 2.0.11 (Larkin et al., 2007). Further adjustments were made by visual inspection. The resulting sequences were concatenated; given the relative high number of haplotypes found by Salmerón-Sánchez et al. (2017), the program Gblocks (Castresana, 2000) was used to trim gapped regions and to remove non informative mutations. Finally, an unrooted haplotype network was constructed using TCS 1.21 (Clement et al., 2000).

On the other hand, the use of ribosomal sequences has allowed to carry out a robust ascription to different clades in the elaboration of molecular phylogenies (Silva et al., 2015), setting *a priori* subdivisions of the populations (Bacchetta et al., 2008). Furthermore, nrDNA sequences are useful in the detection of hybridization events (Widmer & Baltisberger, 1999), as nucleotide additive patterns could be result of recent hybridization events (Aguilar et al., 1999; Plume et al., 2013). It is important to consider the possible existence of intraspecific hybrids and their importance in establishing species management plans (Chan et al., 2019). Ribosomal sequences and ribotypes obtained by Salmerón-Sánchez et al. (2017) were considered in our analyses due to the importance of this type of molecular marker.

3. Results

3.1. Selection of RGUCs

From a total of 1625 AFLP bands, 815 met the rarity requirements (Table 3). Of them, 16 were exclusive to cluster A, 311 were exclusive to cluster B, 110 were exclusive to cluster C, and 62 were exclusive to cluster D. After choosing the PSA for each of the rare bands (Table S1), a total of 115 bands were assigned to cluster A, 336 to cluster B, 173 to cluster C, and 183 to cluster D (1% of the rare AFLP bands were not assigned to any PSA; Table 3). The proportion of rare AFLP bands captured by choosing only one population of the entire range of the species (*i.e.* R-value) was 16.6 %. Considering the different genetic clusters independently, R-values of 22.73 % (cluster B), 36.33 % (cluster C), and 69.20 % (cluster D) were obtained (Table 3 and Fig. 2). Regarding the genetic cluster A, the R-value was not calculated as just one population belongs to this cluster. Based on the PSA distribution of these rare bands and R-values, the optimal proportion of the populations to be sampled within each genetic cluster was 0.31 (cluster B) : 0.26 (cluster C) : 0.43 (cluster D).

With respect to the total number of populations to be sampled, F_{ST} value for the total dataset was 0.323. But considering that cluster A has only one population, F_{ST} value was recalculated excluding this population, an F_{ST} value of 0.312 was obtained. As a result, only four populations are needed (n = 3.95) to gather 99 % of the AFLP bands, whereas six populations are needed (n = 5.95) targeting to 99.9 % the proportion of AFLP bands to preserve.

Giving the optimal proportion of the three clusters, 1.19–1.79 populations from cluster B, 1.07–1.61 populations from cluster C and 1.70–2.56 populations from cluster D should be targeted. The populations with the higher values of genetic diversity within each genetic cluster were chosen: populations 14 and 1 (Nei's GD values of 0.129 and 0.123 respectively) from cluster B, populations 25 and 26 (Nei's GD values of 0.096 and 0.090 respectively) from cluster C, and populations 22, 24, and 21 (Nei's GD values of 0.097, 0.097 and 0.092 respectively) from the cluster D were selected.

The selection proposed gathers 77.54 % of the AFLP bands while the random selection showed a value of 73.33 %. Regarding the number of rare bands found in each genetic cluster, the 100 %, 29.9 %, 27,9%, and 67.4 % of bands were recovered in clusters A, B, C, and D, respectively. Considering all the selected populations, 50.3 % of the rare bands were recovered.

Table 3
Distribution of rare AFLP bands (those with an overall frequency lower than 10 %, and present in less than 20 % of the populations) and RGUCs calculation values in the different genetic clusters (A, B, C and D) considered and throughout the full range of *J. auricula.* PSA (Preferred Sampling Area); R-value (percentage of rare AFLP bands captured by sampling one population within the genetic clusters); n (calculated number of populations to be sampled to include a fixed diversity value; *i.e.*, 99 % and 99.9 %); n values were corrected (see Material & Methods) to adjust the method giving that cluster A has only one population.

	Full range	*A*	*B*	*C*	*D*
Total nº AFLP bands	1625	–	–	–	–
Nº rare AFLP bands	815	115	591	267	227
Exclusive rare AFLP bands	–	16	311	110	62
Nº rare AFLP bands (by PSA)	–	115	336	173	183
% of rare AFLP bands (by PSA)	–	14.11	41.23	21.23	22.45
R-value (%)	16.9	–	22.73	36.33	69.20
Optimal proportion	–	–	0.32	0.26	0.42
n	4.075	–	–	–	–
n (99 % - corrected)	3.955	–	1.24	1.05	1.67
n (99 % - integer)	–	1	2	2	2
n (99.9 % - corrected)	5.95	–	1.87	1.58	2.51
n (99.9 % - integer)	–	1	2	2	3

3.2. Contribution of the populations to global genetic diversity for ex situ conservation

The intra- and inter-population contributions to the total genetic diversity in each area were 78.83 % and 21.17 %, for cluster B, 80 % and 20 % for cluster C, and 76.27 % and 23.73 % for cluster D. The variation of the genetic diversity and distance when removing populations within the cluster B were not significant, being the higher gain of genetic diversity 0.53 % when removing population 3 and the higher loss of genetic diversity of 0.40 % when removing population 14. With respect to the genetic distance, the decrease was 0.87 % when removing population 10 while the increase values were not significant. The optimal contribution calculated for the cluster B included 10 populations (Table 2) being the higher proportional values detected for populations 14, 10, 7, and 11 (with 19.20 %, 16.50 %, 11.80 %, and 11.10 % respectively).

Regarding cluster C, the greatest decrease in genetic diversity (2.12 %) and distance (2.97 %) were found when removing population 25 while the greatest increase in genetic diversity and distance was detected for populations 30 and 31 (1.55 % and 1.00 %, respectively). The optimal contribution calculated for the cluster C included 5 populations (Table 2) being the higher proportional values detected for populations 25, 26, 27, and 32 (with 34.8 %, 18.3 %, 18.3 %, and 14.7 % respectively).

The values calculated for the cluster D showed higher decrease values for genetic distance when removing populations 23 and 24 (4.43 % and 7.99 %, respectively). No significant values regarding genetic diversity were found. The optimal contribution calculated for the cluster D included all the populations (Table 2) being the proportional values detected 37 %, 34.1 %, 15.4 %, and 13.5 % for populations 24, 23, 21, and 22 respectively.

The Nei's genetic diversity values calculated for the synthetic populations in each genetic cluster were 0.149, 0.112, and 0.130 (for clusters B, C, and D respectively) while the random selection of seeds

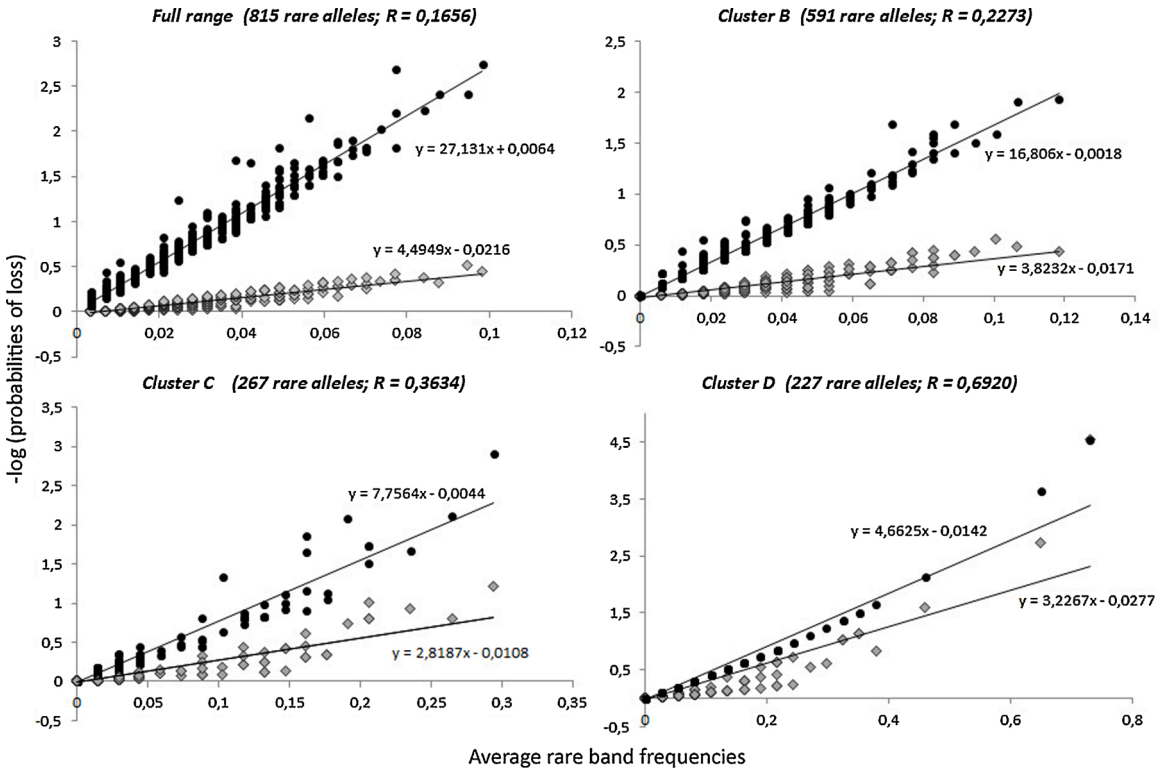

Fig. 2. Regression lines of the average rare bands frequency (x-axis) with the negative logarithms of the observed and expected probabilities of loss [−log(Lo) (grey diamonds) and − log(Le) (black circles)] over the full set of rare AFLP bands and over the genetic clusters (B, C and D) of *J. auricula*. The quotient between the slopes of the observed and the expected regression lines indicates the percentage of rare AFLP bands represented when sampling a single population within the clusters (R-value).

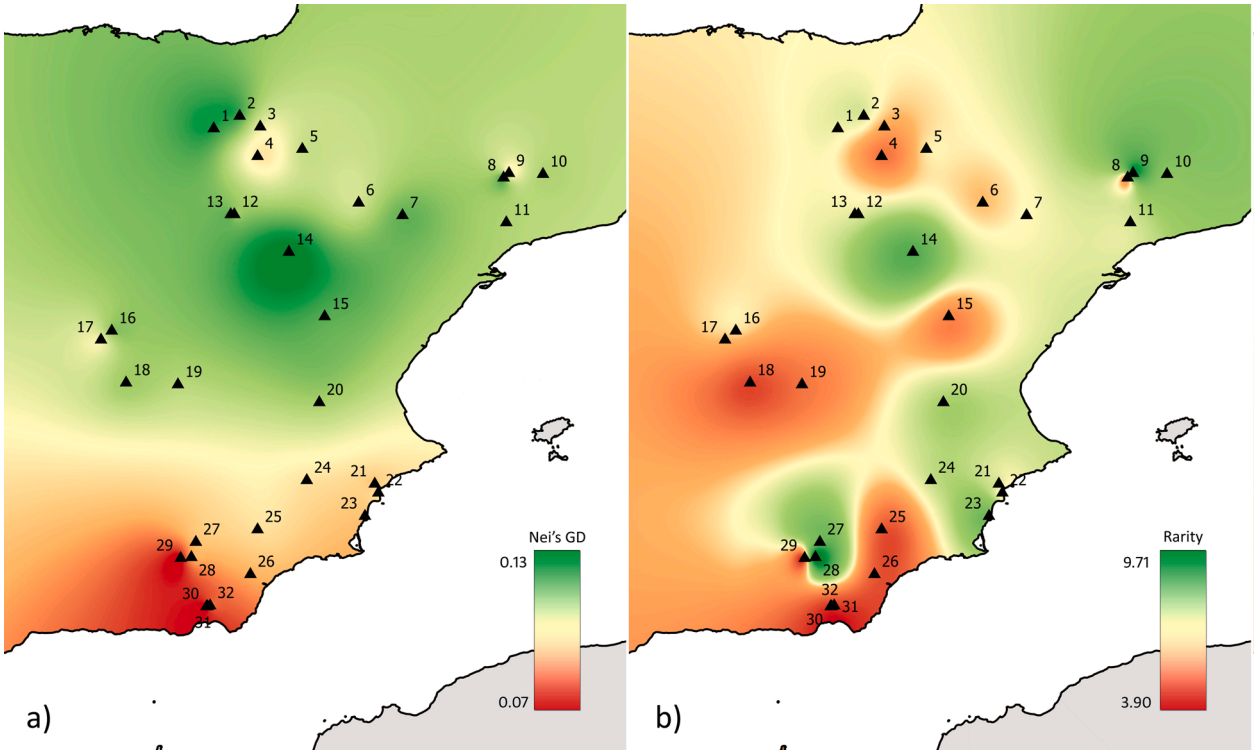

Fig. 3. (a) Nei's gene diversity and (b) rarity patterns (red = low; yellow = medium; green = high). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

from the populations within the clusters would result in values of 0.144, 0.103, and 0.119; this evidences an increase of 3.3 % (cluster B), 7.6 % (cluster C), and 8.1 % (cluster D) of the Nei's genetic diversity values of the proposal with respect to the random selection.

3.3. Map of genetic diversity and rarity patterns

The Nei's gene diversity pattern showed a clear signal of low diversity to the south of the distribution range while the values at the north of the range were higher (Fig. 3a); the most impoverished populations to the south were populations 29 and 30 with a diversity value of 0.74 and 0.71 respectively, while in the north populations 3, 4, and 17 had relative low values of genetic diversity (0.105, 0.098, and 104 respectively). In contrast, populations 1 and 14 held the higher values of genetic diversity to the north (0.123 and 0.129) while populations 22, 24, and 25 held the higher values to the south (0.097, 0.097, and 0.096 respectively).

The rarity pattern (Fig. 3b) also showed the lower values in the southern distributional range (*i.e.*, 3.902, 3.945, and 4.103 in populations 30, 31, and 32 respectively) but also the higher rarity values (7.254 and 9.717 in populations 27 and 28) together with some populations from the north of the Iberian Peninsula (8.672 and 7.637 in populations 9 and 14).

3.4. Plastid and ribosomal DNA pattern

The alignment of the concatenated DNA sequences after the removal of the gaps and uninformative mutations presented 2322 bp which included 33 mutations – eleven of which were considered informative (Table S2). Considering the different regions amplified, six substitutions were found for the *psbA-3'trnK-matK* region, four substitutions were found for the *rpl16* region, whereas the *trnQ-rps16* region only contained two substitutions. These mutations defined a total of 9 haplotypes (Fig. 4). Whereas the central haplotype of the network was distributed along the distributional range, two haplotypes (II and VIII), which differ by only one step from haplotype I, were exclusive to the southern populations. Haplotype IX also differ one step from haplotype VIII and was distributed to the central-south populations but was also found in population 9 to the north. Haplotype III is also one step away from the central haplotype and was found as the main haplotype at the northern populations. Haplotypes IV, V, and VI and are one step away from haplotype III and were exclusive to the northern populations 8, 7, and 10 respectively. Finally, haplotype VII differs by only one step from haplotype V and was also exclusive to three northern populations (*i.e.*, 2, 3, and 6).

Regarding ribosomal sequences, in the phylogeographic study of *Jacobaea auricula* (Salmerón-Sánchez et al., 2017), five different ribotypes were found. This provided evidence of intraspecific hybridization in eight populations (15, 16, 17, 19, 21, 22, 23, and 25). The distribution of ribotypes is shown in Table 1.

4. Discussion

4.1. In situ conservation: selection of Relevant Genetic Units for Conservation (RGUCs)

As Falk & Holsinger (1991) suggest, the highest priority for *in situ* conservation systems is to capture the core of variability present in the species. The method of choosing RGUCs (Pérez-Collazos et al., 2008) allows the selection of the minimum number of populations of *Jacobaea auricula* that should be preserved to mitigate the possible loss of genetic diversity of the species. Our conservation managing proposal would account for a moderate percentage of rare AFLP bands (50 %) when considering the whole distribution of the species. We obtained different results for each genetic group; clusters A and C of our selection accounted for 100 % and 67 % of AFLP rare bands respectively while the results of clusters B and C were lower, capturing 30 % and 28 % of the total number of rare AFLP bands respectively. This is a considerable

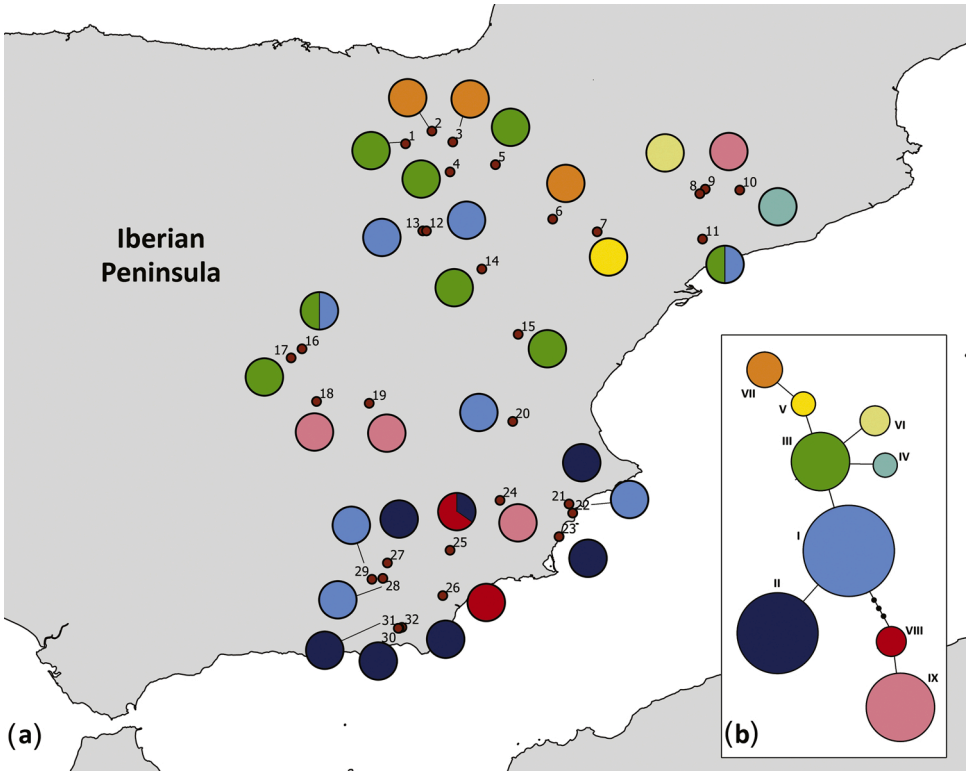

Fig. 4. (a) Geographical distribution of the haplotypes found in *J. auricula*; (b) Haplotype network (size of the circles represents the number of samples; black dots represent haplotypes not found).

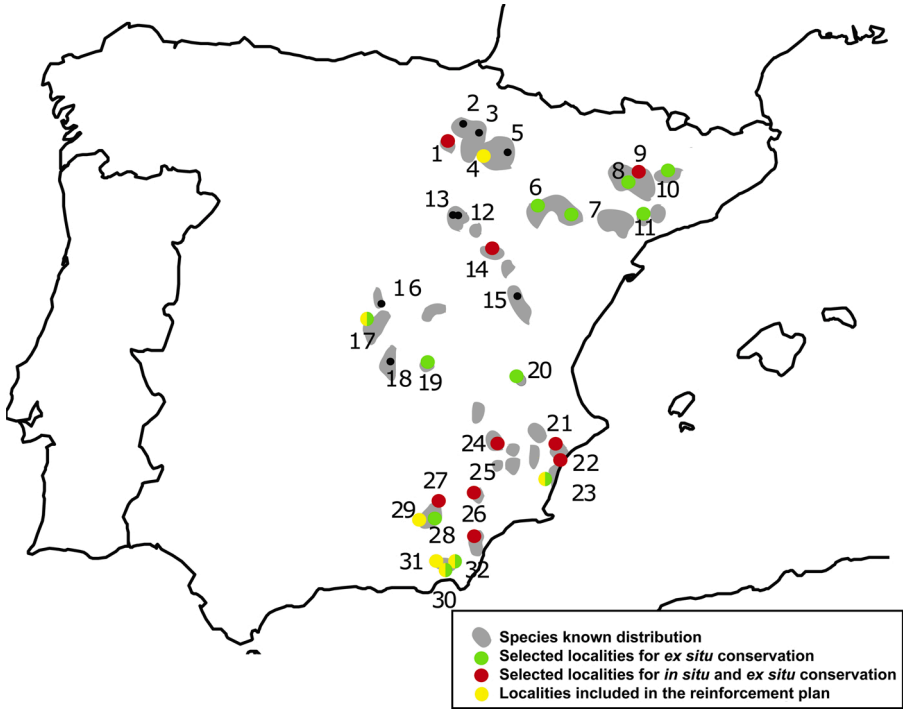

Fig. 5. Geographical distribution of the selected populations for both *in situ* (red) and *ex situ* (green) genetic conservation. Populations selected in the reinforcement plan are colored in yellow. Black dots are non-selected populations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

proportion bearing in mind that, in our proposal, we selected eight populations as RGUCs (see locations in Fig. 5). This is only 25 % of the total number of the populations analyzed. Other authors who have considered the same methodology to create a management proposal required a higher proportion of populations to conserve similar levels of genetic diversity as in *J. auricula* (Caujapé-Castells & Pedrola-Monfort, 2004; Ciofi & Bruford, 1999; Mota et al., 2019; Peñas et al., 2016; Pérez-Collazos et al., 2008; Segarra-Moragues & Catalán, 2010); although it is true that the number of populations to be conserved will depend on the total F_{ST} value for the whole distribution of the species and on the different genetic subdivisions considered. In contrast, the percentage of rare alleles or fragments which would be recovered by selecting the PSA was higher in these studies. This depends on the type of molecular marker used in the proposal of management and the number of rare loci for each species.

In general, when a greater number of populations were selected, the number of rare alleles recovered was greater. For example, in *Androcymbium gramineum* (Cav.) McBride (Caujapé-Castells & Pedrola-Monfort, 2004), eight out of thirteen populations were selected, gathering 97 % of rare alleles. In the case of *Boleum asperum*, four out of eight populations were selected (Pérez-Collazos et al., 2008) which included 85.10 % of the rare bands. Also, in *Borderea pyrenaica* Miégeville, five out of the eleven populations studied were selected (Segarra-Moragues & Catalán, 2010), containing 97.5 % of the rare alleles. In other studies, the results were similar to those obtained in *J. auricula*. Thus, in *Convolvulus boissieri* Steud., where 6 out of the 15 populations studied were selected, around 61 % of the rare fragments were preserved (Mota et al., 2019). Also, these different outputs based on the recovery rate of rare fragments or alleles can be a consequence of the number of molecular markers studied, or of the molecular marker type. Thus, a higher number of rare bands analyzed (815 in *J. auricula,* 273 in *A. edulis*, 102 in *C. boissieri* and 47 in *B. asperum*) were found in the studies based on dominant AFLP markers with respect to those that used codominant markers, such as isoenzymes (38 rare alleles in the *A. gramineum*) or microsatellite markers (24 in *B. asperum*).

Selection of such populations also would allow the capture of most of haplotypes (six out nine), being distributed as follows: haplotype I in population 22, haplotype II in population 21; haplotype III in populations 1 y 14; haplotype VI in population 19; haplotype VIII in populations 25 y 26 and haplotype IX in population 24. This output was similar to the obtained in *C. boissieri* (seven out fifteen; Mota et al., 2019) or in *A. edulis* (five out seven; Peñas et al., 2016) which is considerable as our approach does not take into account haplotypes in the selection of the PSA. Among the non-captured haplotypes, two were exclusive to one single population (haplotype IV and V, located in populations 10 and 7 respectively), and the remaining was present in three populations (haplotype VII, in populations 2,3 and 5).

With respect to ribosomal sequences, all ribotypes except V (exclusive to population 17) were captured. Moreover, we found evidence of intraspecific hybridization in eight of the populations considered (15, 16, 17, 19, 21, 22, 23, and 25; see Table 1). Of them, populations 21, 22, and 25 would be included. Given the controversy regarding the role of intraspecific hybridization in natural populations (Chan et al., 2019), we should be careful in the inclusion of these populations in our management proposal. However, this would be an opportunity for a deeper study of the effect of natural intraspecific hybridization in *J. auricula* that allows us to assess the suitability of hybridization as a conservation tool (as in the case of *Pinus torreyana* Parry ex Carrière; Hamilton et al. (2017)).

Twelve out the 32 populations of J. auricula studied are located within different protected areas (such as micro-reserves, wetlands or natural parks, Sites of Community Importance or Special Areas of Conservation of Natura 2000 network; see Table 1) either partially or in its complete distribution; of them, our RGUC selection included two populations (9 and 22). The fact of already having two populations that are inside protected areas facilitates our work when proposing this *in situ* conservation strategy; in any case, populations within protected areas also need genetic conservation as complementary strategy.

At the present, protection policies applied to *J. auricula* are based on the morphological subdivision of the species (Ascaso & Pedrol, 1991; De

La Torre et al., 1997). As a consequence, only subspecies are included in the different regional and Spanish red lists, under different threats categories (Anthos, 2020). Other protection proposals, such as reserve selection of gypsophile flora (Pérez–García et al., 2011), coincide partially in some localities that we have selected for genetic conservation of *J. auricula* (Table 1 and Fig. 5), but is evident the existence of discrepancies as reserve selection is based on the analysis of the whole gypsophile flora. Our approach, instead, considers the detected genetic groups present in the whole species. From our point of view, present protection could be better targeted and would have a more effective scientific basis if the RGUC concept is followed. Thus, the RGUCs selection would complement present areas of protection of the species throughout its range of distribution.

4.2. *Ex situ conservation: selection for seed bank and reinforcement planning*

Although it appears proven the value of the RGUCs in terms of *in situ* genetic conservation (Peñas et al., 2016; Pérez-Collazos et al., 2008), the conservation proposals focused on passive protection may not be stopping the diversity loss (Fenu et al., 2019). The mere creation of protected areas seems to underestimate the adaptive potential that some populations may contain (Jump et al., 2009; Volis, 2019). Moreover, considering the percentage of rare bands recovered after the selection of eight RGUCs, and due to the restricted distribution of the non-captured ribotype and haplotypes, it is also necessary to establish *ex situ* conservation measures. *Ex situ* and *in situ* techniques should be used in a combined way to maximize the success of the proposal increasing *in situ* protection ability (Engelmann et al., 2007; Hawkes et al., 2000; Li & Pritchard, 2009; Volis & Blecher, 2010). In the creation of a seed bank, collecting most of the diversity is essential to ensure functionality (Caujapé-Castells & Pedrola-Monfort, 2004; Pearse & Crandall, 2004). Traditionally plant material has been collected from several populations from different habitats assuming that diversity was distributed along populations, particularly in wide distribution species (Hamrick & Godt, 1990; Hamrick et al., 1991). Moreover, the capture of alleles present at a very low frequency is unlikely in samples of realistic size when no genetic information is provided (Lawrence et al., 1995). The establishment of systematic strategies to maximize the collection of the genetic diversity and rarity is thus imperative (Farnsworth et al., 2006).

The optimal proportion of seeds calculated in our study provides a direct estimation in order to maximize the genetic diversity in the seed bank within the four different clusters. Also, this method allows the optimization of the sampling effort establishing a lower limit under which we could dismiss the importance of the given population to the whole seed bank (*e.g.*, 2%). At least 21 of the 32 populations (ten from cluster B, six from cluster C and all the populations from clusters D and A) should be selected to create a seed bank for the species. This selection not only gathers all the haplotypes and ribotypes of the species but also those present within the four genetic clusters (see locations in Fig. 5). Further studies are needed to correct the optimal proportions calculated when taking into consideration the differential gemination rates of the different areas and populations (Bacchetta et al., 2008). Moreover, in order to ensure the success of the *ex situ* proposal and giving the self-compatible characteristics of the species (Kunin, 1997), the seed production in self and cross pollination should be studied.

The reinforcement of populations of rare and threatened species has become essential for biodiversity conservation (Armstrong & Seddon, 2008). These proposals aim to increase the survival of a given species (Commander et al., 2018; Volis & Blecher, 2010). The genetic diversity pattern of *J. auricula* shows low values in all the populations in the southern distributional range; a similar pattern has been found for other edaphic endemic species as *Gypsophila struthium* Loefl. (Martínez-Nieto et al., 2013). The most impoverished populations are populations 29–32 (with values under 0.085 Nei's GD). Furthermore, populations 4 and 17 from cluster B and population 23 from cluster D also hold low genetic values when compared with the rest of the populations of the respective clusters (Fig. 5). Our proposal includes the reinforcement of these populations with the optimal proportions calculated here for each cluster. Two considerations must be made regarding the reinforcement of the populations: 1) the risk of inbreeding depression (Barrett & Kohn, 1991; Keller & Waller, 2002), and 2) outbreeding depression (Hufford & Mazer, 2003; Tallmon et al., 2004). Regarding inbreeding depression, the creation of an efficient seed bank that consider the genetic diversity and rarity makes population reinforcements reliable and helps to increase the success of the proposal (Fenu et al., 2019; Lienert, 2004). Outbreeding depression must also be considered given the wide range of *J. auricula*. The ecologic differences of the areas that the plant inhabits makes it probable that the introduction of individuals from different conditions decrease the survival and reproductive ability as local adaptations could have been developed within the areas (Fenster & Galloway, 2000; Lema & Nevitt, 2006). The genetic pattern of four clusters found by Salmerón-Sánchez et al. (2017) reduces the risk of outbreeding depression as the reintroductions are made from populations from the same cluster (Kaulfuß & Reisch, 2017; Shemesh et al., 2018).

5. Conclusions

In situ-based conservation relying on global floristic criteria does not guarantee the conservation of genetic diversity of the different populations of the species. This is even the case where these species appear linked to a very specific habitat, such as outcrops of gypsum and salt-soils. Our proposal would improve the implementation of future genetic conservation measures for *J. auricula* – a model of endemic plants from edaphic habitat islands – allowing us to preserve the highest proportion of the gene pool possible of the species by combining both *in situ* and *ex situ* approaches. This ensures that future translocation and reinforcement planning of the most degraded populations will be possible.

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Declaration of Competing Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jnc.2021.126004.

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