Accepted Manuscript

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PII: S1617-1381(14)00091-0
DOI: http://dx.doi.org/doi:10.1016/j.jnc.2014.08.007
Reference: JNC 25376

To appear in:

Received date: 19-2-2014
Revised date: 10-8-2014
Accepted date: 18-8-2014

Please cite this article as: Alonso, M. Á., Guilló, A., Pérez-Botella, J., Crespo, M. B., and Juan, A.,Genetic assessment of population restorations of the critically endangered *Silene hifacensis* in the Iberian Peninsula, *Journal for Nature Conservation* (2014), http://dx.doi.org/10.1016/j.jnc.2014.08.007

Genetic assessment of population restorations of the critically endangered *Silene hifacensis* in the Iberian Peninsula

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Abstract In order to preserve endangered plant populations and recover their evolutionary potential and ecological behaviour, some restoration measures generally involve the reinforcement of the population size in existing natural populations or the reintroduction of new populations. Genetic monitoring of both natural and restored populations can provide an assessment of restoration protocol success in establishing populations that maintain levels of genetic diversity similar to those in natural populations. The highly threatened Spanish species *Silene hifacensis* (Caryophyllaceae) has only three natural reduced mainland populations in the Iberian Peninsula, following decline and extinction occurred during the late 20th century. Preterit restoration strategies were essentially based on the implantation of new populations and reinforcement of certain existing populations using transplants mostly cultivated in greenhouses. In the present contribution, levels and patterns of genetic variability within natural and restored populations of *S. hifacensis* were assessed using the molecular technique AFLP. Our results pointed out significant genetic diversity differences across the three existing natural populations though their population fragmentation and progressive loss of individuals have not had impact on the global genetic diversity of this species. For restored populations, their levels of genetic diversity were similar and even higher than in natural populations. As a result, the past restoration protocols were successful in capturing similar and even higher levels of genetic diversity than those observed within natural pools. However, inbreeding processes have been detected for two restored populations. Finally, the main source of plant material for the long-time restored transplants appears to be the natural population of Cova de les Cendres. This study demonstrates, once again, how genetic markers are useful tools to be taken in consideration for endangered plant species conservation plans.

Keywords endangered species, AFLP, restoration genetics, conservation, *Silene*, Mediterranean endemics

Introduction

Management plans for preserving endangered plant species generally involve the reinforcement of the natural population size in existing populations or the reintroduction of new populations. Population restoration is a common practice to recover evolutionary potential and autonomous ecological behaviour (Kephart 2004 and Godefroid et al. 2011). Prior to any restoration action, the provenance of plant material and the location of new introduced populations should be defined with accuracy. The origin, variability and relatedness of source material used for both reinforcement and reintroduction should be considered, since significant disadvantages can arise if poorly adapted transplants are used (Vergeer et al. 2004), and to avoid genetic swamping of local gene pool (Potts et al. 2003), and homogenization of the natural genetic structure of the species; all these therefore, resulting in a loss of biodiversity (Krauss and He 2006). The identification of the current genetic variation should be used as a critical element in the design and implementation of restoration activities. Restoration of new populations only established from scarce local plant provenances could be negative (Ritchie and Krauss 2012). The transplants might show a partial and reduced genetic diversity against natural populations, and genetic bottleneck processes might occur facing a higher level of genetic drift (Hufford and Mazer 2003). According to Falk et al. (2001), a reintroduction project would be genetically accurate if it would be able to replicate the original gene pool. Therefore, the genetic variability within and among natural populations should be also considered when conducting any restoration activity. Nevertheless, many former restoration actions have traditionally been undertaken without a sufficient knowledge of the genetic information of each natural population to be reinforced and of those mixtures of transplants to be used.

The Iberian Peninsula is largely considered as a remarkable center of plant biodiversity, due to the high presence of exclusive and endemic flora (Médail and Quézel 1999), which is mostly considered highly threatened and critically endangered (Moreno 2010). This flora is protected under different national and regional law, but the practical conservation of this Spanish threatened flora depends on regional administrations. Most of the official management plans and therefore the functional preservation actions are only developed for regional populations; as defined by political boundaries, which are not always fully coincident with the whole distribution area of a specific endangered plant. Concerning plant species with natural populations in two (or more) regional territories, practical conservation activities are independently achieved by each regional administration. In the particular case of the Valencian Community (East of the Iberian Peninsula), this region houses almost 70 vascular plant species, listed as critically endangered (Laguna et al. 1998). Practical

conservation activities for these plant species were mainly based on storage of plant material (seeds) in different regional germplasm banks (e.g. Botanical Garden of Valencia, Center for Forestry Experimentation and Research-CIEF), ex-situ controlled cultivations, reinforcement measures for existing or extinct populations and establishment of new reintroduced populations (see http//:www.cma.gva.es). This regional management should be accurately analysed using different approaches, including molecular tools.

In the frame of the Iberian region of the Valencian Community, the mentioned reintroduction activities have generally been undertaken without any detailed information about the levels of genetic diversity of the existing natural populations of endangered plant species. Therefore, past long-time restoration and reinforcement protocols might not be preserving the appropriate levels of genetic diversity. Here we study the genetic variation at AFLP loci of the scarce and critically endangered Iberian populations of *Silene hifacensis* Rouy ex Willk. (Caryophyllaceae), to provide a basis for a detailed delineation of genetic variability among natural and reintroduced populations in addition to infer the provenance of used transplants. AFLPs are a versatile tool for addressing spatial genetic structure within populations and delineating provenances for different types of restoration activities (e.g. Bussell et al. 2006; Michalski & Durka 2012). The main aims of this research are (i) to infer the genetic diversity and genetic structure of both natural and restored populations of *S. hifacensis* in the frame of the Iberian Peninsula; (ii) to identify the geographical provenance of the reintroduced samples during past restoration and reinforcement protocols; and (iii) to evaluate the ex situ protocol of plant cultivation at a genetic level for future conservation and management plans of this endangered species.

Material and methods

Study species and distribution area

The species *Silene hifacensis* is a Spanish narrow endemic camephyte up to 50 cm, characterized by pale pink flowers and long calyx tubes, with a notably separation between anther dehiscence and stigma receptivity, and though this is a self-compatible plant, insects seem to be necessary for fruit production (Prentice et al. 2003). The species is confined to vertical calcareous rocky cliffs in two well-isolated areas: (i) Ibiza and some scattered neighboring islets (Balearic Islands); and (ii) the northern coastal region of Alicante

province in the Valencian Community region (Iberian Peninsula) (Blasco et al. 2010). About 700 individuals are reported from 13 different geographical populations in Ibiza in opposition to the approximately 60 individuals reported from only four close localities in Alicante. The population size is not equally distributed, since the number of individuals per population varied from 4 to 200. Both the number of populations and mature individuals were largest in the past decades, since dramatic reductions from 20% to 70% have been identified, and this species was even believed to be totally extinct in the Iberian Peninsula until 1986 (Blasco et al. 2010). Due to the restricted distribution area, the low number of mature individuals per population and the existence of active processes of population regression, this species is listed as Endangered (EN) in the International and National Red Data Books (Blasco et al. 2010, 2011), as Vulnerable (VU) in the Balearic Regional Red Book (Saéz & Roselló 2001), and as in Critical Risk (CR) in the Valencian Regional Catalogue of Threatened flora (Aguilella et al. 2009). Furthermore, two different levels of protection were stated for this species in the Spanish National Catalogue of Threatened Species (Order MAM/2743/2002). The Balearic population is currently considered as "vulnerable", based on the global amount of individuals together with their widespread distribution. Conversely, the Iberian populations of *S. hifacensis* are catalogued as "in peril of extinction", due to the remarkable scarce number of populations, together with the severely low number of individuals per population (mostly less than 25).

Study area and plant material

All the existing populations of *S. hifacensis* in the Iberian Peninsula were studied during 2008, including natural and restored populations. On the one hand, three mainland natural populations named Cova de les Cendres (CC), El Pessebret (PMO) and Morro de Toix (TOIX), plus one insular population (Illot de la Mona, IM), with a global area of occupancy of 19-20 km^2, were located in the northern coast of Alicante (Fig. 1). On the other, three restored populations, named Penyal de Ifac (IFr), Cova de les Cendres (CCr) and Torre del Gerro (TGr), which had previously been established under different management protocols using plant material from any of the mainland populations (Fig. 1, Table 1) were also studied. All three restored populations were established at a time in the early 1990's, and they were successively restored until our sampling (Blasco et al. 2010 and Laguna et al. 1998). IFr and TGr were settled ex novo in sites where populations were respectively either extinct or previously absent. Conversely, CCr was a preexisting wild population, which

was then reinforced. In all cases, plants of *S. hifacensis* grow in rock crevices (mostly the natural populations) or at the basal part (the restored populations) of vertical calcareous cliffs.

A total of 64 samples were collected only from six populations, three natural (CC, PMO, TOIX) and three restored (IFr, CCr, TGr) mainland populations, covering the whole range of the species *S. hifacensis* in the Iberian Peninsula (Fig. 1, Table 1). The insular population of Illot de la Mona (IM) was not included in the present study because this locality was never used as a potential source of plant material for restoration activities prior to 2008 (Laguna et al. 2011). A total of 39 samples (70% of the total) were collected from the three mainland natural populations. 45% of the population of PMO (15 out of 33 individuals) and the entire populations of TOIX and CC (6 and 24 individuals, respectively) were sampled along the vertical sea cliffs, overcoming limitation about the physical inaccessibility of these sites. Provided that these three natural populations could have widely been used as plant material resource for long-past restoration activities, the samples were labeled according to their location along the vertical cliffs, collecting first those individuals close to the path and easy to be sampled. In the case of the restored populations, all members from each population were sampled (total: 25 individuals).

DNA extraction and AFLP protocol

One-two leaves per plant were collected and kept in silicagel before extraction. Total genomic DNA was extracted using a modified method of 2xCTAB protocol (Doyle and Doyle 1987) and total DNA was resuspended in 0.1xTE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). DNA was purified using MOBIO purification kits, according to the manufacturer's protocols. The AFLP technique was carried out in accordance with the AFLP™ Plant Mapping Protocol (Applied Biosystems). A primer trial was carried out with 20 different primer combinations, but only the tested primers EcoR1-ACT + MseI-CAT, EcoR1-ACA + MseI-CTA and EcoR1-AAG+ MseI-CAT showed clearly distinguishable bands. Fluorescently labelled fragments from the selective amplifications were separated by electrophoresis on a 5.0% denaturing polyacrylamide gel using an ABI 3100 automated sequencer. Gel analysis was carried out using Genescan 3.1. and Genotyper 2.0 (Applied Biosystems). Only amplified fragments with sizes ranging from 50 to 500 bases were scored because bands outside this size range cannot be accurately sized.

Statistical analyses

AFLP products were scored by the presence or absence of bands and a binary matrix (0/1) was constructed. For each population, allele frequencies were estimated using the Bayesian approach proposed by Zhivotovsky (1999), based on non-uniform prior distribution of null-allele frequencies. Allele frequencies were then used to calculate polymorphic fragments (P) at 5% level, gene diversity for each population (H), total gene diversity (Ht) and genetic differentiation between populations (Gst) (Lynch and Milligan 1994), using AFLP-SURV (Vekemans et al. 2002). To infer those close related genetic populations, pairwise comparisons between population-based genetic distances of Nei were also conducted with AFLP-SURV. The number of private fragments ($Frag_{priv}$), i.e. those fragments unique to each population, was calculated using the program FAMD 1.30 (Schlüter and Harris 2006). As differences in sampling intensity between populations could bias our comparisons of genetic diversity, we computed both band richness (Br) and polymorphic fragments (Pr) standardized to the smallest sample size by means of a rarefaction method with AFLP-DIV v. 1.0 (Coart et al. 2005). According to Dasmahapatra et al. (2007), the inbreeding coefficient (f_{AFLP}) was obtained using the FAFLPcalc Excel macro (available at http://www.ucl.ac.uk/taxome/kanchon/#publications) to test the inbreeding levels in natural and restored populations. For each measure of genetic diversity, student's t-tests were performed to examine significant differences ($p>0.05$) between natural and restored populations.

To explore the genetic affinity among natural and restored populations (independent and combined matrices), a principal coordinate analysis (PCO) was performed using GenAlex 6 (Peakall and Smouse 2012). In addition, the identity of the genetic structure of the natural and restored populations was inferred using a Bayesian method implemented with the software STRUCTURE v. 2.3.3. (Pritchard et al. 2000) which assigns each studied individual to a genetic population (K) and estimates admixture proportions (Q) for each individual. The proportion of membership for each cluster was calculated without the consideration of sampling localities. The number of clusters, K, needs to be specified a-priori and we used values in the range of 1-4 (natural populations) and 1-7 (natural and restored populations). The final obtained number of K corresponds to genetic populations, which can be or not equivalent to the geographical studied populations. The analyses were performed under the admixture model assuming independent allele frequencies and using a burn-in period of 50000 followed by 100000 Markov Chain Monte Carlo, and the most likely value of K was determined according to Evanno et al. (2005). For each value of K we

repeated STRUCTURE analysis 10 times in order to explore consistency. STRUCTURE HARVESTER v. 0.6.93 (Earl and vonHoldt 2012) was used to visualize the output from STRUCTURE. To facilitate the interpretation of population-genetic clustering results, CLUMPP was also used (Jakobsson and Rosenberg 2007).

Structure results were complemented by the identification of the most likely source of the restored individuals (IFr, CCr and TGr), multilocus assignment tests based on the frequency of AFLP fragments in the predefined natural populations (CC, PMO and TOIX) were performed using AFLPOP v. 1.1 (Duchesne and Bernatchez 2002). To test the assignment of genotypes, allele frequencies were settled as 1/(sample size+1); and the minimum maximum-likelihood difference (MLD) to assign a sample to specific populations was set to 0 or 1. If MLD was 0, individuals were assigned to the population with the highest probability value. With MLD equal to 1, individuals were only assigned to a population if their probability of belonging to that population was at least ten times higher than the probability of their belonging to another population. When the probability of assigning an individual to any candidate population was below a certain threshold (P<0.001), the individual would not belong to any of those populations (Duchesne and Bernatchez 2002).

Results

Population genetic diversity and spatial genetic structure in natural populations

From the three primer-pair combinations here selected, we scored a total of 717 fragments. The genetic diversity showed significant differences among the three natural populations (P<0.0001), with the lowest values in TOIX whereas the highest values corresponded to PMO (Table 1). A total of 104 private-population bands were detected, with a high heterogeneity among natural populations (range: 18-61, TOIX-CC, respectively) (Table 1). After the rarefaction analysis adjusted for sample size, the highest values of Pr and Br corresponded to the CC population (Table 1). The lowest estimated inbreeding levels were found in PMO population (P<0.01), whereas CC and TOIX showed similar values (P>0.05) (average −0.0674). Ht and Gst were 0.2073 and 0.0928, respectively. The highest pairwise Nei genetic distance was found in the southern population TOIX against the northern PMO and CC populations (Table 2).

PCO analysis yielded three principal coordinates accounted for 44.4%, 15.7% and 12.5% of the total variance, respectively. Based on these coordinates, no clear geographical pattern was revealed among the three natural populations (Fig. 2a), though some tendencies could be observed. Samples of PMO and, mainly, TOIX showed a faintly tendency to group separately, but PMO samples clearly overlapped with CC samples. Bayesian clustering analyses using STRUCTURE identified two groups (k=2) (Fig. 3). Similar to PCO, the observed clusters did not correspond to any geographical natural population, indicating the existence of similar genetic structure among all of them. Heterogeneity among populations was reflected in the proportional assignment of their individuals (Fig. 3). Nonetheless, one of the two identified genetic clusters was notably dominant among the three natural populations, whereas the less frequent genetic cluster was well established in the CC population. In fact, certain CC samples were entirely characterized by this less common genetic cluster, which corresponded to those samples collected close to the main path.

Genetic diversity in restored populations

The genetic indexes P, $Frag_{priv}$ and Br were rather similar among all the restored populations, though H values were significantly higher for IFr populations ($P<0.0001$) (Table 1). The total genetic diversity ($Ht = 0.2241$) was higher for restored populations, compared to natural ones ($P<0.0001$). This tendency is also observed between natural and restored samples from Cova Cendres (CC-CCr) ($P<0.0001$), though Pr and $Frag_{priv}$ values are higher for natural populations ($P<0.001$). Higher levels of inbreeding were obtained for the restored populations (average 0.3922; $P<0.0001$) (Table 1). Particularly, CCr and TGr showed the highest values ($P<0.0001$), but no significant differences were only between them ($P>0.05$). Gst value (0.0346) indicates a weak genetic divergence among all restored populations, and Nei genetic distance drops to zero between CCr and TGr populations (Table 2). Finally, band richness was also greater in restored populations compared to natural populations, but this difference did not show any statistical support ($P>0.05$).

Spatial genetic structure between natural and restored populations: genetic assignment

The PCO analysis combining restored and natural populations yielded three principal coordinates (42.1%, 25.1% and 12.7% of the variance, respectively). Samples of any restored population appeared noticeably intermingled among all natural populations without any geographical pattern (Fig. 2b). Nevertheless, most of samples from the restored populations nested close to any natural CC sample. These data are rather coincident with other studied genetic parameters. Among others, minimum values for Nei genetic distance were always obtained between any restored population and the natural population CC. Conversely, the highest values were generally identified for TOIX and PMO against any restored population. STRUCTURE analyses yielded a remarkable genetic heterogeneity among populations (k=2), which pointed out to the existence of closer genetic structure between natural and restored populations (Fig. 3). The three restored populations (CCr, IFr and TGr) showed a similar spatial genetic structure. Most of the individuals are well characterised by the less frequent genetic cluster in natural populations, but it is clearly dominant in those samples collected close to the path in CC.

Analysis of the allocation of the genotypes (AFLPOP) of the three restored populations of *S. hifacensis* assigned most of their individuals (CCr: 6; IFr: 3; TGr: 10) to the natural population CC. Only one individual from CCr and IFr, respectively, plus four samples of TGr matched to the natural population PMO. None of the restored individual matched genetically the southern natural population of TOIX. Consequently, the closest geographical populations CC and PMO could be considered as potential source populations against TOIX. The highest number of individuals assigned to CC would point to regard this natural population as the principal origin of plant material and to a lesser extent, the PMO population.

Discussion

Population genetic diversity and spatial genetic structure in natural populations

Decreasing population size and increased spatial isolation among populations can lead to increased genetic divergence as a result of reduced gene flow, inbreeding and drift (Young et al. 1996 and Lowe et al. 2004). However, the global genetic diversity of the Iberian natural populations of *S. hifacensis* might not be directly influenced by these

mentioned events, because these populations have maintained a high overall genetic diversity, compared to the values reported by Prentice et al. (2003) for the global and regional distribution range of *S. hifacensis* (H_{tot}=0.2030 and H_{reg}=0.1900, respectively). Conversely to other endangered Iberian *Silene* species (López-Pujol et al. 2007 and references cited here), population isolation together with a progressive loss of individuals may not have had a significant impact on the global genetic composition of *S. hifacensis* populations.

Moreover, the typical habitat of *S. hifacensis* involves the usual existence of fragmented and isolated populations in nature, and thus the obtained genetic diversity values compared to data of Prentice et al. (2003) might be also considered as the regular genetic variability for this endemic species. Certain biological factors plus geographical distance could be useful to explain the obtained genetic data. Fruits of *S. hifacensis* are only produced by allogamy, and insect visits are needed for cross-fertilization (Blasco et al. 2010). Besides, the geographical proximity among their natural Iberian populations (over 13 km) may be critical to facilitate the delivery of minimum pollinator services. Furthermore, the maintenance of an active gene flow -via pollen- might avoid inbreeding effects, and genetic diversity levels could be in some extent preserved, overcoming the usual population fragmentation of this narrow endemic species.

Restored populations: genetic diversity and identification of restoration activities

Previous genetic monitoring of plant restoration plans is relatively rare (Young et al. 2005), though one main purpose of species reintroductions is to create populations that closely mimic the characteristics of natural populations (Pavlik 1996). Our findings are very encouraging in regards to the genetic success of the *S. hifacensis* reintroductions. Ex situ plant management and related restoration activities on *S. hifacensis* would warrant maintenance of similar genetic diversity values to those seen in natural populations, as Huffor and Mazer (2003) stated. Since the 1990's, previous ecological restorations were mainly based on ex situ cultivated flowering plants from common greenhouses and nurseries. These plants were kept altogether, and thus cross-pollination would be favored among plants with independence of their geographical origin, which would explain the low genetic differentiation among restored populations. These cyclic activities may enhance the genetic diversity for this threatened species, especially for those reproductive individuals used as transplants. According to Knapp and Dyer (1998), the past ex situ management of cultivated *S. hifacensis* plants closely mimics the genetic characteristics of naturally occurring

populations, since the restored population CCr shows higher genetic diversity than the natural CC population (Table 1), most probably as a result of its long ex situ cultivation history. However, at the same time, this ex situ management could have favored the appearance of severe genetic inbreeding processes (e.g. TGr, CCr). In fact, inbreeding values in CCr population are dramatically higher than those in CC, again related to long-time cultivation management.

Past reintroduction activities for *S. hifacensis* in the Iberian Peninsula were mostly undertaken without any detailed information about the precise seed provenance. To ensure the genetic similarity of introduced and local populations minimizing the probability of outbreeding depression, seed collections should be made near restoration sites (Hufford and Mazer 2003 and Bischoff et al. 2010), but geographical distance between populations is not always the best indicator of population genetic similarity (Montalvo and Ellstrand 2000). The AFLP genetic arrangement and genotype allocation, based on STRUCTURE plus AFLPOP, would show a noticeable meaning in relation to past seed collection actions. Based on these data, the natural population CC would be considered as the potential source of seeds for the past ex situ cultivation activities done in the three restored populations. This genetic information is particularly fairly interesting, since plant reintroduction activities were never recorded in certain populations (e.g. TGr). Nevertheless, selection of potential source populations solely based on a single locality could result in a negative impact on the long-term population viability of transplants due to the probable existence of a reduced genetic diversity and severe genetic bottlenecks (Ritchie and Krauss 2012).

In addition, STRUCTURE data would reveal additional information about the identification of those natural samples widely used as the specific source of seeds. In fact, those *S. hifacensis* individuals first collected close to the path are well characterized by the less frequent cluster, but their genetic information is visibly dominant in most of the reintroduced individuals with independence of the observed restored population. Thus, this fact would point again to consider those peculiar CC individuals as the true source of seeds for most of the past restoration activities. Furthermore, the location of these particular CC individuals along the lower parts of the vertical sea cliff, together with the noticeable ease to collect, might be the main reasons about their recurring selection for long-performed plant collections. This relevant finding might also be related to the importance of the survival of transplants from CC. In fact, transplants from this natural population have shown the highest values of survival against the other natural populations (S. Fos, pers. comm., Ferrer-

Gallego, unpublished data). Nonetheless, seed collections should be made from a large number of individuals to represent population variation adequately (Hufford and Mazer 2003 and Young et al. 2005). Mixing seeds, and even growing reproductive plants altogether, would facilitate the increase of genetic diversity levels among cultivated plants and it consequently would enhance impoverished wild populations of endangered plant species (Young et al. 2005 and Ritchie and Krauss 2012). The recurrent cultivation of an elevated number of reproductive individuals growing together might be originating a permanent and higher gene flow among them. Therefore, this ex situ protocol of controlled plant cultivation of *S. hifacensis* has revealed as an efficient tool for plant restoration management, as it has been shown after comparative genetic analyses between wild and restored samples. However, the original limited seed sourcing might be the main cause of inbreeding depression in most of the restored populations (TGr and CCr), which could likely be overcome using a higher number of seed producer individuals, accordingly to Young et al. (2005).

Recommendations for population conservation

After the establishment of the plan of population management of *S. hifacensis* in Alicante (Generalitat Valenciana 2008), restoration actions are being undertaken using local source of plant material such as seeds and cultivated transplants without previous knowledge of genetic characteristics of populations. Seed collections are adequately monitored and conserved separately according to their provenance, and the number of source plants per population has also been increased. Our obtained data would partially support the independent storage of seeds, though the absence of genetic differentiation between the two closest populations CC and PMO might suggest combined seed collection and storage. Although no ecological differences apparently exist among the sites where natural populations of *S. hifacensis* occur, the genetic divergence found in the TOIX population would suggest that plant samples (seeds or transplants) from the northern populations would initially be collected separately. Restoration actions should be done around close natural populations to ensure genetic similarity of introduced populations, and to avoid outbreeding depression, among other negative consequences, as Hufford and Mazer (2003) stated. This suggestion would be in total accordance with Laguna et al. (2011), who also proposed the establishment of new populations using seed and transplants material from the closest natural population, independent of the population size and number of reproductive individuals. Nevertheless, mixed populations of TOIX and CC might be established in experimental greenhouses for a long-term assessment of eventual genetic improvement of the resulting reproductive individuals, since our findings stress on the importance of the

ex situ particular method of plant production in common nurseries, which would imply the maintenance and improvement of genetic diversity levels for transplants. Similarly to Vergeer et al. (2004), here we would also suggest to increase the use of numerous and unrelated seed producer individuals as the most successful strategy for creating sustainable and viable populations, which at the same time would avoid active inbreeding processes.

Acknowledgments

The work was supported by the project CONSELLERIATERRITORIO4-06i (Conselleria de Territori i Habitage, Generalitat Valenciana). We thank to José Carlos Cristóbal (University of Alicante) and the wardens of the Natural Parks of Montgó and Penyal de Ifac for their help in the fieldwork.

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Fig. 1. Location of *Silene hifacensis* populations in Alicante province (Spain). Black circles correspond to natural populations; grey circles indicate restored populations. Population codes are detailed in Table 1.

Fig. 2. PCO analyses using the scored AFLP phenotypes. Individual samples are represented by a symbol according to their origin. Population codes are detailed in Table 1. (a). Natural mainland populations of *S. hifacensis*; the proportion of the total variance along the two axes was 44.4% and 15.7%, respectively. (b). Natural and restored mainland populations of *S. hifacensis*; the proportion of the total variance along the two axes was 42.1% and 25.1%, respectively.

Fig. 3. Bayesian admixture proportions of individual plants of *S. hifacensis* for a K=2 population model identified by STRUCTURE. Each individual is represented by a vertical line, which is partitioned into K colored segments.

Table 1 Geographical position, sample size (n), and indices of genetic diversity including percentage of polymorphic fragments (P), total genetic diversity of Nei per natural and restored populations (Ht), genetic diversity per each individual population (H), number of private fragments ($Frag_{priv}$), inbreeding coefficient (fAFLP), and after rarefaction analyses band richness (Br) and percentage of polymorphic fragments (Pr) are exposed. Numbers between brackets correspond to standard deviation (SD).

Population	**Latitude/Longitude (N/E)**	**n**	**P**	**Ht/H**	**$Frag_{priv}$**	fAFLP	**Br**	**Pr**
Natural populations		39	49.5	0.2073[a]	104[a]	−0.0674 (0.3934)[a]	1.2798 (0.0654)[a]	52.7[a]
Cova de les Cendres (CC)	38°41.138'/0°9.119'	24	51.0	0.1964 (0.0060)[a]	61[a]	0.1549 (0.1636)[a]	1.343[a]	77.5[a]
El Pessebret (PMO)	38°40.869'/0°9.007'	9	62.2	0.2100 (0.0070)[b]	25[b]	-0.5217 (0.2049)[b]	1.292[b]	52.3[b]
Morro de Toix (TOIX)	38°38.150'/0°0.812'	6	35.4	0.1574 (0.0075)[c]	18[c]	0.1645 (0.6718)[a]	1.188[c]	28.4[c]
Restored populations		25	55.0	0.2241[b]	47[b]	0.3922 (0.2940)[b]	1.3680 (0.0161)[b]	55.0[a]
Torre del Gerro (TGr)	38°49.193'/0°9.581'	14	54.5	0.2019 (0.0061)[d]	19[a]	0.5041 (0.3748)[c]	1.381[a]	64.7[a]
Cova Cendres (CCr)	38°41.138'/0°9.119'	7	58.4	0.2037 (0.0062)[d]	14[b]	0.6139 (0.2925)[c]	1.373[a]	56.5[a]
Penyal de Ifac (IFr)	38°38.131'/0°4.513'	4	52.2	0.2435 (0.0072)[e]	14[b]	0.0587 (0.4278)[d]	1.350[a]	43.8[b]

Statistical comparisons between all natural and all restored populations, and also within natural and within restored populations significant at $P<0.05$. The same symbol or letters mean no significant differences.

Table 2

Pairwise of Nei genetic distance between natural and restored populations of *Silene hifacensis*.

CC	-					
PMO	0.0199	-				
TOIX	0.0268	0.0239	-			
CCr	0.0073	0.0557	0.0510	-		
IFr	0.0302	0.0511	0.0591	0.0186	-	
TGr	0.0068	0.0429	0.0405	0.0000	0.0115	-
	CC	**PMO**	**TOIX**	**CCr**	**IFr**	**TGr**

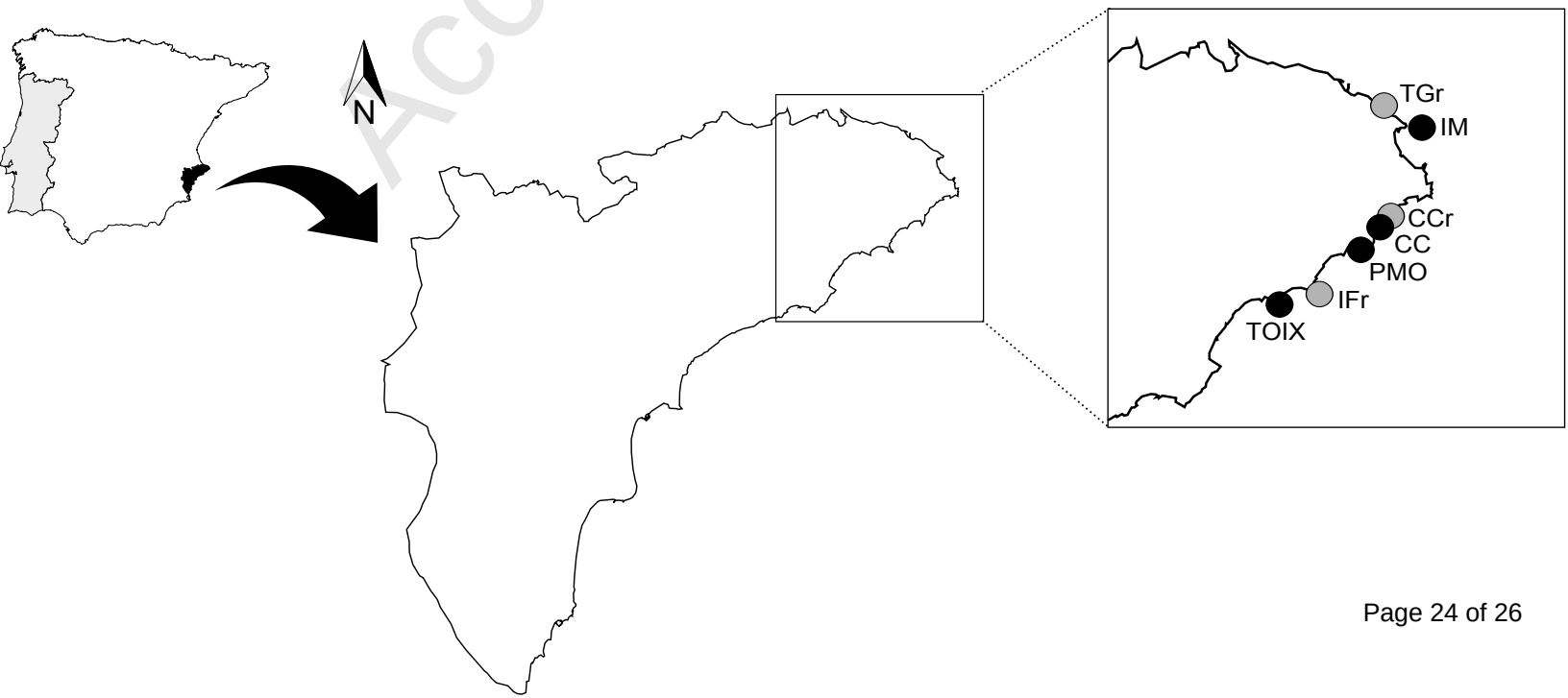

Figure 2

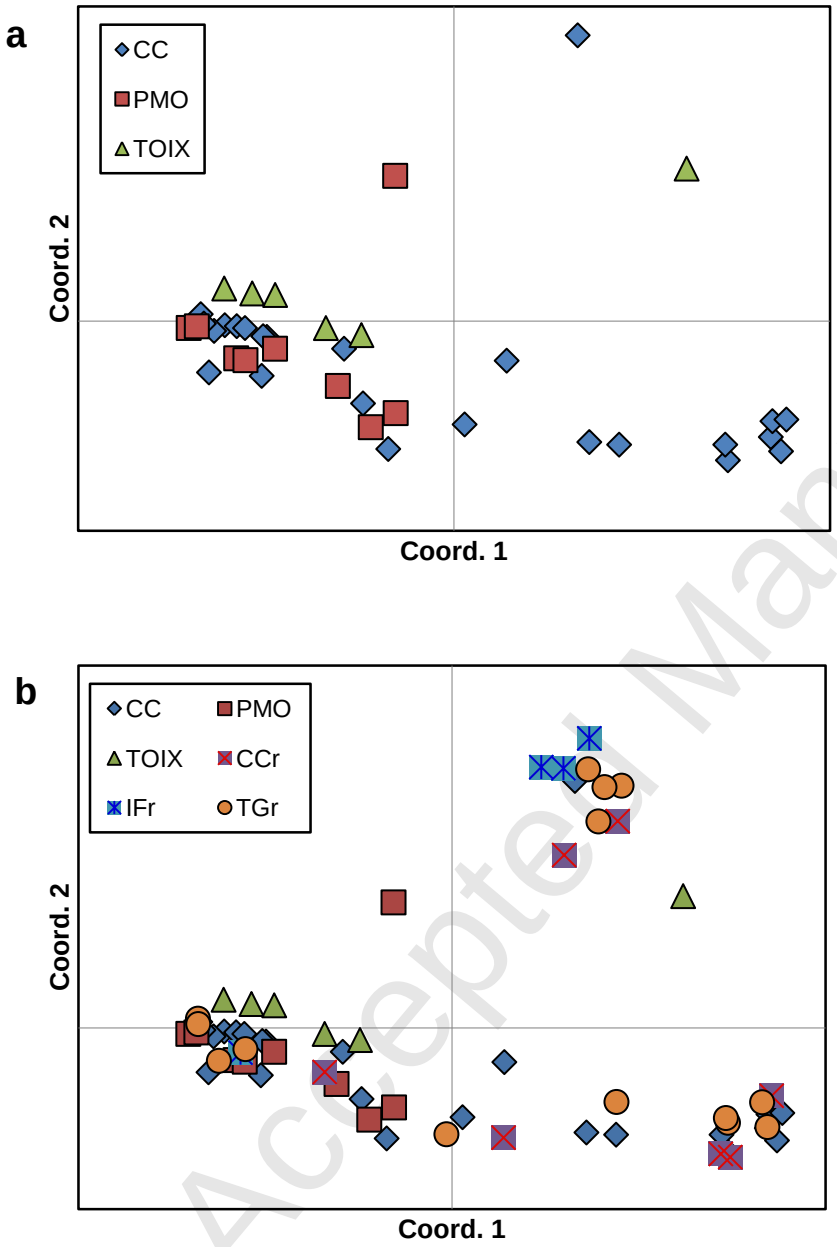

Figure 3