



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 behavior, 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 that 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 *Silene 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 an 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.

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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 behavior (Godefroid et al. 2011; Kephart 2004). 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 & 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 & 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 & 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.

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The Iberian Peninsula is largely considered as a remarkable centre of plant biodiversity, due to the high presence of exclusive and endemic flora (Médail & 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 (Ferrer-Gallego et al. 2013), 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 analyzed using different approaches, including molecular tools.

In the case of the Iberian region of the Valencian Community, the aforementioned 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.

Materials 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 previous 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 (Sáez & Roselló, 2001), and as Critical Risk (CR) in the Valencian Regional Catalog of Threatened flora (Aguilella et al. 2009). Furthermore, two different levels of protection were stated for this species in the Spanish National Catalog 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 cataloged as “in peril of extinction”, due to the remarkable scarce number of

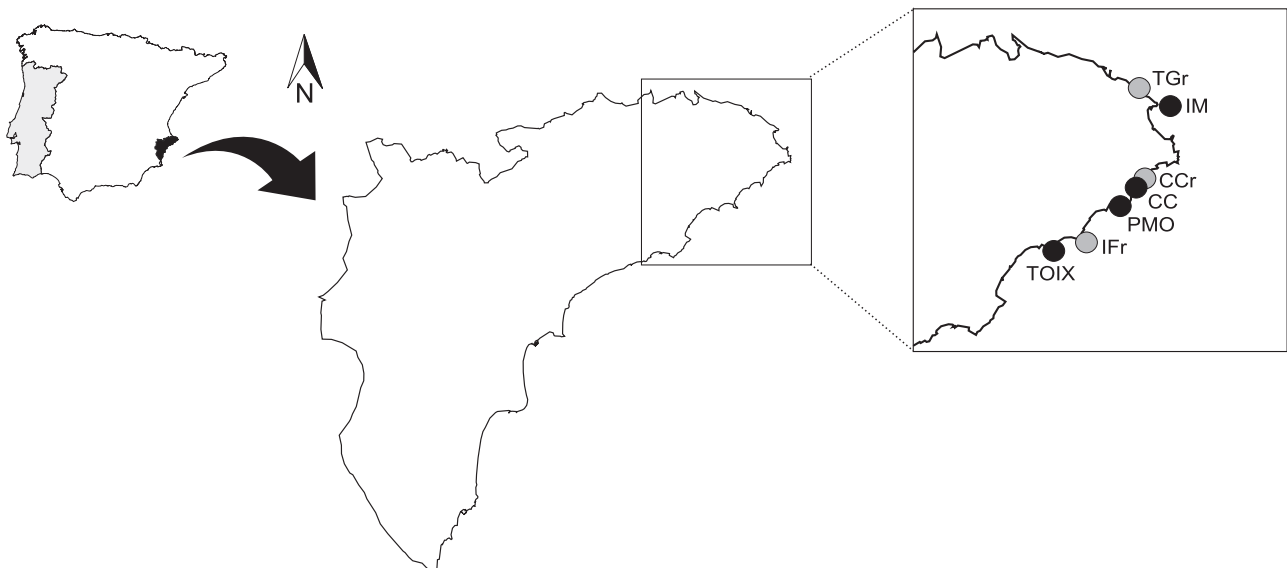


Fig. 1. Location of *Silene hifacensis* populations in Alicante province (Spain). Black circles correspond to natural populations; gray circles indicate restored populations. Population codes are detailed in Table 1.

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