Forest Systems
26 (2), e05S, 10 pages (2017)
eISSN: 2171-9845
https://doi.org/10.5424/fs/2017262-9030
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria O. A., M. P. (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Identification of gene pools used in restoration and conservation by chloroplast microsatellite markers in Iberian pine species

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Abstract

Aim of study: To contribute to the characterization of the origin of material used in afforestation, restoration or conservation activities by using Cp-SSR markers.

Area of study: We used information from the natural range of Iberian pines, from Spain.

Material and methods: We used Iberian pines as an example to undertook gene pool characterization based on a wide Iberian sample of 97 populations from five *Pinus* species (*Pinus halepensis, Pinus pinaster, Pinus nigra, Pinus sylvestris and Pinus uncinata*). Haplotypes from each analyzed tree (derived from nine chloroplast microsatellites markers in *P. halepensis* and six in the rest of the species) were obtained. Based on this information we subdivided each species in regions (considering both genetic structure and its application in afforestation, restoration and conservation programs) and tested the assignation of populations to the different groups based on the genetic distance among samples.

Main results: The rate of successful identification of populations among the different species was very high (> 94 %) for *P. nigra, P. sylvestris* and *P. uncinata*, high (81 %) for *P. pinaster*, and low (< 65 %) for *P. halepensis*.

Research highlights: Chloroplast DNA markers from extensive population datasets can be used to assign the origin of the forest reproductive material in some pine species.

Additional keywords: genetic distance; region of provenance; fingerprinting.

Abbreviations used: cpSSR (chloroplast microsatellite); F_{CT} (variance components of the population permutated among groups); F_{SC} (variance components of genotypes permutated among populations within groups); F_{ST} (variance components of genotypes permutated among populations and among groups); k (number of groups).

Authors´ contributions: Conceived, designed and performed the study: RA, EHT, JH. Analyzed the data: EHT, ZL, RA. Contributed analysis tools: MN. Wrote the paper: EHT, JH, ZL, MN, RA. Coordinated the research project: RA, JH.

Citation: Hernández-Tecles, E.; de las Heras, J.; Lorenzo, Z.; Navascués, M.; Alía, R. (2017). Identification of gene pools used in restoration and conservation by chloroplast microsatellite markers in Iberian pine species. Forest Systems, Volume 26, Issue 2, e05S. https://doi.org/10.5424/fs/2017262-9030

Received: 23 Nov 2016. **Accepted:** 15 Sep 2017.

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Funding: Spanish Ministry of Competitivity and Innovation (RTA2013-00048-C03-01; PCIN-2014-138); Spanish Ministry of Agriculture and Forestry (AEG06-02).

Competing interests: authors have declared that no competing interests exist.

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Introduction

When managing degraded ecosystems the objective is to restore them to normal functioning (Holmes & Richardson 1999), and therefore restoration and conservation activities must be considered complementary. In afforestation and restoration activities, one of the main concerns is the use of a suitable species pool (Jones, 2003), *i.e.* the set of species that can potentially inhabit a site with the local ecological conditions (Zobel *et al.*, 1998; Pärtel *et al.*, 2011; García del Barrio *et al.*, 2013). A special emphasis is being paid to plant species diversification, which involves a better use of the large pool of native species available when using an ecosystem-oriented approach (Bautista *et al.*, 2009). In most cases, it is local species that are promoted, according to basic principles of restoration ecology (Anonymous, 2007) aim towards carbon sequestration and reduction of net CO_2 emissions. Local adaptation has been invoked for the use of local material (Mckay *et al.*, 2005). However, it has been stated that it is seldom the ideal solution (Jones & Monaco, 2009) in degraded or non-productive areas, and that their role is still unclear (*e.g.* the role of local provenance in reintroductions (Sutherland *et al.*, 2006).

Forecasting the performance of forest species used in restoration and conservation activities rely on their intra-specific variability (Langlet, 1971; Van Andel, 1998). Due to the importance of this inter-population differentiation, the core marketing unit of source-identified forest reproductive materials in the certification schemes is the region of provenance, or seed zone (Nanson, 2001). Therefore knowing populations differences both in their levels of diversity for neutral markers and in the variability for important adaptive and performance traits (*e.g*. growth, tolerance to biotic and abiotic stresses) are essential in afforestation and restoration activities (Alía *et al.*, 2009b).

Research has reinforced the idea that the forest material supplied for native woodland creation and restoration should come from a seed source that is both genetically and ecologically proper for the planting site (Mckay *et al.*, 2005; Leimu & Fischer, 2008; Vander-Mijnsbruggea, 2010; Sgrò *et al.*, 2011; Breed *et al.*, 2012; Bucharova *et al.*, 2016), and also would be adapted to the future climatic conditions (Konnert *et al.*, 2015). Considering conservation activities, in particular when managing conservation units or reinforcement activities, the origin of the material is also crucial, as genetic introgression and/or gene flow risk with undesirable origins or non-local material is a big concern for natural populations and their regeneration (Moritz, 1994; Moritz, 1999; Robledo-Arnuncio *et al.*, 2009; Steinitz *et al.*, 2012). Taking into consideration the origin of forest reproductive material is essential for the conservation of forest genetic resources (Koskela *et al.*, 2013), *e.g*. when determining the gene pool that can be used in the vicinities of conservation units (Mckay *et al.*, 2005).

In all these cases, the possibility of checking the origin of the material is essential for an effective control of the marketing of the material used in restoration and conservation activities. Forest tree species present low levels of breeding, and it is hard to have reliable fingerprinting methods to control the use of the correct reproductive material at the population level, or to avoid marketing fraud (Nanson 2001; Degen *et al.*, 2010), despite its extensive use. Some attempts to identify forest reproductive material have been applied to specific materials in pine species (Aragonés *et al.*, 1997; Ribeiro *et al.*, 2002; Deguilloux *et al.*, 2004; Tigabu *et al.*, 2005; Fidler *et al.*, 2006), and also different DNA fingerprinting approaches have been implemented to assign material in *Quercus robur* L. (Degen *et al.*, 2010), following methods already in use for other organisms (Honjo *et al.*, 2008). Also, some methods for origin traceability are being implemented in important tropical timber species (Tnah *et al.*, 2009; Degen *et al.*, 2010; Hong *et al.*, 2010).

Different approaches have been used to identify gene pools based on genetic markers. Especially those derived from Bayesian approaches (*e.g*. Pritchard *et al.*, 2000; Dupanloup *et al.*, 2002). However, these approaches usually group populations with contrasting performance in quantitative or adaptive traits (González-Martínez *et al.*, 2004), and also from different regions of provenance and therefore marketing units.

Extensive studies using chloroplast microsatellites (cpSSRs) covering the distribution range of different species are now available (*e.g*. Soranzo *et al.*, 2000; Gómez *et al.*, 2005; Bucci *et al.*, 2007; Heuertz *et al.*, 2010) . Therefore, it would be interesting to test if these markers constitute a reliable tool for the identification of material to be used in restoration and conservation activities. To our knowledge, there is not an attempt to use extensive information on the geographic variation of forest tree species to test whether it is possible to differentiate among different regions of provenance for important groups of forest species.

We tested here the identification of populations of various pine species with contrasting levels of differentiation (Soto *et al.*, 2010), as an example for the use of extensive marker datasets of populations that are becoming available (*e.g*.: $(GD)^2$ Database: https://gd2.pierroton.inra.fr/gd2/login/login, Demiurge database: http://www.demiurge-project.org/). The species considered were *Pinus halepensis* Mill., *Pinus pinaster* Ait., *Pinus nigra* Arn. subsp. salzamanni (Dunal) Franco, *Pinus sylvestris* L., and *Pinus uncinata* Ram. Forest tree species play an essential role in determining many ecosystem properties and also influence the genetic diversity of associated organisms (Whitham *et al.*, 2006). These species are highly relevant in Europe and along the Mediterranean region as they are broadly used in afforestation, restoration and conservation programs. Moreover, extensive studies on the genetic variation of the species have been done (*P. halepensis*: Morgante *et al.*, 1996; *P. pinaster.* Vendramin *et al.*, 1998; *P. pinaster*: Bucci *et al.*, 2007; *P. halepensis*: Grivet *et al.*, 2009, 2013; *P. pinaster* and *P. sylvestris*: Soto *et al.*, 2010; Unger *et al.*, 2014), and reveal contrasting levels of variation using cpSSR markers. We used haploid cpSSR genetic markers to check the assignation of populations to different groups with application in afforestation, restoration and conservation activities.

We applied the methods to the regions of provenance of the species in the Spanish Iberian Peninsula. The area has a particular climatic regime of cold, wet winters and hot, dry summers, and a long history of human activity, grazing pressure, and fires (Valbuena-Carabaña *et al.*, 2009). Also, reforestation and afforestation activities have a long tradition with more than 2.2 million

of hectares reforested with these pine species, and representing the 74% of the total reforested area during the period 1940-1995 (Montero, 1997).

Firstly, we grouped populations for each species with use in afforestation and conservation programs (Alía *et al.*, 2009b). Secondly, we tested the probability of assignment of different samples to the different groups, based on the Sλ genetic distance (Ribeiro *et al.*, 2002) using a Monte Carlo method among each population and the implemented reference groups through a novel software (Blue Caterpillar).

Material and methods

Plant material and cpSSR determination

We sampled 97 populations (Fig. 1) from five autochthonous *Pinus* species covering their natural distribution range in Spain: *P. halepensis* (14 populations), *P. pinaster* (33 populations), *P. nigra* (19 populations), *P. sylvestris* (26 populations) and *P. uncinata* (five populations). These populations are autochthonous and include the most important regions of provenance and conservation areas with interest for restoration and afforestation (Alía *et al.*, 2009a) or conservation activities (Jimenez *et al.*, 2009).

Different sampled populations have been selected for conservation purposes in these species: three in *P. halepensis*, nine in *P. pinaster*, eight in *P. nigra*, six in *P. sylvestris* and three in *P. uncinata*. These are regions of interest in restoration and conservation activities which are summarized in Figure 1.

Sampling scheme, DNA amplifications and fragment sizing have already been described (Gómez *et al.*, 2005; Soto *et al.*, 2010). Needles were collected from 24 trees in each population, at least 50 m apart from each other. Nine pairs of chloroplast microsatellite

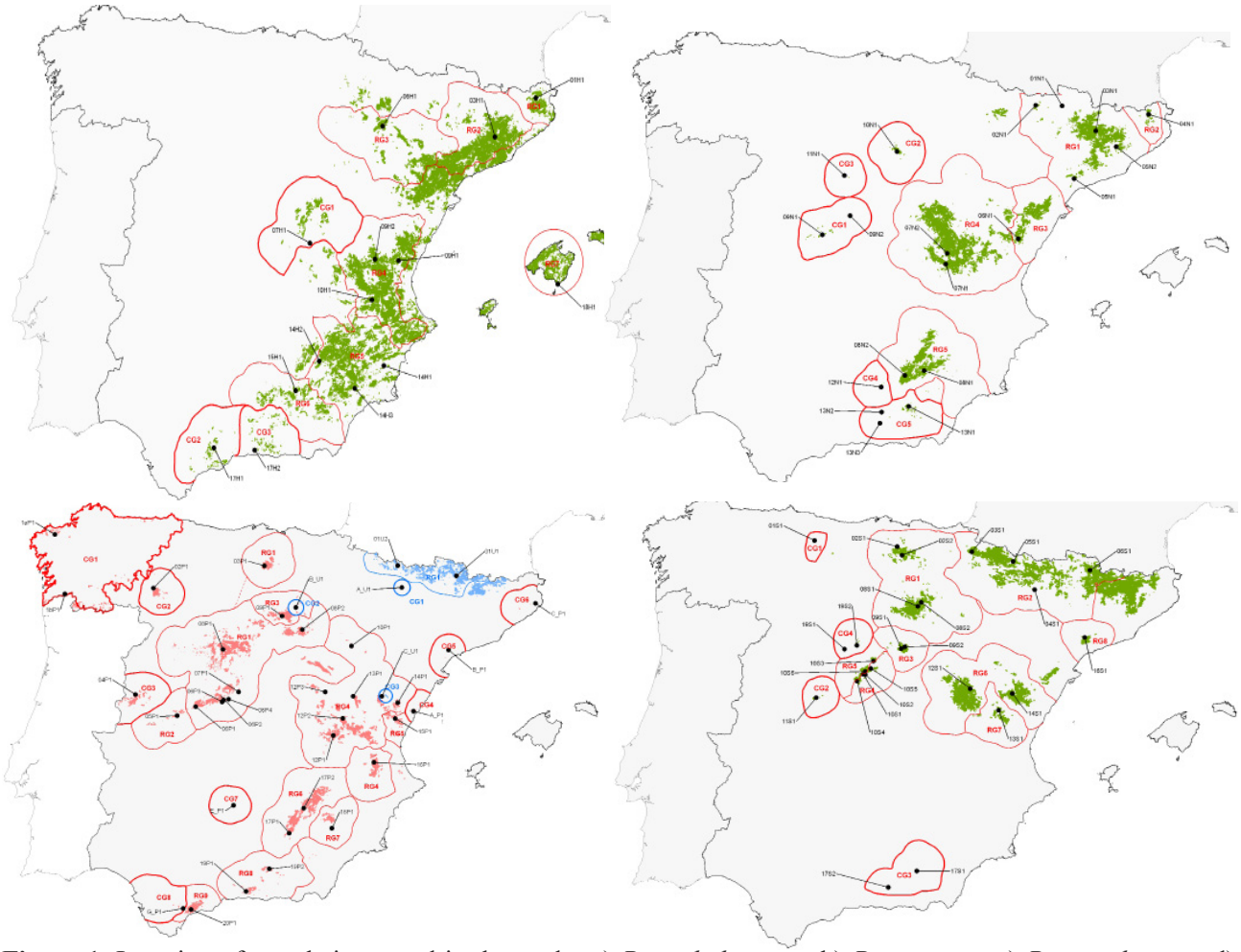


Figure 1. Location of populations used in the study: a) *Pinus halepensis*, b) *Pinus nigra*, c) *Pinus sylvestris*, d) *Pinus pinaster* and *Pinus uncinata*. The autochthonous range of the species is included in green colour, except for *P. pinaster* (red) and *P. uncinata* (blue). The limits of regions of provenance (RG) and conservation areas (C) are included as solid lines.

primers (Pt4825, Pt110048, Pt15169, Pt26081, Pt36480, Pt41093, Pt71936, Pt79951, Pt87268) were used in *P. halepensis*, and six pairs (Pt15169, Pt30204, Pt36480, Pt71936, Pt87268, Pt1254) in *P. pinaster*, *P. nigra*, *P. sylvestris*, and *P. uncinata*. Each chloroplast haplotype was defined as the combination of the cpSSR fragments (nine regions in *P. halepensis* and six regions in the rest of species).

Statistical methods

—*Population grouping*. We firstly grouped the populations according to the region of provenance for each Pinus species in Spain (Alía *et al.*, 2009a). In a second step, we grouped those regions in gene pools based on the similarity established by the cpSSR data. The regions of provenance were grouped together with ressembling genetic groups for each species. For *P. pinaster* and *P. halepensis* we used the classification done by Jaramillo-Correa *et al.*, (2010) Bayesian clustering methods provide one of the best way to assess the genetic structure in cases of unknown genetic origin (Pritchard *et al.*, 2000; Dawson and Belkhir 2001; Corander *et al.*, 2003; Falush *et al.*, 2003). Moreover, they allow testing for panmixia origin of each individual from different reference populations. We performed a STRUCTURE version 2 (Pritchard *et al.*, 2000), without prior information on the locality of origin, allowing the allele frequencies to be correlated among them for the rest of the species. This method is the recommended configuration to be used in the case of limited population structure (Falush *et al.*, 2003). The number of groups (*K*) was set from a minimum of one to a maximum of 10, and ten simulations were run for each *K*-value with a burn-in of 100,000 and with 100,000 iterations each. Theron the mean value of the posterior probability was calculated from the ten simulations for each *K*, and the most likely number of clusters was selected following the methodology proposed by Evanno *et al.*, (2005), implemented in the software STRUCTURE HARVESTER (Earl & von Holdt, 2012). For each species, three groups were defined, but there is a lack of geographical correspondence.

An analysis of molecular variance (AMOVA, Dupanloup *et al.*, 2002) was performed to define the

Table 1. Gene pools defined for conservation (CG), or restoration and afforestation (RG) purposes. For each gene pool the region of provenance defined for the corresponding species is specified.

ID	*P. halepensis*	*P. pinaster*	*P. nigra*	*P. sylvestris*	*P. uncinata*
CG1	07H1	1aP1, 1bP1	09N1, 09N2	01S1	A_U1
CG2	17H1	02P1	10N1	11S1	B_U1
CG3	17H2	04P1	11N1	17S1, 17S2	C_U1
CG4	-	A_P1	12N1	19S1, 19S2	-
CG5	-	B_P1	13N1, 13N2, 13N3	-	-
CG6	-	C_P1	-	-	-
CG7	-	E_P1	-	-	-
CG8	-	G_P1	-	-	-
RG1	01H1	03P1, 06P1, 06P2, 06P3, 06P4, 07P1, 08P1, 08P2	01N1, 02N1, 03N1, 05N1, 05N2	02S1, 02S2, 08S1, 08S2	01U1, 01U2
RG2	03H1	05P1	04N1	03S1, 04S1, 05S1, 06S1	-
RG3	06H1	09P1	06N1	09S1, 09S2	-
RG4	09H1, 09H2, 10H1	10P1, 12P1, 12P2, 12P3, 13P1, 14P1, 16P1	07N1, 07N2	10S1, 10S2, 10S4, 10S5	-
RG5	14H1, 14H2, 14H3	15P1	08N1, 08N2	10S3, 10S6	-
RG6	15H1	17P1, 17P2	-	12S1, 14S1	-
RG7	18H1	18P1	-	13S1	-
RG8	-	19P1, 19P2	-	16S1	-
RG9	-	20P1	-	-	-

distribution of genetic diversity among populations without an explicit a priori definition of population structure. AMOVA defines groups of populations that are geographically homogeneous, and maximally differentiated from each other. The significance of the variance components of the population permutated among groups (F_{CT}), of genotypes permutated among populations within groups (F_{SC}) and of genotypes permutated among populations and among groups (F_{ST}) were tested by 1000 permutations of individuals for each of the hierarchical levels. We tested K = 2 to 10 groups of populations. The number of groups was selected according to the highest FCT value using the sum of squared size differences between haplotypes with 1000 simulated annealing processes.

The gene pools for each species indicating the region/s of provenance included, and the number of populations sampled, are included in Table 1.

—*Assignment of populations to the groups*. A reference population was defined for each group by including all haplotypes of the populations from a given group.

We estimated the genetic distance S_λ (Ribeiro *et al.*, 2002) for each of the sampled populations respect each of the k groups of the different species as:

$$S_\lambda = \sum_{1}^{n} (X_{iR}-X_{i\lambda})^2$$

Where n is the total number of different haplotypes found both in the reference group and in the λ population, X_{iR} is theW frequency of the ith haplotype in the reference group and $X_{i\lambda}$ the frequency of the i^{th} haplotype in the λ population.

Numerical tests based on Monte Carlo methods were used to estimate the significance of the Statistics (Manly, 1997). A given population could be assigned to various reference groups or to none of them. A non-significant $S\lambda$ distance close to 0 indicates that we cannot exclude this reference group as origin of the population. For reference groups with only one population, we measured its distinctiveness in contrast with the rest of populations. This method was implemented in the *Blue Caterpillar* software (https://sites.google.com/site/navascuesresearch/publications-conferences/software/blue-caterpillar).

Table 2. Results of the AMOVA analysis, showing the number of groups distinguished, the differentiation among groups (F_{CT}), among populations within groups (F_{ST}) and within populations (F_{SC}).

	P. halepensis	*P. pinaster*	*P. nigra*	*P. sylvestris*	*P. uncinata*
Populations	14	33	19	26	5
Groups	10	7	8	9	3
FCT	0.100	0.218	0.208	0.220	0.526
FST	0.110	0.290	0.245	0.245	0.598
FSC	0.018	0.092	0.047	0.032	0.152

Table 3. Classification summary. For each type (Conservation, Restoration/Afforestation) we included: number of populations/number of populations well assigned/number of populations incorrectly assigned. For the species incorrectly assigned we included the code of the populations assigned to other group (*), and the code of the populations not assigned to any other group.

	P. halepensis	*P. pinaster*	*P. nigra*	*P. sylvestris*	*P. uncinata*
Conservation	3/3/0	9/8/1	8/7/1	6/5/1	3/3/0
	-	1bP1	13N3	17S1	-
Restoration and afforestation	11/6/5	24/19/5	11/11/0	20/20/0	2/2/0
	09H1, 09H2*, 14H1*, 14H2, 14H3	06P1, 06P3, 06P4, 07P1, 13P1	-	-	-
Total	14/9/5	33/27/6	19/18/1	26/25/1	5/5/0
%	64.3	81.8	94.7	96.2	100.0

Results

All the pine studied species exhibited high rates of differentiation among populations (Table 2), except for *P. halepensis*. Using the analysis of molecular variance (AMOVA), it was possible to distinguish different groups that ranged form three to ten depending on the species.

Those groups were subdivided according to their distinct purpose (afforestation and restoration or conservation) (Table 1). Some of the groups included only one population (8 out of 10 in *P. halepensis*, 12 out of 17 in *P. pinaster*, 5 out of 10 in *P. nigra*, 4 out of 12 in *P. sylvestris* and 3 out of 4 in *P. uncinata*), with most of those populations defined as important in conservation programs.

An assignment summary of either conservation and restoration or afforestation populations into the different genetic groups are presented in. The species with the highest rates of success in assignment were *P. sylvestris* and *P. uncinata*, and the one with lowest rate was *P. halepensis*. In any case, all the species, except *P. halepensis*, present high rates of correct assignments (> 80 %). It is interesting to notice that conservation populations were correctly assigned in most of the cases for all the species.

However, considering that the assignment was correct in most of the cases, it is noteworthy the case of *P. halepensis* and *P. sylvestris* where many groups are genetically close (Table 4) and, therefore, it could bias the assignment success rate.

Discussion

The five Spanish pine species used in this paper exhibit, due to their mating systems, a large level of genetic diversity within populations and a low to intermediate level of differentiation among populations (Soto *et al.*, 2010). These features are known to improve resilience, productivity and recovery from climate extremes and give stability to the ecosystem, therefore being a key issue on the use

Table 4. Mean genetic distance (Sλ) and groups to which correspond (in brackets) that were not statistically significant in the assignment of populations. The same group to which the population belongs was excluded from the analysis.

ID	*P. halepensis*	*P. pinaster*	*P. nigra*	*P. sylvestris*	*P. uncinata*
CG1	0.03 (RG4)	n.a.	n.a.	n.a.	n.a.
CG2	0.065 (RG3, RG6)	0.054 (RG1)	n.a.	n.a.	n.a.
CG3	0.017 (RG5)	n.a.	0.049 (RG1)	n.a.	n.a.
CG4	-	n.a.	n.a.	0.053 (RG3, RG7)	-
CG5	-	n.a.	n.a.	-	-
CG6	-	n.a.	-	-	-
CG7	-	n.a.	-	-	-
CG8	-	n.a.	-	-	-
RG1	0.041 (RG3, RG6)	0.073 (RG5)	n.a.	n.a.	n.a.
RG2	n.a.	n.a.	n.a.	0.060 (CG4, RG1)	-
RG3	0.047(CG2, RG1, RG6)	n.a.	n.a.	0.058 (CG4, RG1)	-
RG4	0.053 (CG1, RG1, RG3, RG6, RG7)	n.a.	0.055 (RG1)	0.054 (RG2)	-
RG5	n.a.	0.084 (RG1, RG10)	0.050 (RG1)	0.049 (CG4, RG1, RG3, RG4, RG7)	-
RG6	0.064 (CG1, CG2, RG1, RG3)	n.a.	-	0.069 (RG5)	-
RG7	0.056 (RG4)	n.a.	-	0.066 (RG2, RG3)	-
RG8	-	n.a.	-	-	-
RG9	-	n.a.	-	-	-
RG10	-	n.a.	-	-	-

n.a.: no other group could be assigned.

of forest reproductive material and conservation programs of the species (Thomson *et al.*, 2009; Isbell *et al.*, 2011; Alfaro *et al.*, 2014).

We present an approach that can assign the origin of material that could be used in afforestation, restoration or conservation activities which are essential in managing the forest genetic resources. The findings showed that the method worked well for two species, *P. sylvestris* and *P. uncinata*, for another two, *P. nigra* and *P. pinaster*, with an exit of assignment over an 85%, being *P. halepensis* the species with the lowest rate of identification. It is noteworthy to say this is the first method that can verify the origin of those species, as research has proven to identify specific populations (Ribeiro *et al.*, 2002; Robledo-Arnuncio *et al.*, 2009), or has been used in the delineation of genetic zones of interest in breeding and conservation activities (Bucci and Vendramin 2000; Bucci *et al.*, 2007). Other methods have been developed based in the identification of both the adult population and the material obtained from it (Deguilloux *et al.*, 2004; Degen *et al.*, 2010).

Although afforestation and restoration activities with these species are usually based on local material, which refers to the same region of provenance, in species with large spatial structure, local material can differ even at short distances. However, in our study the extensive gene flow allows to consider more extensive populations. We could not clearly differentiate populations from some regions of provenance (*e.g.* in *P. halepensis*) showing that only some groups of populations could be distinguished but also, we found that in some cases populations from the same region of provenance were different. Regions of provenance have been defined mostly based on ecological and extensive genetic information (Gil *et al.*, 1996). Information from genetic markers is not the best option to define regions with a similar pattern of variation in traits related to adaptation or growth (*e.g.*, Hamann *et al.*, 2000), therefore redefinition of the limits of some regions of provenance might be needed.

The European Forest Genetic Resource Program (EUFORGEN) has defined different in-situ conservation units of the species, and the conservation program (Jimenez et al., 2009; Koskela et al., 2013) includes the definition of genetic criteria for restoration activities and for monitoring the conservation units (Aravanopoulos 2011; Graudal et al., 2014; Fussi et al., 2016). In our case, we could distinguish almost all the conservation populations considered in the study that will allow a better implementation of activities in Spain.

Conclusions

We demonstrated the usefulness of extensive markers datasets that are becoming available for identification of gene pools at the population level in different species. Nevertheless, results depend on factors such as the genetic diversity and the differentiation within and between populations. The rate of successful identification of populations among the different species was very high (> 94 %) for *P. nigra*, *P. sylvestris* and *P. uncinata*, high (circa 85 %) for *P. pinaster*, and low (< 79 %) for *P. halepensis*. More information is needed in *P. halepensis* and for some areas in *P. pinaster*.

Acknowledgements

We are grateful to the INIA marker database GENFODAT (A. Gómez, A. Soto de Viana, S.C. Gonzalez-Martínez, J.J. Robledo-Arnuncio, P. Jimenez, D. Barba, C. Garcia) for providing the information of the populations. The text has been revised by a professional in English grammar and style, P. C. Grant.

References

Alfaro RI, Fady B, Vendramin GG, Dawson IK, Fleming RA, Sáenz-Romero C, Lindig-Cisneros RA, Murdock, T et al., 2014. The role of forest genetic resources in responding to biotic and abiotic factors in the context of anthropogenic climate change. For Ecol Manage 333: 76-87.

Alía R, Garcia del Barrio JM, Iglesias S, 2009a. Regiones de procedencia de especies forstales de España. Parques Nacionales, Madrid. 361 p.

Alía R, Alba N, Chambel MR, Barba D, Iglesias S, 2009b. Genetic quality of forest reproductive materials in Land restoration programmes. In Innovative approaches in forest restoration; Vallejo VR, Bautista S, Aaronson J (eds), pp: 89-103. CEAM, Valencia.

Anonymous, 2007. Pan-European Recommendations for Afforestation and Reforestation in the context of the UNFCCC. Warsaw, Poland.

Aragonés A, Barrena I, Espinel S, Herrán A, Ritter E, 1997. Origin of Basque populations of radiata pine inferred from RAPD data. Ann Sci For 54 (8): 697-703. https://doi.org/10.1051/forest:19970801

Aravanopoulos FA, 2011. Genetic monitoring in natural perennial plant populations. Botany 89: 75-81. https://doi.org/10.1139/b10-087

Bautista S, Aronson J, Vallejo VR, 2009. Land restoration to combat desertification. Innovative approaches, quality control and project evaluation. CEAM, Valencia.

Breed MF, Stead MG, Ottewell KM, Gardner MG, Lowe AJ, 2012. Which provenance and where ? Seed sourcing strategies for revegetation in a changing environment. Conserv Genet 14 (1): 1-10. https://doi.org/10.1007/s10592-012-0425-z

Bucci G, Vendramin GG, 2000. Delineation of genetic zones in the European Norway spruce natural range: preliminary evidence. Mol Ecol 9 (7): 923-34. https://doi.org/10.1046/j.1365-294x.2000.00946.x

Bucci G, González-Martínez SC, Le Provost G, Plomion C, Ribeiro MM, Sebastiani F, Alía R, Vendramin GG, 2007. Range-wide phylogeography and gene zones in *Pinus pinaster* Ait. revealed by chloroplast microsatellite markers. Mol Ecol 16(10):2137-53. https://doi.org/10.1111/j.1365-294X.2007.03275.x

Bucharova A, Michalski S, Hermann JM, Heveling K, Durka W, Hölzel N, Kollmann J, Bossdorf O, 2016. Genetic differentiation and regional adaptation among seed origins used for grassland restoration: Lessons from a multispecies transplant experiment. J Appl Ecol 54: 127-136. https://doi.org/10.1111/1365-2664.12645

Corander J, Waldmann P, Sillanpää MJ, 2003. Bayesian analysis of genetic differentiation between populations. Genetics 163 (1): 367-374.

Dawson KJ, Belkhir K, 2001. A Bayesian approach to the identification of panmictic populations and the assignment of individuals. Genet Res 78(1):59-77. https://doi.org/10.1017/S001667230100502X

Degen B, Holtken A, Rogge M. 2010. Use of DNA-Fingerprints to control the origin of forest reproductive material. Silvae Genetica 59 (6): 268-273.

Deguilloux MF, Pemonge MH, Petit RJ, Eguillouxa MD, Emongea MP, Etitb RJP, 2004. DNA-based control of oak wood geographic origin in the context of the cooperage industry. Ann For Sci 61: 97-104. https://doi.org/10.1051/forest:2003089

Dupanloup I, Scheneider S, Excoffier L, 2002. A simulated annealing approach to define the genetic structure of populations. Mol Ecol 11: 2571-2581. https://doi.org/10.1046/j.1365-294X.2002.01650.x

Earl DA, vonHoldt BM, 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4(2):359-361. https://doi.org/10.1007/s12686-011-9548-7

Evanno G, Regnaut S, Goudet J, 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol Ecol 14 (8): 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Falush D, Stephens M, Pritchard JK, 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164 (4): 1567-1587.

Fidler F, Burgman MA, Cumming G, Buttrose R, Thomason N, 2006. Impact of criticism of null-hypothesis significance testing on statistical reporting practices in conservation biology. Conserv Biol 20 (5): 1539-1544. https://doi.org/10.1111/j.1523-1739.2006.00525.x

Fussi B, Westergren M, Aravanopoulos F, Baier R, Kavaliauskas D, Finzgar D, Alizoti P, Bozic G, *et al.*, 2016, Forest genetic monitoring: an overview of concepts and definitions. Environ Monit Assess 188 (8): 493. https://doi.org/10.1007/s10661-016-5489-7

García del Barrio JM, Auñon FJ, Sánchez de Ron D, Alía R, 2013. Assessing regional species pools for restoration programs in Spain. New Forests 44 (4): 559-576. https://doi.org/10.1007/s11056-013-9363-y

Gil LA, Diaz-Fernandez P, Jiménez P, Roldan M, Alía R, Agúndez D, Miguel J, Martín S, et al., 1996. Las regiones de procedencia de *Pinus halepensis* Mill. en España. ICONA, Madrid.

Gómez A, Vendramin GG, González-Martínez SC, Alía R, 2005. Genetic diversity and differentiation of two Mediterranean pines (*Pinus halepensis* Mill. and *Pinus pinaster* Ait.) along a latitudinal cline using chloroplast microsatellite markers. Divers Distrib 11 (3): 257-263. https://doi.org/10.1111/j.1366-9516.2005.00152.x

González-Martínez SC, Mariette S, Ribeiro MM, Burban C, Raffin A, Chambel MR, Ribeiro CAM, Aguiar A, et al., 2004. Genetic resources in maritime pine (*Pinus pinaster* Aiton): molecular and quantitative measures of genetic variation and differentiation among maternal lineages. For Ecol Manage 197 (1-3): 103-115.

Graudal L, Aravanopoulos FA, Bennadji Z, Changtragoon S, Fady B, Kjær ED, Loo J, Ramamonjisoa L, et al., 2014. Global to local genetic diversity indicators of evolutionary potential in tree species within and outside forests. For Ecol Manage 333: 35-51.

Grivet D, Sebastiani F, González-Martínez SC, Vendramin GG, 2009. Patterns of polymorphism resulting from long-range colonization in the Mediterranean conifer Aleppo pine. The New Phytologist 184 (4): 1016-28. https://doi.org/10.1111/j.1469-8137.2009.03015.x

Grivet D, Climent J, Zabal-Aguirre M, Neale DB, Vendramin GG, González-Martínez SC, 2013. Adaptive evolution of Mediterranean pines. Mol Phylog Evol 68 (3): 555-566. https://doi.org/10.1016/j.ympev.2013.03.032

Hamann A, Koshy MP, Namkoong G, Ying CC, 2000. Genotype-environment interactions in Alnus rubra : developing seed zones and seed-transfer guidelines with spatial statistics and GIS. For Ecol Manage 136: 107-119.

Heuertz M, Teufel J, González-Martínez SC, Soto A, Fady B, Alía R, Vendramin GG, 2010. Geography determines genetic relationships between species of mountain pine *Pinus mugo* complex in western Europe. J Biogeogr 37 (3): 541-556. https://doi.org/10.1111/j.1365-2699.2009.02223.

Holmes PM, Richardson DM, 1999. Protocols for restoration based on recruitment dynamics, community structure, and ecosystem function: Perspectives from South African fynbos. Restor Ecol 7 (3): 215-230. https://doi.org/10.1046/j.1526-100X.1999.72015.x

Hong L, Leong S, Kit K, Ng S, Faridah Q, Faridah-Hanum I, 2010. Forensic DNA profiling of tropical timber species in Peninsular Malaysia. For Ecol Manage 259 (8): 1436-1446.

Honjo M, Ueno S, Tsumura Y, Handa T, Washitani I, Ohsawa R, 2008. Conservation genetics. V. 9. Kluwer Academic Publishers. https://doi.org/10.1007/s10592-007-9427-7

Isbell F, Calcagno V, Hector A, Connolly J, Harpole WS, Reich PB, Scherer-Lorenzen M, Schmid B, et al., 2011. High plant diversity is needed to maintain ecosystem services. Nature 477 (7363): 199-202. https://doi.org/10.1038/nature10282

Jaramillo-Correa JP, Verdú M, González-Martínez SC, 2010. The contribution of recombination to heterozygosity differs among plant evolutionary lineages and life-forms. BMC Evol Biol 10: 22. https://doi.org/10.1186/1471-2148-10-22

Jimenez P, Díaz-Fernández PM, Iglesias S, Prada A, García-del-Barrio JM, Alba N, Alía R, 2009. National strategy for the conservation and sustainable use of forest genetic resources: a framework for coordinating Central and Autonomous Regional Government activities in Spain. Inv Agrar: Sist Rec For 18 (1): 13-19.

Jones TA, 2003. The restoration gene pool concept: Beyond the native versus non-native debate. Restor Ecol 11 (3): 281-290. https://doi.org/10.1046/j.1526-100X.2003.00064.x

Jones TA, Monaco TA, 2009. A role for assisted evolution in designing native plant materials for domesticated landscapes. Frontiers Ecol Environ 7 (10): 541-547. https://doi.org/10.1890/080028

Konnert M, Fady B, Wolter F, Ducci F, Bozzano M, Maaten T, Kowalczyk J, 2015. Use and transfer of forest reproductive material Use and transfer of forest reproductive material in Europe in the context. European Forest Genetic Resources Programme (EUFORGEN), Bioversity International, Rome, Italy.

Koskela J, Lefèvre F, Schueler S, Kraigher H, Olrik DC, Hubert J, Longauer R, Bozzano M, *et al.*, 2013. Translating conservation genetics into management : Pan-European minimum requirements for dynamic conservation units of forest tree genetic diversity. Biol Conserv 157: 39-49. https://doi.org/10.1016/j.biocon.2012.07.023

Langlet O, 1971. Two hundred years genecology. Taxon 20 (5-6): 653-721. https://doi.org/10.2307/1218596

Leimu R, Fischer M, 2008. A meta-analysis of local adaptation in plants. PLoS ONE 3 (12): 1-8. https://doi.org/10.1371/journal.pone.0004010

Manly BFJ, 1997. Randomization, Bootstrap and Monte Carlo Methods in Biology, 2nd ed. Chapman & Hall, NY.

Mckay JK, Christian CE, Harrison S, Rice KJ. 2005. How local is local? A review of practical and conceptual issues in the genetics of restoration. Restor Ecol 13 (3): 432-440. https://doi.org/10.1111/j.1526-100X.2005.00058.x

Montero G, 1997. L'attività di rimboschimento in Spagna negli ultimi 50 anni. Legno, Cellulosa, Carta 4: 35-42.

Morgante M, Pfeiffer A, Costacurta A, Olivieri AM, 1996. Molecular tools for population and ecological genetics in coniferous trees. Phyton 36: 133-142.

Moritz C, 1994. Defining evolutionarily-significant-units for conservation. Trends Ecol Evol 9: 373-375. https://doi.org/10.1016/0169-5347(94)90057-4

Moritz C, 1999. Conservation units and traslocations: stategies for conserving evolutionary processes. Hereditas 130 (3):217-228. https://doi.org/10.1111/j.1601-5223.1999.00217.x

Nanson A, 2001. The New OECD Scheme for the Certification of Forest Reproductive Materials. Silvae Genetica 50 (5-6): 181-187.

Partel M, Szava-Kovats R, Zobel M, Pärtel M, 2011. Dark diversity: shedding light on absent species. Trends Ecol Evol 26 (3): 124-128. https://doi.org/10.1016/j.tree.2010.12.004

Pritchard JK, Stephens M, Donnelly P, 2000. Inference of population structure using multilocus genotype data. Genetics 155 (2): 945-959.

Ribeiro MM, Le-Provost G, Gerber S, Vendramin GG, Anzidei M, Decroocq S, Marpeau A, Mariette S. *et al.*, 2002. Origin identification of maritime pine stands in France using chloroplast simple-sequence repeats. Ann Forest Sci 59 (1): 53-62. https://doi.org/10.1051/forest:2002100

Robledo-Arnuncio JJ, Navascués M, González-Martínez SC, Gil LA, 2009. Estimating gametic introgression rates in a risk assessment context: A case study with Scots pine relicts. Heredity 103 (5): 385-393. https://doi.org/10.1038/hdy.2009.78

Sgrò CM, Lowe AJ, Hoffmann AA, 2011. Building evolutionary resilience for conserving biodiversity under climate change. Evol Appl 4 (2): 326-337. https://doi.org/10.1111/j.1752-4571.2010.00157.x

Soranzo N, Alía R, Provan J, Powell W, 2000. Patterns of variation at a mitochondrial sequence-tagged- site

locus provides new insights into the postglacial history of European *Pinus sylvestris* populations. Mol Ecol 9 (9): 1205-1211. https://doi.org/10.1046/j.1365-294x.2000.00994.x

Soto A, Robledo-Arnuncio JJ, González-Martínez SC, Smouse PE, Alía R, 2010. Climatic niche and neutral genetic diversity of the six Iberian pine species : a retrospective and prospective view. Mol Ecol 19 (7): 1396-1409. https://doi.org/10.1111/j.1365-294X.2010.04571.x

Steinitz O, Robledo-Arnuncio JJ, Nathan R, 2012. Effects of forest plantations on the genetic composition of conspecific native Aleppo pine populations. Mol Ecol 21 (2): 300-313. https://doi.org/10.1111/j.1365-294X.2011.05394.x

Sutherland WJ, Armstrong-Brown S, Armsworth PR, Tom B, Brickland J, Campbell CD, Chamberlain DE, Cooke AI, *et al*., 2006. The identification of 100 ecological questions of high policy relevance in the UK. J Appl Ecol 43 (4): 617-627. https://doi.org/10.1111/j.1365-2664.2006.01188.x

Thomson JR, Moilanen AJ, Vesk PA, Bennett AF, Nally RM, 2009. Where and when to revegetate: a quantitative method for scheduling landscape reconstruction. Ecol Appl 19 (4): 817-828. https://doi.org/10.1890/08-0915.1

Tigabu M, Oden PC, Lindgren D, 2005. Identification of seed sources and parents of *Pinus sylvestris* L. using visible-near infrared reflectance spectra and multivariate analysis. Trees-Structure and Function 19: 468-476. https://doi.org/10.1007/s00468-005-0408-5

Tnah LH, Lee SL, Ng KKS, Tani N, Bhassu S, Othman RY, 2009. Geographical traceability of an important tropical timber (*Neobalanocarpus heimii*) inferred from chloroplast DNA. For Ecol Manage 258 (9): 1918-1923.

Unger GM, Vendramin GG, Robledo-Arnuncio JJ, 2014. Estimating exotic gene flow into native pine stands: zygotic vs. gametic components. Mol Ecol 23 (22): 5435-5447. https://doi.org/10.1111/mec.12946