

Genetic diversity in peripheral and central populations of the Cantabrian endemism *Genista legionensis* (Pau) M. Laínz (Fabaceae)

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Abstract

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The impact of habitat fragmentation and isolation on the genetic diversity of populations has attracted much attention in studies of plant conservation. The central-peripheral population hypothesis predicts that peripheral populations have reduced genetic variability, so it is often assumed that they deserve higher conservation priority over central populations. In this work, using amplified fragment length polymorphism (AFLP), we studied the genetic diversity of central and peripheral populations of the Cantabrian endemism *Genista legionensis* (Fabaceae). At the species level, percentage of polymorphic bands, Nei heterozygosity and Shannon information index were $PPB = 89.21\%$, $H_e = 0.246$ and $I = 0.377$, respectively. The study revealed that peripheral populations were smaller in number, with lower levels of genetic diversity compared to the central populations. Furthermore, analysis of molecular variance (AMOVA) indicated that most of the variability was partitioned among populations, also supported by principal coordinates analysis. This study indicates that the decrease in diversity from central to peripheral populations could be explained as a result of edge effect and fragmentation through the enhanced inbreeding and genetic drift, and thus supported the view that habitat fragmentation and related edge effect reduce the population genetic diversity. However, the presence of discriminating fragments in the peripheral populations suggests their conservation in order to preserve the genetic diversity in the Cantabrian endemism *G. legionensis*.

Keywords: AFLP, central populations, edge effect, endangered species, endemism, genetic diversity, peripheral populations, plant conservation.

INTRODUCTION

Genista L. is a genus of spiny and non-spiny shrubs centred in the Mediterranean region, which includes over 90 species divided into three subgenera and ten sections (Gibbs, 1996). The genus is also represented throughout most of western and central Europe, extending to the southeast of the former USSR, and Turkey, Syria and North Africa (De Castro & al., 2002; Pardo & al., 2004). In the Iberian Peninsula, the genus is widespread in the Mediterranean part of the country and represented by thirty-nine species in the Iberian flora (see Talavera, 1999). Additionally, it is prevalent at the transition zones between the Mediterranean and Euroatlantic bioclimatic regions. This is the case of *Genista legionensis* (Pau) M. Laínz, an endemic plant that grows on limestone from near sea level to altitudes of 2200 m. Its distribution comprises the central area of the Cantabrian Range (Asturias, Cantabria,

Resumen

Cires, E., Pérez, R., Bueno, A. & Fernández Prieto, J.A. 2013. Diversidad genética en poblaciones periféricas y centrales del endemismo cantábrico *Genista legionensis* (Pau) M. Laínz (Fabaceae). *Anales Jard. Bot. Madrid* 70(1): 91-96 (en inglés).

El impacto de la fragmentación del hábitat y el aislamiento sobre la diversidad genética de las poblaciones, han despertado mucha atención en estudios de conservación de plantas. La hipótesis de la población centro-periferia predice que las poblaciones periféricas reducen la variabilidad genética, por lo que a menudo se asume que merecen una mayor prioridad de conservación respecto a las poblaciones centrales. En este trabajo, hemos empleado los polimorfismos en longitud de fragmentos amplificados (AFLP), para estudiar la diversidad genética de las poblaciones centrales y periféricas del endemismo cantábrico *Genista legionensis* (Fabaceae). A nivel de especie, el porcentaje de bandas polimórficas, la heterocigosidad de Nei y el índice de Shannon fueron $PPB = 89,21\%$, $H_e = 0,246$ y $I = 0,377$, respectivamente. El estudio reveló que las poblaciones periféricas fueron menores en número, con niveles más bajos de diversidad genética si las comparamos con las poblaciones centrales. Además, el análisis molecular de la varianza (AMOVA) indicó que la mayor parte de la variabilidad tuvo lugar entre las poblaciones, también apoyado por el análisis de coordenadas principales. Este estudio indica que la disminución de la diversidad desde el centro hasta las poblaciones periféricas, puede deberse a un efecto de borde y fragmentación acompañado de endogamia y deriva génica, y por ello apoya la idea de que la fragmentación del hábitat y el efecto borde están relacionados con una reducción de la diversidad genética poblacional. Sin embargo, la presencia de fragmentos discriminantes en las poblaciones periféricas sugiere su conservación a fin de preservar la diversidad genética del endemismo Cantábrico *G. legionensis*.

Palabras clave: AFLP, poblaciones centrales, efecto borde, especies en peligro, endemismo, diversidad genética, poblaciones periféricas, conservación de plantas.

León and Palencia), with some isolated locations out of this core (south of Palencia, País Vasco, Cantabria and western Asturias). In regard to its protection status, *G. legionensis* does not appear in the Royal Decree 139/2011 (*Listado de Especies Silvestres en Régimen de Protección Especial y del Catálogo Español de Especies Amenazadas*) neither in the Red List of Spanish Vascular Flora (VV.AA., 2000; Bañares & al., 2004; Moreno, 2008). Llamas & al. (2007) analyzed its frequency in the catalogue of threatened vascular plants of Castilla y León and suggested the criteria of “frequent endemism”. In Asturias, Barreno & al. (1985) and Gómez-Campo (1987) included *G. legionensis* under the category of “not threatened”, and Fernández Prieto & al. (2007) suggested its exclusion from the catalogue of threatened vascular plants of Asturias. On the contrary, in the País Vasco where only one locality has been reported (Monte Lucero, Vizcaya), *G. legio-*

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Table 1. Sampling localities of *Genista legionensis* used in this study.

Code	Population	Locality	Coordinates	Sample size	Voucher specimens
GA	Peripheral	Gamoniteiro, Quirós (Asturias, Spain); 1534 m; AB & JAFP	43° 11' 42.8" N 5° 55' 31.0" W	13	JBAG: 04753
CA	Central	Between Carreño and Ortiguero, Cabrales (Asturias, Spain); 373 m; AB & JAFP	43° 19' 5.0" N 4° 53' 27.8" W	13	JBAG: 04754
ML	Peripheral	Monte Lucero, Muskiz (Vizcaya, Spain); 173 m; AB, AP, EC & JAFP	43° 20' 58.8" N 3° 5' 13.1" W	22	BIO: 5770

Collector abbreviations: AB, A. Bueno; AP, A. Prieto; EC, E. Cires; JAFP, J.A. Fernández Prieto.

nenensis is listed as a species “at risk of extinction” (see B.O.P.V., 1998). Therefore, as most Cantabrian endemic species occurring on habitats exposed to risk of fragmentation, this taxon has interest in the field of conservation biology. The effect of such fragmentation and isolation on the population genetic diversity of this Cantabrian endemism is still unknown.

Understanding the partitioning of genetic variance in peripheral and central populations may shed more light on the effects of genetic drift and gene flow on population genetic structure and, thereby, improve attempts to conserve genetic diversity. There are two opposite scenarios about the genetic diversities in central and peripheral populations. The first view is that, because the peripheral population is small, isolated and at the edge of suitable habitat (Lawton, 1993; Lesica & Allendorf, 1995), it suffers inbreeding and genetic drift; so the genetic diversity of these peripheral isolated populations will be reduced. Another view is that the peripheral population experiences high and various natural selection (Lesica & Allendorf, 1992, 1995); then strong selective pressure may promote differentiation of those populations subjected to selection, and these populations would be the sources for producing new species and should be well preserved as a priority (Höglund, 2009).

In this work, using Amplified Fragment Length Polymorphism (AFLP) (Vos & al., 1995), we studied the genetic diversity of central and peripheral populations of *Genista legionensis*. In particular, we addressed the following questions: (1) what is the level of genetic diversity in populations of *Genista legionensis*? (2) how is the genetic diversity distributed within and between populations?, and finally (3) are the peripheral populations genetically depauperate compared with central populations? As a result of evaluating the genetic status in this Cantabrian endemism, conservation and management strategies are proposed.

MATERIAL AND METHODS

Sampling design and DNA isolation

Genista legionensis specimens were collected in three localities from the Cantabrian Range (see Table 1, Fig. 1A). In the central population “Cabrales” (CA), 13 individuals were randomly collected, with a distance of at least 5 m each other. In the case of peripheral populations, and considering the specific structure of each population, two sampling strategies were carried out: the first one in the population of Monte Lucero (ML), with 22 individuals structured in three subnuclei (Fig. 1B; information provided by Amador Prieto, De-

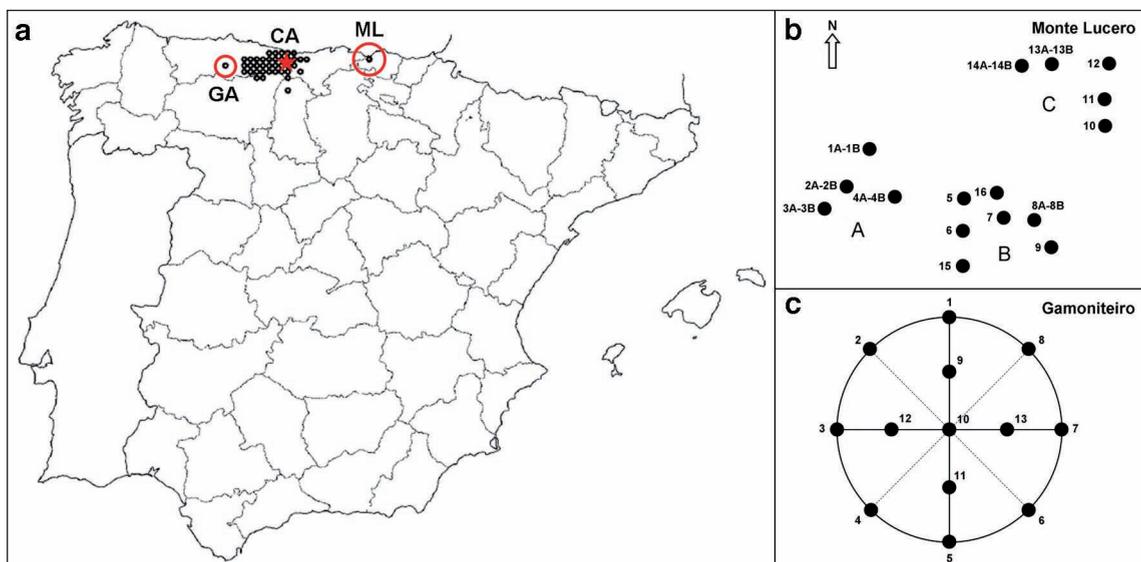


Fig. 1. Distribution map of the examined specimens of *Genista legionensis* (a), and different sampling strategies for the peripheral populations Monte Lucero (b) and Gamoniteiro (c). Populations are coded as in Table 1. Distribution map modified from Vera de la Puente & al. (2002). CA, Cabrales; GA, Gamoniteiro; ML, Monte Lucero.

partamento de Medio Ambiente, Planificación Territorial, Agricultura y Pesca, Gobierno Vasco). In this case all individuals were sampled with the exception of number 6, which was not found. The second sampling strategy was conducted in the population of Gamoniteiro (GA). This peripheral population is a continuous stand of 100 m², so 13 individuals, distributed along two perpendicular diameters and the periphery were targeted for sampling (Fig. 1C). All the collected specimens were dried in silica gel and stored prior to DNA isolation. DNA isolation was carried out using the DNeasy Plant Minikit (Qiagen) following the manufacturer's instructions. In addition, voucher herbarium specimens were collected and kept in the Herbaria of the Atlantic Botanical Garden (JBAG) and University of the Basque Country (BIO).

AFLP analysis

AFLP analysis followed the protocol from Vos & al. (1995) with minor modifications. DNA was isolated from silica gel-dried material collected in the field, digested with restriction enzymes *EcoRI* and *MseI* (New England Biolabs) for 2 h at 37 °C and simultaneously ligated to double-stranded *EcoRI* and *MseI* adapters (Applied Biosystems). Preselective amplifications were performed using primers with one base pair extension. In a second selective amplification, the number of fragments was further reduced by primers with three base pair extension. For this second amplification, four primer combinations (Applied Biosystems) were used (Table 2). For a more detailed explanation of the AFLP technique and quality parameters applied, see Cires & al. (2011). Selective amplification products were sent to the Sequencing Services of the University of Oviedo for fragment analysis: samples were run on an automated DNA sequencer (ABI PRISM® 3100, Applied Biosystems) with an internal size standard GeneScan 500 (ROX™, Applied Biosystems). Raw AFLP data were collected and sized using the Genemapper 4.0 software (Applied Biosystems).

Data analysis

The presence or absence of each band was recorded in a binary data matrix for each individual, assigning a value of 1 or 0 depending on band presence or absence, respectively. The binary data matrix obtained was used to calculate the following parameters assuming Hardy–Weinberg equilibrium: observed number of bands (NB), number of polymorphic bands (NPB), percentage of polymorphic bands (PPB), mean observed

Table 2. Primer combinations used for AFLP amplification and summary of amplified bands.

Primer pairs	NB	NPB	PPB (%)
<i>EcoRI</i> -ACG / <i>MseI</i> -CAT	183	164	89.61
<i>EcoRI</i> -ACG / <i>MseI</i> -CTT	256	219	85.54
<i>EcoRI</i> -ACT / <i>MseI</i> -CAT	241	226	93.77
<i>EcoRI</i> -ACT / <i>MseI</i> -CTT	228	201	88.15
Total	908	810	

NB, number of scorable bands; NPB, number of polymorphic bands; PPB, percentage of polymorphic bands.

number of alleles (A_o), mean effective number of alleles (A_e), observed heterozygosity (H_e), and lastly, Shannon diversity index (I). The numbers of distinguishing markers were quantified for the different populations using the following criteria: “discriminating” fragments - present in all analysed samples of a given population and absent elsewhere, and “private” fragments - restricted to a given population but not present in all of its samples. The hierarchical AFLP frequency distribution was described using the analysis of molecular variance (AMOVA). Components of variance partitioned within populations and among populations were estimated from a Euclidean distance matrix using GenA1Ex 6.4 (Peakall & Smouse, 2006).

A principal coordinate analysis (PCoA) was performed on the Jaccard similarity matrix using Past 1.89 (Hammer & al., 2001) to visualise the genetic relationships among all individual AFLP phenotypes. In addition, a NeighborNet was constructed based on a matrix of p-distances, using the program SplitsTree 4.12 (Huson & Bryant, 2006) to examine genetic structure and reticulation in the AFLP data. Bootstrap support for internal splits was calculated with 2,000 replicates. Fit values ranging from 0 to 100% indicate how well the graph represents the information contained in the data.

RESULTS

The four selected primers generated a total of 908 bands for the 48 *Genista legionensis* samples (Table 2). The number of bands and the percentage of polymorphic bands produced by each primer varied (Table 2). A summary of the genetic diversity for each of the three populations of *G. legionensis*, based on AFLP markers, is given in Table 3. Low levels of genetic diversity were found in peripheral populations: the percentage of polymorphic bands ranged from 36.89% (GA) to 42.07% (ML), the mean observed number of alleles per locus

Table 3. Genetic diversity in populations of *Genista legionensis* based on AFLP markers.

Level	AFLP								
	PPB	$A_o \pm SE$	$A_e \pm SE$	$H_e \pm SE$	$I \pm SE$	A_p	A_d	G_{ST}	Nm
<i>Populations</i>									
GA	36.89	1.368 ± 0.482	1.181 ± 0.308	0.109 ± 0.171	0.167 ± 0.249	36	6		
CA	75.44	1.754 ± 0.430	1.383 ± 0.357	0.229 ± 0.186	0.351 ± 0.259	139	0		
ML	42.07	1.420 ± 0.493	1.211 ± 0.326	0.126 ± 0.180	0.192 ± 0.261	33	1		
Average	51.46	1.514	1.258	0.154	0.236				
<i>Species</i>	89.21	1.892 ± 0.310	1.414 ± 0.365	0.246 ± 0.185	0.377 ± 0.249			0.3922	0.3874

PPB, percentage of polymorphic bands; A_o , observed mean number of alleles per locus; A_e , effective mean of alleles per locus; H_e , expected heterozygosity; I , Shannon diversity index; A_p , number of private alleles; A_d , number of discriminating alleles; G_{ST} , coefficient of genetic differentiation among populations; Nm , gene flow.

Table 4. Analysis of molecular variance (AMOVA) for the central and peripheral populations of *Genista legionensis* based on AFLP markers (d.f., degrees of freedom; SS, sums of squares; MS, mean sums of squares; VC, variance components). Level of significance are based on 9999 iteration steps.

Source of variation	AFLP					
	d.f.	SS	MS	VC	%	p
Among populations	2	2491.542	1245.771	74.246	52.11	<0.001
Within populations	47	3206.458	68.223	68.223	47.89	<0.001
Total	49	5698.000		142.469		

ranged from 1.368 (GA) to 1.420 (ML) while the mean effective number of alleles per locus ranged from 1.181 (GA) to 1.211 (ML). The Nei's gene diversity ranged from 0.109 (GA) to 0.126 (ML), and the Shannon's information index ranged from 0.167 (GA) to 0.192 (ML). In the case of the central population (CA), the percentage of polymorphic loci was higher (75.44%). The mean observed number of alleles per locus was 1.754; while the mean effective number of alleles reached 1.383. The Nei's gene diversity and the Shannon's index were 0.229 and 0.351, respectively. At species level, high levels of genetic diversity were revealed (PPB = 89.21%, $H_E = 0.246$ and $I = 0.377$). The estimated number of migrants per generation (Nm) was 0.3874 (Table 3), which suggested a low rate of gene flow among populations. It is also noteworthy the presence of 208 private fragments in the three populations sampled (36 for GA, 139 for CA, 33 for ML), as well as the existence of 1 and 6 discriminating fragments in the populations of Monte Lucero (ML) and Gamoniteiro (GA), respectively.

The two-level AMOVA analysis from the distance matrices for the individuals studied (Table 4) showed that the percent of genetic variance explained by differences between groups detected by AFLP was 52.11% ($P < 0.001$), whereas the amount of variation within populations was 47.89% ($P < 0.001$). In the principal coordinate analysis (PCoA) the first three axes explained 43.62, 14.30 and 5.21% of the total variation respectively, and revealed three groups (Fig. 2). In accordance with the PCoA analysis, the NeighborNet (Fig. 3)

illustrates three major splits corresponding also to the populations studied.

DISCUSSION

The genetic diversity of a population is usually related to the degree of isolation. Low levels of genetic diversity can be expected in peripheral populations as a result of low levels of immigration and high levels of genetic drift (e.g. Lesica & Allendorf, 1995; García-Ramos & Kirkpatrick, 1997). Our results support this scenario since the two peripheral populations of *Genista legionensis* exhibited lower genetic diversity than the central population. Declining genetic diversity towards the periphery of the species ranges has been found in many European/American species, such as: *Alnus glutinosa* (King & Ferris, 1998), *Arabidopsis thaliana* (Kuittinen & al., 1997), *Calluna vulgaris* (Mahy & al., 1997), *Stipa* spp. (Wagner & al., 2012; Durka & al., 2013) or *Thuja occidentalis* (Pandey & Rajora, 2012). Several mechanisms at peripheral populations may have led to the lower genetic diversity observed, such as genetic drift, that can provoke clines of genetic variation due to reduction of both habitat quality and quantity toward the periphery; or spatial isolation, with a lack of gene exchange between periphery populations (Eckert & al., 2008).

The extremely low levels of genetic variation within peripheral populations of *G. legionensis* in the Cantabrian Range involve important implications for the conservation of this species. One of the major goals of conservation biology is to preserve the evolutionary potential of species (Höglund, 2009). In order to assess if a differentiation process is ongoing, as the adaptation of peripheral populations to different environments often reflects a stepping stone to speciation (Mayr, 1970), more detailed studies such as genome scan based on AFLP could be proposed (e.g. Paris & Despres, 2012; Wang & al., 2012). An increased sampling population would be needed, and by this data acquisition, more information would assist on determining the plausible scenarios: i) peripheral populations are under strong directional selection; ii) peripheral populations are at higher risk of genetic drift than central populations, and iii) habitats of the peripheral populations are often marginal near the species border and selection is severe, thus only a small number of genotypes survive (Lesica & Allendorf, 1995).

Therefore, the interest of the peripheral populations of *G. legionensis* for conservation purposes would then mainly rely on their genetic uniqueness, and priority should be given to the preservation of these genetic pools. The loss of peripheral populations may decrease the species ability to adapt to future environment changes, and then reduce its potential for speciation (Channell, 2004). In conclusion, and according to

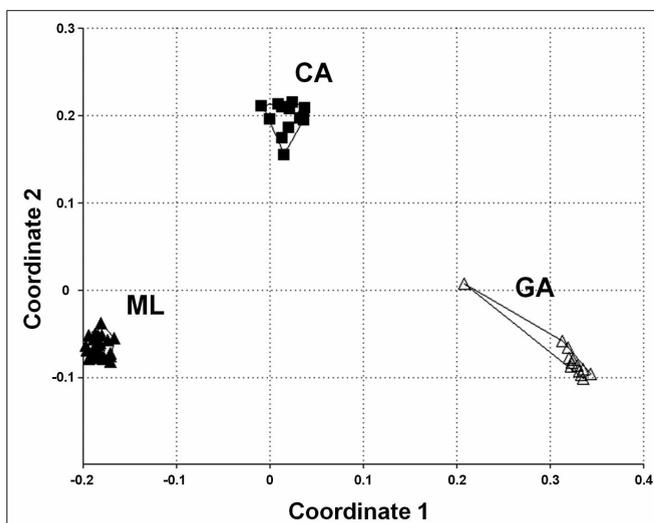


Fig. 2. Principal Coordinates Analysis (PCoA) from *Genista legionensis* populations based on the correlation matrix of presence/absence of AFLP data: CA, filled square (■); GA, open triangle (Δ); ML, filled triangle (▲).

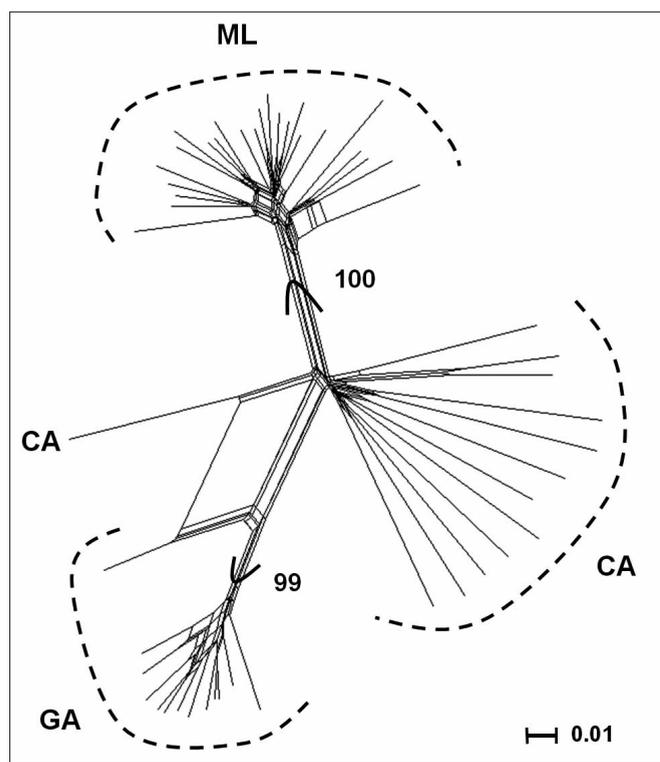


Fig. 3. NeighborNet derived from AFLP data of *Genista legionensis*. Numbers along branches are bootstrap values from neighbor-joining analysis (2,000 replicates). The least squares fit index for the split network has a value of 97.01%. CA, Cabrales; GA, Gamoniteiro; ML, Monte Lucero.

the results presented, genetic monitoring should be kept, as long as fragmentation can have an effect on genetic variation in the long term, and we recommend the conservation of peripheral populations of *Genista legionensis* (i.e. Monte Lucero and Gamoniteiro), without meaning a global protection for the species in the Cantabrian Range as this endemic species is common in its distribution area.

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