Molecular Ecology (2007) **16**, 2155–2171 doi: 10.1111/j.1365-294X.2007.03280.x

Ecology matters: Atlantic–Mediterranean disjunction in the sand-dune shrub *Armeria pungens* (Plumbaginaceae)

ROSALÍA PIÑEIRO,* JAVIER FUERTES AGUILAR,* DAVID DRAPER MUNT† and GONZALO NIETO FELINER*

**Real Jardín Botánico de Madrid, CSIC, Plaza de Murillo 2, 28014 Madrid, Spain, †Natural History Museum Botanical Garden. University of Lisbon, R. Escola Politécnica 58, 1250-102 Lisboa, Portugal*

Abstract

Inferring the evolutionary history of Mediterranean plant lineages from current genetic, distributional and taxonomic patterns is complex because of a number of palaeoclimatic and geological interconnected factors together with landscape heterogeneity and human influence. Therefore, choosing spatially simplified systems as study groups is a suitable approach. An amplified fragment length polymorphism (AFLP) study using two restriction enzyme combinations (*Eco*RI/*Mse*I and *Kpn*I/*Mse*I) was carried out to estimate the structure of genetic variation throughout the range of *Armeria pungens*. This species has a West Iberian-Corso/Sardinian disjunct distribution on coastal sand-dune ecosystems. Bayesian, AMOVA **and genetic distance analyses of the AFLP data revealed the same distinguishable genetic groups, which do not match the main geographical disjunction. Corso-Sardinian populations were found to be genetically closer to southwest Portuguese than to those from the Gulf of Cadiz (the closest geographically). Eastwards long-distance dispersal is therefore invoked to explain this geographical disjunction. A GIS analysis based on bioclimatic envelope modelling aiming to characterize the current locations of *A. pungens* found strong similarities between the Portugal and Corsica-Sardinia sites and less so between these areas and the Gulf of Cadiz. This coincident pattern between AFLP and climatic data suggests that the geographical disjunction is better explained by climatic factors than by the likeliness of a stochastic dispersal event. Such a combined phylogeographical–GIS modelling approach proves to be enlightening in reconstructing the evolutionary history of plant species.**

Keywords: AFLP, bioclimatic envelope, coastal plants, continental islands, GIS, long-distance dispersal, Mediterranean phylogeography

Received 4 September 2006; revision received 15 November 2006; accepted 8 January 2007

Introduction

The Mediterranean flora has high levels of plant diversity and endemism (80% of European plant endemics are Mediterranean) combined with a striking variety of species distribution patterns including frequent disjunct distributions (Blondel & Aronson 1999; Médail & Quézel 1999; Thompson 2005). The historical interpretation of such diverse patterns is often difficult, since they reflect the overlapping effects of several processes that operated at different spatial and temporal scales. From a spatial perspective, the Mediterranean landscapes provide high habitat variability (Blondel & Aronson 1999). At a temporal scale, different palaeoclimatic and geological processes have played a role in the evolution of Mediterranean plant communities. Most often invoked are the isolation of microplates resulting from Tertiary tectonic movements, the Messinian salinity crisis at the Miocene–Pliocene boundary, 5.96–5.33 million years ago (Hsü *et al*. 1977; Krijgsman *et al*. 1999) the establishment of a Mediterranean climate type at the Plio–Pleistocene boundary, 3.2–2.8 million years ago (Suc 1984), and sea-level changes associated with Pleistocene glaciations (Hewitt 2000). The negative impacts of humans on certain plant communities during the last several thousand years may have been a further relevant factor (Martínez & Montero 2004).

Correspondence: Rosalía Piñeiro Portela, Fax: +34 91420 0157; E-mail: pineiro@rjb.csic.es

Systems that simplify the spatial component, such as coastal species with linear distribution ranges, allow identifying influential changes in a more clear way. Phylogeographical studies of some of these systems stress the importance of ecological requirements of each species modulated by Pleistocene climatic oscillations to explain differences in their genetic structures (Clausing *et al*. 2000; Kadereit *et al*. 2005). Other linear systems are best interpreted in terms of the geological history of their habitats (e.g. *Hypochaeris salzmanniana* in Tremetsberger *et al*. 2004). Many more comparative phylogeographical studies of plants in the Mediterranean are needed to clarify the relative contribution of historical vs. ecological factors in shaping present-day species distributions.

The present phylogeographical study examines the coastal plant species *Armeria pungens*, which has a linear geographical distribution with two significant disjunctions (Fig. 1b). *Armeria pungens* is a perennial shrub that grows on sandy maritime dunes (exceptionally on sandy soils over limestone maritime cliffs) with a main geographical distribution along a 500-km coastal stripe in southwest Iberia, from the mouth of the Tagus river to the Gibraltar Strait. It also occurs on two continental archipelagos: in the Atlantic, on the Cíes Islands (offshore Galician coast, Northern Spain), and in the Mediterranean, on South Corsica and North Sardinia).

Taxonomic boundaries within *Armeria* are sometimes difficult to establish because of frequent hybridization, even between distantly related species (Bernis 1954; Nieto Feliner 1990; Nieto Feliner *et al*. 2001; Tauleigne-Gomes & Lefèbvre 2005). However, this is not the case for *A. pungens*. All taxonomic treatments of this genus have recognized *A. pungens* as a distinct species with a disjunct distribution pattern (Bernis 1955; Arrigoni 1970; Pinto da Silva 1972; Nieto Feliner 1990). Thus, the fact that the Iberian and Corsican populations were initially described as separate species was only due to the circumstance that they were discovered and described almost simultaneously in the beginning of the 19th century (see Bernis 1955; Arrigoni 1970).

An interesting feature of our study system is the different geological histories of the two disjunct areas, which opens several a priori possibilities for the occurrence of *A. pungens*. While Cíes archipelago was recently isolated from the mainland coasts after the last glaciations (Luaces & Toscano 1998) and is only 2.5 km apart, the isolation of Corsica and Sardinia from eastern Spain and southern France dates back to the Tertiary (c.16 million years ago, Krijgsman 2002). Land bridges with North Italy have been proposed during the Messinian salinity crisis and during the glaciations, although the latter are considered very unlikely by some authors (Gamisans 1991). The strong affinities of Corso-Sardinian floras with other territories, mainly with the Balearic Islands but also with Provence, Alps, Pyrenees and Iberian Peninsula, have traditionally been explained by the position of the Corso-Sardinian plate before the Miocene (Briquet 1910; Cardona & Contandriopoulos 1979; Gamisans 1991). In contrast, the relative contribution of long-distance dispersal to the flora of Corsica and Sardinia has been insufficiently addressed in classic Mediterranean biogeography. Recent molecular phylogenies suggest that dispersal has been important in shaping contemporary distribution patterns in many plant groups (Winkworth *et al*. 1999; Brochmann *et al*. 2003; Schönswetter *et al*. 2004; de Queiroz 2005). The presence of early divergent lineages in Corsica and Sardinia has been confirmed with molecular data for some plant groups (e.g. *Doronicum corsicum*, Álvarez Fernández *et al*. 2001) but not for others (e.g. *Erodium maritimum* and *Erodium corsicum*, Fiz *et al*. 2006).

The well-defined taxonomic status of *A. pungens*, its linear geographical distribution, together with the well-known geological history of the islands where it occurs provide a simple model to test scenarios of vicariance (old presence favoured by land connections) vs. long-distance dispersal (recent arrival). For this purpose, we assessed the current genetic variation across *A. pungens* populations and examined them in the context of the extant distribution patterns and geological history.

Predictive species–climate modelling has become very popular in recent years as a powerful tool to assess biodiversity patterns, species distribution, and increasingly the potential impacts of climate change (Huntley *et al*. 1997; Guisan & Thuiller 2005). Bioclimatic envelope models (BEM), describing the relationship between a species' observed distribution and climate, are supported on the niche theory of Hutchinson (Luoto *et al*. 2005). Climate envelopes based on empirical correlation between species' distributions and selected climate variables allow characterizing eco-physiological conditions in particular habitats without experimental analyses, unpractical in natural populations (Bakkenes *et al*. 2002). In this work, we use this technique as a way of comparing genetic and ecological data from *A. pungens* in the West Mediterranean. This combined approach has been applied from a phylogenetic perspective to assess the role of ecology on speciation within several groups (Peterson *et al*. 1999; Graham *et al*. 2004; Yesson & Culham 2006).

Specifically, the following issues were addressed: (i) Are disjunct populations on continental islands in the Atlantic and the Mediterranean the result of past fragmentations involving tectonic landmass movements or are they the result of long-distance dispersal? (ii) If due to long-distance dispersal, which is the main source area? To address these questions we needed to determine the genetic structure of *A. pungens*, that is to recognize genetic groups of populations and clarify the similarities among them,

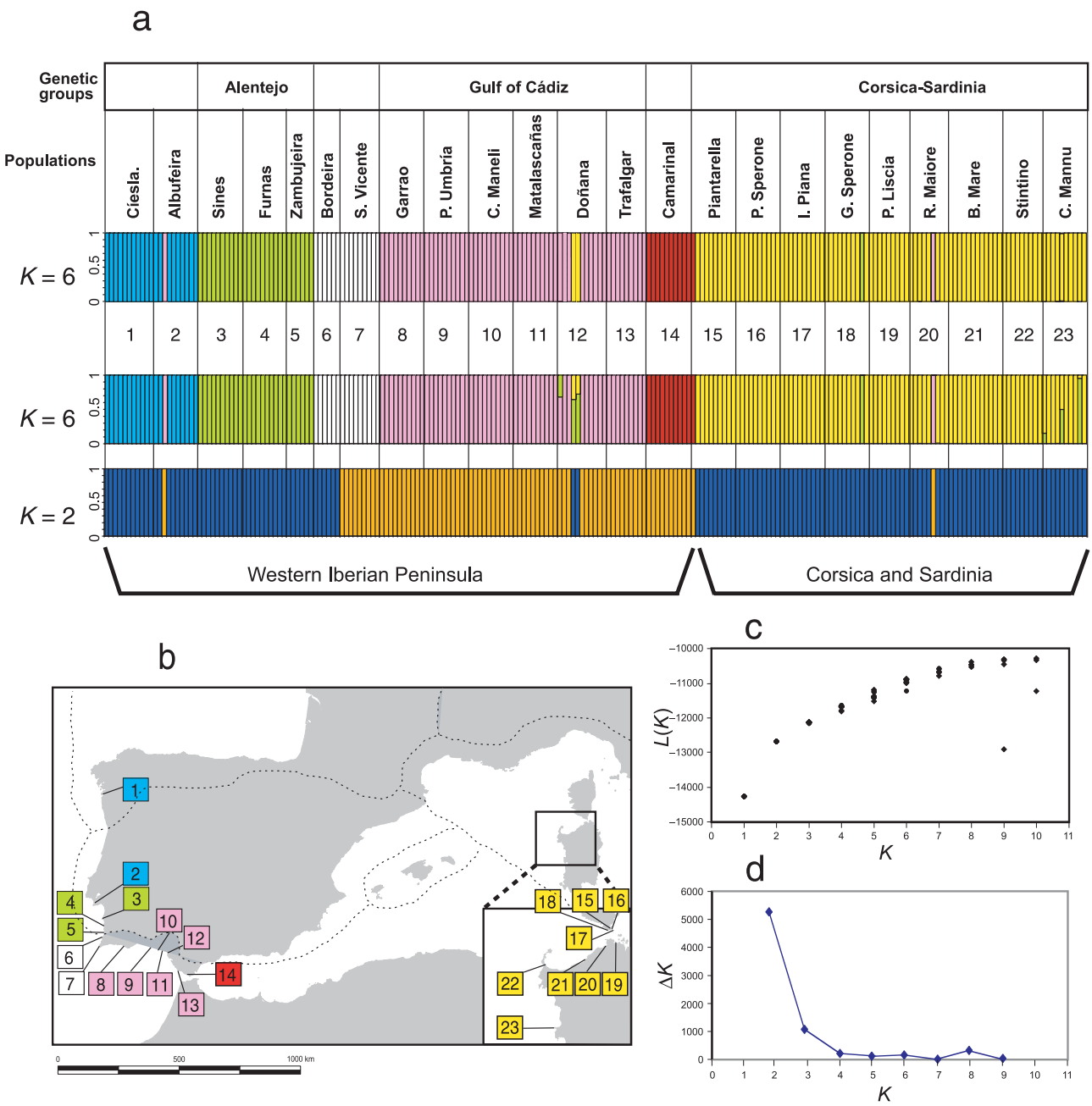

Fig. 1 Genetic structure of *Armeria pungens* inferred by Bayesian clustering of AFLP data. (a) Assignment of 221 individuals into *K* genetically distinguishable groups. Each individual is represented by a vertical bar coloured according to the assigned group(s). The 23 populations are identified by name and number following Table 1. The upper band represents the highest probability partition yielded by BAPS. The middle and lower bands show the most stable and likely assignments estimated by STRUCTURE (at $K = 2$ and $K = 6$) (see text). (b) Geographical location of sampled populations of *A. pungens*, numbered as in Table 1 and colour-coded following Bayesian clustering results for six groups in Fig. 1(a). Dotted lines mark approximate boundaries of floristic provinces in Takhtajan (1986). (c) Log probability of data $L(K)$ as a function of K for 10 STRUCTURE runs at $K = 1$–6 or 5 runs at $K = 7$–10. (d) Rate of change in the probability between successive runs, ΔK, as a function of K (see Evanno *et al*. 2005).

considering the distribution of the genetic variation within and among populations. (iii) Finally, we assessed the contribution of bioclimatic factors to the current distribution of *A. pungens*. For this purpose, using BEM tools, we modelled the climatic conditions of the *A. pungens* present range, as well as those of their genetically characterized groups of populations, to explore their potential distribution habitats.

Materials and methods

The species

Armeria pungens belongs to section *Macrocentron* Boiss., a small but morphologically well-differentiated group with long-spurred calyces represented by 10 species in the Iberian Peninsula (Nieto Feliner 1990), two in North Africa (Nieto Feliner 2002) and possibly one in the Eastern Mediterranean (Pinto da Silva 1972). In Corsica and Sardinia, *A. pungens* is the only representative of the section (Arrigoni 1970). Chromosome counts on specimens from all main geographical areas have always revealed a diploid number, $2n = 18$ (Arrigoni 1970; Lago & Castroviejo 1993; and references therein), as reported in most counts for the genus.

Although reproductive biology of *A. pungens* has not been studied in detail, it is well documented that, with just some exceptions mainly in the Arctic and North and South America (Baker 1966; Moore & Yates 1974), almost all species of *Armeria* are obligate outcrossers, as determined by an incompatibility system that involves a pollen-stigma dimorphism (Baker 1966). Pollination by unspecific insects has been reported for the close relative *Armeria velutina* (Herrera 1988) as well as for the widespread *Armeria maritima* (Eisikowitch & Woodell 1975; Woodell & Dale 1993). Wind pollination has also been suggested (Woodell & Dale 1993) but it seems unlikely to be effective because of the low number of pollen grains per anther (Tauleigne-Gomes & Lefèbvre 2005; G. Nieto Feliner, unpublished data). Parachute-like calyces containing the fruit suggest wind dispersal although recorded distances are low in *A. maritima* (Philipp *et al*. 1992) and animal dispersal may also occur facilitated by stiff hairs of fruiting calyces. *Armeria* fruits lack any special mechanism for water transport such as corky tissues.

Sampling, DNA extraction

Leaves and ripe fruits were collected in 23 populations of *A. pungens* spanning the whole species distribution (Table 1). Where possible, a distance of 10 m between individuals was kept. Voucher specimens of all sampled individuals were collected and deposited in the herbarium of the Royal Botanical Garden of Madrid (MA). Leaves were dried and stored at −80 °C, whereas fruits were germinated with giberelic acid after a cold treatment of 1 month. Seedlings were cultivated in a greenhouse in the Botanical Garden of Madrid from 2002 to 2004. Most amplified fragment length polymorphism (AFLP) fingerprints were obtained from young fresh leaves grown from seeds belonging to separate individuals in the field. When germination failed, frozen dried leaves were used instead. An average of 10 individuals per population were sampled (Table 1). Genomic DNA was extracted from dried or fresh leaves using DNeasy Plant Minikit (QIAGEN) according to the manufacturer's instructions. The approximate quantity and quality of the isolated DNA was determined by 1.5% Tris-Acetate-EDTA (TAE)-agarose electrophoresis and ethidium bromide staining. Negative controls were performed to monitor contamination.

AFLP protocols: Eco*RI*/Mse*I and* Kpn*I*/Mse*I*

In order to cover as many genomic regions as possible, two different restriction enzyme pairs were combined: *Kpn*I/*Mse*I and *Eco*RI/*Mse*I. The restriction with *Kpn*I, insensitive to methylation, in addition to the extensively used *Eco*RI, only affected by overlapping methylation (5-methylcytosine in CpG formed with the adjacent sequence) reduces polymorphism because of different levels of methylation (Roberts *et al*. 2005). By this strategy, we minimized tissue-specific or ecotype-related effects associated to gene expression (Reyna-López *et al*. 1997; Xiong *et al*. 1999; Cervera *et al*. 2002).

An initial screening of 37 combinations of selective primers on four individuals from different geographical areas was performed. Three combinations were selected that yielded clear and evenly distributed bands: (6-FAM)*Eco*RI + acc/*Mse*I + cacc, (6-FAM)*Eco*RI + acg-*Mse*I + ctac, (6-FAM) *Kpn*I + atc/*Mse*I + cag (MWG-Biotech AG). To prevent mismatch amplifications with the *Mse*I primer with four selective nucleotides (Vos *et al*. 1995), preselective primers with two selective bases were used.

Restriction and ligation of genomic DNA with *Eco*RI and *Mse*I were performed according to Gaudeul *et al*. (2000) with few modifications. Double digestion with *Kpn*I and *Mse*I was performed independently from the ligation. The restriction reaction was incubated at 37 °C for 2.5 h and consisted of 0.2 μL NE1 buffer, 0.1 μL 1 mg/mL BSA, 1 U *Mse*I, 5 U *Kpn*I (New England BioLabs) and 10 μL template DNA (final volume 20 μL). Ligation, also incubated for 2.5 h at 37 °C, comprised 2.0 μL 10× T4 DNA ligase buffer, 1.0 μL BSA (1 mg/mL), 2.0 μL 10 μM *Mse*I adapter pair, 2.0 μL *Kpn*I adapter pair, 2 U T4 DNA ligase, and 15 μL restriction reaction (final volume 25 μL). *Kpn*I adapter sequences 5′-CTCGTAGACTGCGTACAGTAC-3′ and 5′-TGTACGCAGTCTAC-3′, as well as the adapter-matching primers 5′-GACTGCGTACAGTACCA and 5′(6-FAM)-GACTGCGTACAGTACCATC-3′ were designed following the rules for good primer design (Dieffenbach *et al*. 1993). Restriction-ligation products were diluted 10 times in purified H_2O. Pre-amplification and selective amplification were performed as described in Gaudeul *et al*. (2000) with small modifications. Restriction-ligation and amplification reactions were incubated in a thermocycler GeneAmp PCR System 9700 (PE Biosystems). The fragments were separated using an ABI PRISM 3700 sequencer (Applied

Table 1 Collection data of 23 populations of *Armeria pungens* included in the AFLP study. *N*, sampling size (approximately 10 individuals per population were included). The geographical location of each population is represented in Fig. 1(b). Coordinates of each population were recorded as presence data for the climatic model

Population code	Site location, collector (year)	Coordinates latitude/longitude	*N*
1-Cíes	Spain, Pontevedra, Illas Cíes, RPP, IMA, IMF, LMR, MSA & PGM (2003)	42.2245/−8.89666	11
2-Albufeira	Portugal, Estremadura, Lagoa de Albufeira, GNF & JFA (2002) and RPP, AC & PEG (2003)	38.5111/−9.17712	10
3-Sines	Portugal, Baixo Alentejo, Sines-Cercal, RPP, AC & PEG (2003)	37.9160/−8.78885	10
4-Furnas	Portugal, Baixo Alentejo, Vilanova de Milfontes, Praia das Furnas, RPP, AC & PEG (2003)	37.7266/−8.78937	10
5-Zambujeira	Portugal, Baixo Alentejo, Zambujeira do Mar, GNF & JFA (2002) and RPP, AC & PEG (2003)	37.5372/−8.77858	6
6-Bordeira	Portugal, Algarve, Praia do Bordeira, GNF & JFA (2002)	37.2038/−8.90348	6
7-S. Vicente	Portugal, Algarve, Cabo de São Vicente, GNF & JFA (2002) and RPP, AC & PEG (2003)	37.0235/−8.98239	9
8-Garrrao	Portugal, Algarve, Praia de Garrao, GNF & JFA (2002) and RPP, AC & PEG (2003)	37.0739/−8.07122	10
9-P. Umbría	Spain, Huelva, Punta Umbría, Playa de Punta Umbría, GNF & JFA (2002)	37.1956/−6.98823	10
10-C. Maneli	Spain, Huelva, Mazagón-Matalascañas, 'cuesta Maneli', GNF & JFA (2002)	37.0729/−6.68781	10
11-Matalascañas	Spain, Huelva, Torre de la Higuera, Playa de Torre la Higuera, GNF & JFA (2002) and RPP, AC & PEG (2003)	37.0164/−6.56591	10
12-Doñana	Spain, Huelva, Doñana National Park, pr. El Inglesillo, SG (2002)	36.9678/−6.39901	11
13-Trafalgar	Spain, Cadiz, Cabo de Trafalgar, GNF & JFA (2002) and RPP, AC & PEG (2003)	36.1839/−6.03606	9
14-Camarinal	Spain, Cadiz, Punta Camarinal, GNF & JFA (2002)	36.0798/−5.79252	10
15-Piantarella	France, Corsica, Bonifacio, Piantarella, GNF & JFA (2002)	41.3761/9.22254	10
16-P. Sperone	France, Corsica, Bonifacio, Petit Sperone, CBNMP (2002)	41.3691/9.22269	10
17-I. Piana	France, Corsica, Bonifacio, île Piana, CBNMP (2002)	41.3735/9.23012	10
18-G. Sperone	France, Corsica, Bonifacio, Grand Sperone, CBNMP (2002)	41.3682/9.21417	10
19-P. Liscia	Italy, Sardinia, Porto Pozzo, Porto Liscia, GNF & JFA (2002)	41.2000/9.31667	9
20-R. Maiore	Italy, Sardinia, S of Santa. Teresa di Gallura, Spiaggia di Rena Maiore, GNF & JFA (2002)	41.1441/9.13725	9
21-B. Mare	Italy, Sardinia, Badesi Mare, GNF & JFA (2002)	40.9667/8.88333	12
22-Stintino	Italy, Sardinia, Pr. Porto Torres, Stintino, S Stagno di Pilo, GNF & JFA (2002)	40.9372/8.22750	9
23-C. Mannu	Italy, Sardinia, NW of Oristano, Capo Mannu, between the cape and Su pallosu, GNF & JFA (2002)	40.0750/8.37129	10

Abbreviations of collectors: AC, A. Costa; CBNMP, Conservatoire Botanique National Mediterranéen de Porquerolles; GNF, G. Nieto Feliner; IMA, I. Martínez Arcos; IMF, I. Martínez Fernández; JFA, J. Fuertes Aguilar; LMR, L. Muriel Ríos; MSA; M. Souto Alonso; PEG, P. Escobar García; PGM, P. García Meijide; RPP, R. Piñeiro Portela; SG, S. Gatelier.

Biosystems) using 1 μL of polymerase chain reaction (PCR) product and GeneScan-500 ROX size standard.

A reproducibility test was performed by re-extracting DNA from one individual per population, in 22 of the 23 populations, and repeating the whole AFLP procedure. The error rate was calculated for every primer combination as the number of phenotypic differences related to the total number of phenotypic comparisons, and subsequently averaged over the three combinations. This rate was further used to evaluate the quality of the fingerprints.

AFLP analysis

Amplified bands were aligned with the internal size standard using the ABI PRISM GENESCAN Analysis Software version 3.1 (Applied Biosystems). Subsequently, fragments of each primer combination were scored automatically with GENOGRAPHER version 1.6.0 (Montanta State University, http://hordeum.oscs.montana.edu/genographer/) either as present (1) or absent (0), and manually corrected. Peaks were recorded in a range from 50 to 500 bp.

Genetic distance-degree of similarity between individuals and populations. To distinguish genetically similar groups of individuals in our AFLP data set and clarify the similarity among them, first a descriptive comparison of phenotypes was made by constructing a pairwise similarity matrix between all individuals using Dice's coefficient and subjecting it to a principal coordinates analysis (PCoA). Secondly, a pairwise distance matrix was computed between

populations using net nucleotide differences between populations (Nei & Li 1979, Equation 25), as implemented in ARLEQUIN 3.0 ('Nei's net average number of differences between populations'; Excoffier & Schneider 2005), and visualized through a neighbour-joining (NJ) tree. The PCoA and the NJ analyses were performed with NTSYS pc 2.0 (Rohlf 1998).

Genetic differentiation — the distribution of genetic variation among populations and regions. We also used a Markov chain Monte Carlo (MCMC) Bayesian clustering method to identify genetically similar groups, that is having distinctive allele frequencies, using STRUCTURE version 2.0 (Pritchard *et al*. 2000). This approach assumes Hardy–Weinberg and linkage equilibrium within groups. Following user's manual recommendations for dominant markers, a missing value was added to each marker and the ancestry model of no admixture was chosen. For the allele frequency model, we set the default option of correlated allele frequencies (Falush *et al*. 2003) as advised by the manual when they are expected to be similar in the different groups. Since clustering of individuals was different across independent runs and even very long chains of 10^6 did not stabilize the results, we run 10 simulations for each number of groups, K, from $K = 1$–6 and five replicates for $K = 7$–10, using a burn-in period of 10^5 and runs lengths of 10^6. We applied two criteria to choose the best value of K in our data set: the estimated posterior log probability of the data, $L(K)$, and the stability of assignment patterns across runs. Since $L(K)$ continued to grow slightly with increasing values of K, the criterion of selecting the K that maximizes the probability of the data was difficult to apply. We therefore calculated another *ad hoc* quantity based on the rate of change in the probability between successive K, ΔK, as proposed by Evanno *et al*. (2005). The results were compared with those provided by another Bayesian clustering method, BAPS 3.2 (Corander *et al*. 2006), which uses stochastic optimization instead of MCMC to find the optimal partition (with highest estimated probability). Given the faster performance of this algorithm, the simulation was started from $K = 2$ to $K = 23$ as the maximum number of diverged groups, with three replicates for each K.

In order to quantify the genetic differentiation with an alternative method not assuming Hardy–Weinberg equilibrium or independence of markers, the proportion of molecular variance within and among populations, AMOVA, was analysed with ARLEQUIN 3.0. AMOVAs based on pairwise distances between individuals were carried out for the whole data set considering three levels, among regions, among populations within regions and within populations. For subsets of the data, two levels, within and among populations, were considered.

Genetic diversity within populations. The amount of genetic variation was measured for each population: (i) in terms of allele richness, by the direct observation of percentage of polymorphic loci (P), that is variable across the data set; (ii) in terms of similarity, with Shannon's index (H_{SH}) (Shannon 1948); and (iii) in terms of allele frequencies, assuming Hardy–Weinberg equilibrium for each locus, using Nei's unbiased gene diversity H_S (Nei 1978). Since every AFLP band represents a different location in the genome, both Shannon's and Nei's indices were calculated for every locus and then averaged over all loci as implemented in POPGENE version 1.32 (Yeh & Boyle 1997) and TFPGA version 1.3 (Miller 1997), respectively. Shannon index was standardized to the lowest sampling (six individuals).

Bioclimatic envelope model

The potential distribution of *A. pungens* according to climatic factors was determined through a GIS modelling analysis undertaken with IDRISI Kilimanjaro software (Clark Laboratories) and DIVA-GIS (Hijmans *et al*. 2001). The BIOCLIM algorithm was used to build the distribution model (Nix 1986; Busby 1991; McMahon *et al*. 1995). This algorithm first characterizes the environmental conditions of the actual distribution of the species and then identifies additional sites that fall within the already defined environmental hyperspace (Barry & Elith 2006). We considered as presence data the geographical location of our own sampling sites (Table 1), which represent most of the known populations of the species. These geographical data were supplemented with those obtained from herbarium specimens (Table 2). Altogether, it is considered that presence data used are representative for characterizing the environmental conditions of a species with a linear distribution range and confined to a restricted ecological habitat.

Only climatic variables were used to construct the distribution model because we were not only interested in characterizing the environmental conditions of *A. pungens* with respect to other species but also to explore if geographically cohesive ecological subsets could be identified corresponding to genetic subgroups. For this purpose, environmental variables such as substrate (sand), elevation above sea level and the sea influence through salt accumulation are largely invariant across the distribution range of the species. Among the possible climatologic variables, we chose three with a more likely direct physiological significance on seed germination as well as plant growth and survival: monthly minimum temperature (T_{min}), monthly maximum temperature (T_{max}) and monthly precipitation (Médail & Quézel 2003). Monthly minimum temperature may represent a threshold for over-winter survival and for seed germination. Monthly maximum temperature is critical during Mediterranean summers when it is associated to drought. Monthly precipitation was chosen as an estimate of moisture availability for both seed germination

Table 2 Presence data of *Armeria pungens*, based on herbarium specimens, used to build the climatic model in addition to the populations sampled for AFLP (Table 1). All voucher specimens are from the Royal Botanical Garden of Madrid (MA)

	Site location, collector, date (voucher)	Coordinates latitude/longitude
a	Portugal: Baixo Alentejo, Peninsula de Troia, pr. Malha da costa, Malato-Beliz *et al.* 27.6. 1971 (MA 306078)	38.4300/−8.83323
b	Portugal: Baixo Alentejo, Praia de Melides, M. Henna & L. Loidi, 19.5. 1990 (MA 486568)	38.1324/−8.78824
c	Portugal: Baixo Alentejo, Sines, pr. lighthouse, E. Monasterio, F. Muñoz Garmendia & J. Pedrol, 10.4. 1988 (MA 448985)	37.9612/−8.87979
d	Portugal: Baixo Alentejo, Cabo Sardao, Malato Beliz & J.A. Guerra, 17.6. 1978 (MA 238063)	37.6004/−8.82370
e	Portugal: Baixo Alentejo, Praia do Carvalhal, Malato Beliz & J.A. Guerra, 16.5. 1984 (MA 421137)	37.5012/−8.78999
f	Portugal: Algarve, Odeceixe, F. Bernis, 24.4. 1949 (MA 394446)	37.4381/−8.80147
g	Portugal: Algarve, Praia de Esteveira, A. Herrero, 27.3. 1991 (MA 649267)	37.4291/−8.81279
h	Portugal: Algarve, Praia de Monte Clérigo, F. Bellot, 19.4. 1968 (MA 498827)	37.3930/−8.82418
i	Portugal: Algarve, Ponta de Sagres, V.J. Arán & M.J. Tohá, 28.3. 1999 (MA 643449)	37.0235/−8.99363
j	Portugal: Algarve, Praia de Armançao de Pêra, Praia grande, L. Medina, S. Nisa & M. Pardo, 7.6. 2001 (MA 691371)	37.0937/−8.32977
k	Portugal: Algarve, Praia do Ançao, Faro-Ferreiras, A. Moura, 28.5. 1986 (MA 394453)	37.0285/−8.03805
l	Spain: Huelva, La Rábida, Pinar del Palo, 29.4. 1949, F. Bernis (MA 394442)	37.2127/−6.93144
m	Spain: Huelva, Mazagón, Fernández Casas, 15.8. 1969 (MA 413843)	37.0918/−6.73224
n	Spain: Huelva, La Barra, C. Vicioso, 21.4. 1943 (MA 145356)	37.1745/−7.37170

and plant growth. These three environmental predictors were obtained from WorldClim data set (www.worldclim.org), which contains records from 1960 to 1990, with 30 s resolution (Hijmans *et al*. 2005). Since each of these variables was considered for 12 months, the models were based on 36 data layers. For each of the 36 variables, we considered the ranges between (i) maximum and minimum values; (ii) mean ± 1 standard deviation; (iii) 95% of the confidence interval; (iv) mean ± 1.96 standard deviation, and combined them either in an excluding way (AND) or in an inclusive way (OR). We chose to use the range between maximum and minimum values and the 'AND' combination, which of the ones recovering the actual distribution area was the most selective of the four criteria. Thus, localities were selected as matching the climatic conditions of the presence data only when their values for each of the 36 variables fell within the ranges determined by the maximum and minimum values on the basis of the presence data.

In accordance with the two purposes pursued here with the species distribution modelling, two approaches were followed. To explore whether the current distribution of *A. pungens* covers most of its environmental envelope or, alternatively, suitable sites are found elsewhere, all presence data were used to construct the model. To explore if geographically cohesive climatic profiles could be identified within the actual range of *A. pungens* matching the genetic subgroups, different subsets of the whole data in Tables 1 and 2 were used to construct the models. These subsets were (i) Gulf of Cadiz, that is, data from the Southern coasts of Iberia from S. Vicente Cape to Gibraltar; (ii) Atlantic populations north of São Vicente Cape; and (iii) Corso-Sardinian populations.

Results

AFLP profiles

Highly reproducible AFLP patterns were obtained for all 22 replicates undertaken in the reproducibility test. An average error rate of 4.7% was estimated across all three primer pairs, in agreement with previous reports below 5% (Blears *et al*. 1998; Bonin *et al*. 2004; Skrede *et al*. 2006). Seven unreliable fragments, nonreproducible in at least 5 out of 22 allelic comparisons, were removed leading to a total data set of 223 bands. Only one of the bands was monomorphic across the 221 individuals assayed and no identical multilocus phenotypes were found between individuals or populations. The average number of fragments per individual was 56.82, and 80.33 per primer combination. Number of private and rare (< 10% of the individuals) fragments per population as well as the percentage of fragments shared between populations are provided as Supplementary material.

In order to further assess the quality of the data, we recalculated the error rate after discarding potentially unreliable bands following four different criteria: (i) slight size differences among putative homologous bands across individuals, (ii) low intensity bands, (iii) changing intensity of one band across samples, and (iv) bands of high (upper 10%) or small (lower 10%) molecular weight (Bagley *et al*. 2001; Bonin *et al*. 2004). Bands of changing intensity across samples resulted to be the least reproducible, but discarding them implied the loss of too much information (an average of 20.5 bands per primer combination) while only achieving an improvement in reproducibility of less than 1%. We therefore decided to keep the entire

data set of 223 bands, since we considered it preferable to maximize the phylogeographical signal by sampling more loci even if maintaining a small amount of noise.

Genetic groups vs. geographical disjunctions

Using the increase in the probability of the data and the stability of the assignments as criteria, STRUCTURE revealed that two groups are most appropriate to interpret our AFLP data set, which can be further subdivided into a total of six groups (Fig. 1a). Most individuals were assigned with high probability to one of the clusters and individuals from the same population were always assigned to the same cluster, with the exception of a few outliers (assigned to a different cluster than the remaining individuals from the same population).

The largest increase in the posterior probability of the data occurred at $K = 2$. In estimations of more than two groups, it continued to increase slightly. This can be observed directly through the graphic representation of $L(K)$ over 10 or 5 runs for each K value (Fig. 1c) and is even more clear when representing ΔK, which exhibited a maximum value at $K = 2$ (Fig. 1d).

The assignment of individuals across replicate runs only provided stable results for $K = 2$ and $K = 6$ (results not shown). At $K = 2$, all 10 runs inferred exactly the same two clusters not matching the main geographical disjunction (Fig. 1a): (i) Gulf of Cadiz (pops. 7–14) and (ii) Cíes Islands, Portugal and Corsica-Sardinia (pops. 1, 2–6 and 15–23). Five outliers were obtained. At $K = 6$, the six subgroups identified are compatible with the two groups revealed under $K = 2$, with the exception of São Vicente Cape population which represents the geographical boundary between the two $K = 2$ groups (Fig. 1a,b): (i) Cíes Islands with Albufeira (pops.1 and 2), (ii) Alentejo (pops. 3–5), (iii) S. Vicente Cape with Bordeira (pops. 7 and 6), (iv) Gulf of Cadiz (pops. 8–13), (v) Camarinal (pop. 14), and (vi) Corsica-Sardinia (pops. 15–23). These six clusters were consistent in 8 out of 10 simulations; the two remaining runs produced different solutions but showed much lower log likelihood. Seven individual outliers were obtained, but they seem not meaningful since the assignment of five of them varied across runs. For K higher than six, clustering was very unstable and basically split Corso-Sardinian individuals into several extra groups lacking any pattern. BAPS optimal partition estimate (Fig. 1a) coincided with STRUCTURE by showing the same six groups.

Also, the results of AMOVA (Table 2) were consistent with the groupings inferred by the STRUCTURE and BAPS packages. In fact, when considering the two main STRUCTURE groups (Corsica-Sardinia and Cíes Islands together with Portugal in one group vs. Gulf of Cadiz in the other), the proportion of variance explained by differences between regions was 17.16%. This percentage is larger than that obtained analysing the two main geographical groups: Atlantic vs. Mediterranean (13.86%) and even larger than considering the three main disjunct areas: Cíes Islands vs. Southwest Iberian Peninsula vs. Corsica-Sardinia (16.94%), which indicates that the link between Corsica-Sardinia and Portugal is strong. The grouping of $K = 6$ is also highly supported. This population clustering maximizes the among regions variance component (23.18%), relative to the among-populations/within-regions component (21.14%). In at least 10 other designs we tried, variation was always higher among populations/within regions than among regions (results not shown), as was in the two clusterings at $K = 6$ exhibiting low likelihood.

Genetic distance among groups

The same six genetic groups were revealed both after the PCoA of individuals (Fig. 2) and the NJ tree of populations (Fig. 3). In the Iberian Peninsula, the southernmost populations, in the Gulf of Cadiz, formed a highly compact and distinct group, whereas the remaining populations showed a decreasing degree of similarity towards the North: São Vicente-Bordeira group, followed by Alentejo group and, finally, Albufeira-Cíes Islands group. Therefore, within the Iberian range, the genetic structure of *Armeria pungens* was shown to be strongly congruent with the linear distribution of the species along the coast, that is increasing the geographical distance among populations leads to a decrease in the genetic distances. In contrast, the disjunct Corso-Sardinian group did not follow this trend of genetic distance correlated with geographical distance since it fell between Alentejo group and Albufeira-Cíes Islands group in the PCoA and close to Albufeira-Cíes Islands in the NJ tree. Finally, although the Camarinal population was most closely related to the Gulf of Cadiz group it stood well apart from all other populations (see Discussion below).

Genetic diversity and differentiation

The estimates for within-population diversity of *A. pungens* were 28.86% (P), 0.11 (H_S) and 0.12 (H_{SH}). The value for AMOVA-derived among-population variation, Φ_{ST}, was 0.41.

The partitioning of genetic variation within and among populations revealed different patterns in the different areas. Among-population variation resulted to be higher in the Atlantic than in the Mediterranean. Separate AMOVA analyses for every disjunct area or genetic subcluster (Table 3) showed that Corso-Sardinian populations are the least differentiated (23.76%), as compared to the Iberian ones. This was also true when the distinct population of Camarinal was excluded from the analysis (results not shown). Shorter branches between Corso-Sardinian populations in the NJ (Fig. 3) support this conclusion. Finally, the percent of variance explained by differences among the

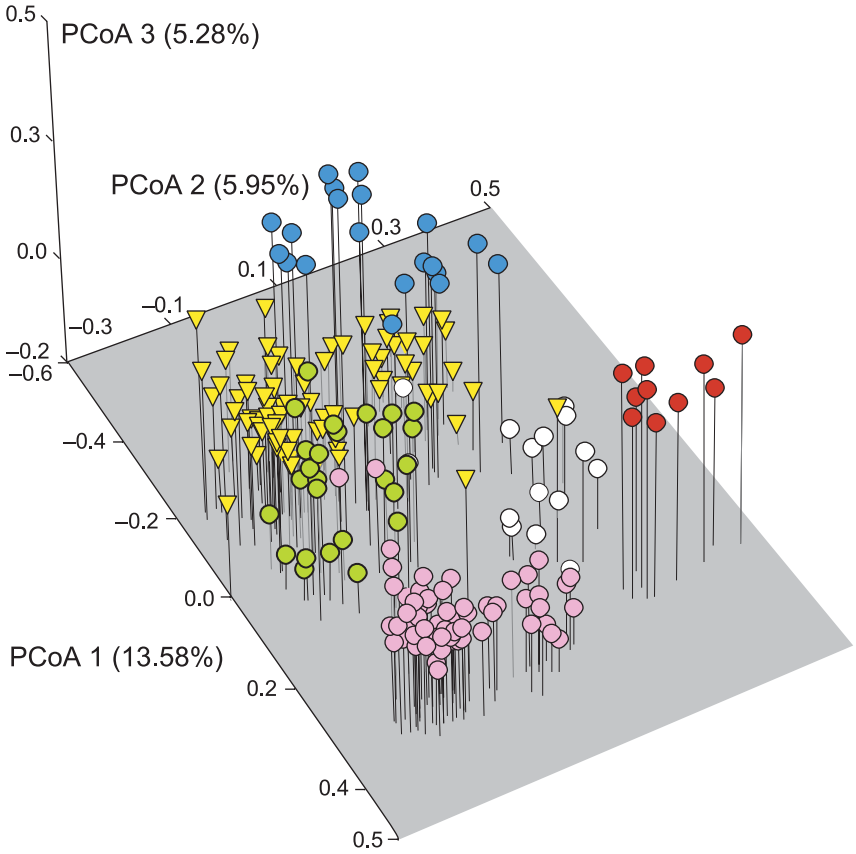

Fig. 2 Genetic distance among individuals of *Armeria pungens* based on AFLP. Principal coordinate analysis based on a pairwise similarity matrix between individual phenotypes using Dice. Scatterplot of 221 individuals against the first three principal axes indicating the percentage of the variance explained by each axis. Colour codes of each individual according to its assignment to any of the six genetic groups depicted in Fig. 1.

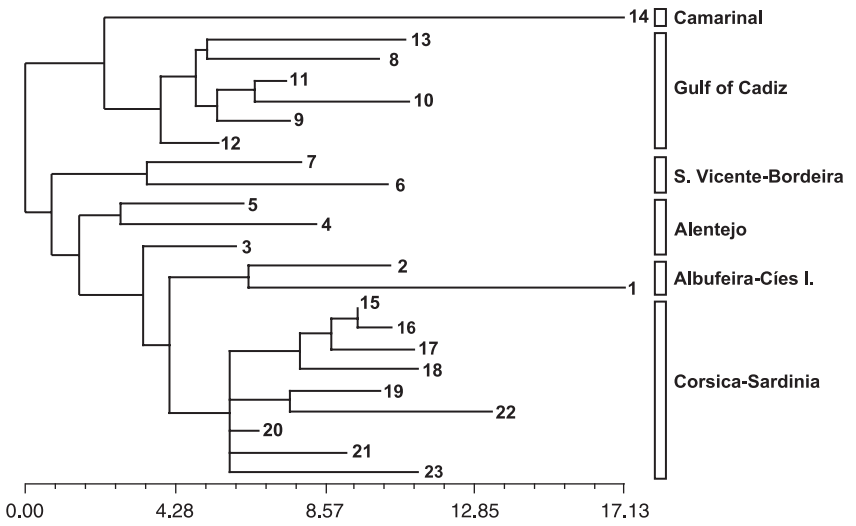

Fig. 3 Genetic distance among populations of *Armeria pungens* based on AFLP. Neighbour-joining tree built from a pairwise distance matrix between 23 populations based on Nei & Li distance (1979).

Cíes Islands and Albufeira is high (42.25%). These results suggest that even if the genetic proximity of these two populations is indicated by both Bayesian and genetic distance analyses, they are still significantly differentiated.

With respect to within-population diversity (Table 4), the lowest average estimates corresponded to the Gulf of Cadiz area (P = 21.45%, $H_S = H_{SH}$ = 0.08) as compared to the remaining areas. The populations from Corsica and Sardinia on the other hand appeared to be as much or even more variable (P = 33.08%, H_S = 0.12, H_{SH} = 0.14) than the Iberian. No apparent influence of population size was observed. For very small populations like Albufeira (pop. 2), and Piantarella (pop. 15, the smallest in Corsica, with only 34 individuals cf. Paradis & Culioli 2003), remarkable

Table 3 Analysis of molecular variance (AMOVA) of *Armeria pungens* based on AFLP considering three hierarchical levels for the whole AFLP data set and two levels for various subsets of the data. The significance of variance components and Φ-statistics was $P < 0.0001$ for all tests. Genetic groups are defined based on Bayesian and distance analyses. Geographical groups are defined on the basis of disjunctions

	Percentage of molecular variance		
Tested groups	Among groups	Among populations within groups	Among individuals within populations
Genetic groups			
2 groups: Gulf of Cadiz vs. Cíes Islands + Portugal + Corsica-Sardinia	17.16	29.25	53.59
6 groups: Cíes-Albufeira/Alentejo/S. Vicente-Bordeira/ Gulf of Cadiz/Camarinal/Corsica-Sardinia	23.18	21.14	55.68
Geographical groups			
2 groups: Iberian Penisula vs. Corsica-Sardinia	13.86	31.35	54.78
3 groups: Cíes Islands/SW Iberian Peninsula/Corsica-Sardinia	16.59	28.86	54.54
Genetic groups			
2 groups			
Gulf of Cadiz (8 populations)	—	39.54	60.46
Cíes Islands + Portugal + Corsica-Sardinia (15 populations)	—	33.30	66.70
6 groups			
Cíes Islands + Albufeira (2 populations)	—	42.25	57.75
Alentejo (3 populations)	—	27.82	72.18
S. Vicente + Bordeira (2 populations)	—	28.72	71.28
Gulf of Cadiz (6 populations)	—	32.10	67.90
Corsica-Sardinia (9 populations)	—	23.76	76.24
Camarinal (1 population)	—	—	—
Geographical groups			
2 groups			
Iberian Peninsula (14 populations)	—	44.37	55.63
Corsica-Sardinia (9 populations)	—	23.76	76.24
3 groups			
Cíes Islands (1 population)	—	—	—
SW Iberian Peninsula (13 populations)	—	44.81	58.19
Corsica-Sardinia (9 populations)	—	23.76	76.24
Total range (23 populations)	—	41.17	58.83

levels of genetic diversity were obtained. Cíes Islands populations showed to be less diverse ($P = 26.46\%$, $H_S = H_{SH} = 0.09$) than the closest populations from Alentejo and São Vicente-Bordeira ranges, but still more diverse than those of the Gulf of Cadiz. The divergent population of Camarinal was one of the most diverse of the Iberian range ($P = 34.53\%$, $H_S = H_{SH} = 0.14$; see Discussion). As expected, Shannon's index estimates were slightly higher than Nei's unbiased gene diversity for most populations. The general trend was the same for all the three indices, with only minor differences found among them.

Bioclimatic envelope model

Total data approach. The analysis of the whole data (Fig. 4a) shows that the current distribution of *A. pungens* covers most of its environmental envelope. Localized spots of climatically potential sites are also found in Minorca, Majorca and Algeria.

Subsets of the total data. When only the data from the Southern coasts of Iberia from São Vicente Cape to Gibraltar (Gulf of Cadiz) are used to build the model (Fig. 4b), the analysis failed to find any other similar area within the Western Mediterranean. This result indicates that the Gulf of Cadiz has different environmental conditions than the rest of the current geographical range of *A. pungens*. When only the Atlantic populations north of São Vicente are considered (Fig. 4c), populations from the Gulf of Cadiz are not selected by the envelope whereas those from Corsica and Sardinia are. This result is reciprocal since when only Corso-Sardinian populations are used as input (Fig. 4d), Atlantic populations north of São Vicente are selected but those from the Gulf of Cadiz are excluded.

An inspection of monthly mean values for the variables helps us to identify the parameters responsible for differences and similarities among the climatic profile of *A. pungens* across populations of its range. When looking at the monthly variation of the analysed climatic variables we

Table 4 Within-population genetic diversity of *Armeria pungens* based on AFLP. Populations arranged according to main genetic groups based on Bayesian and distance analyses

Population	P	H_S	H_{SH}
I. Cíes-Albufeira			
1-Cíes I.	26.46	0.09	0.09
2-Albufeira	28.70	0.10	0.13
mean	27.58	0.10	0.11
II. Alentejo			
3-Sines	36.77	0.13	0.12
4-Furnas	25.56	0.11	0.10
5-Zambujeira	26.46	0.12	0.13
mean	29.60	0.12	0.12
III. Bordeira-S. Vicente			
6-Bordeira	26.46	0.11	0.14
7-S. Vicente	32.29	0.12	0.15
mean	29.37	0.12	0.15
IV. Gulf of Cadiz			
8-Garrao	19.28	0.07	0.09
9-P. Umbría	29.60	0.11	0.12
10-C. Maneli	19.28	0.06	0.06
11-Matalascañas	17.49	0.07	0.07
12-Doñana	24.66	0.10	0.09
13-Trafalgar	18.39	0.06	0.09
mean	21.45	0.08	0.09
V. Camarinal			
14-Camarinal	34.53	0.14	0.14
VI. Corsica-Sardinia			
15-Piantarella	43.95	0.17	0.14
16-P. Sperone	25.11	0.10	0.11
17-I. Piana	36.77	0.14	0.16
18-G. Sperone	34.08	0.12	0.16
19-R. Maiore	34.98	0.14	0.14
20-P.Liscia	34.53	0.13	0.17
21-B. Mare	31.84	0.11	0.14
22-Stintino	28.25	0.10	0.14
23-C. Mannu	28.25	0.11	0.13
mean	33.08	0.12	0.14

Notes: P, percentage of polymorphic loci; H_S, Nei's unbiased gene diversity; H_{SH}, Shannon's index standarized to six individuals.

observe that while T_{min} do not exhibit any clear covariation between Corso-Sardinian and Atlantic populations, T_{max} and precipitation show differences between Atlantic-Corso-Sardinian populations on one side and Gulf of Cadiz populations on the other. For precipitation, the almost total lack of rainfall in the Gulf of Cadiz during July (0–1 mm, mean 0.7) and August (1–3 mm, mean 1.9) differs from range values in Atlantic populations (1–24 mm, mean 4.5 in July; 1–6 mm, mean 3.6 in August) and Corso-Sardinian populations (3–33 mm, mean 7.4 in July; 3–16 mm, mean 9.6 in August) (Fig. 5a). A similar pattern is observed in the same months for the maximum temperature where the Gulf of Cadiz populations reach 24.7–30.5 °C (July, mean 28.3) and 24.9–30.5 °C (August, mean 28.6) while the range values in Atlantic populations is 23.1–28.9 °C (July, mean 27.2) and 23.2–29.2 °C (August, mean 27.5). Similarly, Corso-Sardinian values for maximum temperature are 25.8–28.6 (July, mean 27.2) and 26.4–28.9 (August, mean 27.6) (Fig. 5b). The patterns shown by both variables underline the fact that summer drought is the most important factor segregating the Gulf of Cadiz climatic conditions from those of the remaining populations of *A. pungens*.

Discussion

Long-distance dispersal of Armeria pungens *into continental islands*

Corsica and Sardinia. It is very likely that populations of *Armeria pungens* from Corsica and Sardinia are the descendants of individuals dispersed from Portugal. Such a link is suggested by the fact that Portugal together with Corsica, Sardinia and Cíes Islands harbour one of the two main genetic lineages of *A. pungens*, the other one being currently located in the Gulf of Cadiz. This is inferred from the most likely Bayesian partition of the AFLP data at $K = 2$ (Fig. 1) and supported by the AMOVA showing a higher molecular variance between the two genetic lineages than among the main disjunct geographical areas (Table 3). Interestingly, the indument of the leaves correlates this genetic pattern: glabrous or subglabrous leaves in the Gulf of Cadiz (except for the puberulent plants from Camarinal) as compared to leaves with ciliate middle veins in the remaining areas (R. Piñeiro *et al.*, unpublished data). Within Portugal, populations from Alentejo region are the closest genetically to the Corso-Sardinian ones, as indicated by the distance analysis (Figs 2 and 3).

Genealogical relationships among alleles and thus the direction of dispersal cannot be directly inferred with unordered AFLP (Schaal & Olsen 2000). However, the distribution of the genetic variation among populations suggests that the dispersal took place from Alentejo, with higher AFLP differentiation among populations, into Corsica or Sardinia, with a lower level of population differentiation. Previously obtained nuclear ribosomal internal transcribed spacer (ITS) data is consistent with this hypothesis. ITS sequence variation follows a clear geographical structure in *Armeria* that is independent of taxonomy (Fuertes Aguilar *et al*. 1999; Fuertes Aguilar & Nieto Feliner 2003). This consists of (i) the occurrence of the same or very similar ITS sequences in different species within the same geographical area, and (ii) the presence of different ITS sequences, depending on the geographical origin, in species with wider geographical distributions. This pattern has been interpreted as due to extensive gene flow among congeners, favoured by weak internal reproductive barriers, and biased concerted evolution (Nieto Feliner *et al*. 2001). *Armeria pungens* from Corsica and Sardinia presents the same

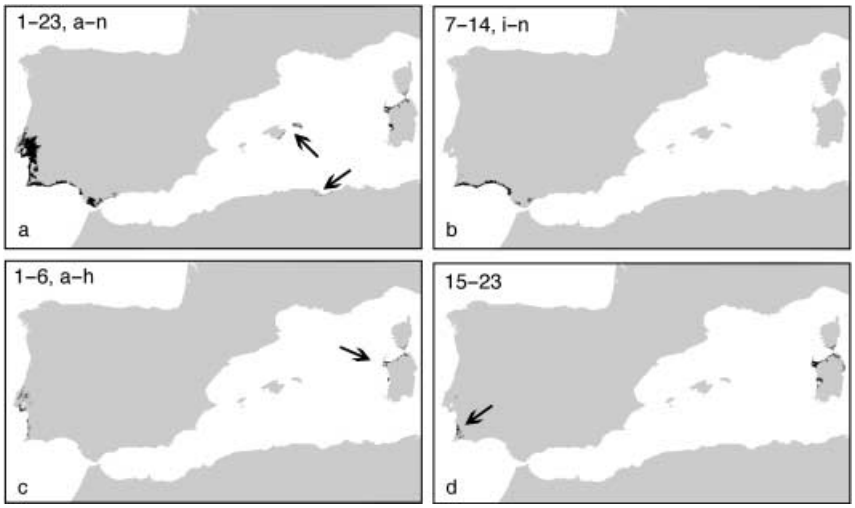

Fig. 4 Potential distribution modelling of *Armeria pungens* according to climatic factors based on: (a) all presence data; (b) Gulf of Cadiz data; (c) Atlantic locations north of São Vicente Cape; and (d) Corso-Sardinian data. The locations used to build each model are indicated by numbers (those from which individuals have been sampled in this study, see Table 1) and letters (those taken from the literature or herbaria, see Table 2). Areas selected by BIOCLIM algorithm are coloured in black; those with an arrow in cases a, c and d fall apart from the range defined by presence data.

ITS sequence as the Iberian populations (R. Piñero, unpublished data) instead of the ITS found in four of the Corso-Sardinian endemics of *Armeria* (Fuertes Aguilar & Nieto Feliner 2003). This exception to the geographical structure of ITS variation suggests that contact between *A. pungens* and the other species of *Armeria* has not occurred, probably due to a relatively recent arrival of *A. pungens* into the archipelago.

The distribution of the relatives of *A. pungens* is also consistent with this direction of dispersal into Corsica or Sardinia. The Southwest of the Iberian Peninsula constitutes the main geographical range of *A. pungens* and its congeners of section *Macrocentron*. They often occur in sympatry or parapatry, suggesting that the divergence from a common ancestor took place in this area. In contrast, the remaining species of *Armeria* from Corsica and Sardinia are not related to section *Macrocentron* (Bernis 1955). All of them, except for the coastal species *Armeria soleirolii*, have very similar morphology and occur on ecologically isolated sites, never in sympatry (Arrigoni 1970).

Taking together the dating of the counter-clockwise rotation of the Corsica-Sardinia microplate from the continent (ended *c*. 16 million years ago; Krijgsman 2002) and the low genetic distinctness of Corso-Sardinian populations of *A. pungens* with respect to their Iberian conspecifics, the conclusion is that migration from Portugal was probably by long-distance dispersal. Land bridges with North Italy proposed during the Messinian salinity crises (*c*. 5 million years ago) and during the Pleistocene glaciations, do not seem to have played any role in the evolutionary history of *A. pungens* since neither this species nor any representative of section *Macrocentron* occur in the Italian Peninsula.

The remarkable within-population variation in Corsica-Sardinia does not fit population genetics predictions. Colonized islands are expected to be less diverse than their continental sources since they harbour only a subset of the initial variation carried by immigrants (Wright 1931). However, no evidence for founder events was detected in our case. This is an unexpected result, which has several possible causes. Island colonization by several founders is likely to have contributed to a comparatively high genetic diversity within the Corso-Sardinian archipelago (Brown & Marshall 1981). In fact, this is a prerequisite for strictly allogamous plants like *Armeria* to colonize islands (Baker's rule, Baker 1955). But the question concerning whether subsequent colonization or gene flow has taken place following the initial establishment cannot be answered with our data. A number of molecular studies support reduced diversity in colonized islands (Schwaegerle & Schaal 1979; Glover & Barret 1987; Inoue & Kawahara 1990; Richardson *et al*. 2003). But there are also examples of introduced plants having equal or even more genetic diversity than source areas, for which different causes have been proposed. Several independent introductions are one of them, as suggested for *Rubus alceifolius* in Madagascar (Amsellem *et al*. 2000). Others include a bottleneck in the source area, as in *Pinus luchuensis* (Chiang *et al*. 2006), a scenario that does not seem to hold for *A. pungens*.

Cíes Islands. The genetic link of the isolated population on offshore Cíes Islands with the closest mainland population of Albufeira revealed by the Bayesian and distance analyses, suggests that the former originated from the latter (currently 500 km apart) by long-distance dispersal. The low genetic diversity of this population might indicate a founder effect.

Dispersal agents. Once the distribution of genetic diversity excludes a vicariance scenario, identifying the dispersal agents that caused the disjunction may be relevant. Given the characteristics of the fruits of *A. pungens*, dispersal may

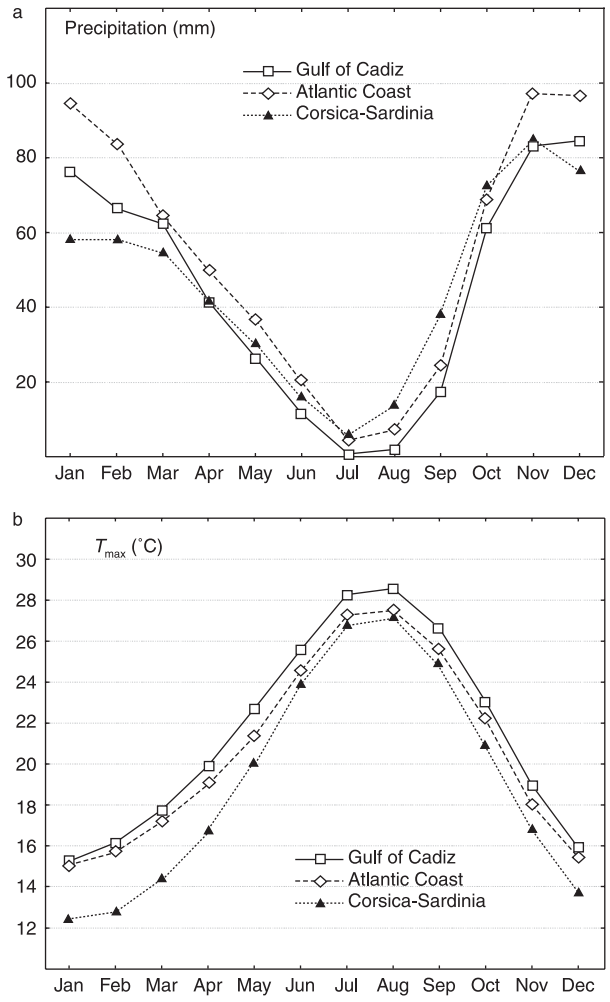

Fig. 5 Monthly variation of (a) mean precipitation and (b) mean maximum temperature of pooled populations of *Armeria pungens* from the Atlantic coasts of Iberia, Corsica-Sardinia and the Gulf of Cadiz.

have taken place by seabird movements along the Atlantic and the Mediterranean. This scenario is consistent with the establishment of important colonies of seabirds on offshore islands close to the areas of introduction, for example Cíes Islands themselves (Viada 1998), Piana Island (Lavezzi archipelago) in Corsica and Asinara Island (Maddalena archipelago) in Sardinia (Monbailliu & Sultana 1993). Alternatively, humans may have played the role of dispersers. The first known connections between Corsica-Sardinia and Iberia are the trade routes established by Phoenician colonies since the end of the Bronze Age (Morgenroth 1999; González Ruibal 2004). In Cíes Islands, there are also remains of stable settlements since the end of the Bronze Age (Luaces & Toscano 1998) and contacts between this area and the Mediterranean increased from the end of the second millennium onwards (González Ruibal 2004).

Isolation by distance in Southwest Iberian Peninsula

An important feature of our results is the occurrence of the main phylogeographical break of *A. pungens* in the Southwest of the Iberian Peninsula. The two main genetic lineages meet at São Vicente Cape: the Gulf of Cadiz lineage, on the one hand, and populations north of this cape, on the other. Within both lineages, further genetic groupings can be distinguished. All of them strongly conform to the linear distribution of *A. pungens* along the Southwest coast of Iberia, indicating that there is isolation by distance (Wright 1943). Climatic conditions correlate the genetic and morphological (glabrous or subglabrous leaves) distinctness of the populations from the Gulf of Cadiz (see below; Figs 4 and 5).

Excluding the Camarinal population, which is discussed below, the Gulf of Cadiz lineage has experienced genetic drift, as shown by the considerable differentiation between populations apparent in the NJ tree of populations (Fig. 3) and the low amounts of within-population diversity shown by genetic diversity parameters, which yielded even lower values than in the isolated population on the Cíes Islands (Table 4). Genetic drift might be a consequence of reduced population sizes and/or founder events in the Gulf of Cadiz associated with the glaciations and postglacial period or destructive episodic tsunamis. Tsunamis have been reported in this area every 1500–2000 years according to sedimentary and historical records, and were likely associated with a subduction zone beneath the Gulf of Cadiz and Gibraltar Strait (Luque *et al*. 2002 and references therein; Gutscher 2005).

Introgression in the Camarinal population

We propose that the distinctness of the population from Punta Camarinal, detected on the basis of the AFLP data is due to introgression from another species of section *Macrocentron*, namely *Armeria macrophylla*. This hypothesis explains the exceptionally high level of genetic diversity harboured by this population and is consistent with data from plastid as well as nuclear ribosomal sequences (Piñeiro *et al*., unpublished data). A morphological character that is absent in the remaining populations of *A. pungens* (puberulent leaves and scapes) occurs both in the Camarinal population of *A. pungens* and in *A. macrophylla*. While both species occur on sandy soils, *A. pungens* is mostly of sand dunes beaches while *A. macrophylla* usually occurs in pine forest understorey or shrubland. The introgressive explanation is also spatially feasible since the Camarinal population occurs on a fossil dune only a few hundred metres apart from a population of *A. macrophylla*.

Interpreting genetic structure with bioclimatic modelling

AFLP analysis strongly supports recent long-distance dispersal between Portugal and Corsica-Sardinia but does

not provide clues to understand either the mechanisms involved or the explanation for the current absence of *A. pungens* in intermediate sites (Mediterranean Iberian coast, Balearic Islands). Another question, which AFLPs do not help to solve, is why populations from the Gulf of Cadiz were not the source for the colonization of the Thyrrenian Islands despite being the closest geographically. The answers to these questions are provided by our modelling studies. These firstly conclude that bioclimatic conditions of Corso-Sardinian populations match those of the Portuguese populations from the Iberian Peninsula and deviate from the Gulf of Cadiz (Figs 4 and 5), in concordance with the genetic structure of *A. pungens* based on AFLP data (Figs 1–3). Summer drought seems to be a key factor for this similarity pattern (Fig. 5). The bioclimatic model also shows that climate conditions where populations thrive are not found nowadays along the West Mediterranean coast except for a few spots in South Minorca, Southeast Majorca and Gulf of Bejaia (Algeria) (Fig. 4). Causes for the current absence of *A. pungens* in these sites include the lack of specific habitats (sand dunes) which are not included in the model and/or the failure of diaspores to arrive there but also suggest the possibility of a past occurrence. It has been pointed out that BEM has limitations in explaining potential species distributions when plant–animal interactions and human impact are involved (Hampe 2004; Pearson *et al*. 2006). However, the strong correspondence between genetic lineages and bioclimatic groups supports the confidence on the bioclimate modelling as an accurate way of describing the habitat variation within *A. pungens*.

Dispersal agents sometimes provide convincing explanations for disjunct species distribution patterns but this is usually so inasmuch as they depart from stochasticity, for example constant winds (Muñoz *et al*. 2004). We have examined the 'habits' of potential dispersal agents to try to elucidate why dispersal of *A. pungens* involved the farthest populations. However, we are not aware of any specific link for potential dispersers (seabirds, winds, sea currents and routes of commerce) between Portugal and Corsica-Sardinia that excludes the Gulf of Cadiz. Therefore, the occurrence of populations in Corsica-Sardinia and their absence in the intermediate sites are more likely due to selection of successful genotypes in similar habitats than to inability of dispersers to bring diaspores from the Gulf of Cadiz or to disseminate them in such intermediate sites. This is consistent with previous classical and recent studies stressing the role of the Gibraltar Strait as a biogeographical barrier not just between Iberia and North Africa but also between Atlantic and Mediterranean lineages (Takhtajan 1986; Rivas-Martínez *et al*. 2002; Kadereit *et al*. 2005). Altogether, our data suggest that the climate is one of the main factors shaping the current genetic structure in *A. pungens* and that bioclimate envelope analysis may be a useful tool to explore the present role of environmental factors even in places with long history of human influence.

During the last years, understanding of complex Mediterranean plant phylogeography has mostly relied on our ability to accommodate available knowledge of historical events (glaciations, salinity crisis), organisms' capabilities for dispersing, palaeopalynological data on distribution and reconstruction of genetic relationships. In addition to these approaches, our study shows how modelling of ecological conditions, in this case through bioclimate envelope modelling, can help to explain the causes of plant distribution patterns.

Acknowledgements

We are especially grateful to Inger Skrede, Pernille Bronken Eidesen, Siri Kjølner, Virginia Mirré and other members of Christian Brochmann's group at the National Centre for Biosystematics in Oslo for training in AFLP technique and analysis methods. We also thank Alejandro González for contributing to the discussion and figures, to Pedro Escobar and Andrea Costa for help during fieldwork, to François Boillot (Conservatoire Botanique National Méditerranéen de Porquerolles) and Sébastien Gatelier (Estación Biológica de Doñana) for providing seeds and to Cristina Tauleigne-Gomes for information on localities in Portugal. Juan J. Aldasoro, Javier Francisco Ortega, Alex Widmer and three anonymous reviewers provided helpful comments on the draft manuscript. The following natural reserves provided collecting permits: Parque Nacional das Illas Atlánticas, Conservatoire Botanique National Méditerranéen de Porquerolles and Estación Biológica de Doñana. This work was supported by the Spanish Dirección General de Enseñanza Superior e Investigación Científica (grant BOS2001–1839).

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This work is part of the PhD dissertation of Rosalía Piñeiro on phylogeography and reticulate evolution of island species of *Armeria*. David Draper's research focus is on plant conservation using GIS techniques. Javier Fuertes Aguilar is interested in application of molecular markers to plant evolution at different levels from phylogeny of families to the intraspecific level as well as on the effects of polyploidy. Gonzalo Nieto Feliner's current interests are the phylogeography of Mediterranean groups and the effects of reticulation on the evolution of plant lineages.

Supplementary material

The following supplementary material is available for this article:

Table S1 Number of total, private and rare AFLP fragments per population of *A. pungens*. Populations arranged according to main genetic groups based on Bayesian and distance analyses. Calculations were obtained using AFLPdat (Ehrich 2006). f_{tot} = total number of fragments; f_p = number of private fragments; f_r = number of rare fragments (< 10% individuals).

Table S2 Percentage of shared fragments between populations. Calculations were obtained from the presence-absence matrix of populations.

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