Low Genetic Diversity in the Rare Madeiran Endemic *Armeria maderensis* (Plumbaginaceae)

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Abstract The genetic diversity and possible geographic structure of the Madeiran endemic *Armeria maderensis* have been assessed with AFLP. Its scarce distribution (less than 3 km between the two most distant localities) and restricted habitat (vertical pastures on the highest elevations of Madeira), at least in part due to grazing by goats, recommend an evaluation of its conservation status. Diversity estimates obtained for *A. maderensis* were evaluated through comparison with reference values of AFLP diversity for outcrossing plants and, in order to correct for phylogenetic constraints, with a widespread congener analyzed with the same AFLP markers. Our results reveal that low levels of genetic diversity and a weak intraspecific genetic structure underlie the restricted distribution of *A. maderensis*.

Keywords AFLP, Bayesian clustering analyses, Conservation biology, Island endemic

Running title: Low genetic diversity in *Armeria maderensis*

Introduction

Propensity to extinction of island plants has been well documented. The reviews by Primack (1998) and WCMC (1992) indicate that most species gone extinct between 1600 and the 1990s occurred on islands (Frankham et al. 2002). This susceptibility seems to be due to the particular demographic conditions endured on islands, often involving small effective population sizes, isolation and low migration rates, colonization through founding events and/or habitat destruction by humans or introduced animals (Coblentz 1978; Rieseberg and Swensen 1996; Frankham et al. 2002; Holmgren 2002; Cowie and Holland 2006; Cuevas and Le Quesne 2006).

An important matter when assessing the conservation status of vulnerable island plants is to test whether the rare distribution of the species is paralleled by low levels of genetic diversity (Frankham 1997). In order to properly assess the amount of genetic diversity, the species under study needs to be compared with values of reference. However, interspecific comparisons might be biased by differences in *i*) the biology of the species compared, specifically the particular phylogenetic position or life history traits, as well as in *ii*) the analytical procedures across studies, i.e., sampling strategy, type of genetic marker, lab protocol and diversity parameters used (Loveless and Hamrick 1984; Felsenstein 1985; Hamrick and Godt 1989; Stoneburner et al. 1991; Gitzendanner and Soltis 2000; Culley et al. 2002; Nybom 2004; Petit et al. 2005).

In plants, several approaches have been proposed to obtain reliable comparisons of genetic diversity across species. In the classic approach, the estimate for a particular species is compared to average diversity values, accounting for the type of marker, the life history traits category and the population genetic parameter used (Hamrick and Godt 1989: protein markers; Nybom 2004: RAPD, AFLP or SSR). However, this method does not control for phylogenetic constraints and, therefore, when a phylogeny is available, other approaches have been applied. The first one is the comparison with the most closely related widespread congener, as suggested by Gitzendanner and Soltis (2000), who found empirical similarity of population genetic parameters within related taxa. This approach has been used to study a large number of plant endemics, including taxa restricted to islands (Young and Brown 1996; Frankham 1997; Soltis et al. 1997; Dodd and Helenurm 2002; Ellis et al. 2006; Moreira

da Silva et al 2007). Another possibility is to perform phylogenetically independent contrasts (PIC; Felsenstein 1985). Aguinagalde et al. (2005) used the latter method to assess chloroplast-DNA-based G_{st} trends in European temperate trees and shrubs and found little association with life history traits when phylogenetic position was accounted for, thus challenging the conclusions of the classic meta-analyses by Hamrick and Godt (1989) and Nybom (2004), Petit et al. (2005).

Our study focuses on the island endemic plant *Armeria maderensis* Lowe*,* which has an extremely restricted distribution in rocky areas exposed to humid winds in the mountains of Madeira (Vieira 1992; Press and Short 1994; Jardim and Francisco 2000). Madeira, a volcanic island originated within the African plate in the Tertiary (approximately 5.3 MYA; Geldmacher et al. 2000), comprises 1226 described vascular plant species, of which 234 are Macaronesian endemics and 157 of these endemics are exclusive to the island (Vieira 1992; Press and Short 1994; Jardim and Francisco 2000). *Armeria maderensis* occurs only above 1600 m, i.e., strictly in the supratemperate belt, spanning a small area between the highest peaks, Ruivo (1862 m) and Areeiro (1818 m) (Mesquita et al. 2004). This area is part of the Natural Park of Madeira, a geological and high altitude vegetation reserve, also part of a Special Protected Area (SPA) included in the Natura 2000 Network as a Community Important Site (CIS). Climax vegetation in this belt includes several rupicolous communities, which may develop where tree cover and grazing are absent. One of them is the *Armerio maderensis-Parafestucetum albidae***,** dominated by *Armeria maderensis* Lowe, *Deschampsia maderensis* (Hack. & Bornm.) Buschm., *Parafestuca albida* (Lowe) Alexeev*, Anthoxanthum maderensis* Teppner, and *Anthyllis lemmaniana* Lowe. Moderate grazing of this community seems to result in the replacement by the association *Viola rivianae-agrostietum castellanae*, dominated by non-endemics (Costa et al. 2004; M. Silva, D. Menezes, S. King, E. Menezes de Sequeira, M. Menezes de Sequeira, unpubl.). In locations exposed to heavy grazing and soil erosion, even poorer annual communities are established (*Leontodo longirrostris-Ornithopetum perpusilli*).

Grazing seems to be an important factor determining the restricted distribution of the species. In 2003, the regional government completed the program of removal of goats in the Madeiran Mountains initiated in 1994 in the frame of the EU Habitats Directive 92/43/EEC. From that moment on, *Armeria maderensis* has experienced a

spectacular increase of its populations, colonizing more accessible alpine pastures (*Viola rivianae-agrostietum castellanae* and *Leontodo longirrostris-Ornithopetum perpusilli*) in addition to the inaccessible rock crevices that were the exclusive habitat before. Moreover, a general increase of floristic diversity in Madeiran Mountais could be observed (M. Silva, D. Menezes, S. King, E. Menezes de Sequeira, M. Menezes de Sequeira, unpubl.).

Like other Macaronesian endemics (reviewed in Juan et al. 2000; Emerson 2002; Carine et al. 2004), *A. maderensis* appears to have its closest relatives in the Western Mediterranean. Nuclear ribosomal ITS phylogenies are consistent with this general trend and reveal a substantial divergence (Nieto Feliner et al. 2001; Fuertes Aguilar and Nieto Feliner 2003). Unluckily, accurate estimation of isolation time and as well as identification of its sister species are precluded by the low level resolution of the ITS tree, due to the frequent interspecific hybridization in natural populations of *Armeria* and the likely recent diversification of the genus (Gutiérrez Larena et al. 2002; Fuertes Aguilar and Nieto Feliner 2003; Tauleigne-Gomes and Lefèbvre 2005).

This study uses AFLP data to assess the genetic structure and diversity levels of *Armeria maderensis,* to interpret its evolutionary history and to evaluate its conservation perspectives. Our sampling was performed in 2003. Therefore, it provides an accurate description of the genetic structure of the species just before goats were definitively removed, after almost 600 years of grazing (Sousa 2003).

In order to correct for phylogenetic bias, following Gitzendanner and Soltis (2000), we have evaluated the levels of genetic diversity in *A. maderensis* in comparison with a previous AFLP study on the western Mediterranean widespread congener *A. pungens* (Piñeiro et al. 2007). *Armeria pungens* is disjunctly distributed in the Atlantic and the Mediterranean. It has a main area along a 500 km stripe in southern Atlantic Iberia, from the mouth of the Tagus River to the Gibraltar Strait. It also occurs on two disjunct archipelagos: in the Atlantic, in the Cíes islands (offshore Galician coast, northern Spain), and in the Mediterranean in southern Corsica and northern Sardinia. Previous morphological and molecular data reveal that *A. pungens* presents a diverse ancestral lineage occurring in the Atlantic coasts of SW Portugal (Piñeiro et al. 2007; Piñeiro et al., unpubl.), while introgression events (Piñeiro et al., unpubl.) may explain the relatively high diversity in the Cíes islands. The remaining populations in the Southern

part of the Atlantic range (Gulf of Cadiz) as well as the disjunct Mediterranean populations in Corsica and Sardinia are the result of recent expansions from the original Portuguese area. Colonization events meant the loss of genetic diversity, especially in the Gulf of Cadiz, probably due to the different environmental conditions in this area.

Specifically, in this study the following questions are investigated: *i*) Is the restricted distribution of *A. maderensis* paralleled by a low genetic diversity? *ii*) Is genetic variation geographically structured in *A. maderensis*? *iii*) Which might be the factors influencing current genetic structure and levels of diversity? *iv*) What are the implications of the current genetic structure and diversity for the conservation of the species?

Material and Methods

Study System

Armeria maderensis is the only endemic representative of the genus in Macaronesia. Morphologically, the most unique feature of this species within the genus is the lack of imbrication of involucre bracts, which are few, narrow and seemingly arranged in a single row so that it can be hardly said that they constitute an involucre. The insertion of the flower pedicel on the calyx is frequently more truncate and thus less spurred than in other congeners. Another apparent feature is the patent arrangement of the inner flower in each spikelet within the glomerule. These morphological characters are useful to identify *A. maderensis* but to the extent that they are not shared with other species, they do not help in finding its closest relatives.

Sampling Strategy, DNA isolation, AFLP protocol

The sampling was performed in 2003 in the small distribution area between peaks Ruivo and Areeiro before goats were removed from the island (Table **1**, Fig. **1**). To our knowledge, no previous field studies had attempted to localize or quantify the

populations of *A. maderensis.* Nine sites (nr. 1–9) were found, the most distant ones being less than 3 km apart. We succeeded at collecting fruits from eight of these sites (Table **1**). An additional inaccessible site, harbouring a few individuals on a vertical north-facing wall (close to site nr. 8, on the path to Torrinhas peak), was also detected.

Sites 2, 4, 5 and 6 corresponded to consistent populations, whereas sites 1, 3, 7 and 8 consisted of very few scattered individuals (Table **1**, Fig. **1**). The remote and inaccessible status of populations limited the number of individuals collected at each site (e.g. population nr. 4 had to be sampled by equipped climbers). In total, we were able to sample ripe fruits from 44 separate mother plants.

Three replicates per mother plant were germinated after a cold treatment of one month. Seedlings were cultivated in the greenhouse at the Botanical Garden of Madrid between October 2003 and June 2005 in order to provide fresh leaves for DNA isolation. This is a limitation of our study, because we have actually assessed the genetic diversity in a potential future generation.

DNA was extracted with Plant DNeasy Minikit (Qiagen). The AFLP protocol was performed according to Gaudeul et al. (2000) for *Eco*RI/*Mse*I enzyme combination and Piñeiro et al. (2007) for *Kpn*I/*Mse*I. The following three primer combinations were used: (6-FAM)*Eco*RI+ acc/*Mse*I + cacc, (6-FAM)*Eco*RI + acg/*Mse*I + ctac, (6-FAM)*Kpn*I + atc/*Mse*I + cag. Protocols and selective primers were the same used in the phylogeographic study of the congener *A. pungens* (Piñeiro et al. 2007). The fact that AFLP markers have been obtained using two different enzyme combinations increased the genomic regions represented in the fingerprints (Vos et al 1995; Ulrich et al. 1999). In total, 31 individuals were successfully genotyped. The remaining individuals collected did not survive in the greenhouse or failed for amplification with at least one primer combinations. Sampling of more than one individual from the same mother plant was avoided. The sampling, although small, might appropriately represent the genetic variation of *A. maderensis* given its extremely restricted distribution. Voucher specimens are kept at MA.

A reproducibility test was performed for each primer pair by re-extracting DNA and repeating the whole procedure (7, 6 and 5 individuals were re-amplified using the three primer combinations given above, respectively). The error rate was calculated as the total number of loci differences relative to the total number of loci comparisons

and subsequently averaged over the three combinations. To further assess and eventually refine the quality of the AFLP data, potentially non-homologous bands were checked 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% and 20%) or small (lower 10% and 20%) molecular weight (Bagley et al. 2001; Bonin et al. 2004). Once identified the bands falling into any of those categories, the error rate improvement was calculated after removing bands from each category (Piñeiro et al. 2007).

Genetic Structure of Armeria maderensis

Bayesian clustering analyses were performed with STRUCTURE 2.1 (Pritchard et al. 2000; Falush et al. 2007) and BAPS 3.2 (Corander et al. 2006). With STRUCTURE we used run lengths of 10^6 following a burnin of 10^5. Long burnin periods were necessary to reach convergence of the alpha statistic before the end of the burnin phase. Each phenotype was coded by a single allele and a missing datum according to the indications in the manual (1-missing for dominant markers and 2-missing for recessive). We selected the model of correlated allele frequencies, appropriate in cases where weak genetic structure is expected, and tested both the no-admixture (ten repetitions at each *K*) and the admixture (five repetitions) ancestry models. Simulations from *K*=1 to *K*=8 were run, i.e. the maximum number of genetic groups tested equalled the number of geographical sites. The number of genetic groups (*K*) in our data set was inferred taking into account the estimated posterior log probability of the data, *L(K)*, as well as the stability of the assignment patterns of individuals into *K* groups across repetitions. BAPS simulations were run from *K*=1 to *K*=8 as the maximum number of groups, with four replicates at each *K*.

Dice similarity among individuals was calculated and visualized with a principal coordinates analysis (PCoA; NTSYSpc 2.1; Rohlf 1998). In addition, Jaccard and simple matching coefficients were calculated. A minimum spanning tree (MST) based on Dice was imposed on the PCoA to detect local distortions. Nei and Li distance (1979) was also calculated with PAUP 4.0b10 (Swofford 2002). Correlation between Nei and Li

distances and geographic distances was calculated by performing a Mantel test with NTSYSpc 2.1. Significance was tested by randomization (1000 permutations).

Genetic Diversity of Armeria maderensis

Three genetic diversity estimates were computed at the total species level and at the within-populations level: *i*) allelic richness, from the percentage of polymorphic loci, *P* (POPGENE 3.2; Yeh and Boyle 1997); *ii*) gene diversity of Nei (1973), *H* (POPGENE 3.2); and *iii*) allele similarity using Shannon index (1948), *Sh* (POPGENE 3.2).

To calculate the global genetic diversity within *A. maderensis*, all genotyped individuals were pooled. In contrast, calculations on a per-population basis were challenged by the fact that only three out of eight collected sites are well-separated populations (pops. nr. 2, 5 and 6). Therefore, within-population genetic diversity was reported as the average of the estimates for these three sites.

Standard deviations of diversity estimates are reported. Monomorphic and polymorphic loci were included in the calculations. Nei's & Shannon's measures were calculated for each locus and averaged over loci. Since the concept of heterozygosity cannot be applied to dominant markers, average Nei's gene diversity (*H*) is simply a measure of genetic variation. We also calculated Nei's diversity estimate using Lynch & Milligan's method (1994) implemented with TFPGA (Miller 1997), which attempts to correct the bias generated by dominant markers by pruning frequent loci for the estimation of allele frequencies.

Comparison with the Widespread Congener Armeria pungens

For the western Mediterranean widespread congener *A. pungens,* the same three genetic diversity estimates were calculated as described above. The comparison of the genetic diversity values of *A. maderensis* with *A. pungens*, in addition to the type of marker, life history traits category and population genetic parameters, corrects for phylogenetic bias and homogenizes the AFLP protocol in both species, including the selective primers used.

Still, the AFLP study on *A. pungens* involved 221 individuals from 23 well defined populations spanning a range of hundreds of kilometres, which implies an important difference with the sampling strategy and spatial scale of the current assessment of *A. maderensis* (Piñeiro et al. 2007). In order to account for such differences, a per-population comparison of *A. pungens* and *A. maderensis* would be desirable. However, the scattered distribution of *A. maderensis* individuals challenges the reliability of within-population estimates. We have thus made a comparison on the basis of the genetic lineages detected within *A. maderensis* using Bayesian and genetic distance methods with those previously found in the widespread *A. pungens* (Piñeiro et al. 2007): Cíes islands, Portugal, Vicente-Bordeira, Gulf of Cadiz and Corsica-Sardinia (hear called I, II, III, IV, VI, respectively). An additional lineage (lineage V, Camarinal population) was not included, given its low sample size and hybrid origin (Piñeiro et al. 2007; Piñeiro et al., unpubl.).

For the comparison across intraspecific genetic lineages, independent presence/absence matrices were edited from the complete AFLP matrices for *A. maderensis* (present study) and for each lineage of *A. pungens* (from the original data in Piñeiro et al. 2007). For each genetic lineage, two matrices were edited, one at the population and another at the total genetic lineage level, and diversity parameters were recalculated from them. According to the lowest sample sizes, the number of individuals was standardized to n=6 at the population level and to n=15 at the total genetic lineage level. This was achieved by random exclusion of individuals. Loci absent in all individuals of one lineage were removed to avoid underestimation of the genetic variability.

Comparison with Reference Values of AFLP Diversity in Plants

Nei's unbiased gene diversity (1978) was also calculated for *A. maderensis* with TFPGA (Miller 1997) at the species level (H_t) and within populations (H_s). This estimate was chosen since it was the one used by Nybom (2004) for comparison of within-population parameters across studies based on dominant markers. It corrects for different sampling sizes by multiplying the index per $2n/2n$-1, where n is the sample size (Nei 1987: equation 8.4). The bias is effectively reduced for sampling sizes <50 and

large number of loci available, which fits our sampling strategy. Following Nybom's approach, unbiased Nei's diversity is here reported only for polymorphic bands.

The comparison of Nei's unbiased gene diversity values for *A. maderensis* with the reference values reported in Nybom (2004) based on dominant markers for perennial and outcrossing plants accounts for the type of marker, life history traits and population genetics parameters.

Results

AFLP Profiles

By recalculating the error rate after removing each of the categories of potentially not reproducible bands, those bands changing intensity across samples were observed to be the least reliable (Table **2**). Discarding them meant a decrease of the error rate below 5% (3.1%). Accordingly, 67 unreliable bands were discarded. An additional unreliable band, non reproducible in most comparisons, was eliminated. Subsequently, 16 individuals that were not amplified for one of the primer combinations were removed, which meant the loss of 32 bands. A final data set of 31 individuals and 90 markers was retained for analysis. Markers spanned from 56 bp to 447 bp and only 58 (64.44 %) were polymorphic. No identical phenotypes among individuals were detected.

Genetic Structure

BAPS and STRUCTURE inferred a single Bayesian cluster comprising all sampled individuals of *A. maderensis*, revealing the weak genetic structure in the AFLP data (Fig. **2a**). BAPS found the optimal partition at *K*=1 (results not shown). In the STRUCTURE analysis, individuals were evenly assigned to the *K* groups in simulations from *K*=2 to *K*=8 for both admixture and no-admixture models (Fig. **2a**). As stated in the STRUCTURE manual, this situation, when the proportion of the sample assigned to each population is roughly symmetric, is indicative of no population structure. Consistently, *L(K)* did not show a maximum value at any specific *K*. Instead, runs at *K*=1, *K*=2, *K*=6, *K*=7 and *K*=8

yielded very similar posterior probabilities between *L(K)*=-589.3 and *L(K)*=-586.7 (Fig. **2b**).

In contrast, the PCoA representing Dice similarities among individuals (Fig. **3**) showed some degree of genetic structure at local scales, since genotypes specific to different populations could be distinguished. Nonetheless, the absence of discontinuities in the PCoA scatterplot confirmed the low level of genetic divergence and weak structuring of the overall genetic variation within *A. maderensis*, as pointed by Bayesian clustering analyses. Jaccard and simple matching coefficients gave almost identical results as Dice (r=0.99 with Jaccard and r=0.98 with simple matching). The Mantel test (r=-0.01608; P random Z<observed Z=0.6134) corroborated the lack of overall linear correlation between genetic and geographic distances.

Genetic Diversity

When the total diversity estimates of *A. maderensis* are compared with each of the genetic lineages of the widespread congener *A. pungens*, diversity of *A. maderensis* resulted to be lower than that in the lineage with the lowest diversity of *A. pungens* (lineage IV; Table **3**). This pattern holds for the percentage of polymorphisms, Nei´s gene diversity (H_t; Nei 1973) and Shannon index (*Sh*). The remaining lineages of *A. pungens* showed significantly higher diversity levels for all three diversity parameters, especially lineages I, II and III, followed by intermediate levels of diversity in lineage VI. In every case, correction of Nei's index for dominant markers using estimation of allele frequencies by Lynch and Milligan's (1994) (results not shown) method gave almost identical results as those based on the squared root of the frequency of the recessive allele (Nei 1973).

The conclusion of a lower diversity of *A. maderensis* as compared to lineages within *A. pungens,* drawn from the total diversity measures, hold for measures on a per-population basis, as shown in Table **4**. For comparison, we estimated the diversity levels of the different lineages of *A. pungens* including also those bands that were absent in all individuals of one lineage. In this case, the diversity levels of the different lineages of *A. pungens* slightly decreased but were still higher than in *A. maderensis* (results not shown).

Finally, the unbiased within-population gene diversity of Nei (1978) for *A. maderensis* averaged over populations 2, 5 and 6 was H_s=0.19. Pooling all genotyped individuals, it was H_t=0.14.

Discussion

Is Armeria maderensis Genetically Depauperate?

In her review of population genetics studies based on dominant markers, Nybom (2004) reported an average within-population unbiased gene diversity of Nei (1978) of H_s=0.27 for outcrossing plants and of H_s=0.25 for perennials. The lower and upper limits of AFLP diversity in plants were H_s=0.15 and H_s=0.31, respectively (average H_s=0.23). In this context, *A. maderensis,* with H_s=0.19 at the population level and H_t=0.14 at the total species level, exhibits significantly low levels of genetic diversity. Nonetheless, this comparison should be taken with caution for three reasons. First, although life history traits are considered, phylogenetic constraints are not controlled for. Second, Nybom´s estimates are given in a per-population basis, whereas this is difficult to obtain for *A. maderensis* given its particular geographical distribution. Third, Nybom´s calculations were based on polymorphic loci, but considering only polymorphic markers in a plant like *A. maderensis*, with a high percentage of monomorphic loci (52.23%, see Table **3**), probably leads to an overestimation of the genetic diversity.

The comparison with the widespread congener *A. pungens* is more reliable because it is corrected for both biological (life history traits and phylogeny) and methodological constraints and because the phylogeographic history of *A. pungens* is well known based on morphological and molecular data (Piñeiro et al. 2007; Piñeiro et al., unpubl.). Our genetic diversity estimates for *A. maderensis* are shown to be, both at the total genetic lineage level (Table **3**) and at the population level (Table **4**), even lower than for the extremely impoverished lineage IV of *A. pungens* that has recently colonized the Gulf of Cadiz. Still, these comparisons with *A. pungens* must be also considered cautiously. On the one hand, the comparison on the basis of genetic lineages does not fully account for the different spatial scales of *A. pungens* and *A.*

maderensis, still larger in *A. pungens* lineages (maximal distances within lineages from 21 km to 413 km) that in *A. maderensis* (maximal distance about 3 km). On the other hand, the average within-population diversity for *A. maderensis* is only based on calculations for three sites. Another possibility, is to consider the fact that diversity estimates for the whole range of *A. maderensis* (H_t=0.08, *Sh*=0.14, *n*=15; Table **3**) are below the lowest within-population estimates of *A. pungens* (H_s=0.10–0.19, *Sh*=0.15–0.27, *n*=6; Table **4**). This assessment, at comparable spatial scales, definitively confirms the reduced level of genetic diversity within *A. pungens* beyond any reasonable doubt.

Besides the information provided by the population genetic estimates, the infraspecific genetic structure within *A. maderensis* is weak and shown to be restricted to local scales.

Threats for Armeria maderensis and Future Prospects

We considered the possibility that the reduced genetic diversity in *A. maderensis* is actually reflecting a shift to autogamy during establishment after long-distance dispersal. The breakdown of self-incompatibility is observed in *Armeria* in circumpolar areas. It has been hypothesized that this mechanism favours establishment after long distance dispersal or in areas poor in pollinators (Baker 1966). However, two different pollen-stigma morphs that are associated to the incompatibility system in *Armeria* have been detected in *A. maderensis* plants (Nieto Feliner, unpubl.), suggesting that incompatibility has been maintained after colonisation. Mechanisms favouring outcrossing have been reported for other Macaronesian species (Francisco-Ortega et al. 2000).

Therefore, once discarded the influence of the breeding system, the genetic impoverishment of *A. maderensis* seems to result from historical events. The significant increase of the distribution of *A. maderensis* into accessible horizontal pastures since the removal of goats in 2003, points at grazing as one of the factors responsible for the reduced genetic diversity of the species. Isolated evolution in Madeira precluding gene flow, long-term small population sizes (Frankham et al. 2002), founder effects during the colonization of Madeira by mainland ancestors (Winkworth et al. 1999; Charbonnel

et al. 2002; Cowie and Holland 2006) or destructive effect of volcanic eruptions might also be considered.

Although no fragmentation of the range has taken place, the observed level of genetic diversity may increase the risk of inbreeding depression, which could reduce survival and fecundity in the short term. However, to assess accurately the amount of inbreeding, co-dominant markers would be required. Long-term adaptation to environmental changes might be also compromized (Frankham et al. 2002; Peterson and McCracken 2005). To prevent these risks, we recommend monitoring the populations of *A. maderensis* in the following years, to survey its recovery in the absence of goats. Future genetic assessments of *A. maderensis* may be performed and the present genetic study used as a reference of the diversity levels immediately before eradication of grazing. If the evolution of the populations was observed to be non appropriate, taking into account the observed lack of genetic structure, a reinforcement of populations could be designed in order to reduce the danger of outbreeding depression. Germination rates of seeds are usually high in *Armeria* (Woodell and Dale 1993), and are maximized when seeds follow a cold treatment (personal observation).

Aside from the practical application of our genetic study to the conservation of *A. maderensis,* it provides the opportunity to address in the near future the theoretical question of how the genetic variation parallels the recovery in number of individuals after a population bottleneck. This might complement the available reports of increase of the cover of vegetation or specific richness following the removal of herbivores (Mueller-Dombois and Spatz 1975; Coblentz 1977; Schofield 1989; Lorvelec and Pascal 2005).

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This research complies with the current laws of the countries in which it was performed.

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Table 1 Details of sample sites of *Armeria maderensis* for the AFLP study (all in Madeira; see also Fig. **1**)

Site nr.	Site, habitat (altitude)	Latitude	Longitude	*n* collected/ genotyped
1	Pico Areeiro, stony pastures in the summit (1779 m)	32º44'16.6'' N	16º55'42.5'' W	2/2
2	Pico Areeiro, rock crevices (1668 m)	32º44'21.5'' N	16º55'55.3'' W	12/6
3	From Pico Areeiro to Pico Ruivo, rock crevices (1753 m)	32º44'23.7'' N	16º55'58.8'' W	1/1
4	From Pico Areeiro to Pico Ruivo, Manga Grande, rock crevices (ca. 1650 m)	32º44'12.7'' N	16º56'03.3'' W	5/4
5	From Pico Areeiro to Pico Ruivo, pr. Pico do Gato, rock crevices (1779 m)	32º44'35.5'' N	16º56'17.5'' W	12/8
6	Pr. Pico Areeiro, western slope of Pico do Gato, rock crevices (1599 m)	32º44'39.3'' N	16º56'13.4'' W	10/8
7	From Pico Ruivo to Pico das Torrinhas, stony open shrubs and pastures (1739 m)	32º45'42.2'' N	16º56'41.2'' W	1/1
8	From Pico Ruivo to Pico das Torrinhas, rocky crevices in walls (1741 m)	32º45'42.2'' N	16º57'02.9'' W	1/1

Collectors: A. Costa; G. Nieto Feliner; J. Fuertes Aguilar, M. Menezes de Sequeira, and R. Lansac. Date: July 2003

Table 2 Error rate in the AFLP data set of *Armeria maderensis*, calculated as the total number of loci differences relative to the total number of loci comparisons, and subsequently averaged over the three primer combinations

	Nr. bands retained	% error rate
All bands included	190	7.1
Type of bands removed:		
Different size among samples	147	6.4
Low intensity	144	6.6
Changing intensity among samples	123	**3.1**
Upper 10% molecular weight	172	6.9
Lower 10% molecular weight	172	6.7
Upper 10% molecular weight + Lower 10% molecular weight	154	6.4
Upper 20% molecular weight	154	7.1
Lower 20% molecular weight	154	6.1
Upper 20% molecular weight + Lower 20% molecular weight	132	5.6

Error rates were calculated for the whole data set and after removing potentially non-homologous bands according to 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 or small molecular weight (Bagley et al. 2001; Bonin et al. 2004; see the text)

Table 3 Comparison of the total AFLP diversity in *Armeria maderensis* with five lineages detected within the widespread congener *Armeria pungens* according to Bayesian clustering methods (recalculated from Piñeiro et al. 2007)

***n*=15**	***P***	H_t	***Sh***
A. maderensis	**47.77**	**0.08** (0.14)	**0.14** (0.20)
A. pungens lineage I (mainland+ island)	73.91	0.21 (0.18)	0.33 (0.26)
A. pungens lineage II (mainland)	81.81	0.21 (0.17)	0.33 (0.23)
A. pungens lineage III (mainland)	83.9	0.24 (0.18)	0.37 (0.24)
A. pungens lineage IV (mainland)	**51.85**	**0.14** (0.18)	**0.22** (0.26)
A. pungens lineage VI (Island)	77.44	0.19 (0.18)	0.30 (0.25)

P – percentage of polymorphic loci; H_t – Nei's gene diversity (1973) at the species/lineage level; *Sh* – allele similarity, Shannon index (1948). Sample size was standardized to 15 individuals according to the size of the smallest lineage. Standard deviation is reported in brackets. The lineage of *A. pungens* with the lowest diversity level is highlighted.

Table 4 Within-population AFLP diversity of *A. maderensis* and the five lineages detected within the widespread congener *Armeria pungens* according to Bayesian clustering methods (recalculated from Piñeiro et al. 2007)

***n*=6**	***P***	H_s	***Sh***
A. maderensis			
Pop. 2	17.78	0.06 (0.14)	0.09 (0.20)
Pop. 5	24.44	0.07 (0.14)	0.11 (0.20)
Pop. 6	27.78	0.06 (0.11)	0.12 (0.19)
Mean	**22.33**	**0.06**	**0.11**
***A. pungens* lineage I**			
Pop. 1	43.47	0.14 (0.19)	0.21 (0.28)
Pop. 2	50.43	0.18 (0.26)	0.20 (0.29)
Mean	**46.95**	**0.16**	**0.20**
***A. pungens* lineage II**			
Pop. 3	51.51	0.18 (0.20)	0.27 (0.29)
Pop. 4	35.61	0.11 (0.17)	0.17 (0.25)
Pop. 5	43.18	0.13 (0.18)	0.21(0.26)
Mean	**43.43**	**0.14**	**0.22**
***A. pungens* lineage III**			
Pop. 6	50.00	0.18 (0.20)	0.26 (0.29)
Pop. 7	54.24	0.19 (0.20)	0.28 (0.29)
Mean	**52.12**	**0.19**	**0.27**
***A. pungens* lineage IV**			
Pop. 8	26.67	0.09 (0.17)	0.14 (0.25)
Pop. 9	38.52	0.14 (0.19)	0.20 (0.28)
Pop. 10	23.70	0.09 (0.17)	0.13 (0.24)
Pop. 11	20.00	0.07 (0.16)	0.11 (0.23)
Pop. 12	34.07	0.11 (0.18)	0.17 (0.25)
Pop. 13	25.18	0.09 (0.18)	0.14 (0.25)
Mean	**28.02**	**0.10**	**0.15**
***A. pungens* lineage VI**			
Pop. 15	50.00	0.16 (0.19)	0.25 (0.27)

Pop. 16	29.27	0.11 (0.18)	0.16 (0.26)
Pop. 17	39.02	0.13 (0.18)	0.19 (0.26)
Pop. 18	28.66	0.10 (0.18)	0.15 (0.26)
Pop. 19	42.07	0.15 (0.19)	0.22 (0.28)
Pop. 20	42.68	0.15 (0.19)	0.22 (0.28)
Pop. 21	35.37	0.13 (0.19)	0.19 (0.27)
Pop. 22	35.98	0.13 (0.19)	0.20 (0.28)
Pop. 23	31.71	0.11 (0.17)	0.16 (0.25)
Mean	**37.20**	**0.13**	**0.19**

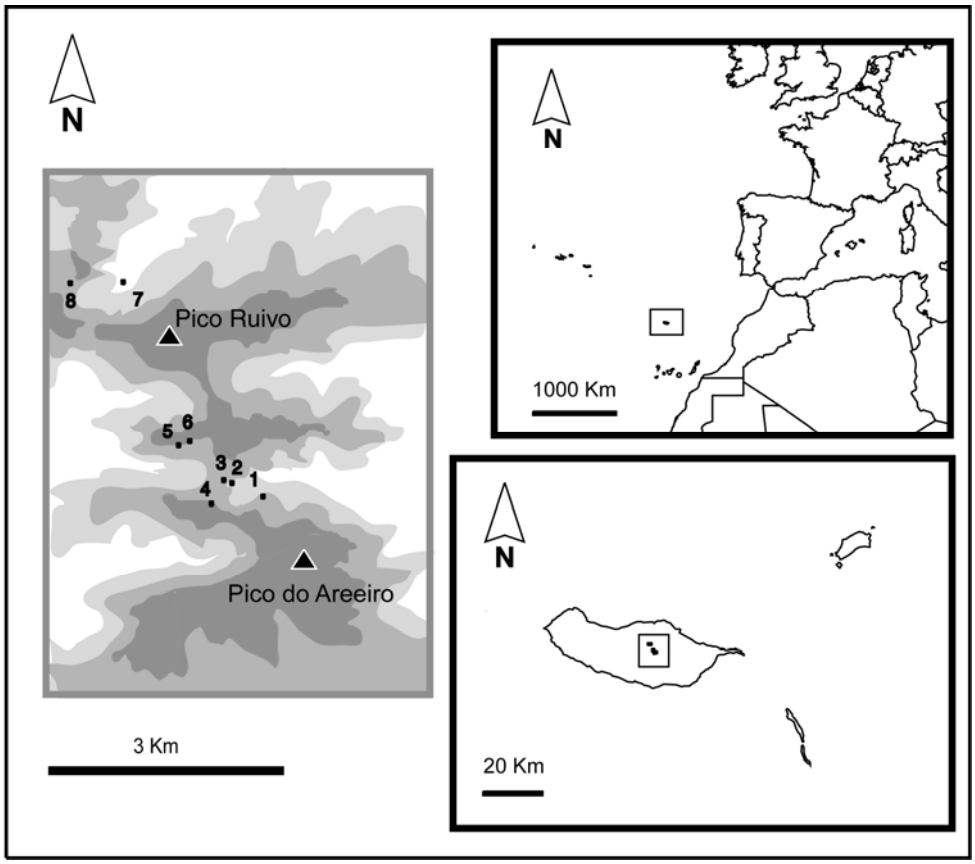
P – percentage of polymorphic loci; H_s – Nei's gene diversity (1973) at the population level; *Sh* – allele similarity, Shannon index (1948). Population sample sizes were standardized to six individuals. Standard deviation is reported in brackets. The lineage of *A. pungens* with the lowest diversity level is highlighted

FIGURE CAPTIONS

Fig. 1 Location of sampled sites of *Armeria maderensis* for the AFLP study, numbered as in Table **1**

Fig. 2 AFLP structure of *Armeria maderensis* estimated with Bayesian clustering using STRUCTURE. **a** Assignment of 31 individuals into *K* groups. Every individual is represented by a vertical bar divided in stripes of different colour corresponding to the estimated assignment probabilities to each group. Sampled sites are numbered as in Table **1**. Ten replicates at each *K* produced nearly identical assignment patterns; the highest probability runs at each *K* are represented here. **b** Log probability of the data as a function of *K* averaged over 10 STRUCTURE runs from *K*=1 to *K*=8.

Fig. 3 Principal coordinates analysis (PCoA) representing Dice similarities between AFLP phenotypes of *Armeria madarensis*. Plot of 31 phenotypes each corresponding to a single individual against the first and second principal axes is shown, with a minimum spanning tree (MST) superimposed. The percentage of variance accounted for by each axis is indicated.


N
8
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Pico Ruivo
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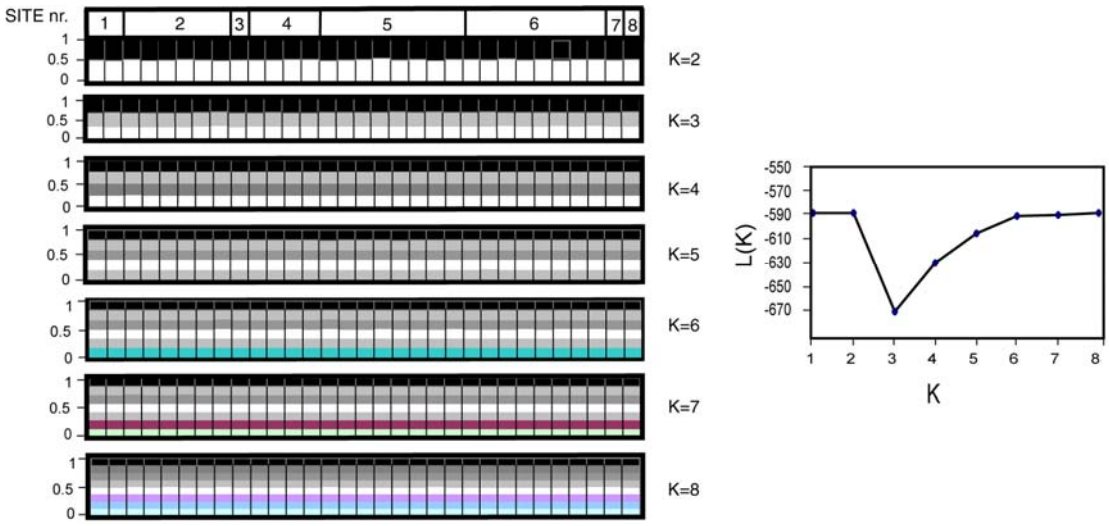
SITE nr.
1
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-650
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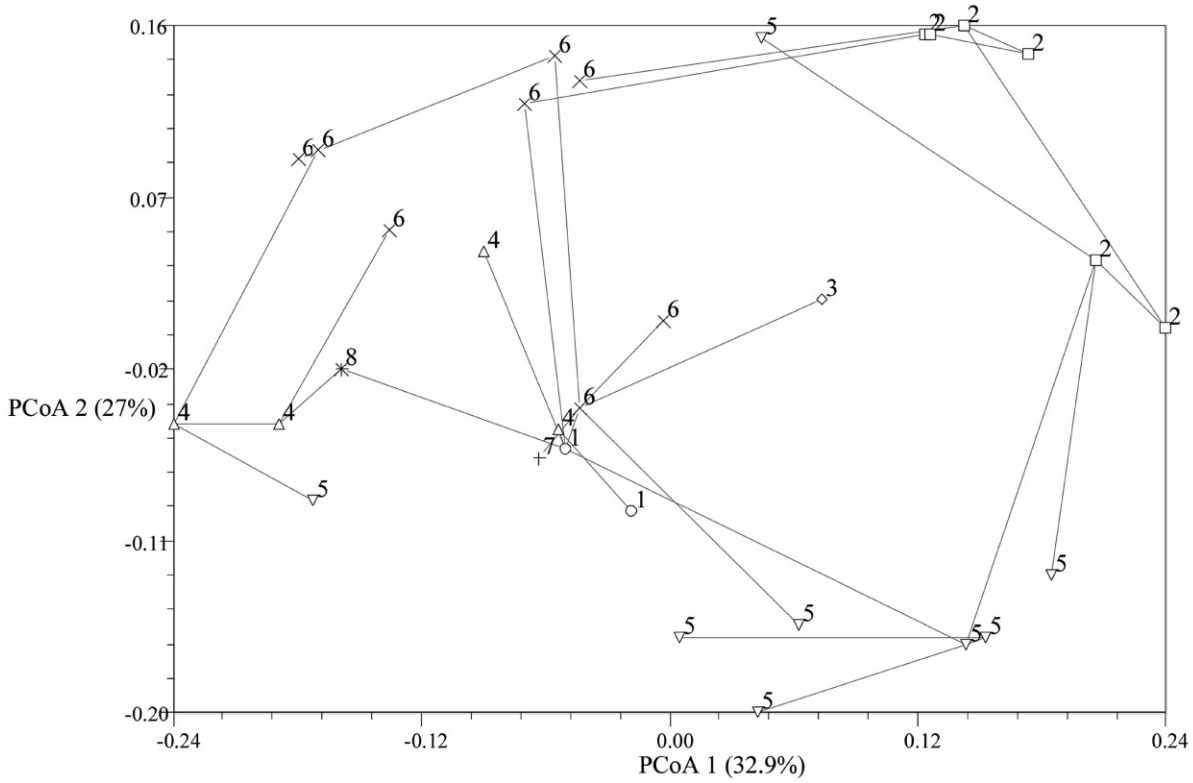
0.16
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PCoA 2 (27%)
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PCoA 1 (32.9%)