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RESEARCH PAPER

Erodium maritimum (Geraniaceae), a species with an uneven and fragmented distribution along the Western Mediterranean and European Atlantic coasts, has a weak genetic structure

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ABSTRACT

***Erodium maritimum* L. is an annual species presenting heterogeneous, sometimes very small, and distant populations, distributed along a discontinuous coastal strip of the European Atlantic and the central and western Mediterranean basin. The aim of this study is to investigate genetic variation and geographic structure changes across its large distribution. Fourteen populations of *E. maritimum* were studied using AFLP fingerprints, together with their population sizes, reproductive systems and flower visitors. AFLP markers revealed the genetic structure of the species to be weak. Many individuals from one population clustered together with those of other populations, showing a high degree of genetic admixture. Despite having a self-compatible reproductive system, populations (especially the largest ones) showed high levels of genetic polymorphism, and the majority of genetic variation was contained within populations. The low genetic structure suggests high levels of gene flow, which might be explained through the dispersability of the species' fruits. Finally, recommendations are provided for management strategies to facilitate the conservation of this endangered species.**

INTRODUCTION

A considerable number of European and African coastal plants have now been studied to draw distribution patterns. Strong human impact, coupled with the natural instability of coastal ecosystems place coastal taxa at an elevated risk of extinction. The recent anthropogenic deterioration of coastal habitats may have increased the fragmentation and decline of some plant populations.

The spatial options for the migration for these plants appear limited, which could facilitate the reconstruction of their past distributions (Clausing *et al.* 2000). Plants occupying European coastal habitats show a striking diversity of phylogeographic patterns. For some species, Atlantic Ocean and Mediterranean Sea clusters have been identified, and differences in the genetic diversity of these clusters observed (generally higher in Mediterranean clusters; Table 1). These patterns have been interpreted in terms of species-specific distribution limits after the last glacial maximum, and with respect to more recent gene flow barriers caused by sea currents. For other species, however, little phylogeographic structure has been detected (Arafeh & Kadereit 2006; see revision in Escudero *et al.* 2010). Indeed, long-distance seed dispersal seems to be a major factor shaping the geographic distribution of several coastal species (Arafeh & Kadereit 2006; Fievet *et al.* 2007; Weising & Freitag 2007). Also plant attributes such as autogamous breeding system and a short life span may have also exerted an influence (Prinz *et al.* 2009).

Erodium maritimum L. is an annual selfer, found in discontinuous and generally small populations on the Atlantic coast of the British Isles, NW France and Galicia (Spain), and in the western Mediterranean, *e.g.* on Dragonera Island (Balearic Islands, Spain), in Corsica (France), in the Tuscan Archipelago, in Sardinia (Italy) and on Zembra Island (Tunisia) (Fig. 1). It is sister to *E. corsicum* [a perennial mixed-mating species, distributed along the coasts of Corsica and Sardinia (Fiz-Palacios *et al.* 2010; Alarcón *et al.* 2011)], which together form part of *Erodium* sect. *Malacoidea* (Fiz *et al.* 2006). The diversification of this section and the divergence of species may have occurred in the Mediterranean Basin during the late Miocene and Pliocene (Fiz-Palacios *et al.* 2010).

The distribution pattern and the contrasting population sizes found in *E. maritimum* may suggest reiterated episodes of migration and colonisation along the species' range. Also, admixture and extinction events among these migrating lineages may be expected. As high levels of selfing occur in *E. maritimum* (Alarcón *et al.* 2011), population subdivision and founder effects can exert strong effects on the magnitude and distribution of genetic diversity (Ingvarsson 2002). Determining the genetic structure of the populations of *E. maritimum* may provide insight into the gene flow, distribution of genetic diversity and historic changes in the geographic distribution experienced by the species. It may also provide data important for its protection. Historic distribution data indicate that many populations of *E. maritimum* may have recently retreated from sites on the western British and Spanish coasts (Walker 2007; Silva-Pando *et al.* 2008).

Table 1. Phylogeographic patterns shown by European coastal plant species (local studies excluded).

Species	Molecular marker	Differentiation between Mediterranean and Atlantic lineages	Phylogeographic structure of Atlantic coastal populations	Phylogeographic structure of Mediterranean coastal populations	Genetic diversity in the Atlantic (SI: Shannon index; N: Nei's nucleotide diversity index, Hs: Nei's expected diversity)	Genetic diversity in the Mediterranean (SI: Shannon index; N: Nei's nucleotide diversity index, Hs: Nei's expected diversity)
Cakile maritima	AFLP (Kadereit *et al.* 2005)	Yes	Differentiation between Baltic and Atlantic populations	Differentiation between four regions: SW Spain, W Mediterranean, Adriatic and Aegean Sea	Wide (SI: 0.334)	Wide (SI: 0.379)
Calystegia maritima	AFLP (Arafeh & Kadereit 2006)	No	No differentiation in Atlantic populations	No differentiation in Mediterranean populations	Little (Hs: 0.103)	Little (Hs : 0.116–0.130)
Carex extensa	cpDNA AFLP nSSR (Escudero *et al.* 2010)	No	No differentiation in Atlantic populations	Two lineages: one in southern Spain and Corsica (the same as on the Atlantic coast), the other in Corsica, Greece and Turkey	Little (Hs: 0.101)	Little (Hs: 0.129)
Crithmum maritimum	AFLP (Kadereit *et al.* 2005)	Yes	No differentiation in Atlantic populations; populations of E Spain clustered with Atlantic populations	Differentiation between four regions: W Mediterranean (two clusters), Adriatic and Aegean Sea	Little (SI: 0.118)	Intermediate (SI: 0.192)
Erodium maritimum	AFLP (this study)	Yes	No clear differentiation but the populations of the British Isles generally have genetic clusters different to those of France	Weak differentiation in Mediterra nean populations	Little–Wide (Hs: 0.164–0.243)	Wide (Hs: 0.213–0.290)
Eryngium maritimum	AFLP (Kadereit *et al.* 2005)	Yes	Differentiation between C and N Europe, Cantabrian and Portu guese populations	Differentiation between two regions: Mediterranean, and Aegean Sea	Wide (SI: 0.247)	Wide (SI: 0.296)
Halimione portulacoides	AFLP (Kadereit *et al.* 2005)	Yes	No differentiation in Atlantic populations	Differentiation between five regions: E Spain, S France, C Mediterranean, Adriatic and Aegean Sea	Little (SI: 0.117)	Little (SI: 0.125)
Hordeum maritimum	Cp DNA and cpSSR (Jakob *et al.* 2007)	Yes	No differentiation in Atlantic populations	Differentiation between two regions: Iberian Peninsula, and the rest of Mediterranean	Little (N: 0.00)	Intermediate–Wide (N: 0.25–0.86)
Posidonia oceanica	nSSR (Arnaud-Haond *et al.* 2007)	–	–	Differentiation between two regions: E and W Mediterranean	–	Wide (Hs: 0.3–0.53)
Salsola kali	AFLP (Kadereit *et al.* 2005)	Yes	No differentiation in Atlantic populations	Differentiation between three regions: W Mediterranean, Adriatic and Aegean Sea	Little (SI: 0.102)	Little (SI: 0.148)
Triglochin maritimum	AFLP (Lambracht *et al.* 2007)	–	Two lineages: one on the Spanish, French and British coasts, the other on N Atlantic coasts and in inland Europe	–	–	–

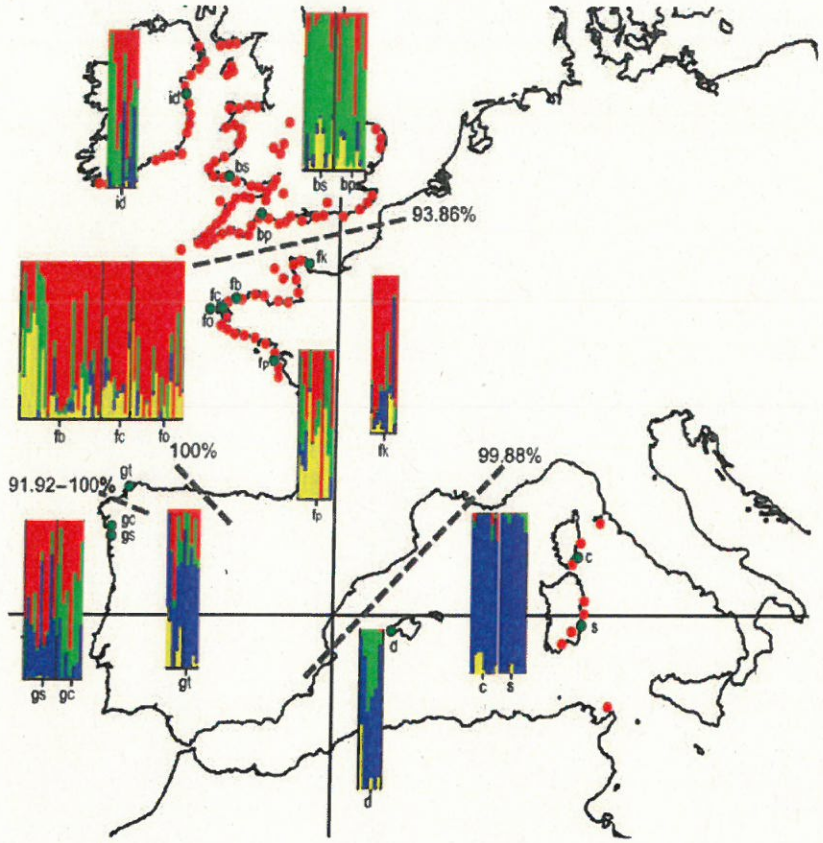


Fig. 1. Distribution and Structure analysis of *Erodium maritimum*. Green spots: populations studied in this work, red spots: localities of plants seen in herbaria. Superimposed are the acronyms for the populations studied, id: Dalkey Island (Ireland), bs: Swansea coast (Wales, UK), bp: Polperro (Cornwall coast, UK), fb: Islet du Mouton, Islet de Stagadon plus Islet de Enez-Vrach (Brest, France), fo: Ile de l'Ouessant (Brest), fc: Le Conquet coast (Brest), fp: Pillier Island (Noirmoutier, France), fk: Cherbourg, France, gt: San Andrés do Teixido (La Coruña, Galician coast, Spain), gs: Sálvora Island (Pontevedra, Galician coast), gc: Cíes Islands, (Pontevedra, Galician coast), d: Dragonera Island (Baleares, Spain), c: Bastia (Corsica, France), s: Santa Teresa de Gallura (Sardinia, Italy). Dotted lines in black indicate significant Barrier boundaries with their bootstrap value.

Small population sizes are reflected by the conservation status of *E. maritimum*, which is classified as 'Critically Endangered' in Spain (Gómez-Orellana & Sáez 2004).

The present study investigates the population genetic structure of *E. maritimum* by using AFLP finger printing to find out whether it depends on (i) geographic distances between populations and (ii) population sizes. The factors involved in the observed pattern are discussed, together with implications for conservation of this endangered species.

MATERIAL AND METHODS

Plant material

Fourteen populations of *E. maritimum* were sampled, including a total of 156 individuals. Population sizes were very small, *e.g.* three populations contained fewer than 81 small individuals (Cíes, Le Conquet and Swansea). Consequently, at these sites, the number of sampled plants was limited. For each population, leaves from 12 individuals (when available) 10–15 m apart along several transects were collected, numbered, dried and conserved in silica gel. Voucher specimens of all sampled individuals were collected and deposited in the herbaria of the Madrid Botanical Garden (MA) and the Barcelona Institute of Botany (BC) (Table 2).

Reproductive system

Data on flower visitors were obtained in four localities (Dragonera Island, Spain; Brest, France; Swansea, UK; and Dalkey Island, Ireland) from observations in the field. The absolute number of visits per unit time was recorded in April–May 2008 and April 2011 (total census time 16 h). Visiting insects were collected and identified in the laboratory. To test for self-compatibility, around five inflorescences from eight to 10 cultivated plants were bagged but not manually self-pollinated, and the number of viable seeds in the mature mericarps counted. A similar number of field non-bagged plants were used as a control.

Seed dispersal by animals

Rabbit and gull dung samples were picked up from burrows or sites close to the nests at Dragonera (Spain), Dalkey (Ireland), Brest (France) and Swansea (Great Britain) sites by means of faecal collecting bags. The material was sun-dried in a greenhouse and then kept at 4 °C for at least 2 weeks. After grinding the pellets, the *E. maritimum* fruits were counted and spread out over sterile compost in seed trays. Trays were kept well watered in a glasshouse for 4 month, and emerging seedlings were recorded.

DNA extraction and AFLP protocol

DNA was extracted from herbarium and field collections (Table 2) using the DNeasy Plant Mini Kit (Qiagen Laboratories, Crawley, West Sussex, UK). Silica-dried leaves (20–25 mg) were ground using an automatic mortar (model RETSCH Mixer Mills MM200 Retsch GmbH, Haan, Germay) and the genomic DNA extracted according to the kit manufacturer's instructions.

Table 2. Material sampled from populations, and P, private fragments and Hj results following AFLP analysis.

Population	Abbreviation	Coordinates N	Number of individuals/ population	Number of individuals sampled	Number and % (in brackets) of polymorphic fragments over a total number of 1215 fragments (P, calculated using rarefaction)	Hj (SE)	Number of private fragments
Corsica, Bastia (Mediterranean, France)	c	42° 42′ 39″ N; 09° 27′ 00″ E	560	7	635 (48.0)	0.213 (0.0063)	1
Sardinia, Santa Teresa de Gallura (Mediterranean, Italy)	s	42° 14′ 06″ N 09° 16′ 20″ E	>20,000	10	860 (69.9)	0.290 (0.0059)	13
Dragonera (Mediterranean, Balearic Islands, Spain)	d	39° 35′ 05″ N 02° 19′ 24″ E	741	6	678 (49.0)	0.230 (0.0064)	1
Salvora Island (S Galicia, Spain)	gs	42° 27′ 57″ N 09° 00′ 46″ W	31,000	10	765 (58.1)	0.238 (0.0059)	3
Cies Islands (S Galicia, Spain)	gc	42° 13′ 24″ N 08° 54′ 14″ W	20	8	577 (39.1)	0.164 (0.0057)	1
S. Andrés de Teixido (N Galicia, Spain)	gt	43° 42′ 36″ N 7° 58′ 56″ W	239	10	784 (60.5)	0.243 (0.0059)	2
I. du Pilier (Nantes, France)	fp	47° 03′ 00″ N 02° 22′ 00″ W	690	11	783 (56.6)	0.223 (0.0058)	1
I. du Mouton, I. de Enez-Vrach and I. de Stagadon (Brest, France)	fb	43° 37′ 16″ N 04° 35′ 37″ W	1120	27	754 (74.1)	0.216 (0.005)	6
I. de l'Ouessant, Lampaul and Stiff (N Atlantic, Brest, France)	fo	48° 29′ 27″ N 05° 03′ 23″ W	240	18	714 (58.8)	0.191 (0.0053)	0
Le Conquet (N Atlantic, Brest, France)	fc	42° 14′ 06″ N 04° 44′ 51″ W	81	10	817 (57.2)	0.227 (0.0057)	0
Cherbourg (France)	fk	49° 39′ 00″ N 01° 39′ 00″ W	-	9	755 (57.1)	0.218 (0.0057)	0
Polperro (Cornwall, British Isles)	bp	50° 19′ 52″ N 04° 31′ 11″ W	143	10	548 (45.1)	0.172 (0.0056)	0
Swansea (Wales, British Isles)	bs	51° 35′ 00″ N 03° 54′ 01″ W	50	10	679 (48.2)	0.190 (0.0057)	3
Dalkey (Ireland, British Isles)	id	53° 16′ 34″ N 06° 06′ 14″ W	128	10	657 (50.0)	0.196 (0.0059)	0

AFLP analysis (see Vos *et al.* 1995) was performed using the AFLP Plant Mapping Kit (PE Applied Biosystems, Warrington, Cheshire, UK) following the manufacturer's recommendations: http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_040959.pdf. An initial screening of 32 combinations of selective primers involving five individual plants from different geographic areas was performed. Four combinations were selected that yielded clear and evenly distributed bands: 1-*Eco*RI$_{6\text{-FAM}}$ ACC/*Mse*I-CAT; 2-*Eco*RI$_{6\text{-FAM}}$-ACC/*Mse*I-CCT; 3-*Eco*RI$_{VIC}$ AGG/*Mse*I-CTG, and 4-*Eco*RI$_{VIC}$ -AGG/*Mse*I-CAA. These combinations produced polymorphic AFLP profiles with clearly defined fragments. The DNA sample was digested with the enzymes *Eco*RI and *Mse*I and linked to the primers *Eco*RI (5′-CTCGTAGACTGCGTACC-3′/5′AATTGGTACGCAGTCT AC-3′) and *Mse*I (5′- GACGATGAGTCCTGAC-3′/5′-ATCT CAGGACTCAT-3′). Fragments of each primer combination were processed and analysed using GeneMapper software (Applied Biosystems). Polymorphic bands were scored as present (1) or absent (0) to produce a binary character matrix. The number of private fragments for a population was also counted. Weak bands were not scored, nor were those where fragments of similar mobility made homology assessment doubtful. Bands of 100–500 bp for all samples were compared; shorter fragments were not scored to avoid potential homoplasy. These matrices were compared with the corresponding electrophoretograms; unclear bands were corrected manually. As recommended by Vekemans *et al.* (2002), the correlation between AFLP band size and frequency among samples was also tested. The reliability of data was assessed by comparison of duplicates, using one or two individuals for each population (24 reproducibility tests). The reproducibility obtained was 94–100%, with a mean of 97.2%.

AFLP data analysis

The resulting combined AFLP fragment presence/absence matrix was analysed using AFLPsurv 1.0 software (Vekemans 2002; Vekemans *et al.* 2002) to estimate Nei's heterozygosity (Hj), the F_{ST} and the bootstrapped Nei's genetic distance

matrix (Nei & Li 1979; Lynch & Milligan 1994). In these analyses, the default Bayesian method was used assuming self-fertilisation (Zhivotovsky 1999). The number of private fragments for populations was also examined (Table 2). A regression between the percentage of polymorphic fragments per population (P) or the Hj and the logarithm of the population size was performed using Statistica software (StatSoft, Tulsa, OK, USA). For calculating the percentage of polymorphic fragments, we used rarefaction method using AFLPDiv 1.1 software (Coart *et al.* 2005).

Genetic distances between individuals, populations and geographic groups were calculated. To genetically distinguish groups of individuals in the AFLP dataset, a comparison was made by constructing a pair-wise similarity matrix for all individuals. This was performed by calculating Dice's coefficient and subjecting the resulting matrix to principal components (PCO) analysis using Ntsys v.2.1 software (Rohlf 1998). A neighbour-joining (NJ) analysis of AFLP data was also performed, both for individuals and populations using SplitsTree 4.10 software (Huson & Bryant 2006). To quantify the amount of genetic differentiation attributable to geographic and population subdivision, a hierarchical analysis of molecular variance was performed (AMOVA; Excoffier *et al.* 2005) using Arlequin v.3.0 software (Table S1).

To test for isolation by distance, correlations between genetic (measured as F_{ST}) and spatial distances between pairs of populations were calculated using the Mantel permutation procedure (Mantel 1967). The genetic distance matrix used was based on the fragment presence/absence matrix; the geographic distance matrix was based on distances between the geographic coordinates for each collected population. Structure v. 2.2 software was used to examine the structure of the populations. This software uses a Bayesian clustering approach for dominant markers [Pritchard *et al.* 2000 (available at http://pritch.bsd.uchicago.edu/structure.html); Falush *et al.* 2007], with an admixture model involving correlated allele frequencies between populations. The degree of admixture alpha was set to be inferred from the data. Preliminary analyses determined that stationarity of parameters (alpha, ln likelihood, F_{ST}) was achieved by 20,000 generations (data not shown). Thus, for all Structure analyses, we used a total run length of 100,000 generations, including a burn-in of 25,000 generations, testing $K = 1$–6 with ten repetitions. The most accurate value of K was chosen following Evanno *et al.* (2005). We plotted the mean ln likelihood of the data *versus* K, and ΔK *versus* K for all runs, and examined all groupings at each K (Fig. S1).

Barrier v. 2.2 software (Manni & Guérard 2004) was used to identify possible geographic locations acting as major genetic barriers among groups of populations, based on their genetic distances. The significance of barriers identified was tested by means of 5000 bootstrapped distance matrices obtained using AFLPsurv software.

Finally, we performed assignment tests in order to show: (i) whether within a group of populations, the number of miss-assigned individuals within each group was different in the Mediterranean, Galician, French or British groups; and (ii) whether the number of individuals miss-assigned to a population outside its group depends on the groups. Using the Bayesian clustering method implemented in Structure, we assumed that our sample consisted of 14 populations and that prior migration between populations was rare ($v = 0.05$). Prior population information (population or geographic group) was incorporated into the analysis for all individuals from the 14 potential source populations. Individuals were then assigned to the population in which their posterior probability was highest.

RESULTS

Insect visitors and dispersers of *E. maritimum*

When weather conditions were favourable and the wind moderate, there was a relatively large number of visits by ants and flies. The production of seeds in visited and non-visited flowers was similar, since the flowers are self-compatible and seed production is generally high (73–95%; Table 3).

The consumption of *E. maritimum* fruits by rabbits was recorded at Dalkey and Brest, where 9–10% of all fruits collected in faeces contained viable seeds. No endozoochorous dispersal by gulls was observed, and gull faeces contained no *E. maritimum* fruits. No seedlings were obtained in the trays of gull droppings (studied in Dalkey, Swansea and Dragonera populations).

Geographic and genetic relationships

A total of 156 individuals from 14 populations were surveyed for AFLP variation (Table 2). With the four selective primer combinations used, 1215 fragments were scored, 548–860 of which were polymorphic.

Table 3. Location, characteristics and sampling effort for the *Erodium maritimum* populations studied with respect to insect visitors.

Locality	Date	Mean number of visits/min/ 20 flowers	Time (min)	Viable seed production in cultivated plants that were bagged % (SD)	Viable seed production in field plants, that were not bagged (spring), % (SD)	Groups of insects
Spain, Dragonera Is.	April 17, 2011	0.05	200	79 (3.1)	80.2 (4.0)	Formicidae: 100%
France, Brest, I. du Mouton	May 27, 2008	0.29	240	88 (5.5)	89 (7.3)	Muscidae: 14.2%, Formicidae: 85.8%
United Kingdom, Wales, Swansea	June 16, 2008	0.11	240	92.1 (4.5)	73.7 (2.5)	Muscidae: 19%, Formicidae: 61%, other Hymenoptera: 20%
Ireland, Dalkey Is.	June 12, 2008	0.08	280	88.2 (6.5)	95.1 (8.2)	Muscidae: 19%, Formicidae: 61.1%, other Hymenoptera: 19.8%

Neighbour-joining analysis returned four main, not supported, clusters with intermingled populations: (i) France plus some British island individuals, (ii) southern Galicia (71% BS, mainly southern populations), (iii) other France plus British island individuals, and (iv) all of the Mediterranean (70% BS, but with two southern Galician individuals lying within), plus the northern Galician and a few British and French individuals (Fig. 2). PCO analysis using the Dice index returned three groups also with many intermingled populations, corresponding to (i) France and the British Isles (red and green, respectively), (ii) Galicia populations (orange) and (iii) the Mediterranean group (blue) (Fig. S2).

Structure analysis suggested that the optimal value of K was four (Fig. S1). The main clusters (Fig. 1) are shown in blue (Corsica and Sardinia), green (mainly Great Britain), yellow (France) and red (abundant in France, but present in most Atlantic populations). All populations except those for Corsica and Sardinia showed a considerable admixture.

A Mantel test involving geographic and genetic distances returned a significant result ($r = 0.727$, $P = 0.000$; Fig. S3). Barrier analysis revealed four major boundaries separating five geographic groups of populations: Mediterranean, southern Galicia, northern Galicia, France and British Isles (Fig. 1).

Individual membership using assignment by Structure and prior population information (Table 4) suggests that the proportions of miss-assigned individuals in populations are different if the population belongs to the Mediterranean, Galician, French or British groups. Indeed, this proportion is higher for French and British groups. Populations from these two groups seemed to receive more migrants than populations from the Mediterranean and Galician groups. However, if we consider a miss-assigned individual as an individual assigned to a population outside the group, the proportion of miss-assigned individuals did not differ among groups, suggesting that the populations received the same amount of long-distance migrants whatever their group.

Population-level analyses, polymorphism and genetic diversity

The AFLP variation within populations, estimated as P and Hj, varied among localities. The P ranged from 39.1% to 74.1%, and the Hj from 0.164 to 0.290. The population of small islets close to Brest (Mouton, Enez-Vrach and Stagadon) showed the highest P (74.1%) value, and that of Sardinia the highest Hj (0.290) value (Table 2). In general, populations showed few private AFLP fragments, but Brest and Sardinia showed six and 13, respectively (Table 2). Moreover, for any population, the relationship between the geographic and genetic distance to Sardinia was significant (MS model = 0.0219, F = 11.52, P = 0.00599).

The sizes of populations varied heavily and presented an uneven distribution. The largest populations were Salvora and Sardinia (more than 20,000 individuals), while those of Corsica, Pilier, Dragonera and Brest were of medium size (500–1100 individuals), and the remaining populations were quite small (<250 individuals; Table 2). P and Hj were signif-

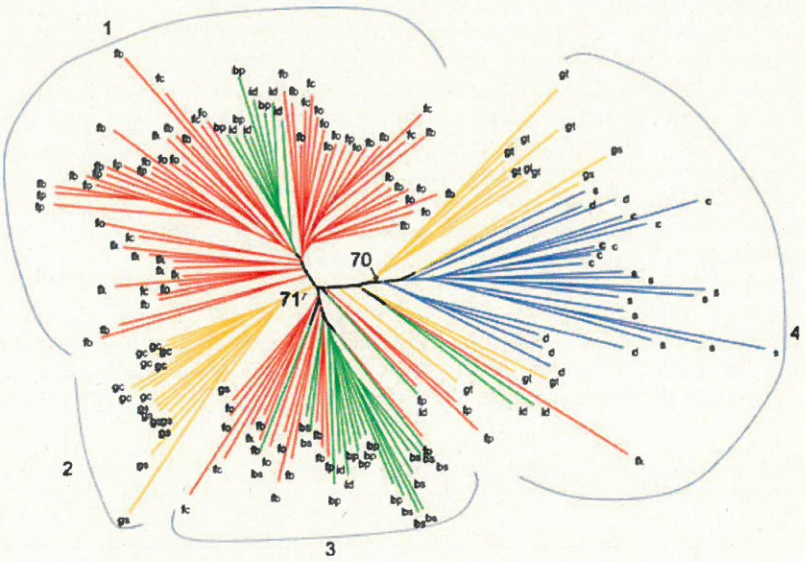


Fig. 2. Neighbour-joining tree produced from the AFLP data for *E. maritimum*. Bootstrap values were lower than 50% for all branches. Colours indicate groups of populations, green: British Isles, red: French coast and islands, orange: Galician coast and islands (Spain), blue: Mediterranean islands (Dragonera, Sardinia and Corsica). Abbreviations for the populations as in Fig. 1.

Table 4. Number of individuals correctly and incorrectly assigned to their population and geographical group, using the chi-square test of independence.

	Mediterranean	Galicia	France	British Is.	χ^2(df), P
Number of individuals correctly assigned to their population	12	19	20	7	18.7109 (df = 3,
Number of individuals miss-assigned to their population	11	9	55	21	P-value = 0.00003137)
Number of individuals assigned to a population inside the geographic group (Mediterranean, Galicia, France or British Is.)	18	23	59	18	5.0839 (df = 3, P-value = 0.1658)
Number of individuals assigned to a population outside the geographic group	5	5	16	12	

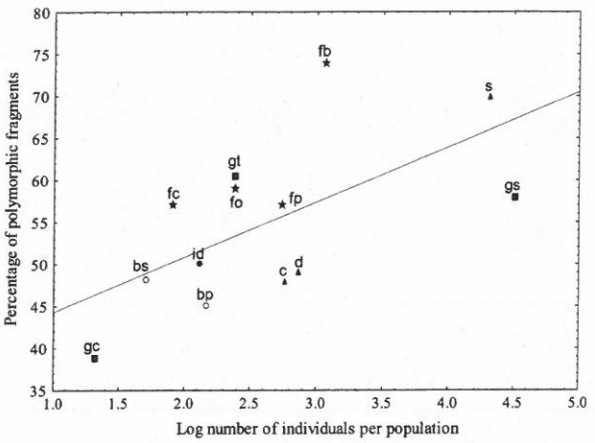


Fig. 3. Regression of P against the logarithm of population size in *E. maritimum*. Symbols indicate areas, stars: France; open circles: Great Britain; filled circles: Ireland; filled squares: Galicia; triangles: Dragonera, Corsica and Sardinia (population abbreviations as in Fig. 1).

icantly correlated with the number of individuals ($r = 0.619$, $P = 0.024$ and $r = 0.63$, $P = 0.02$, respectively; Fig. 3).

AMOVA showed the intra-population variance for the Mediterranean (12.61%) and more southern Atlantic groups (17.19%) was greater than that of the northern populations (9.1% for British Isles and 5.41% for France) (Table S1). In an analysis of all populations, about 82.5% of the overall diversity was assigned to variation within populations.

DISCUSSION

We failed in finding a strong genetic structure for *E. maritimum*. The high degree of genetic admixture shown by AFLP fingerprints indicates multiple connections between currently fragmented populations. In addition, certain populations show particular features related to long-distance gene flow dispersal.

Genetic structure and diversity in *E. maritimum*

The results of the Mantel analyses indicated that long-distance dispersal might have been able to homogenise the genetic structure of this species. Certainly, *E. maritimum* showed a weak genetic structure, with many individuals from one population clustering in others in neighbour-joining analysis. Further, PCO identified just three intermingled assemblages, and Bayesian analysis confirmed a high degree of admixture for most populations, the exceptions being Corsica and Sardinia. Bayesian analyses revealed four groups: Mediterranean islands, France (two groups) and Great Britain. However, the populations of Galicia, Ireland and several localities in France suffered higher levels of admixture. The Barrier results showed the population of northern Galicia was separated from the two southern ones, and from those located in France and the Mediterranean. Thus, our data suggest that Galicia may have been a crossroads for *E. maritimum* heading north and south through European coasts. Two main conclusions can be drawn from the assignment analyses: (i) within groups, populations are connected by gene flow differently depending on the group: populations from France and the British Isles seem to receive more migrants than those of the Mediterranean and Galicia; and (ii) among groups, the populations receive the same amount of gene flow whatever the group.

Genetic diversity values were heterogeneous but generally high, especially in the larger populations. The population of Sardinia presented high genetic variability, accumulated a considerable number of private fragments, and the relationship between geographic and genetic distance from any population to Sardinia was significant, pointing to its ancestral origin (Stehlik *et al.* 2002; Schönswetter & Tribsch 2005). The present results are comparable to those of Kadereit *et al.* (2005), Kadereit & Yaprak (2008) and Escudero *et al.* (2010), and suggest an ancient isolation centred on the Mediterranean Basin, congruent with an early east-to-west colonisation and later dispersal along the Atlantic coasts. Also, several authors have reported differentiation between the Atlantic and Mediterranean populations of European coastal species, with the latter usually showing a stronger phylogeographic structure, more clusters and greater genetic diversity (Table 1). However, our results show that the genetic structure is stronger in the Atlantic (four clusters) than in the Mediterranean (only two clusters) (Fig. 1).

As mentioned above, despite having a highly self-compatible reproductive system (Table 3; Alarcón *et al.* 2011), the majority of genetic variance of *E. maritimum* was contained within populations, and the diversity levels were moderate to high. When selfing occurs within populations, colonisation and migration processes may reduce the genetic variation within and increase the differentiation between populations (Ingvarsson 2002). Although some outcrossing mediated by ants and/or flies may occur in this species, pollen migration should occur at rates that are too low to compensate for reductions in within-population diversity caused by repeated colonisation (Ingvarsson 2002; Chauvet *et al.* 2004). Thus, seed rather than pollen migration must account for the observed levels of genetic diversity in this species.

Granivorous birds and rabbits actively eat and transport seeds of *Erodium* spp. (Ridley 1930; Soholt 1973; Skorupa *et al.* 1980; Shmida & Ellner 1983; Lane *et al.* 1999; Valera *et al.* 2005; Verdú *et al.* 2009). Endozoochory, together with epizoochory, as suggested by fruit morphology, may have played an important role in long-distance dispersal of *E. maritimum* seeds. Indeed, viable seed from rabbit faeces only (not from bird droppings) confirms the potential of seed dispersal by endozoochory. However, rabbits are now scattered along the coastline and were introduced by humans into European countries (other than Portugal and Spain), indicating that they are unlikely responsible for long-distance dispersal in this species. Therefore, epizoochory should be further investigated to find causes related to long-distance gene flow.

Population size and conservation implications

Erodium maritimum has been described as rare/restricted in Scotland (Jardine *et al.* 2011), Ireland (Green & Fitzpatrick 2008) and some regions of France (Lacroix *et al.* 2008; Muséum national d'Histoire naturelle 2011), and critically endangered in Spain (Gómez-Orellana & Sáez 2004). Historical distribution data demonstrate that several populations have recently declined or disappeared from the Atlantic coasts dra-

matically altered by human actions (Walker 2007; Green 2008). According to these authors, *E. maritimum* lost about two-thirds of its habitat in southeast England during the period 1987–2000.

Genetic diversity and polymorphism values were heterogeneous and significantly correlated with the number of individuals. However, genetic diversity presented only modest decreases for smaller populations. Population size predicts genetic diversity and may be an important factor for maintaining diversity (Ingvarsson 2002), and low number of individuals per population may have been a detrimental factor during recent environmental alterations or could have caused genetic bottlenecks. Thus, the relatively high diversity could in some cases be a remnant of populations that were more widely distributed and dispersed in the recent past.

Other coastal taxa are currently facing habitat reduction and increasing risks of genetic depauperation or extinction due to the combined effects of the fragmentation of populations, the strong human impact and invasion by naturalised aliens. Consequently, active initiatives of *in situ* conservation, such as monitoring, fencing and limitation of invasive species (already adopted for *E. maritimum* in Galicia), are advocated here to protect such unique remnants in the French, British and Mediterranean coasts, especially in the well preserved habitats of small coastal islets of the Atlantic, the Channel Islands and the small Mediterranean archipelagos. As gene flow is high within groups (Table 4), there is a high need to preserve population size (see Fig. 3), which currently does not require any assisted migration programme.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Right *y*-axis: Log-likelihood value of the data [Ln $P(D)$] as a function of the value of K (1–6) over ten runs. Left *y*-axis: second-order rate of change of the likelihood function with respect to K according Evanno *et al.* (2005). Maximum ΔK values correspond to the presumed true number of K clusters.

Figure S2. PCO diagram of *E. maritimum* populations, obtained using the Dice index. The percentage eigenvalue for axis 1 was 55.66%, for axis 2 it was 3.33%, and for axis 3 it was 2.35%. Colours indicate groups of populations, green: British Isles, red: French coast and islands, orange: Galician coast and islands (Spain), blue: Mediterranean islands (Dragonera, Sardinia and Corsica).

Figure S3. Regression of $F_{ST}/1 - F_{ST}$ against geographic distances for *E. maritimum* populations.

Table S1. Analysis of molecular variance (AMOVA) for *Erodium maritimum* AFLP data.

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