Title: Phylogeographic insights into *Artemisia crithmifolia* (Asteraceae) reveal several areas of the Iberian Atlantic coast as refugia for genetic diversity.

Running title: Phylogeography of the coastal species *Artemisia crithmifolia*

Alfredo García-Fernández^{1, 2 *}, Daniel Vitales^{1, 3}, Jaume Pellicer⁴, Teresa Garnatje¹, Joan Vallès³

¹*Institut Botànic de Barcelona (IBB-CSIC-ICUB), Passeig del Migdia s.n., Parc de Montjuïc, 08038 Barcelona, Catalonia, Spain.*

²*Biodiversity and Conservation Area, Universidad Rey Juan Carlos, C/ Tulipán SN, 28933, Móstoles, Spain*

³*Laboratori de Botànica, (UB), Unitat associada al CSIC, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Av. Joan XXIII s.n., 08028 Barcelona, Catalonia, Spain.*

⁴*Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, United Kingdom.*

*** Corresponding author: Alfredo García-Fernández**

Email: alfredo.garcia@urjc.es

Telephone: +34914888290

Abstract

The study of fragmentation processes should be tackled using different approaches, in order to obtain solid and robust evidence that could help in identifying potential barriers and threats for species. In this study, we have evaluated the spatial fragmentation patterns in *Artemisia crithmifolia* (Asteraceae) along its current distribution along the Atlantic coastlines of Europe, from Portugal to the Netherlands and the United Kingdom. Niche modelling analyses considering current and past climatic conditions, combined with plastid markers, have been used to evaluate the disconnected distribution pattern of the species, genetic flow between populations and habitat suitability for present and past populations.

Plastid markers showed very low variability, while suggesting that the westernmost populations from the Atlantic coast of the Iberian Peninsula are partially genetically isolated, due to the existence of a potential barrier for gene flow on the northern Spanish coast. However, niche models identify this area as a potential habitat for the species, even during the last glacial maximum (20,000 years ago). The combination of both techniques allows identification of potential refugia for the species, highlighting the most likely recolonisation routes and distribution patterns which resulted in the overall low levels of genetic diversity. Anthropogenic activities (urban sprawl, beach expansions etc.) are most likely behind local extinctions, thus preventing the establishment and expansion of new populations.

Keywords: Asteraceae, Coastal dune ecosystems, Conservation, European Atlantic coasts, Fragmented distribution, Niche modelling.

Introduction

Marine ecosystems have been recognised among the habitats that are at higher risk from global climate change. This includes those areas located in coastal and estuarine zones (e.g. Poff et al. 2002; Feagin et al. 2005). Coastal regions are especially fragile environments, and so are the species that inhabit them, since they live close to their tolerance thresholds (Wetthey et al. 2011). Certainly, the existence of several environmental gradients associated with both biotic and abiotic variables contributes to their unique characteristics (e.g. salinity, humidity, competition, etc. Helmuth et al. 2002; Occhipinti-Ambrogi 2007). Unfortunately, coastal dune ranges have been repeatedly altered, suffering from both climatic fluctuations and human activities (tourism, infrastructures, urban sprawl, Heslenfeld et al. 2004). In addition to this, the presence of invasive species, which are often linked to the above-mentioned economic activities and are in direct competition with native flora (Doody 2013), frequently poses a challenge to maintaining the ecosystem's equilibrium. Consequently, several coastal dune habitats have been included in the Habitats Directive of the European Union with the aim of promoting their conservation and sustainable use (Habitat Directive, European Commission 1992).

The study of the phylogeographic patterns of coastal plant species has seen increasing interest over recent years (Weising and Freitag 2007).

Representing a narrow distribution (i.e. the shoreline), these habitats become an ideal scenario to test biogeographical hypotheses concerning the existence of barriers to genetic exchange (e.g. transport routes, straits or oceanic courses), climate change or geological events (Ruiz et al. 2000; Kadereit et al. 2005). Southern European peninsulas (i.e. Balkan, Italian and Iberian Peninsulas) have been recognised as potential refugia for many plants and animals during glacial periods (Hewitt 1999; Médail and Diadema 2009; Patsiou et al. 2014). This hypothesis has been widely tested in many plant species (reviewed in Nieto Feliner 2014) occurring in a diverse range of habitats (alpine (Patsiou et al. 2014; Kyrkou et al. 2015), boreal (Gussarova et al. 2015) and riparian (King and Ferris 1998)). However, until recently, our understanding of the influence of Quaternary oscillations over marine and inter-tidal habitats and the species inhabiting them was relatively limited (Weising and Freitag 2007). The latest developments in climatic modelling and studies using isotopes have therefore provided significant insights into the impact of abiotic factors (such as temperature changes or alterations of sea level and coastal line) in shaping plant diversity in coastal habitats in recent millennia (Lang 1994; Frau et al. 2014).

The genus *Artemisia* comprises more than 500 species (Oberprieler et al. 2007). Many of the species are landscape dominant, showing expansive distributions as the result of their adaptations to a wide range of habitats and environmental conditions (Vallès et al. 2011). Most species are wind-pollinated, and, although seeds are lacking pappi, their relatively small size and light weight allows long-range dispersal to take place frequently (Laursen et al. 2007). *Artemisia crithmifolia* L. (*A. campestris* L. subsp. *maritima* (DC.) Arcang.)

is an ideal system in which to investigate the genetic consequences of late Pleistocene climatic oscillations in a coastal plant with a wide distribution and significant long-range dispersal capability. The species occurs in coastal sand dune habitats of Atlantic European shores, along a fragmented distribution range of more than 3,000 km, from the southwest coast of Spain to the Netherlands, including two isolated populations on the coasts of England and Wales (Pellicer et al. 2009). In its current distribution, the northern distributional limit of *A. crithmifolia* matches precisely the December/January/February mean temperature isotherm of 4°C (van Andel 2002, Fig. 1), suggesting the importance of colder temperatures for its distribution. In this context, we posed the following questions aimed at gaining further insights into the phylogeographic history of this taxon, combining genetic studies with present and past species distribution models: i) Do plastid markers allow inference of genetic structure in *A. crithmifolia* populations, and could structure be associated with long distance dispersal (LDD) events? ii) Is there any evidence suggesting a pattern of southern refugium occupation based on the genetic structure of the species? iii) Is there congruence between the past distribution modelling and current genetic structure? iv) What are the likely origins of the British populations?

Material and methods

Population sampling

A total of 133 specimens from 48 populations of *A. crithmifolia* from Spain, Portugal, France, Belgium, the Netherlands and United Kingdom were

collected and analysed for this study (Table 1, Fig. 1). Populations were sampled to cover the whole distribution area of the species and capture most of the inter-population genetic diversity and unique haplotypes. Two to three individuals were collected per population. To minimise excessive sampling from within the progeny of a single maternal plant, the individual samples were collected from plants at least 10 m apart. Further details about the studied material (population origin, geographic coordinates, number of analysed individuals, collectors and herbarium vouchers) are shown in Table 1 and Figure 1.

DNA extraction, chloroplast regions and PCR conditions

Leaf tissue dried in silica gel (approximately 20 mg) was used for DNA extraction using the CTAB protocol (Doyle and Doyle 1990) with minor modifications. Quality and quantity of DNA extracts were checked with a Nanodrop 1000 spectrophotometer and in 0.8% agarose gel stained with SYBR (Invitrogen, the Netherlands).

After a pilot study involving eleven nuclear and chloroplast markers (ADH gene, LEAFY gene, *psbA-trnH* spacer, *rbcL* gene, *rpl32-trnL* spacer, *rps12-rpl20* spacer, *rps16* gene, *trnK* gene, *trnS-psbC* spacer, *trnS-trnC* spacer and *trnS-trnfM* spacer), two chloroplast regions, *rpl32-trnL* and *psbA-trnH*, were chosen for this study because of their successful sequencing and the greater level of variability found. The first region, *rpl32-trnL*, was amplified with *rpl32* and *trnL* (UAG) primers (Shaw et al. 2007). The primer combination *psbA* (forward) and *trnH* (reverse) was used for the *psbA-trnH* region (Hamilton 1999). PCR reactions were performed in a 25 µl reaction mixture, containing 2.5

µl of genomic DNA (approximately 50 ng), 12.8 µl of sterile water, 2.5 µl of 2 mM MgCl₂, 2.5 µl of 10X Gene Taq Universal buffer, 2.5 µl of 2.5 mM dNTPs mixture, 1 µl of each primer (5 pmol/µl), 0.2 µl of AmpliTaq DNA polymerase (Applied Biosystems, Carlsbad, CA, USA). The reactions were carried out in a thermocycler (S 1000TM Thermal Cycler, BIORAD Laboratories Inc, Hercules, CA, USA) under the following conditions: i) *rp/32-trnL* spacer - 3 min at 95°C, followed by 34 cycles of 94°C for 40 s, 54°C for 40s and 72°C for 1min, and a final elongation step of 10 min at 72°C. ii) *psbA-trnH* region - 3 min at 95°C, followed by 33 cycles of 94°C for 30s, 55°C for 30 s and 72°C for 1 min, with a final elongation step of 10 min at 72°C. PCR reactions were checked on 1.2% agarose gel and the amplification product was sequenced using a BigDye Terminator Cycle Reaction Kit v3.1 (PE Biosystems, Foster City, CA, USA) using the forward primers for each region. DNA sequencing was performed in an ABI PRISM 3730 XL DNA analyser (PE Biosystems) at the Centres Científics i Tecnològics of the University of Barcelona.

Molecular data analysis

The *psbA-trnH* and *rp/32-trnL* sequences were assembled with BioEdit ver. 7.1.3.0 (Hall 1999), aligned with ClustalW Multiple Alignment v. 1.4 (Thompson et al. 1994) and adjusted manually. DnaSP v5 (Rozas et al. 2003) was used to estimate the genetic variation shown by the data (including polymorphic sites, parsimony informative sites, number of haplotypes and total haplotype diversity).

Plastid haplotypes were determined from nucleotide substitutions and insertions/deletions (indels) in a combined data set that included both the *psbA-*

trnH and *rp/32-trnL* regions. Indels were treated as single-mutation events. The evolutionary relationships among haplotypes were inferred based on a parsimony network constructed using TCS version 1.21 (Clement et al. 2000). The maximum number of differences resulting from single substitutions among haplotypes was calculated with 95% confidence limits. According to previous phylogenetic reconstructions of the genus (Garcia et al. 2011; Riggins and Seigler 2012), *Artemisia dracunculus* L. and *A. leucophylla* (Turcz. ex Besser) Turcz. ex C.B. Clarke were included in the analysis as outgroups due to their phylogenetic relatedness (Genbank accession numbers: *psbA-trnH* (JN862129 and JN862055) and *rp/32-trnL* (JN862129 and JN862152).

Finally, haplotype spatial genetic structure was analysed with SAMOVA 1.0 (Dupanloup et al. 2002), carrying out a simulated annealing approach to identify populations clusters (K). These groups are maximally differentiated from the others but geographically similar. K values ranged from 2 to 20, starting from 100 random initial conditions for each simulation. An F_{CT} index of genetic differentiation was estimated among initial K groups, followed by an iterative simulated annealing mechanism to obtain the best group configuration and final F_{CT} values, which was replicated 1000 times. The obtained configuration with the largest F_{CT} value among the 100 tested was conserved as the best grouping of populations.

Species distribution modelling

We performed species distribution modelling (hereafter, SDM) to evaluate the potential distribution of *A. crithmifolia* under present climatic conditions. We used MAXENT v3.3 (Phillips and Dudík 2008) software and

employed the maximum entropy algorithm. Nineteen bioclimatic variables (at 30s resolution) for Western Europe under current conditions were obtained from the database of WorldClim website (www.worldclim.org; Hijmans et al. 2005). All these 19 variables (see Online Resource 1) were used together as predictors to calibrate the species distribution model with MAXENT. In the occurrence data set, we employed the 48 georeferenced localities corresponding to the sampled populations of *A. crithmifolia* (Fig. 1; Table 1). These occurrences were randomly split into training data (75%) and test data (25%) and 10 subsampled replicates were run for model evaluation. To reconstruct past suitable areas for *A. crithmifolia*, the distribution model under current conditions was extrapolated to the last glacial maximum (hereafter LGM, c. 20 kyr BP) using paleoclimatic layers simulated under two general atmospheric circulation models: the community climate system model (CCSM, Collins et al. 2006) and the model for interdisciplinary research on climate (MIROC, Hasumi and Emori 2004). For comparative purposes, we performed an alternative SDM approach, with the same methods and parameters explained above but using only the most ecologically meaningful and best performing variables inferred from the first analysis (i.e. bio4, bio 6, bio7 and bio 15, see Online Resource 1).

RESULTS

Genetic diversity

The sequences of *psbA-trnH* and *rpl32-trnL* intergenic spacers were aligned in two matrices containing 362 bp and 663 bp, respectively (see Online

Resource 2 for Genbank accession numbers, Online Resource 3 and 4 for the alignments). Low levels of variation in these plastid regions were detected (five nucleotide substitutions - four of them parsimony informative - and one indel of 11 bp). Based on these polymorphic sites, we were able to identify seven haplotypes among the *A. crithmifolia* populations, and total haplotype diversity (Hd) was estimated to be 0.69. Most populations (42) contained only one haplotype, while five of them exhibited some diversity among individuals. With the exception of haplotype E (only found in population PI4 from the southern Iberian Peninsula, Fig. 1), the remaining chlorotypes were shared by several populations. Haplotype A showed the highest frequency (41.4%), followed by haplotype C (36.8%), whereas the rest of them showed much lower frequencies (6.8–0.8%). Haplotype A was found in 21 populations – the only one found in 18 of them– distributed along the coast of France, Belgium, the Netherlands and in one of the two known populations from the United Kingdom (UK1). Haplotype B was found in four populations from France and one population from the Netherlands. Haplotype C was detected in 18 populations, of which it was the only one detected in 17 of them. Haplotype C was mainly distributed across the Iberian Peninsula, and in the second population from United Kingdom (UK2). Haplotype D was present in three different populations from France –the only haplotype found in two of them– and in the unique population from the northern coast of the Iberian Peninsula (PI20). Finally, haplotypes F and G were only detected in three populations from the Iberian Peninsula, one of them sharing both haplotypes (PI5).

The haplotypic evolutionary relationships are shown in the parsimony network (Fig. 1). This network implies that most of the haplotypes are

connected by a single mutation step, haplotype E being the only one separated by two nucleotide substitutions from its genetically closest neighbours (haplotypes B and C). The highest root probability was assigned by TCS to haplotype C ($P = 0.33$), which was central within the network, showed the second highest frequency (36.8%) and was widespread on the Iberian Peninsula. The outgroup species, *A. dracunculus* and *A. leucophylla*, were separated by 10 and 16 mutation steps, respectively, from haplotype F.

The results of the spatial analysis of the genetic structure carried out with SAMOVA showed similar results to the previous analyses. Five was proposed as the most likely number of clusters that grouped all populations ($\Phi_{CT} = 0.51$, $p < 0.01$). Each group was characterised by one of the haplotypes described previously in the network. The populations with the haplotypes E (PI4) and F (PI8) were grouped with the haplotype C in one group.

Species distribution modelling

The species distribution model for current conditions (Fig. 2) spanned the current distribution range of *A. crithmifolia* (from the Southern Iberian Peninsula to the Northern Netherlands), plus additional areas, such as isolated points on the Northern African coast (where the species is no longer present). The mean area under the receiver-operating characteristic curve (AUC; a measure of model fitness) for testing data was high (0.982), which supported the predictive power of the model. The standard deviation of the 10 replicates was low (0.005) and the omission rate using maximum training sensitivity plus specificity threshold was 4.44%. According to jackknife testing, the environmental variables with highest gain when used in isolation were bio4 (temperature

seasonality), bio6 (minimum temperature of coldest month) and bio7 (annual temperature range), which therefore appeared to be the most informative (see Online Resource 1 and 5). The environmental variable that reduces the gain the most when omitted is bio15 (precipitation seasonality), therefore appearing to have the most information that is not present in the other variables (see Supplementary Information).

The CCSM and MIROC models for the LGM yielded almost identical inferences for the *A. crithmifolia* paleodistribution. The CCSM model (Fig. 2b) inferred a restricted potential distribution in the southern half of the Atlantic coast of the Iberian Peninsula and the Atlantic coast of North Africa. This model did not support the occurrence of *A. crithmifolia* outside the Iberian coasts during the LGM. The MIROC model (Fig. 2b) inferred a larger distribution of suitable areas, also including the northern coast of the Iberian Peninsula and the southernmost part of the Biscay Bay. Both CCSM and MIROC models did not support the presence of *A. crithmifolia* in the northernmost areas of the European coastline.

The alternative SDM approach, using only the best performing variables (i.e. bio4, bio 6, bio7 and bio 15), resulted in similar projections (Figs. 2d-f) to the models inferred with 19 variables (Figs. 2a-c). The main difference between both approaches was in relation to the potential distribution of the species during the LGM, where the model using the four most informative variables inferred a larger distribution of suitable areas northwards up to the Biscay Bay. In this alternative SDM, the average test AUC for the ten replicate runs was

0.962, and the standard deviation was 0.011. The average omission rate using maximum training sensitivity plus the specificity threshold was 5.83%.

DISCUSSION

In spite of the intense and representative sampling effort, the inter- and intra-population diversity was low and multiple haplotypes were found in only five populations (12.8% of the total), probably due to the number and type of regions considered, which could only partially cover the genetic variability of the species. This low intra-population variability and the well-defined genetic groups might suggest that the colonisation pattern during the range expansion along the coast line would have been the result of a mixture of punctual founder events, following a stepping stone pattern. For this range expansion along the coastal line, the formation of specialised structures in seeds (e.g. for water floating or promoting zoochorous dispersal) and/or asexual vectors would be necessary (Vallès 1989). The breeding mechanism of the species is also an important factor for the colonisation of new habitats and the survival of the population if a solitary individual reaches a new territory (Richards 1997). Self-compatibility would promote an important advantage for the coloniser (Baker's Law, Baker 1955) although such a mechanism (or partial compatibility), albeit one that has been described in many congeners, is unknown in *A. crithmifolia* (McArthur 1994; Tkach et al. 2008).

One of the genetic consequences of these dispersal mechanisms (only one seed or propagule to generate the whole population) is the occurrence of reduced genetic diversity, bottlenecking and allele fixation, that can be associated with founder events during range expansions (Frankham et al. 2002;

Roman and Darling 2007) as observed in this study. It is thus essential to perform further studies using highly variables markers (e.g. AFLP, microsatellite or NGS approaches) to confirm these patterns of low genetic variability and the presence of barriers to gene flow.

Finally, the low inter-population diversity along the coastline and the stepping stone model found in this study not only corroborate previous findings in other plant species along the European Atlantic coasts (Kadereit et al. 2005), but also in other species of the genus *Artemisia*, such as the alpine *A. eriantha* Ten. (Sanz et al. 2014) or *A. halodendron* Turcz. ex Besser (Huang et al. 2013).

Phylogeographic patterns in A. crithmifolia

Haplotype networks and the SAMOVA groups had a clear geographic distribution (excluding the British populations). All the populations from the eastern Iberian Peninsula shared four haplotypes (two SAMOVA groups), whilst populations from Northern Spain, France, Belgium and the Netherlands shared three other different haplotypes (three SAMOVA groups). This pattern suggested the existence of a barrier in the southern part of the Biscay Bay (Fig. 1), which had already been proposed for other species (i.e. Lundy et al. 2000; Fontaine et al. 2007). In addition, the absence of populations on the north coast of Spain (with the exception of PI20) despite the presence of suitable habitats for *A. crithmifolia* (Fig. 2a), would also support the existence of such a barrier. Notwithstanding this, alternative explanations attributed to local factors, including urban sprawl - leading in some cases to the total disappearance of the suitable habitat for the taxon- or plant-plant competition, cannot be discarded (Aseginolaza et al. 1985; Gredilla 1914). In the case of the British populations,

which shared haplotypes with continental species, their origin could be inferred as the consequence of sporadic occurrences of long distance dispersal that might be mediated by water flows (Pingree 1993). However, as hypothesised by Smith (2005), their actual origin is not completely clear, and at least the English population (UK1) could have arrived on seed mixtures from residual mounds of a close pumping station.

Although the numbers of detected haplotypes were similar on each side of the Biscay Bay, some differences in haplotypic diversity and structure could be pinpointed. Haplotype C (i.e. the most common in the Iberian Peninsula) showed the highest probability of being the most ancestral haplotype of *A. crithmifolia* (according to Castelleo and Templeton 1994; Avise 2000). Central positions in networks are generally interpreted as ancestral haplotypes. Additionally, haplotype F (endemic from the southern Iberian Peninsula and closer to haplotype C) could be the most closely related to the outgroup species, suggesting as well a putative ancestral origin for this haplotypic form. According to both rooting approaches and the presence of two scarce haplotypes (F and G), we highlight the importance of the south-eastern Iberia as centre of diversity for this species. Conversely, in the north group, although haplotype A is the most common, haplotypes D and B are also very frequent. A recolonised region can have either low or high diversity depending on whether it is derived from a single population expanding its range, or from the admixture of various populations, but it is expected to have a low proportion of private haplotypes (Petit et al. 2003; Maggs et al. 2008a). These differences in the richness, rarity and structure of the haplotypes of each group could be associated with the potential role of genetic refugia that have been proposed for

the South European Peninsulas (i.e. Hewitt 1999; Petit et al. 2003), including for marine-coastal organisms (Maggs et al. 2008b). The Atlantic coastline of the Iberian Peninsula, from the Algarve to Biscay bay, has been recognised as a potential refugium for coastal species during Quaternary glaciations (Maggs et al. 2008b; Nieto Feliner 2014), although it has not always been considered the starting point for northern colonisation (e.g. Hoarau et al. 2007; Provan and Maggs 2012). Other potential glacial refugia have been considered for several marine species (i.e. Western of the English Channel or the Southwest of Ireland, Coyer et al. 2003; Maggs et al. 2008b; Provan and Maggs 2012) but the absence of private alleles in northern populations, the lack of coastal dune habitats in these localisations and the small differences between haplotypes found may suggest that these refugia were not suitable for *A. crithmifolia*.

Past and present species distribution modelling

The result of the species distribution modelling according to current conditions (Figs. 2a and 2d) closely mirrored the current distribution pattern of *A. crithmifolia* (Fig. 1), except for certain peripheral areas, such as North African or Mediterranean coastlines (i.e. continental and island), where *A. crithmifolia* is absent despite their environmental suitability, most likely due to the limitations of SDMs.

The results of cpDNA-based phylogeographic analyses are in line with the species paleodistribution modelling according to simulations of LGM climate, with a significant reduction of suitable areas in northern Europe for *A. crithmifolia* during the LGM (Fig. 2b, 2c and 2e). The CCSM-based models showed paleodistribution patterns particularly consistent with the refuge of *A.*

crithmifolia in the southern Atlantic coasts of the Iberian Peninsula during the LGM (Figs. 2b and 2e). Subsequently, the species would have colonised (or recolonised) northern European localities from the refugial area proposed by the CCSM model, giving rise to the potentially derivative haplotypes that occur in France, Belgium and the Netherlands. In contrast, the MIROC-based models supported various potential refugia for the species: the southern Atlantic coast of the Iberian Peninsula, the southern Bay of Biscay (Fig. 2c and 2f) and the northern Bay of Biscay (Fig. 2f). These latter models might indicate that southern haplotypes (C, E, F and G) may have persisted along Southern Iberian Peninsula coastlines, whereas northern haplotypes (A, B and D) could have survived in potential refugia close to the Biscay Bay. In this scenario, the respective colonisation (or recolonisation) of northern Iberia and northern European coastlines could have occurred from those separate refugia independently. This scenario would also support the differences found between the genome sizes of the groups (Pellicer et al. 2009), thus highlighting the importance of glacial refugia in shaping plant genome diversity.

CONCLUSIONS

Iberian coasts have been recognised as potential refugia for many European species. The combination of several approaches (i.e. niche modelling, genetic markers, genome sizes), strengthens the potential conclusions and highlights the importance of these areas for species conservation, as has occurred with *A. crithmifolia*. In spite of the low levels of genetic diversity found, the combined analyses allow the interpretation of the phylogeographic patterns of the species. Future phylogeographic studies should

try to include different methodologies to ensure the detection of these refugia in order to foster their conservation.

ACKNOWLEDGEMENTS

The authors thank S. Garcia, D. Guest, C. Ibarria, A. Jones, Ph. Smith, J.D. Twibell, M. Wilcox and J. Woodman for their help during field sampling and S. Pyke and A. Plant for language editing. The study has been supported by projects CGL2007-64839-C02-01/-02, CGL2010-22234-C02-01.02/BOS and CGL2013-49097-C2-2-P (Spanish government) and 2014SGR514 (Catalan government).

References

- Aseginolaza C, Gómez D, Lizaur X, Montserrat G, Morante G, Salaverria M, Uribe-Echebarria P, Alejandro J (1985) Catálogo florístico de Álava, Vizcaya y Guipúzcoa. Gobierno Vasco Vitoria-Gasteiz
- Avise JC (2000) Phylogeography: the history and formation of species. Harvard University Press,
- Baker HG (1955) Evolution of reproductive isolation in plants. *Evolution* 9:37-349
- Castelloe J, Templeton AR (1994) Root probabilities for intraspecific gene trees under neutral coalescent theory. *Molec. Phylogen Evol* 3:102-113.
doi:doi:10.1006/mpev.1994.1013
- Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molec Ecol* 9:1657-1659. doi:10.1046/j.1365-294x.2000.01020.x
- Collins WD, Bitz CM, Blackmon ML, Bonan GB, Bretherton CS, Carton JA, Chang P, Doney SC, Hack JJ, Henderson TB (2006) The community climate system model version 3 (CCSM3). *Journal of Climate* 19:2122-2143. doi:10.1175/JCLI3761.1
- Commission E (1992) Council directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Communities. Series L, vol 206.
- Coyer J, Peters A, Stam W, Olsen J (2003) Post-ice age recolonization and differentiation of *Fucus serratus* L.(Phaeophyceae; Fucaceae)

populations in Northern Europe. *Molec Ecol* 12:1817-1829.

doi:10.1046/j.1365-294X.2003.01850.x

Doody JP (2013) Alien Plant Invasion. In: Sand Dune Conservation, Management and Restoration, vol 4. Coastal Research Library. Springer Netherlands, pp 177-199. doi:10.1007/978-94-007-4731-9_8

Doyle JJ, Dolye JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13-15

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Molec Ecol* 11:2571-2581. doi:10.1046/j.1365-294X.2002.01650.x

Ehlers J, Gibbard PL (2004) Quaternary glaciations-extent and chronology: part I: Europe, vol 2. Elsevier,

Feagin RA, Sherman DJ, Grant WE (2005) Coastal erosion, global sea-level rise, and the loss of sand dune plant habitats. *Frontiers in Ecology and the Environment* 3:359-364. doi:10.1890/1540-9295(2005)003[0359:cegsra]2.0.co;2

Fontaine M, Baird S, Piry S, Ray N, Tolley K, Duke S, Birkun A, Ferreira M, Jauniaux T, Llavona A, Ozturk B, A Ozturk A, Ridoux V, Rogan E, Sequeira M, Siebert U, Vikingsson G, Bouquegneau J-M, Michaux J (2007) Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in Old World waters. *BMC Biol* 5:30. doi:10.1186/1741-7007-5-30

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, New York

- Frau D, Eizaguirre BO, Arbeiza CG, de la Peña JAJ (2014) The role of the hydrodynamic regime in the distribution of the invasive shrub *Baccharis halimifolia* (Compositae) in Oyambre Estuary (Cantabria, Spain). *Limnetica* 33:1-12
- Garcia S, McArthur ED, Pellicer J, Sanderson SC, Vallès J, Garnatje T (2011) A molecular phylogenetic approach to western North America endemic *Artemisia* and allies (Asteraceae): Untangling the sagebrushes. *Amer J Bot* 98:638-653. doi:10.3732/ajb.1000386
- Gredilla F (1914) Itinerarios botánicos de Dn Javier de Arízaga y relacion detallada de dos nuevos manuscritos. Diputación foral de Álava Vitoria
- Gussarova G, Allen GA, Mikhaylova Y, McCormick LJ, Mirré V, Marr KL, Hebda RJ, Brochmann C (2015) Vicariance, long-distance dispersal, and regional extinction–recolonization dynamics explain the disjunct circumpolar distribution of the arctic-alpine plant *Silene acaulis*. *Amer J Bot* 102:1703-1720. doi:10.3732/ajb.1500072
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nuclear Acids Symposium Series* 41:95-98
- Hamilton MB (1999) Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molec Ecol* 8:521-523. doi:citeulike-article-id:7325958
- Hasumi H, Emori S (eds) (2004) K-1 coupled model (MIROC) description. K-1 Technical Report 1. University of Tokyo, Tokyo

- Helmuth B, Harley CD, Halpin PM, O'Donnell M, Hofmann GE, Blanchette CA (2002) Climate change and latitudinal patterns of intertidal thermal stress. *Science* 298:1015-1017. doi:10.1126/science.1076814
- Heslenfeld P, Jungerius P, Klijn J (2004) European coastal dunes: ecological values, threats, opportunities and policy development. In: *Coastal dunes*. Springer, pp 335-351
- Hewitt GM (1999) Post-glacial re-colonization of European biota. *Biol J Linn Soc* 68:87-112. doi:10.1111/j.1095-8312.1999.tb01160.x
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25:1965-1978. doi:10.1002/joc.1276
- Hoarau G, Coyer J, Veldsink J, Stam W, Olsen J (2007) Glacial refugia and recolonization pathways in the brown seaweed *Fucus serratus*. *Molec Ecol* 16:3606-3616. doi:10.1111/j.1365-294X.2007.03408.x
- Huang J, Cang H, Liu Q, Shao J (2013) Environment friendly inhibitor for mild steel by *Artemisia halodendron*. *Int J Electrochem Sci* 8:8592-8602.
- Kadereit JW, Arafteh R, Somogyi G, Westberg E (2005) Terrestrial growth and marine dispersal? Comparative phylogeography of five coastal plant species at a European scale. *Taxon* 54:861-876. doi:10.2307/25065473
- King RA, Ferris C (1998) Chloroplast DNA phylogeography of *Alnus glutinosa* (L.) Gaertn. *Molec Ecol* 7:1151-1161. doi:10.1046/j.1365-294x.1998.00432.x
- Kyrkou I, Iriando JM, García-Fernández A (2015) A glacial survivor of the alpine Mediterranean region: phylogenetic and phylogeographic insights into

Silene ciliata Pourr. (Caryophyllaceae). PeerJ 3:e1193.

doi:10.7717/peerj.1193

Lang G (1994) Quartäre Vegetationsgeschichte Europas: Methoden und Ergebnisse. Spektrum Akademischer Verlag,

Laursen S, Reiners W, Kelly R, Gerow K (2007) Pollen dispersal by *Artemisia tridentata* (Asteraceae). International Journal of Biometeorology 51:465-481. doi: 10.1007/s00484-007-0086-7

Lundy CJ, Rico C, Hewitt GM (2000) Temporal and spatial genetic variation in spawning grounds of European hake (*Merluccius merluccius*) in the Bay of Biscay. Molec Ecol 9:2067-2079. doi:10.1046/j.1365-294X.2000.01120.x

Maggs CA, Castilho R, Foltz D, Henzler C, Jolly MT, Kelly J, Olsen J, Perez KE, Stam W, Väinölä R (2008a) Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. Ecology 89: S108-S122. doi:10.1890/08-0257.1

Maggs CA, Castilho R, Foltz D, Henzler C, Jolly MT, Kelly J, Olsen J, Perez KE, Stam W, Väinölä R, Viard F, Wares J (2008b) Evaluating Signatures of Glacial Refugia for North Atlantic Benthic Marine Taxa. Ecology 89 :S108-S122. doi:10.1890/08-0257.1

McArthur ED (1994) Ecology, distribution, and values of sagebrush within the Intermountain region. In Proceedings—ecology and management of annual rangelands. USDA Forest Service General Technical Report

Médail F, Diadema K (2009) Glacial refugia influence plant diversity patterns in the Mediterranean Basin. J Biogeogr 36:1333-1345. doi:10.1111/j.1365-2699.2008.02051.x

- Nieto Feliner G (2014) Patterns and processes in plant phylogeography in the Mediterranean Basin. A review. *Perspect Pl Ecol Evol Syst* 16:265-278.
doi:10.1016/j.ppees.2014.07.002
- Oberprieler C, Himmelreich S, Vogt R (2007) A new subtribal classification of the tribe Anthemideae (Compositae). *Willdenowia* 37:89-114.
doi:/10.3372/wi.37.37104
- Occhipinti-Ambrogi A (2007) Global change and marine communities: alien species and climate change. *Mar Pollut Bull* 55:342-352.
doi:10.1016/j.marpolbul.2006.11.014
- Patsiou TS, Conti E, Zimmermann NE, Theodoridis S, Randin CF (2014) Topo-climatic microrefugia explain the persistence of a rare endemic plant in the Alps during the last 21 millennia. *Global Change Biol* 20:2286-2300.
doi:10.1111/gcb.12515
- Pellicer J, Garcia S, Garnatje T, Vallès J (2009) Changes in genome size in a fragmented distribution area: the case of *Artemisia crithmifolia* L.(Asteraceae, Anthemideae). *Caryologia* 62:152-160.
doi:10.1080/00087114.2004.10589680
- Petit RJ, Aguinagalde I, de Beaulieu J-L, Bittkau C, Brewer S, Cheddadi R, Ennos R, Fineschi S, Grivet D, Lascoux M, Mohanty A, Müller-Starck G, Demesure-Musch B, Palmé A, Martín JP, Rendell S, Vendramin GG (2003) Glacial Refugia: Hotspots But Not Melting Pots of Genetic Diversity. *Science* 300:1563-1565. doi:10.1126/science.1083264
- Phillips SJ, Dudík M (2008) Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography* 31:161-175.
doi:10.1111/j.0906-7590.2008.5203.x

- Pingree RD (1993) Flow of surface waters to the west of the British Isles and in the Bay of Biscay. Deep-Sea Res 2,: Topical Stud. Oceanogr. 40:369-388. doi:10.1016/0967-0645(93)90022-F
- Poff N, Brinson MM, Day J (2002) Aquatic ecosystems and global climate change. Pew Center on Global Climate Change, Arlington, VA 44
- Provan J, Maggs CA (2012) Unique genetic variation at a species's rear edge is under threat from global climate change. Proc Roy Soc Biol Sci Ser B 279:962-8452. doi:10.1098/rspb.2011.0536
- Richards AJ (1997) Plant breeding systems. Garland Science,
- Riggins CW, Seigler DS (2012) The genus *Artemisia* (Asteraceae: Anthemideae) at a continental crossroads: Molecular insights into migrations, disjunctions, and reticulations among Old and New World species from a Beringian perspective. Molec Phylogen Evol 64:471-490. doi:10.1016/j.ympev.2012.05.003
- Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22:454-464. doi:10.1016/j.tree.2007.07.002
- Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496-2497. doi:10.1093/bioinformatics/btg359
- Ruiz GM, Fofonoff PW, Carlton JT, Wonham MJ, Anson HH (2000) Invasion of Coastal Marine Communities in North America: Apparent Patterns, Processes, and Biases. Annual Rev Ecol Syst 31:481-531. doi:10.2307/221741

- Sanz M, Schönswetter P, Vallès J, Schneeweiss GM, Vilatersana R (2014) Southern isolation and northern long-distance dispersal shaped the phylogeography of the widespread, but highly disjunct, European high mountain plant *Artemisia eriantha* (Asteraceae). Bot J Linn Soc 174:214-226. doi:10.1111/boj.12132
- Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Amer J Bot 94:275-288. doi:10.3732/ajb.94.3.275
- Smith P (2005) Botanical interest of Crosby sand-dunes.
<http://activenaturalist.org.uk/mbb/node/363>.
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl Acids Res 22:4673-4680. doi:10.1093/nar/22.22.4673
- Tkach NV, Hoffmann MH, Röser M, Korobkov AA, Von Hagen KB (2008) Parallel evolutionary patterns in multiple lineages of arctic *Artemisia* L. (Asteraceae). Evolution 62:184-198. doi:10.1111/j.1558-5646.2007.00270.x
- Vallès J (1989) Dades sobre la biologia d'especies iberico-baleariques d'*Artemisia* L. Collect Bot 17:237-245
- Vallès J, Garcia Giménez S, Hidalgo O, Martín J, Pellicer J, Sanz M, Garnatje T (2011) Biology, genome evolution, biotechnological issues and research including applied perspectives in *Artemisia* (Asteraceae). Advances Bot Res 60:349-419. doi:10.1016/B978-0-12-385851-1.00015-9

- van Andel TH (2002) The Climate and Landscape of the Middle Part of the Weichselian Glaciation in Europe: The Stage 3 Project. *Quatern Res* 57:2-8. doi:10.1006/qres.2001.2294
- Weising K, Freitag H (2007) Phylogeography of halophytes from European coastal and inland habitats. *Zoologischer Anzeiger - A Journal of Comparative Zoology* 246:279-292. doi:10.1016/j.jcz.2007.07.005
- Wetthey DS, Brin LD, Helmuth B, Mislan K (2011) Predicting intertidal organism temperatures with modified land surface models. *Ecol Modelling* 222:3568-3576. doi:10.1016/j.ecolmodel.2011.08.019

Figure legends

Figure 1. A) Geographical distribution of cpDNA haplotypes and studied populations of *Artemisia crithmifolia*. The population codes correspond to those in Table 1, and the pie charts represent the percentage of each haplotype in each population. Grey areas along the coastline refer to *A. crithmifolia* distribution. The fragment on the north coast of Spain coloured in red corresponds to an area with very few records of the species, where a potential genetic barrier could be situated. B) Statistical parsimony network showing the relationships of the seven plastid haplotypes. Each line between haplotypes indicates a mutational step, and black dots represent extinct or unsampled haplotypes. Dashed line represents December/January/February mean temperature isotherm of 4°C.

Figure 2. Potential distribution of *Artemisia crithmifolia* generated using Maxent on the 19 bioclimatic variables (A-C) and on the four best-performing variables (D-F). The different projections display the potential distribution of the species in the present (A and D) and during the last glacial maximum according to two different general circulation models: CCSM (B and E) and MIROC (C and F). The grey areas show the approximate extent of the ice sheets during the LGM (Ehlers and Gibbard 2004)

Online Resource

Online Resource 1. Description of the environmental (BIOCLIM) variables used to construct ecological niche models in Maxent.

Online Resource 2. Genbank reference numbers for the sequences obtained for each marker.

Online Resource 3. Alignment of *psbA-trnH* region used for the analysis of *Artemisia crithmifolia*

Online Resource 4 . Alignment of *rpl32-trnL* region used for the analysis of *Artemisia crithmifolia*.

Online Resource 5. Results of the jackknife test on the importance of the environmental variables, showing the gain in predictive (regularised training gain) when the different variables are used in isolation and the decrease in the gain when each of them is omitted.