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Artemisia herba alba pdf

Skip Nav Destination Artemisia herba-alba is an important component of Mediterranean dry steppe floras, being widely distributed in arid areas of the Iberian Peninsula and North-West Africa. In this study, we use genetic, cytogenetic and niche modelling tools to investigate the natural history of the species, focusing particularly on the role played by polyploidization to explain current diversity patterns throughout the main distribution range of the plant. Our sequencing data indicate a complex phylogeographical structure showing similar haplotype diversity patterns on both sides of the Strait of Gibraltar and no clear signals of genetic refugia. According to our cytogeographical results, we inferred multiple polyploidization events, which probably took place on the Iberian Peninsula and in North Africa independently. Environmental niche modelling suggested stable potential distributions of A. herba-alba on both sides of the Mediterranean Sea under present and past Last Glacial Maximum conditions, which could be related to the intricate genetic and cytogenetic patterns shown by the species. Finally, environmental modelling comparison among cytotypes revealed that the niche of tetraploids is narrower and nested in that of diploids, a result that could indicate environmental specialization and could potentially explain recurrent establishment success of tetraploids. The Mediterranean region is considered one of the 25 world biodiversity hotspots, especially given its high plant species richness and level of endemism (Myers et al., 2000). Consequently, the Mediterranean Basin has long attracted the attention of biologists interested in understanding the diversification processes of plants (Médail & Diadema, 2009). Remarkable phylogeographical patterns explaining the outstanding diversity of the region and neighbouring areas have principally been reported for species from the Mediterranean islands and southern European peninsulas (Weiss & Ferrand, 2007). In contrast, studies on taxa distributed in North Africa are much scarcer, and the evolutionary histories of these taxa have been considerably less deeply explored to date (Nieto Feliner, 2014; Médail & Baumel, 2018). The still limited phylogeographical research of North African plants has mainly focused on the Strait of Gibraltar, emphasizing the importance of this biogeographical area in understanding the emergence and maintenance of the rich Mediterranean biota (Hewitt, 2011). Intrinsic characteristics of this region such as the heterogeneous relief, the constantly changing geographical barrier or a spatially diverse but locally stable climate have been inferred as key features to explain the high degree of genetic and taxonomic distinctiveness found in the Mediterranean area (e.g. Jaramillo-Correa et al., 2010; García-Aloy et al., 2017; Herrando-Moraira et al., 2017; Massó, López-Pujol & Vilatersana, 2018). These geographical and climatic factors would have facilitated processes including vicariance, long-distance dispersal, hybridization and ecological adaptation (Thompson, 2005), jointly shaping the current plant diversity of North Africa and southern Europe. However, phylogeographical studies focusing on widespread plant species distributed on both sides of the Mediterranean Basin are still limited [for recent examples with representative sampling from African and European distribution ranges, see Magri et al. (2007) in Quercus suber L.; Guzmán et al. (2017) in Chamaerops humilis L.; Villa-Machío et al. (2018) in Lavatera maritima Gouan], and other potentially important evolutionary processes could have been overlooked as a result. Polyploidy is recognized as one of the major evolutionary forces driving plant diversification by promoting adaptation to new ecological niches or conferring reproductive isolation (Otto & Whitton, 2000). In the Mediterranean region, examples of species experiencing polyploidization are numerous (Marques et al., 2018), and a few of them have been thoroughly explored from taxonomic, phylogenetic and ecological points of view (e.g. Jakob, Ihlow & Blattner, 2007; Balao et al., 2010; Bardy et al., 2010; Zozomová-Lihová, Marhold & Španiel, 2014) and these studies emphasized the importance of auto- and allopolyploidization in the richness of the Mediterranean flora. Biogeographical processes, including historical patterns of origin or migration, interactions among cytotypes and divergence in levels of environmental tolerance have typically been reported as the main factors determining the success of populations with different ploidies (Husband, Baldwin & Suda, 2013). However, the mechanisms of establishment and spread of polyploid complexes in the Mediterranean region are still poorly known (Marques et al., 2018). In this sense, more studies combining cytogeographical information, phylogeographical data and environmental niche modelling (ENM) in mixed-ploidy species are necessary to better understand the role of whole-genome multiplications in Mediterranean plant diversity. Artemisia herba-alba Asso (Asteraceae, Anthemideae) belongs to Artemisia L. subgenus Seriphidium (Besser ex Less.) Rouy and it has been referred to as a species complex from the Mediterranean region, with closely related taxa considered as independent species in the Irano-Turanian region, or as a single species (e.g. Vallès, 1987; Ouyahya & Viano, 1988; Vallès et al., 2011; Podlech, 2013; Bougoutaia et al., 2014). Recent phylogenetic studies (e.g. Malik et al., 2017) indicate that A. herba-alba s.s. would better constitute a single species basically distributed in the Iberian Peninsula and North-West Africa, whereas other Irano-Turanian taxa formerly included in the complex (e.g. A. inculta Delile, A. ol023iana J.Gay ex Besser and A. sieberi Besser; often treated as synonyms of A. herba-alba) were inferred to be evolutionarily distinct from A. herba-alba and should be considered as separate species. This perennial small shrub is an important component of Mediterranean dry steppe floras, being the main forage species in chamaephytic steppes of North Africa, where it covers c. 10 million ha (El Aich, 1992; Le Houérou, 2001). Consequently, A. herba-alba has been well studied from ecophysiological and grazing management perspectives (e.g. Escudero et al., 2000; Houmani, Houmani & Skoula, 2004). The species has also been commonly used in folk medicine, mainly in North Africa, and the biochemical diversity and activity of the plant have also been widely explored (Mighri et al., 2010; Mohamed et al., 2010; Younsi et al., 2018). From an evolutionary point of view, a few studies have focused on the cytogenetic diversity of A. herba-alba (e.g. Vallès, 1987; Ferchichi, 1997; Torrell & Vallès, 2001; Torrell et al., 2003; Betina, Khalfallah & Khelil, 2007; Bougoutaia et al., 2014, 2016), revealing the existence of two ploidies in the species in both European and North African populations. The distribution of diploid and polyploid (tetraploid) cytotypes of A. herba-alba in Algeria was explained as resulting from a process of genome differentiation, which could be related to environmental and biogeographical factors (Bougoutaia et al., 2016), but the limited sampling and missing phylogeographical context hampered further inferences on the role of ploidy during the evolutionary history of the species. The main objective of this study is to obtain a deeper and detailed knowledge of the natural history of A. herba-alba as a key species of the dry steppes from the Iberian Peninsula and North Africa. We hypothesize that the Strait of Gibraltar played an important role on shaping the genetic diversity of this species. Given the occurrence of distinct cytotypes, we also expect to find a genetic structure associated with ploidies, which could illuminate the origins of whole genome duplication in A. herba-alba. Finally, we suggest that current ecological differentiation among diploid and polyploid cytotypes could have driven the establishment and spread of this species in the western Mediterranean region. To test these hypotheses, we applied a multidisciplinary approach combining current genetic and cytogenetic analyses with ENM. Specifically, we: (1) used flow cytometry to assess the genome size and infer the ploidy of 185 individuals of A. herba-alba on both sides of the Mediterranean Sea; (2) reconstructed a phylogeographical framework for this species from 388 sequences of plastid DNA regions, which have been analysed as particularly useful markers when investigating heteroploid plant systems (Záveská et al., 2019); and (3) performed species distribution modelling, under present and past climatic scenarios, and calculated environmental differences between cytotypes. MATERIAL AND METHODS Sampling, genome size estimates and DNA sequencing Forty populations of A. herba-alba were sampled, covering the main distribution range of the species on the Iberian Peninsula (12 populations) and in North Africa (28 populations). Leaf material from five to ten plants per population was collected and stored fresh (for genome size analysis) and dried in silica gel (for DNA sequencing procedures). Voucher specimens were deposited at the herbarium BCN, of the Centre de Documentació de Biodiversitat Vegetal (Universitat de Barcelona). To minimize 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, geographical coordinates, number of analysed individuals and herbarium vouchers) are given in Table 1 and in the Supporting Information (Table S1). Table 1.Sampling information, estimated ploidy and haplotypes (Hd, haplotype diversity; π, nucleotide diversity) of studied Artemisia herba-alba populations Population code* . Collection data . N . Ploidy† . Haplotype(s) . Hd . π . P3 Algeria, Djelfa: Ben-Hamed 5 4x H1(5) 0 0 P4 Algeria, B. B. Arreridj: El-Esch 5 4x H1(5) 0 0 P2 Algeria, M'sila: Zerarka 5 2x/4x H1(2), H2(3) 0.600 0.0004 P7 Algeria, M'sila: Mohamed Boudiaf 5 2x H1(5) 0 0 P9 Algeria, M'sila: Oued Slimane 4 2x H1(2), H2(1), H3(1) 0.833 0.0007 P11 Algeria, Laghouat: Sebgag 5 4x H1(4), H4(1) 0.400 0.0027 P12 Algeria, Sétif: Hammam soukhna 5 4x H1(5) 0 0 P14 Algeria, Tiaret: Rechaiga 4 4x H1(4) 0 0 P15 Algeria, Tiaret: Ain Dheb 5 4x H1(5) 0 0 P17 Algeria, Biskra: Baniane 5 2x H2(1), H5(4) 0.400 0.0016 P19 Algeria, Batna: Arris 5 4x H1(5) 0 0 P26 Algeria, Tébéssa: Oum Ali 5 4x H1(2), H5(3) 0.600 0.0020 P31 Algeria, Souk-Ahras: Taoura 5 4x H1(5) 0 0 P33 Algeria, Saida: Ain Skhouna 5 4x H1(3), H6(2) 0.600 0.0004 P38 Algeria, S.B. Abbès: Marhoum 5 4x H1(5) 0 0 P40 Algeria, Tlemcen: El-Aricha 4 4x H1(4) 0 0 P60 Algeria, Tamanrasset: Tazrouk 7 2x H19(7) 0 0 P42 Tunisia, Sidi Bouzid: Jemla 5 4x H3(2), H5(2), H7(1) 0.800 0.0024 P43 Tunisia, Medenine: Neffatia 5 2x H1(5) 0 0 P44 Tunisia, Medenine: IRA 5 – H1(5) 0 0 P45 Tunisia, Medenine: Oued El Fedje 5 2x H8(5) 0 0 P59 Morocco, Marrakaech: Imagdal 3 2x H16(3) 0 0 P61 Morocco, Ouarzate: Taliouine 6 2x H20(6) 0 0 P62 Morocco, Ouarzate: Skoura 5 4x H4(3), H20(2) 0.600 0.0057 P63 Morocco, Midelt: Kerrandou 5 4x H4(5) 0 0 P64 Morocco, Guercif: Outat El Haj 5 4x H16(3), H21(1), H22(1) 0.700 0.0022 P65 Morocco, Nador: El Massira 5 4x H16(2), H23(3) 0.600 0.0004 P66 Morocco, Midar: Tafersite 5 2x/4x H16(2), H19(1), H24(2) 0.800 0.0009 P46 Spain, Aragon, Zaragoza: Bujaraloz 5 4x H9(1), H10(1), H11(3) 0.700 0.0009 P47 Spain, Aragon, Zaragoza: Calatayud 5 4x H9(4), H12(1) 0.400 0.0003 P48 Spain, Aragon, Zaragoza: Alhama de Aragón 2 4x H9(2) 0 0 P49 Spain, Madrid: Aranjuez 5 2x H13(1), H14(3), H15(1) 0.700 0.0027 P50 Spain, La Mancha, Ciudad Real: Argamasilla de Alba 5 2x H13(3), H17(2) 0.600 0.0020 P51 Spain, Andalusia, Jaén: Carchelejo 5 2x H13(3), H16(2) 0 0 P52 Spain, Andalusia, Granada: Cúllar 5 4x H13(3), H16(1), H17(1), H18(1) 0.900 0.0031 P53 Spain, Andalusia, Almería: Níjar 5 4x H16(5) 0 0 P54 Spain, Valencia, Castelló: Sonta 5 2x H9(5) 0 0 P56 Spain, Valencia, Castelló: Castellfollit de Riubregós 5 2x H10(4), H11(1) 0.800 0.0005 P57 Spain, Catalonia, Lleida: Arbeca 4 4x H10(4) 0 0 The genome size of 39 populations was estimated by flow cytometry at the Centres Científics i Tecnològics, Universitat de Barcelona (CCiTUB), following the procedures explained by Bougoutaia et al. (2016). Five individuals were analysed in most populations, except for P40 (four individuals), P19, P38, P45 (three individuals) and P48 (two individuals) due to availability of fresh material. Two independent replicates of each individual were performed. The analyses for a given population were all performed on the same day. Petunia hybrida Vilm. 'PxPc6' and Pisum sativum L. 'Express Long' (Marie & Brown, 1993) were used as internal standards. Seeds of the standards were provided by the Plateforme de cytométrie d'Imagerie-Gif, CNRS – I2BC (Gif-sur-Yvette, France). Nuclear DNA contents (2C) were calculated by multiplying the known DNA content of the standard by the quotient between the peak positions (mode) of the target species and the standard in the histogram of fluorescence intensities, assuming a linear correlation between the fluorescence signals from the stained nuclei of the unknown specimen, the known internal standard and the DNA amount (Doležel, 1991). The genome size values of 12 populations from Algeria (see Supporting Information, Table S1) were obtained from Bougoutaia et al. (2016). Leaf tissue dried in silica gel (c. 20 mg) was used for DNA extraction using the CTAB protocol (Doyle & Doyle, 1987) with minor modifications. The quality and quantity of DNA extracts were checked with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The plastid intergenic regions rpl32-trnL and ndhC-trnV were amplified and sequenced for all samples. The amplification procedure was performed as described by Malik et al. (2017). Direct sequencing of the amplified DNA segments was performed with Big Dye Terminator Cycle Sequencing v.3.1 (PE Biosystems, Foster City, CA, USA) at the Unitat de Genòmica (CCiTUB) on an ABI PRISM 3700 DNA analyser (PE Biosystems). The sequence primers used were the same as those for amplification. Sequences were edited and aligned using Chromas Lite v.2.01 (Technelysium PTY, Tewantin, Queensland, Australia) and Bioedit v.7.0.9 (Ibis Biosciences, Carlsbad, CA, USA). The alignment was conducted in Clustal W (Thompson, Higgins & Gibson, 1994) and adjusted manually. GenBank accession numbers are provided in the Supporting Information (Table S1). Genetic analyses Plastid haplotypes were determined for nucleotide substitutions in a combined data set that included both the rpl32-trnL and the ndhC-trnV regions. Gaps resulting from indels and mononucleotide repeat units were treated as missing data. The evolutionary relationships among haplotypes were inferred based on a parsimony TCS network constructed using PopArt (Leigh & Bryant, 2015) with default settings. Haplotype (Hp) and nucleotide (p) diversities were calculated for each population using DnaSP v.5.0 (Rozas & Rozas, 1999). The same indices were estimated for groups of samples according to ploidy level (i.e. diploid and tetraploid populations) and geographical origin (i.e. Iberian and African populations). Haplotype richness [R(n)] was computed with RAREFAC (Petit, el Mousadik & Pons, 1998), software that uses a rarefaction approach to standardize the haplotype richness to a fixed sample size to facilitate comparisons across groups of samples. In this case, the rarefaction value was set according to the sample size of the smallest groups of populations at ploidy and geographical levels (i.e. diploid group and Iberian group). The molecular phylogenetic reconstruction of A. herba-alba haplotypes was performed by Bayesian inference with MrBayes v.3.2 (Ronquist et al., 2012) based on the DNA sequences of the haplotypes obtained from the previous procedures. Artemisia annua L. and A. chitralensis Podlech were chosen as outgroups according to a phylogenetic study on Artemisia subgenus Seriphidium (Malik et al., 2017). Partitioning strategies and models of molecular evolution were selected with PartitionFinder v.2.1.1 (Lanfear et al., 2017). A scheme with two independent partitions (GTR model) was applied for both rpl32-trnL and ndhC-trnV intergenic spacers. Two independent Markov chain Monte Carlo (MCMC) analyses with four Metropolis coupled chains each were run for 10 million generations, sampling every 1000 generations. The first 25% of the trees were discarded as 'burn-in', after confirming that the average standard deviation of the split frequencies was < 0.01, and the potential scale reduction factor approached 1.0 for all parameters. The remaining trees were pooled to construct 50% majority-rule consensus trees that approximate the posterior probabilities. The existence of phylogeographical structure was evaluated by the permutation test between GST and NST (coefficients of genetic differentiation) implemented in PERMUT2.0 with 1000 permutations (Pons & Petit, 1996). The relationship between the genetic differentiation [DxyNei (1987), estimated through DnaSP] and the geographical distance per population pairs was determined through Mantel tests using three datasets: (1) all populations, (2) only Iberian populations and (3) only North African populations. Pairwise correlations between distance matrices were computed using 10 000 permutations with the function mantel available in the package 'vegan' (Oksanen et al., 2019) of R v.3.5.2 (R Core Team, 2018). Haplotype spatial genetic structure was further analysed with SAMOVA2 (Dupanloup, Schneider & Excoffier, 2002), carrying out a simulated annealing approach to identify population clusters. We explored K values (i.e. numbers of groups of populations) from 2 to 20, starting from 100 random initial conditions for each simulation, and chose the number of groups that gave the highest ΔFCT (i.e. FCT differences between groups). Finally, we also conducted analysis of molecular variance (AMOVA) in Arlequin v.3.5 with 10 000 replicates (Excoffier & Lischer, 2010) to measure variation among populations and to test the genetic differentiation between groups of populations according to: (1) SAMOVA clustering, (2) the main geographical regions (i.e. Iberian Peninsula and North Africa) and (3) ploidy. Ecological niche analyses We employed ENM to analyse the potential distribution of A. herba-alba under present climatic conditions. We used MaxEnt v.3.3 (Phillips, Anderson & Schapire, 2006) software and employed the maximum entropy algorithm. Nineteen bioclimatic variables (at 30-s resolution) under current conditions and an elevation layer were obtained from the database of the WorldClim website (Fick & Hijmans, 2017) and clipped to cover the Iberian Peninsula and North Africa. After a combination of a correlation analysis in a random sample of 1000 points within the study area plus jackknife and per cent contribution analyses to evaluate the relative importance of each variable, 11 relatively uncorrelated (r < |0.85|) variables were selected [bio1 (annual mean temperature), bio2 (mean diurnal range); bio3 (isothermality); bio4 (temperature seasonality); bio6 (minimum temperature of the coldest month); bio8 (mean temperature of the wettest quarter); bio9 (mean temperature of the driest quarter); bio12 (annual precipitation); bio15 (precipitation seasonality); bio18 (precipitation of the warmest quarter); and elevation]. Two soil variables (pH and organic content measured at 15 cm depth) were downloaded from ISRIC (World Soil Information: www.isric.org) and added to the dataset. These 13 variables were used together as predictors to calibrate the species distribution model. In the occurrence dataset, we used the original geographical coordinates corresponding to the sampled populations of A. herba-alba (Fig. 1; Table 1). These occurrences were randomly split into training data (80%) and test data (20%), and 100 subsampled replicates were run for model evaluation, with the threshold obtained under the maximum training sensitivity plus specificity rule. The distribution model under current conditions was projected to the Last Glacial Maximum (LGM; c. 21 kyr BP) under two models: the community climate system model (CCSM; Collins et al., 2006) and the model for interdisciplinary research on climate (MIROC; Watanabe et al., 2010). Because no scenarios are available for the LGM performance of elevation and soil variables, they were discarded from the LGM projection models. Open in new tabDownload slideA, geographical distribution of the cytotypes and the plastid DNA haplotypes (see Table 1 for population codes) found in Artemisia herba-alba. B, statistical parsimony network of relationships between the haplotypes. Black bars represent unsampled intermediate haplotypes, one base mutation distant. The size of the circles represents the number of individuals. The ploidy of population P44 could not be assessed. To compare the ecological niche between cytotypes, independent ENM analyses were performed for each subset of populations. In these cases, we used the same 13 variables and MaxEnt settings employed for the whole dataset under current climatic conditions. To calculate the differences on geographically suitable areas between cytotypes, the maximum sensitivity plus specificity (MSS) logistic threshold was used, a metric recommended as being robust with all data types (Liu, Newell & White, 2016); MSS was used as the 'cut-off' value to transform the continuous value outputs of MaxEnt to binary maps (absence/presence). Niche similarity between these two groups of populations was assessed by estimating Hellinger-derived I and Schoener's D indices (Warren, Glor & Turelli, 2008) calculated with the niche overlap test implemented in the software ENMTools v.1.4.3 (Warren, Glor & Turelli, 2010). A test with 100 pseudo-replicates was calculated to generate a distribution of the expected values of each index. Histograms were constructed after performing both tests to visualize the niche differentiation. The differences on niche breadth of the different cytotypes was measured using the 'inverse concentration' B1 (Levins, 1968) and the 'uncertainty' B2 metrics in ENMTools, using 100 subsample iterations from MaxEnt to account for model uncertainty. Niche differences between diploid and tetraploid plants were also evaluated by a principal components analysis (PCA) approach using the same 13 variables. A three-dimension environmental space for each group of plants was generated based on the observed occurrences defined by the first three axes that were identified by the PCA. Finally, we used Wilcoxon signed-rank and Levene tests to explore the environmental differences (of medians and variances, respectively) between diploid and tetraploid cytotypes. These last statistical analyses (i.e. PCA, Wilcoxon signed-rank and Levene tests) were performed in R v.3.5.2 (R Core Team, 2018) with the 'Rcmdr' package (Fox & Bouchet, 2020). RESULTS Cytogenetic and genetic data Nuclear DNA amount data for the 39 studied populations (185 individuals) of A. herba-alba are presented in the Supporting Information (Table S1). Average nuclear DNA amounts ranged from 5.93 to 7.76 pg for diploid accessions and from 11.53 to 13.84 pg for tetraploids (Table S1). The relatively wide dispersion of genome size (GS) estimations within each ploidy could be due to genuine cytogenetic variation. For instance, the presence of B chromosomes has already been reported in A. herba-alba (Torrell et al., 2003), potentially causing slight differences in nuclear DNA amount. However, although the coefficient of variation (CV) of the 2C peaks was always < 5%, we cannot be certain that technical issues (e.g. sample conservation) affected the precise GS estimation of certain populations. Therefore, we only employed the results of flow cytometry assessments to infer ploidy of specimens. These GS estimates revealed that 23 of the analysed populations contain only tetraploid plants and 14 only contain diploid plants. In two North African populations (P6 and P66), diploid and tetraploid individuals were intermixed. Diploid and tetraploid populations were present on both sides of the Mediterranean Sea, although showing a scattered geographical distribution in each continent (Fig. 1). The sequences of the rpl32-trnL and ndhC-trnV intergenic spacers were aligned in two matrices containing 812 and 810 nucleotides, respectively. Both plastid DNA regions showed a noticeable level of polymorphism among the 194 specimens of A. herba-alba analysed in this study. Specifically, 27 and 11 polymorphic (segregating) sites were observed for the rpl32-trnL and ndhC-trnV markers, respectively. Based on analysis of the concatenated matrix, we were able to identify 24 haplotypes across the 40 studied populations (Table 1). The TCS parsimony revealed a relatively complex evolutionary structure (Fig. 1), from frequent (e.g. H1; 71 individuals) to rare haplotypes (e.g. H7, H12, H15, H18, H21 and H22; one individual each) connected by one to six mutation steps. Only one haplotype (H16) was found both in the Iberian Peninsula and in North Africa; the rest occurred only in one of the regions (15 haplotypes in North Africa and ten on the Iberian Peninsula). However, haplotypes from different sides of the Strait of Gibraltar were intermixed according to the evolutionary relationships shown by the parsimony network (Supporting Information, Fig. S1). Regarding the ploidy of populations, the haplotype network was not structured according to cytotype distribution: most haplotypes (13) were shared by diploid, tetraploid and/or populations showing both ploidies (Fig. S2). Half of the populations harboured only one haplotype, and the others showed different levels of haplotype and nucleotide diversity (Table 1). From a phylogeographical point of view, the samples from North Africa contained a greater number of haplotypes (15) than those from the Iberian Peninsula (10), but the latter showed higher genetic variability in terms of haplotype and nucleotide diversity (Table 2). Haplotype richness calculated after rarefaction [R(56)] was higher in North Africa than on the Iberian Peninsula. Regarding the genetic variability among cytotypes, tetraploid populations contained more haplotypes (19) compared with diploid ones (14), but haplotype and nucleotide diversity, as well as haplotype richness [R(10)] were higher in diploids than in tetraploids (Table 2). The phylogenetic reconstruction of A. herba-alba haplotypes (Fig. 2) inferred the existence of several strongly supported monophyletic lineages, most of them in derived positions of the tree. In contrast, early-diverging haplotypes were not grouped in statistically supported lineages. With plastid haplotypes being, most of them, exclusive to the geographical distribution or to the ploidy of populations. Table 2.Genetic variability values for the geographical and cytogenetic groups of populations defined in the study . No. of sampling sites . N . Hp . Hd . R (n) . π . Iberian Peninsula 12 56 10 0.866 9.000 0.00327 North Africa 28 138 15 0.840 10 914 0.00265 Diploid populations 14 60 14 0.921 6.092 0.00325 Tetraploid populations 23 119 19 0.751 4.361 0.00262 Both ploidies 2 10 5 0.867 4.000 0.00218 All populations 40 194 24 0.836 – 0.00300 Open in new tabDownload slidePhylogenetic tree of Artemisia herba-alba haplotypes based on Bayesian inference of the combined plastid regions. Posterior probability (PP) values are shown above branches. The colour of the squares placed over the terminal branches indicates the geographical distribution of the haplotypes. The rings in greyscale indicate the ploidy level of populations containing each haplotype. The permutation test showed that haplotypes sampled from within populations are phylogenetically closer than haplotypes sampled from different populations (NST = 0.731, GST = 0.651; P < 0.01), indicating the existence of phylogeographical signal (Pons & Petit, 1996). The Mantel test found a significant correlation between the pairwise genetic differentiation and the geographical distance of populations from North Africa (r = 0.4736; P < 0.005), but not for the whole A. herba-alba dataset (r = 0.1723; P > 0.005) or for the Iberian populations (r = 0.1284; P > 0.005). Spatial genetic analyses of plastid DNA haplotypes using SAMOVA indicated that the largest increase of FCT values occurred between K = 2 and K = 3 (Supporting Information, Fig. S3). The genetic structure shown by K = 2 clustered the populations from the Iberian Peninsula and Morocco plus two populations from Algeria (i.e. P17 and P26) in one group and the remaining populations from Algeria (Fig. S4). The structure depicted by K = 3 showed one group constituted by Iberian and Moroccan populations, a second group with Iberian, Moroccan and Algerian populations, and a third cluster entirely constituted by the majority of populations from Algeria (Fig. S4). The results of AMOVAs studying the partitioning of genetic diversity are summarized in Table S2. Non-hierarchical AMOVA showed that 78.02% of the variation was explained by differences among populations, and 21.98% was explained by differences within populations. Hierarchical AMOVA according to the phylogeographical structure inferred by SAMOVA showed that genetic differences among K = 2 clusters explained 59.94% of the variance, and genetic differences among K = 3 clusters explained 71.23% of the variation. Running a hierarchical AMOVA in which the two main geographical regions were considered (North Africa and Iberian Peninsula) showed that differences among those groups represented 28.36% of the genetic variance. Hierarchical analysis with ploidy defining two groups of populations revealed that only 2.00% of the genetic variation was attributable to the between-cytotype component. Ecological niche analyses The distribution model of A. herba-alba under current conditions (Fig. 3) spanned its current distribution in the western Mediterranean Basin, including most sampled localities of the species. The mean areas under the receiver operating characteristic curve (AUC; a measure of model fitness) for testing data was high (0.927), supporting the predictive power of the model. The standard deviation of the 100 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 bio12 (annual precipitation), bio1 (annual mean temperature) and the soil organic content, which therefore appeared to be the most informative (see Supporting Information). The environmental variables that reduced the gain the most when omitted was the pH of the soil, therefore appearing to have the most information that is not present in the other variables (see Supporting Information). The CCSM and MIROC models for the LGM yielded almost identical inferences for the palaeodistribution of A. herba-alba as the model constructed using the same ten variables from the present (Fig. 3). In all cases, AUC values were reasonably high (> 0.80), the most important variables being bio12 (annual precipitation), bio6 (minimum temperature of coldest month) and bio18 (precipitation of warmest quarter). The two LGM models predicted only minor area changes (i.e. an increase of c. 7%) compared to the present distribution (Supporting Information, Table S3). Open in new tabDownload slidePotential distribution maps of Artemisia herba-alba obtained with MaxEnt under different modelling conditions. A, present model with 13 environmental variables; C, Last Glacial Maximum CCSM model with ten environmental variables; D, Last Glacial Maximum MIROC model with ten environmental variables. Dots indicate sampled populations for this study. Independent ENM analyses predicted the potential current distribution ranges for each subset of populations showing different ploidies (Fig. 4A, B). Both models provided reasonably good estimates of the potential range distributions of the two cytotypes, when compared with their current occurrence in the studied area. The area under the curve values were high (> 0.80) in both cases (Supporting Information, Table S3), indicating a strong predictive power for the models (Loo, Mac Nally & Lake, 2007). Both models supported the occurrence of A. herba-alba in similar regions of the Iberian Peninsula and North Africa, but the total predicted area for tetraploid populations was lower (~55%) than for diploid populations (Fig. 4C, D), indicating that the environmental niches are equivalent. Conversely, according to both estimated B1 and B2 metrics, niche breadth less showed significant differences between ploidies (Fig. 4E, F), suggesting that the environmental niche of diploid cytotypes is larger than that of tetraploid populations. PCA using the 13 environmental variables captured 75.2% of the variance in the first three components (PC1: 41.3%, PC2: 21.4%, PC3: 12.5%; Table S4). The 3D scatterplot for the first three components (Fig. S5), showing the ellipsoid that contains 50% of the data, showed that the environmental space of both cytotypes partially overlaps, with the niche of diploids being larger and containing that of the tetraploids. We did not find obvious environmental niche shifts between diploid and polyploid populations based on independent Wilcoxon signed-rank and Levene tests (Table S6; P > 0.05 in all cases). However, Levene tests indicated that diploid populations show larger variances for bio12 (i.e. annual precipitation), organic content of the soil and pH of the soil than tetraploid populations (P < 0.05; see Table S6). Open in new tabDownload slideNiche comparison analyses between ploidies of Artemisia herba-alba populations. Independent environmental niche modelling for diploid (A) and tetraploid (B) populations of the species. Observed niche overlap values for the Hellinger-derived I index (C) and Schoener's D index (D) compared with a null distribution. In all cases, the similarity score (red arrow) is contained within the distribution predicted by the null hypothesis for niche equivalency, indicating that the environmental niches are equivalent. Niche breadth test according to the B1 index (E) and B2 index (F) indicating that the environmental niche of diploid cytotypes is significantly larger than that of tetraploid populations. DISCUSSION Comparably intricate phylogeographical patterns of A. herba-alba on both sides of the Strait of Gibraltar Phylogeographical studies on plants distributed in both the northern and the southern parts of the Mediterranean Basin have frequently revealed European lineages to be nested in African clades and higher genetic diversity and differentiation at the intraspecific level in North Africa than in southern Europe (e.g. Guzmán & Vargas, 2009; Casimiro-Soriguer et al., 2010; Veríssimo et al., 2016; García-Aloy et al., 2017; Bobo-Pinilla et al., 2018; Villa-Machío et al., 2018). Conversely, a few plant species occurring on both sides of the Strait of Gibraltar have shown the opposite pattern, i.e. the Iberian Peninsula is home to the most ancestral lineages and higher genetic diversity values (e.g. Escudero et al., 2008; Jaramillo-Correa et al., 2010). The complex phylogeographical structure we found in A. herba-alba does not clearly fit either of the two previous patterns. First, levels of haplotype and nucleotide diversity were similar between the Iberian Peninsula and North Africa (Table 2). Second, early-diverging haplotypes were observed in Iberian and North African populations (Fig. 2). Indeed, the evolutionary relationship between the haplotypes indicated the occurrence of several lineages on both sides of the Strait, independently of their phylogenetic distance (Fig. 1). Finally, partitioning schemes inferred by the spatial analyses of molecular variance explained a large proportion of the genetic variability of the species (K = 2, 59.94%; K = 3, 71.23%), but the biogeographical structure they showed was not related to the division of the Mediterranean Basin (Supporting Information, Fig. S4). Conversely, differentiation across the continents only accounted for 28.36% of the total molecular variance (Table S2), this result providing further evidence that the Strait is not a major phylogeographical barrier for this species. To decipher the phylogeographical signals of a species we need to consider its biological features and how past climatic changes affected its particular distribution (Hewitt, 2004). Range expansions and contractions associated with Quaternary climatic oscillations are regularly related to extinction and recolonization processes, resulting in contrasting genetic patterns between refugia (e.g. higher diversity and endemism) and recently colonized regions (e.g. lower diversity and higher uniformity). In contrast, phylogeographical studies under climatic stability scenarios usually report high broad-scale diversity and spatial genetic complexity (Bilton et al., 1998; Qu et al., 2014; Faye et al., 2016). Specifically, cold-tolerant taxa are inferred to have exhibited widespread populations in continental lowlands of the Iberian Peninsula during full glacial stages, promoting the mixture of lineages or greater shifts in the spatial location of populations (Abellán & Svenning, 2014). ENM of A. herba-alba, which could be considered a chilling-tolerant species (Lyons, 1973) according to the values of minimal temperature of the coldest month (i.e. bio 6) in our studied localities, indicated the occurrence of similarly stable niches on the Iberian Peninsula and in North Africa under present and past LGM conditions (Fig. 3). Therefore, the intricate genetic structure of A. herba-alba, appearing unrelated to the geographical split established by the Strait of Gibraltar, fits the ecological stability inferred by our niche modelling results. Despite the lack of a clear phylogeographical signal related to the division of the Mediterranean Basin, our analyses show certain genetic distinctiveness of the Algerian and Tunisian populations from the Moroccan and Iberian populations. Excluding the isolated Tamanrasset population, samples from Algeria to Tunisia have exclusive haplotypes (H1, H2, H3, H5, H6, H7 and H8) of this area (Fig. 1), and they only have one haplotype (P11, H4) which is shared with two populations of Morocco (P62 and P63). On the other hand, Morocco and the Iberian Peninsula share H16 between eight populations (P59, P64, P65, P66, P51, P52, P53 and P54). This east–west separation is also observed in SAMOVA with K = 2 (Supporting Information, Fig. S4a), being also supported by Mantel tests showing significant spatial auto-correlation for North African populations but not for the whole dataset including populations from both continents. The Tamanrasset population (P60), which is approximately as distant from the rest of the Algerian populations as from the Moroccan populations, shares H19 with one population of Morocco (P66) and could be the result of a long-distance dispersal event to the south of Algeria. The Moroccan–Algerian phylogeographical split in A. herba-alba is consistent with some other intraspecific diversification patterns on western Mediterranean plants (Terrab et al., 2008; Naciri, Caval & Jeanmonod, 2010; Talb et al., 2020). This east–west disjunction in North Africa could be explained by a vicariance model with geographical breaks such as the Atlas range (e.g. Coque-Castells & Jansen, 2003; Andrés-Sánchez et al., 2015) or the Rifan corridor crossed by the Moulouya river (e.g. Beddek et al., 2018) having stronger effects as barriers than the Strait of Gibraltar. Alternatively, our results could reflect ancient separate origins of the Moroccan and Algerian genetic pools (e.g. Magri et al., 2007; Sánchez-Robles et al., 2014). Solving this question would require molecular dating and biogeographical analyses that include other Mediterranean taxa closely related to A. herba-alba. Besides the geological and climatic context during the evolutionary history of A. herba-alba, other biological characteristics probably played a role in shaping the phylogeographical patterns we have reported above. Most Artemisia spp. are wind-dispersed, and although achenes are lacking pappi, their relatively small size and light weight allow long-range dispersal to take place frequently (Laursen et al., 2007). Species with propagules dispersed by wind have been reported to occur disproportionately on both sides of the Strait of Gibraltar (Lavergne, Hampe & Arroyo, 2013). Artemisia herba-alba is also an ecosystem dominant species, showing expansive distributions in dry steppes of the Iberian Peninsula and North Africa (Vallès, 1987; Le Houérou, 2001). Large population sizes, together with the stable habitats as suggested by our ENM results, have generally been associated with retention of ancestral polymorphisms (Schaal et al., 1998). Indeed, the importance of incomplete lineage sorting mechanisms during the evolution of Artemisia subgenus Seriphidium has already been suggested by Malik et al. (2017). The complex mosaic-like haplotype distribution in A. herba-alba, in which some areas are dominated by certain lineages occasionally intermingled with other genetically unrelated haplotypes, could therefore be related to the dispersal and demographic characteristics of the species (for additional details see Vallès, 1989). Further studies using highly variable nuclear markers (e.g. microsatellite or next-generation sequencing approaches) would be necessary to confirm the role played by these and other factors in the evolutionary history of A. herba-alba. Absence of a clear genetic structure related to the diploid–polyploid cytotype distribution Polyploidy is one of the available literature recently reported that geographical barriers seem to play a major role in driving the emergence and establishment of polyploid complexes in the Mediterranean flora (Marques et al., 2018). In contrast, diploid and tetraploid populations of A. herba-alba were distributed without a clear geographical pattern, being equally well represented on both sides of the Strait of Gibraltar (Fig. 1). From a phylogeographical point of view, many haplotypes were found in both diploid and tetraploid populations, and evolutionarily early-diverging haplotypes were present in populations showing either of the two cytotypes (Fig. 2). Moreover, AMOVA indicated that the ploidy of populations was not significantly associated with the genetic structure of the species (Supporting Information, Table S2). These results support the hypothesis that multiple events of whole genome duplication, giving rise to tetraploid populations from diploid populations, occurred during the evolutionary history of A. herba-alba, probably on both sides of the Mediterranean Sea. Shared genetic background between co-occurring cytotypes is usually associated with repeated in situ formation of autopolyploids via unreduced gametes (Kolář et al., 2017). Regarding A. herba-alba, autopolyploidy is also supported by morphological homogeneity (Vallès, 1987) as well as similar karyotypes (Vallès & Siljak-Yakovlev, 1997) between diploid and tetraploid populations. Recurrent autopolyploidization events have been inferred in many other plant species showing similar combinations of phylogeographical and cytogeographical patterns (e.g. Segraves et al., 1999; Yamane, Yasui & Ohnishi, 2003; Mairal et al., 2018). Specifically, multiple origins of autopolyploids have also been reported in Artemisia tridentata Nutt. (Richardson et al., 2012), an evolutionarily distant congener showing noticeable ecological diversity across the species studied here. The alternative hypothesis of frequent crosses between diploid and tetraploid genotypes as a source of haplotype diversity in A. herba-alba tetraploids is unlikely due to the lack of autotetraploids (tetraploids in which diploid genotypes of A. herba-alba tetraploids is unlikely due to the lack of autotetraploids (triploids, 3x) would be expected as a mediator of gene flow (Kolář et al., 2017)]. The vast majority of populations we studied (all except two) exclusively showed either diploid or tetraploid individuals, suggesting the existence of reproductive barriers between the two cytotypes and the occurrence of frequency-dependent exclusion by minority cytotype disadvantage (Levin, 1975; Husband, 2000]. The recurrent origins of autotetraploids, together with the high production of anemochorous dispersed achenes of A. herba-alba (Vallès, 1989), could explain the broad occurrence of both diploid and polyploid cytotypes across the distribution range of the species, but it is not sufficient to explain their maintenance. Under a scenario of minority disadvantage, newly originating cytotypes would experience frequency-dependent selection and they would be excluded by drift from the populations of the progenitors in a few generations (Levin, 1975). Therefore, changes in environmental requirements, promoting eco-spatial segregation and within-cytotype mating, would be necessary for the establishment and persistence of populations with different ploidies (Felber, 1991). The presence of within-cytotype gene flow in A. herba-alba is supported by the frequency of diploid and tetraploid populations showing more than one haplotype (Fig. 1). Regarding the ecological differentiation, our modelling comparisons did not support a significant shift in the environmental niche of either cytotype, but the analyses revealed a significant variation in their niche breadths (Fig. 4). Despite the similarly wide range of distribution of both cytotypes, a larger environmental space in diploid than in tetraploid populations was also inferred by their predicted potential areas (Supporting Information, Table S3). Finally, PCA results illustrate that the tetraploid niche is fully nested in the niche breadth of diploids (Fig. S5). Niche comparisons between cytotypes in which the niche of one ploidy is narrower and nested in the niche of the other cytotype have been interpreted as indicating environmental specialization (e.g. Parisod & Broennimann, 2016; Castro et al., 2019). Therefore, to establish successfully, tetraploids of A. herba-alba could be thriving in particular areas of the landscape where they would outcompete the progenitor diploids and, thus, avoid the minority cytotype exclusion. According to our statistical tests to explore the environmental variable differences between ploidies, tetraploid populations specifically showed narrower ranges in annual precipitation, organic content of the soil and pH of the soil (Supporting Information, Fig. S5). The narrower variance shown by tetraploid A. herba-alba for the two studied soil variables, particularly for the organic content, could indicate this specialization in disturbed habitats. However, to test this hypothesis, reciprocal transplant experiments would be necessary to confirm that tetraploids are more or less able to develop than diploid under certain environmental conditions. CONCLUSIONS To our knowledge, this is the first study combining DNA sequencing, genome size assessments and niche modelling on a plant species widely distributed on both sides of the Mediterranean Sea. Such an integrative approach enabled the inference of various unexpected phylogeographical and cytogeographical patterns. Our results suggest that the Strait of Gibraltar did not play a major role in shaping the genetic diversity and structure of A. herba-alba, and we did not find evidence of any particular area acting as a genetic reservoir or refugium for this species. Likewise, multiple polyploidization events were inferred to have occurred in different regions during the evolutionary history of the plant. According to our environmental modelling results, palaeoecological stability, together with the dispersal and demographic characteristics of the species, was hypothesized as a potential driver of the intricate geographical distribution of genotypes and cytotypes reported here. The environmental comparisons among cytotypes indicated that polyploidization did not cause a significant shift in the niche of A. herba-alba, but tetraploids showed narrower ecological preferences that could explain their frequent and successful establishment. Overall, as we suggested for the evolution of Artemisia subgenus Seriphidium (Malik et al., 2017), our study highlights a complex natural history that underlies the morphological uniformity in this key species of the Mediterranean dry steppes.

Cytogenetic studies in the genus Artemisia L. (Asteraceae): fluorochrome-banded karyotypes of five taxa, including the Iberian endemic species Artemisia barrelieri Besser. Canadian Journal of Botany: –. , , , , , , , , , , , . Pleistocene diversification in Morocco and recent demographic expansion in the Mediterranean pond turtle Mauremys leprosa. Biological Journal of the Linnean Society: –. , , , , . Out of North Africa by different routes: phylogeography and species distribution model of the western Mediterranean Lavatera maritima (Malvaceae). Botanical Journal of the Linnean Society: –. , , , , , , , , , , , , , . Improved climate simulation by MIROC5: mean states, variability, and climate sensitivity. : –. , , , . Intraspecific cpDNA Variations of diploid and tetraploid perennial buckwheat, Fagopyrum cymosum (Polygonaceae). American Journal of Botany: –. , , , , . Relationship between chemotypic and genetic diversity of natural populations of Artemisia herba-alba Asso growing wild in Tunisia. : –. , , , , , , . Multiple auto-and allopolyploidisations marked the Pleistocene history of the widespread Eurasian steppe plant Astragalus onobrychis (Fabaceae). Molecular Phylogenetics and Evolution: . , , , , . Taxonomy and evolutionary history of Alyssum montanum (Brassicaceae) and related taxa in southwestern Europe and Morocco: diversification driven by polyploidy, geographic and ecological isolation. : –. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (, which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com Original Articles artemisia herba alba essential oil. artemisia herba alba asso. artemisia herba-alba tea. artemisia herba alba medicinal uses. artemisia herba-alba seeds. artemisia herba-alba oil. artemisia herba-alba extract. artemisia herba alba pdf

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