Genetic and morphologic diversity of European fan palm (*Chamaerops humilis* L.) populations from different environments from Sicily

ANTONIO GIOVINO[1], SILVIA SCIBETTA[1], SERGIO SAIA[1]* and CARMINE GUARINO[2]

[1]*Consiglio per la ricerca e la sperimentazione in agricoltura (CRA), Unità di ricerca per il recupero e la valorizzazione delle specie floricole mediterranee (CRA-SFM), S.S. 113 – km 245 500, I90011 Bagheria (PA), Italy*
[2]*Department of Biological and Environmental Sciences, University of Sannio, Via Port'Arsa, 11 I82100 Benevento, Italy*

Chamaerops humilis is decreasing in abundance in Mediterranean Europe, which has induced the European community to call for its protection in Special Areas of Conservation. However, information about its genetic and morphological variability, which is crucial to the development of any conservation strategies, is insufficient. The present study aimed to investigate the genetic and morphological variability of *C. humilis* in Sicily, which was selected as a model because of the high number of dense populations. The relationships between morphological traits and climatic variables were studied to highlight patterns of adaptation to the environment, along with the genetic similarity among the populations. Ten natural populations were sampled, analyzed using 28 specifically designed SSR primers, and evaluated based on 29 morphological traits. The populations were clustered similarly based on genetic and morphological traits. Heterozygosity was high and inbreeding coefficients were low. These results, along with higher intra- than inter-population differentiation, suggest that *C. humilis* populations in Sicily differentiated from a common ancestor and that inter-population variation arose from secondary evolution processes induced by ecological adaptation. The correlations between climatic variables and morphological traits suggest that the morphological adaptation to arid environments depends more on summer temperatures than on evapotranspiration or rainfall and that autumn and winter temperatures are determinants of the species establishment at new sites. Considering the response of *C. humilis* to seasonal temperatures, the present results indicate this species as a candidate for tracking climatic changes in Europe. Further studies are needed to highlight the adaptation of *C. humilis* to cold environments. Palaeo-climatological and -ecological studies could help clarify its strategies for the colonization of new sites.   © 2014 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2014, **176**, 66–81.

ADDITIONAL KEYWORDS: arid environment – climatic variables – drought tolerance – ecology – Mediterranean – morphological traits – structure – SSR.

INTRODUCTION

Chamaerops humilis L. (European fan palm) is the most northerly occurring palm in Europe, where it plays an important ecological role in the semi-arid Mediterranean vegetation system, especially in xerophytic shrub communities and degraded ecosystems,

*Corresponding author. E-mail: sergio.saia@entecra.it

as a result of its ability to grow in harsh conditions such as rocky or poor soils or Mediterranean semi-arid and arid climates. In addition, *C. humilis* is one of the most cold-tolerant palm species and ranges all along the Mediterranean seacoast up to the Portofino promontory (Liguria, northern Italy), presently its northernmost natural population. Despite the wide Mediterranean distribution, the occurrence of natural populations of *C. humilis* is progressively declining as

a result of several factors, such as anthropic disturbance (fire, plant and seed collection for ornamental use, agricultural land reclamation, etc.), climate change, and wild and domesticated animal grazing, all comprising stresses that result in both the fragmentation of the habitats and a reduction in the number of specimens per population, particularly those on small Mediterranean islands. These factors have motivated some European regions to call for protection for the species in the form of Special Areas of Conservation as defined in the European Union Habitats Directive (92/43/EEC). However, many European regions still lack such a protection law, and the available information about the genetic and morphological variability of the natural populations of the European fan palm is insufficient. The quantification of inter-population diversity is an important issue in many diversity hotspots, especially when natural plant populations are threatened. This information is pivotal for estimating the extent of divergence among populations, identifying evolutionarily significant units, and preserving their genomes through the conservation of remnant populations of threatened species and the establishment of management priorities for the unthreatened ones. Presently, only two varieties of *C. humilis* are formally described from a botanical point of view (Govaerts & Dransfield, 2005), depending primarily on leaf colour. However, the Kew Checklist of Palms still reports > 20 unaccepted subspecific synonymic epithets among varieties, subvarieties, and formae, which implies that *C. humilis* classification is variable and that the numerous taxa described cannot be upheld.

Molecular approaches have been successfully used in many plant studies to estimate population variability (Juan *et al*., 2011). Recently, a specific SSR primer set for *C. humilis* var. *humilis* has been developed (Arranz *et al*., 2013), representing a suitable tool for a genetic diversity evaluation of the natural populations of this taxon.

The present study aimed to investigate the genetic and morphological variability of ten natural *C. humilis* populations and their relationship with the environment, to support a conservation strategy for this species, to highlight the importance of the genetic origin and the environment for the expression of important morphological traits that could be used as botanical descriptors, and to evaluate whether this species can be used as a bioindicator of global climatic change. To achieve these goals, Sicily was chosen as a model because: (1) it is the area with the highest number of natural populations in Europe; (2) *C. humilis* populations still include a high number of individuals, which ensures the collection of a genetically stable assortment of plants; (3) the island has high environmental variability and is a genetic

hotspot, which may have contributed to a high differentiation of the populations; and (4) some populations are from small isolated islands, receiving only rare influxes of genetic material from the main island.

MATERIAL AND METHODS

GERMPLASM COLLECTION AND CHARACTERIZATION OF THE COLLECTION SITES

Samples were collected from *C. humilis* individuals in natural populations distributed across Sicily, Italy (Fig. 1). According to APAT indications concerning germplasm collection from uniform populations, ten individuals, half male and half female, were randomly sampled from each population for morphological analysis. Seven hundred and five individuals were sampled for the genetic analysis. For each population, the sample size (N) varied between 27 and 123 individuals, with a mean of 70.5 (Table 1).

The samples were collected during flowering and fruit setting. Leaves, inflorescences, and fruiting spates were collected for further analyses. A seed bank and an *in vivo*, *ex situ* germplasm collection were created at the Unità di ricerca per il recupero e la valorizzazione delle Specie Floricole Mediterranee (CRA-SFM), Bagheria, PA (Italy). Voucher specimens from each population were also collected and deposited at the CRA-SFM. For molecular analysis, fresh young leaves were collected, frozen in liquid nitrogen and stored at –80 °C for further analyses.

For each collection site, the main long-term climatic parameters were obtained from data collected by the closest weather station with at least 30 years of consistent data, as reported by Cartabellotta *et al*. (1998). The climatic data considered were rainfall (R); mean maximum, mean minimum, and overall mean temperatures (T) on a per season basis; the number of rainy days per year; and mean maximum, mean minimum, and overall mean potential evapotranspiration (ETP) on a per-year basis. The climatic indices calculated were: the FAO-UNEP aridity index as R/ETP (AI); the Lang index (Ir), as R/T; the de Martonne index (Ia), as $R/(T + 10)$; the Thornthwaite index (Im), as $(R \times ETP)/ETP \times 100$; the Emberger index (Iq) as $[R/(TH^2 – TC^2)] \times 100$, where TH and TC are the mean maximum temperatures of the hottest and mean minimum temperatures of the coldest months, respectively. The soil on the island of Lipari was classified as Xerands according to the USDA Soil Taxonomy. The soils from the other collection sites were classified as Xerorthents.

MORPHOLOGICAL ANALYSIS

Morphometric descriptors were selected from those reported by the IPGRI (2005) because of their
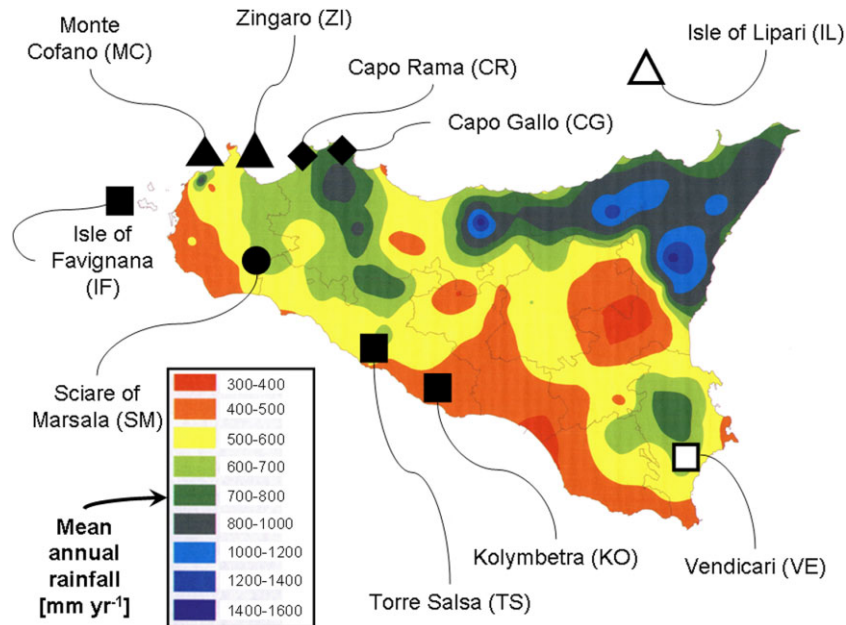
Figure 1. Locations of the collection sites of the ten *Chamaerops humilis* populations in Sicily, Italy. Mean annual rainfall is shown. Sites with the same symbol are from the same cluster as defined in the CA.

discriminating efficiency. Twenty-nine plant morphological traits were measured: height and diameter of whole plant, trunk, and crown, length of petiole and lamina, depth of leaflet split, leaf hair density, mean number of thorns on a whole petiole, thorn density on the petiole base (10 cm from the insertion on the trunk) and on the apex (10 cm from the insertion of the leaflets), mean length of thorns, mean number and size of inflorescences, infructescence size, fruit shape, ratio of trunk height to plant height, ratio of crown height to plant height, ratio of crown height to trunk height, ratio of crown diameter to crown height, crown and trunk volumes, ratio of depth of leaflet split to the total lamina length, leaf area, and leaf margin. For seeds and fruits (retrieved for only six of the ten populations sampled), dimensions were taken, and the area, perimeter, major and minor axis lengths, elongation, roundness, Feret diameter (which is calculated as the ratio between actual perimeter and π) and compactness were measured on at least 80 fruits per population. This relatively high number of plant morphological descriptors was needed to dissect the relationship between morphological and environmental variables.

MORPHOLOGICAL AND ENVIRONMENTAL DATA ANALYSIS

All of the variables corresponding to proportions were arcsine transformed before analysis to better fit a Gaussian distribution. Two separate principal component analyses (PCA) were performed on the plant morphological data from the ten populations and on the fruit and seed data from only six populations considered in the present study (Princomp procedure, SAS Institute, 2002) to highlight the importance of different traits in explaining multivariate polymorphisms. The populations from Torre Salsa (TS), Capo Gallo (CG), Favignana islands (IF), and Lipari (IL) were not included in the fruit analysis because no fruit was retrieved from these collection sites. Before the PCAs, the values for each trait were standardized to a mean of 0 and an SD of 1. The correlations among variables were calculated and, when two or more variables were highly correlated ($r > 0.70$), only one was retained in the analysis to avoid element-weighting distortion, as suggested by Pengelly & Maass (2001). Those principal components exhibiting an eigenvalue higher than or equal to 1 were retained for cluster analyses (CA; Cluster procedure, SAS Institute, 2002) according to Kaiser's criteria (Kaiser, 1960). Euclidean distances were used as a measure of dissimilarity, and the average method was used as the clustering algorithm. The number of clusters was estimated using pseudo F and t^2 statistics (Milligan & Cooper, 1985). Linear correlations among environmental and morphological traits were calculated (Corr procedure; SAS/STAT, 2002). A canonical correspondence analysis (CCA; Ter Braak, 1986) was performed in the SAS environment (SAS/STAT, 2002) to

Table 1. Names, acronyms and climatic variables of the ten sites where the *Chamaerops humilis* populations under study were collected

Collection site	Favignana Island	Kolymbetra	Lipari Island	Marsala's Sciare	Nature Reserve of Capo Gallo	Nature Reserve of Capo Rama	Nature Reserve of Monte Cofano	Nature Reserve of Torre Salsa	Nature Reserve of Vendicari	Nature Reserve of Zingaro
Acronym	IF	KO	IL	SM	CG	CR	MC	TS	VE	ZI
N	51	27	37	82	71	65	123	69	70	110
Latitude	37°55.952N	37°17.520N	38°28.389N	37°52.576N	38°12.943N	38°08.297N	38°05.966N	37°22.091N	36°48.223N	38°05.868N
Longitude	12°18.964E	13°34.884E	14°54.082E	12°31.417E	13°17.719E	13°03.546E	12°39.688E	13°21.290E	15°06.145E	12°47.930E
Mean annual ETP	919	912	955	881	954	954	970	912	924	970
Minimum annual ETP	852	858	875	828	851	851	898	858	872	898
Maximum annual ETP	1028	1127	1221	1000	1033	1033	1180	1127	1085	1180
Mean annual rainfall	450	516	620	678	683	683	491	516	452	491
Minimum annual rainfall	252	263	338	303	246	246	204	263	162	204
Maximum annual rainfall	793	1021	908	1162	1173	1173	770	1021	791	770
Number of rainy days per year	60	59	72	77	72	72	61	59	42	61
% Winter rainfall	36.7	40.9	37.5	39.7	39.8	39.8	39.1	40.9	38.1	39.1
% Spring rainfall	22.8	22.7	23.0	23.9	21.5	21.5	22.3	22.7	14.8	22.3
% Summer rainfall	3.0	2.9	5.9	3.9	3.9	3.9	2.4	2.9	5.0	2.4
% Fall rainfall	37.6	33.5	33.6	32.4	34.9	34.9	36.2	33.5	42.1	36.2
Mean winter temperature	12.33	12.08	13.53	10.88	11.83	11.83	13.28	12.08	12.45	13.28
Mean spring temperature	14.75	14.50	15.70	14.50	15.12	15.12	15.40	14.50	14.63	15.40
Mean summer temperature	24.77	24.73	25.12	24.45	25.55	25.55	25.47	24.73	25.17	25.47
Mean fall temperature	20.03	21.47	20.95	18.47	19.90	19.90	21.15	21.47	19.93	21.15
Maximum winter temperature	15.17	15.50	16.50	14.27	15.13	15.13	15.93	15.50	15.77	15.93
Maximum spring temperature	19.57	19.90	19.87	20.20	20.63	20.63	19.83	19.90	20.07	19.83
Maximum summer temperature	28.57	29.97	28.60	29.80	30.57	30.57	29.43	29.97	29.87	29.43
Maximum fall temperature	23.30	24.03	24.30	22.57	24.00	24.00	24.43	24.03	23.27	24.43
Minimum winter temperature	9.50	8.67	10.57	7.50	8.53	8.53	10.63	8.67	9.13	10.63
Minimum spring temperature	9.93	9.10	11.53	8.80	9.60	9.60	10.97	9.10	9.20	10.97
Minimum summer temperature	20.97	19.50	21.63	19.10	20.53	20.53	21.50	19.50	20.47	21.50
Minimum fall temperature	16.77	18.90	17.60	14.37	15.80	15.80	17.87	18.90	16.60	17.87
Number of days with temperature > 35°	351	309	324	284	273	273	316	309	321	316
Lang index	25.00	29.00	33.00	39.00	37.12	37.12	26.00	29.00	25.00	26.00
De Martonne index	16.00	18.00	21.00	25.00	24.05	24.05	17.00	18.00	16.00	17.00
Emberger index	57.00	58.00	78.00	75.00	72.10	72.10	56.00	58.00	51.00	56.00
Thornthwaite index	−51.00	−43.00	−35.00	−23.00	−28.41	−28.41	−49.00	−43.00	−51.00	−49.00
Humidity index	0.49	0.57	0.65	0.77	0.72	0.72	0.51	0.57	0.49	0.51

ETP, potential evapotranspiration.

examine the relationships between noncorrelated population traits and environmental variables.

MOLECULAR ANALYSIS

Leaf samples were freeze-dried (LIO5P; 5Pascal). Approximately 40 mg of dried tissue was disrupted by homogenization (Speed Mill P12; Analitik Jena). Genomic DNA was isolated using the cetyltrimethyl-ammonium bromide method (Doyle & Doyle, 1987). A set of 28 microsatellite primer pairs developed for *C. humilis* var. *humilis* (Arranz *et al*., 2013) was used to genotype the samples (see Supporting information, Table S1). Polymerase chain reaction (PCR) amplification was conducted in a total volume of 8.0 µL containing 0.2 mM dNTPs, 10 mM Tris-HCl, pH 9.0, 0.3 U of Taq DNA polymerase (AmpliTaq Gold PCR Mastermix, Applied Biosystems), 0.3 µM each primer, and 20 ng of template DNA. The PCR was performed with an EpGRADIENT (Eppendorf) thermal cycler. The cycling conditions for touchdown PCR were: 94 °C for 3 min; followed by 10 cycles of 94 °C for 30 s, 65 °C for 60 s with a temperature reduction of 1 °C per cycle, and 72 °C for 60 s; 25 cycles of 94 °C for 30 s, 55 °C for 60 s, and 72 °C for 60 s; and a final step of 72 °C for 7 min. Forward primers were labelled with the fluorescent dyes NED, PET, VIC or 6-FAM (Applied Biosystems). The PCR products were analyzed using an ABI 3130 Genetic Analyzer (Applied Biosystems) in accordance with the manufacturer's instructions. GeneScan 500(-250) LIZ was used as the internal size standard, and allele sizes were determined using GENEMAPPER, version 4.0 software (Applied Biosystems).

MOLECULAR DATA ANALYSIS

The effective number of alleles (N_E), observed (H_0), and expected (H_E) heterozygosities and inbreeding coefficient (F_{IS}) were estimated using GENALEX, version 6 (Peakall & Smouse, 2006). Maximum-likelihood estimates of null allele frequencies were obtained using the expectation-maximization algorithm using FREENA (Chapuis & Estoup, 2007), and the adjusted allele frequencies were then used to recompute the expected heterozygosity values [H_E(null)]. The estimation of allelic diversity affected by differences in sample size, the allelic richness (A_R), was calculated using FSTAT, version 2.9.3 (Goudet, 1995). An unbiased estimate of Wright's fixation index theta (F_{ST}) was calculated with FSTAT. The significance of the genetic differentiation among pairs of populations was tested by permuting the genotypes among samples 1000 times. This method of permutation does not assume Hardy–Weinberg equilibrium. F_{ST} may be overestimated when null alleles exist

(Chapuis & Estoup, 2007). We estimated the potential effect of null alleles on genetic differentiation by calculating F_{ST} values using the excluding null allele (ENA) method implemented in FREENA. In addition to F_{ST}, we also estimated genetic differentiation by R_{ST}, which accounts for differences in allele size and assumes a stepwise mutation model (Slatkin, 1995). The presence of phylogeographical structure was tested by comparing R_{ST} with its value after permutating allele size within loci (pR_{ST}) using SPAGeDi, version 1.2 (20 000 permutations) (Hardy & Vekemans, 2002). According to Hardy & Vekemans (2002), if R_{ST} is significantly higher than pR_{ST}, then allele mutations contributed to the population differentiation and can be interpreted as the presence of phylogeographical structure. An analysis of molecular variance (AMOVA) was performed using GENALEX. This analysis was conducted at three hierarchical levels (among populations, among individuals, and within individuals) to determine whether the variance of the genetic structure is in agreement with the geographical distribution of populations. The Mantel test was also performed using GENALEX on matrices of dissimilarity between populations considering genetic distances and geographical distances. The matrix of geographical distances was calculated from the Euclidean distances between populations using coordinates of latitude and longitude. The genetic structure of *C. humilis* var. *humilis* populations was investigated using two Bayesian clustering methods. First, we used STRUCTURE, version 2.1 (Pritchard, Stephens & Donnelly, 2000). Second, we used GENELAND, version 3.14 (Guillot, Mortier & Estoup, 2005) because it may provide a better definition of spatial genetic units by integrating the spatial coordinates of samples. In STRUCTURE, we used the admixture model, which estimates the fraction of ancestry from each cluster for each individual, and ran the analyses with correlated allele frequencies. We performed ten independent runs for each K value, which ranged from 1 to 15. We used a burn-in period of 10000 generations and 105 Markov-chain Monte Carlo (MCMC) replications. The most likely number of structured populations, K, was then inferred from the estimation of ΔK (Evanno, Regnaut & Goudet, 2005). In GENELAND, all of the parameters (including K) were simultaneously processed using the MCMC algorithm. We ran the MCMC five times (to verify the consistency of the results), allowing K to vary, with the parameters: 500 000 MCMC iterations, maximum rate of the Poisson process fixed at 500, uncertainty attached to spatial coordinates fixed at 1 km (i.e. the precision of our sample locations; see above), minimum K fixed at 1, maximum K fixed at 15, and maximum number of nuclei in the Poisson–Voronoi tessellation fixed at 200. Other parameters
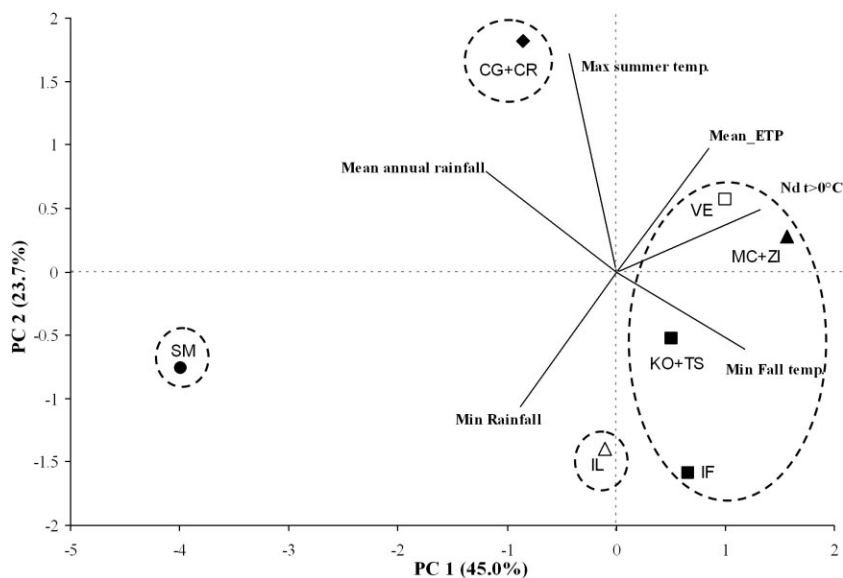
Figure 2. Principal components (PCs) biplot of the characteristics of *Chamaerops humilis* collection sites from Sicily, Italy. Percentage of total variance accounted for by each principal component is shown in parentheses. The seven lines intersecting at (0,0) represent the original variables. The length of each vector is proportional to its contribution to the PCs. Sites with the same symbol are from the same cluster as defined in the CA. The oval-shaped dotted lines represent the second order clustering defined in the CA using the pseudo F and t^2 statistics.

remained similar to those of the runs with a variable K. We calculated the mean logarithm of posterior probability for each of the 100 runs and selected the ten runs with the highest values. The posterior probability of population membership for each pixel of the spatial domain was then computed for each of these ten runs (using a burn-in of 50 000 iterations). The number of pixels was set to values similar to those recommended, 150 pixels along the x-axis and 100 along the y-axis (values chosen to avoid having two individuals in the same pixel). We then computed the posterior probability of population membership for each pixel of the spatial domain and the modal population of each individual. Finally, we checked the consistency of the results across these ten runs. We also compared these results with those of three runs with one million MCMC each to verify their consistency with longer run lengths. In addition, we performed a spatial analysis of molecular variance (SAMOVA), which groups populations to maximize the differentiation among groups. The SAMOVA was used to identify groups of populations that are geographically homogeneous and maximally differentiated from each other. The method searches for K groups of adjacent populations to maximize among-group genetic variation (F_{CT}). The underlying assumption is that maximum genetic differentiation among K groups best circumscribes spatially homogeneous population clusters. We tested for $K = 1$–15,

performing 100 independent simulated annealing processes.

RESULTS

ENVIRONMENTAL TRAITS

The climatic data (on a 30-years basis) indicated high variability among the collection sites (Table 1); however, some climatic traits were common to all of the sites. In particular, all of the collection sites experienced an ETP much higher than rainfall, as shown by the Thornthwaite and humidity indices, and a number of rainy days ranging from 42 to 77. Spring and summer rainfall accounted for 26% of the mean annual rainfall and ranged from 90 to 189 mm.

The similarity among the environments of the collection sites was studied using PCA and CA (Fig. 2). The environments were classified into six different groups: the collection sites Torre Salsa, Kolymbetra, and Favignana were characterized by low autumn temperatures and a relatively high number of rainy days per year. The environments of Monte Cofano, Zingaro, and Vendicari were characterized by high mean and maximum temperatures throughout the whole year, especially during the winter, and high ETP. The environments from Capo Gallo and Capo Rama were characterized by high altitude, high spring and summer temperatures, and a low number of days with temperatures higher than 35 °C. Finally,
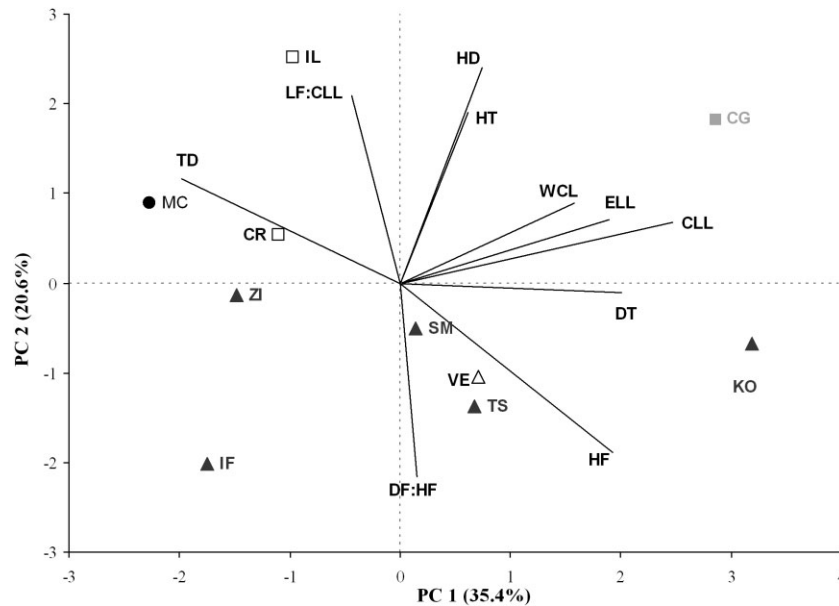
Figure 3. Biplot for principal component (PC)1 versus PC2 calculated based on plant morphology for the ten natural *Chamaerops humilis* populations collected in Sicily, Italy. Percentage of total variance explained by each principal component is shown in parentheses. For the population acronyms, see Table 1. Populations with the same symbol are from the same cluster as defined in an *ad hoc* CA (Fig. 2). Lines intersecting at (0,0) represent the original variables. The length of each vector is proportional to its contribution to the PCs. The acronyms for the vectors are: HT, trunk height; HF, crown height; DT, trunk diameter; CLL, median leaflet length; WCL, width of median leaflet; ELL, external leaflet length; HD, hair density; DF : HT, crown diameter to crown height ratio; LF : CLL, depth of leaflet split to the total lamina length; TD, thorn density.

the environment of Marsala's Sciare was in a group by itself and was not characterized by any of the evaluated traits. A second-order clustering was defined in the CA using the pseudo F and t^2 statistics. Taking into account the second-order clustering, the collection sites of Vendicari, Monte Cofano, Zingaro, Kolymbetra, Torre Salsa, and Favignana experienced a relatively arid environment compared with the other collection sites, especially those of the Capo Gallo and Capo Rama reserves.

PLANT AND FRUIT MORPHOLOGICAL TRAITS

Ten of the 29 morphological traits evaluated were retained for the cluster analysis (Fig. 3, Table 2). Four PCs exhibited an eigenvalue higher than 1 and together accounted for 80% of the total cumulative variance. High correlations were observed between the observed traits and the four selected PCs. PC1 mostly correlated with the median leaflet length (CLL), PC2 with hair density (HD), PC3 with the depth of leaflet split to the total lamina length (LF : CLL), and PC4 with the median leaflet width (WCL).

Fruit and seed morphologies were also used to discriminate among the studied populations. Seeds were found in only six of the populations. Five of the 17 fruit and seed traits were retained for the PCA (Fig. 4). Only the first two PCs exhibited an eigenvalue higher than 1. PC1 had an eigenvalue of 2.01 and primarily correlated with seed area ($r = 0.65$), fruit width ($r = 0.41$), and fruit elongation ($r = 0.51$), whereas PC2 showed an eigenvalue of 1.66 and was highly correlated with seed elongation ($r = 0.72$) and seed roundness (0.60). Notably, no correlation was observed between seed and fruit elongations ($r = -0.05$). The populations in which seeds were retrieved were clearly differentiated by the PCA and CA based on the fruit and seed traits. The population from Marsala's Sciare was characterized by large fruit and seeds and a high flesh to fruit ratio, those from Zingaro and Capo Rama were characterized by high seed roundness, and those from Vendicari, Monte Cofano, and Kolymbetra were characterized by small fruit and seeds, relatively low seed roundness, and a low flesh to fruit ratio.

Two separate cluster analyses were performed using the data on plant morphology (Fig. 5) and on the seed and fruit morphologies (see Supporting information, Fig. S1), and two dendrograms of similarity among populations were constructed. Based on the plant morphological traits, five clusters were identified. The

Table 2. Eigenvalues, variance (both as single and cumulative) of the first four principal components (PCs) and correlation coefficients between the PCs and the ten selected plant morphological characters basing on the correlation analysis of the plant morphological traits

| Acronym	Trait	PC1	PC2	PC3	PC4
	Eigenvalue	3.59	2.57	1.98	1.47
	Variance explained	0.30	0.21	0.17	0.12
	Cumulative variance	0.30	0.51	0.68	0.80
HT	Trunk height	0.41	0.22	−0.26	−0.27
HF	Foliage height	0.37	0.01	0.28	−0.23
DT	Trunk diameter	0.39	−0.05	0.14	0.38
Mean L	Mean number of leaflets per leaf	0.44	0.00	0.20	0.10
WCL	Width of central leaflet	0.29	0.29	0.24	0.33
HD	Hair density	0.02	0.07	−0.42	0.50
AT	Number of thorns in apical petiole	0.06	0.52	0.07	−0.36
LT	Mean length of thorns	−0.26	0.33	0.28	0.45
HF : HT	Foliage height to trunk height ratio	−0.23	−0.07	0.57	−0.02
DF : HF	Foliage diameter to foliage height ratio	−0.10	−0.15	0.37	−0.11
LF	Depth of leaflet split to the total lamina length	0.01	0.54	0.06	−0.03
TD	Thorn density	−0.36	0.39	−0.10	−0.05
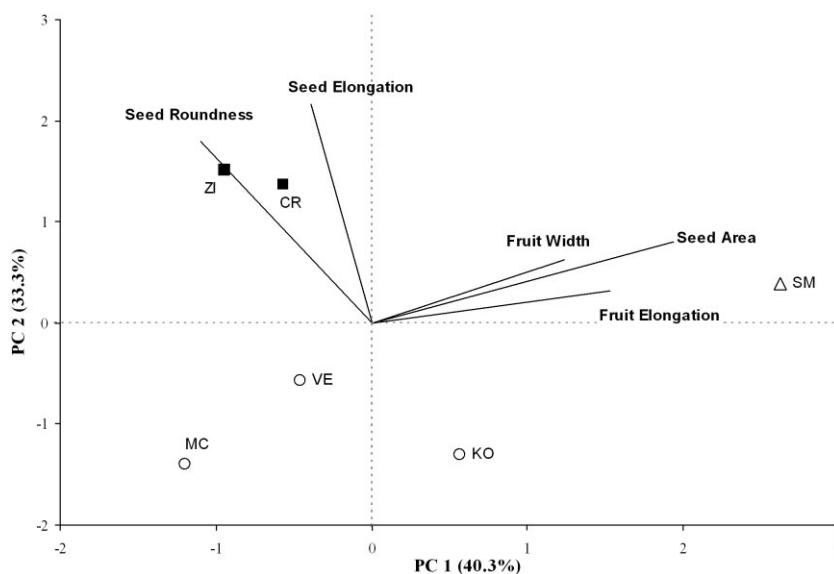
Figure 4. Biplot for principal component (PC)1 versus PC2 calculated on the basis of fruit and seed traits for the six natural *Chamaerops humilis* populations collected in Sicily. Percentage of total variance explained by each principal component is shown in parentheses. For the population acronyms, see Table 1. Populations from Torre Salsa, Capo Gallo, Favignana, and Lipari were not included in the analysis because no fruit was retrieved from these collection sites. Populations with the same symbol are in the same cluster as defined in an *ad hoc* CA (Fig. 2). Lines intersecting at (0,0) represent the original variables.

populations from Capo Rama and Lipari clustered together (Fig. 5, Cl. 2) and were characterized by a high ratio of depth of leaflet split to the total lamina length, high thorn density, and low crown volume. The populations from Marsala's Sciare, Torre Salsa, Zingaro, Kolymbetra, and Favignana also grouped together (Fig. 5, Cl. 3) and were characterized by a high crown diameter to crown height ratio, low hair density, and low ratio of the depth of leaflet split to the total lamina length. Finally, the Capo Gallo, Monte Cofano, and Vendicari populations were grouped into three one-population clusters. Capo Gallo
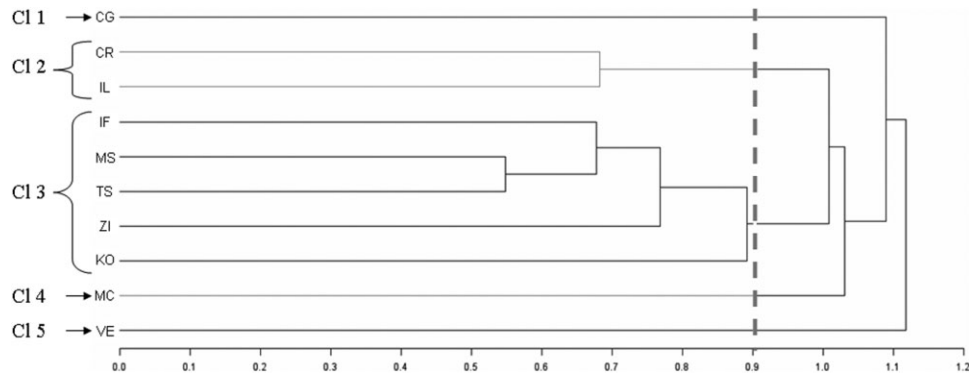
Figure 5. Dendrogram classification based on the plant morphology of *C. humilis* populations clustered with the average method, truncated at the five-group level (vertical dotted line), according to pseudo *F* and t^2 statistics. For the population acronyms, see Table 1.

was characterized by a high width of median leaflet and high external leaflet length, Monte Cofano was characterized by a high thorn density and low crown height, and Vendicari was characterized by a low thorn density and high crown height. The CA performed using the first 2 PCs calculated using the fruit and seed data distributed the six populations from which fruits were retrieved in three different clusters (see Supporting information, Fig. S1). The first cluster included the Capo Rama and Zingaro populations; the second cluster included Kolymbetra, Monte Cofano, and Vendicari; and the third cluster only included the Marsala's Sciare population.

RELATIONSHIPS AMONG ENVIRONMENTAL CHARACTERISTICS AND MORPHOLOGICAL TRAITS

In general, few morphological traits were related to climatic characteristics (see Supporting information, Table S2). Significant and negative correlations were observed between the climatic traits related to drought stress (ETP, seasonal temperatures) and those indices related to plant vegetative growth, such as the crown height to trunk height ratio and crown height to plant height ratio. Similar results were found for the morphological indices related to plant growth during the juvenile stage (trunk diameter to trunk height ratio). The morphological indices related to drought tolerance/resistance (hair and thorn densities) positively correlated with mean ETP but not with any of the climatic indices calculated.

A CCA (Fig. 6) was performed on the morphological and environmental variables selected before the PCAs. The first canonical axis accounted for 77.76% of the total variation (Eigenvalue = 0.020248; *P* < 0.01) and primarily correlated with the mean ETP (*r* = +0.42) and the number of days with temperatures higher than 0 °C (*r* = +0.31), whereas CA2 accounted for 11.17% (Eigenvalue = 0.002909; *P* < 0.01) of the

total variation and correlated primarily with the maximum summer temperature (*r* = 0.22), mean ETP (*r* = +0.16), and mean *R* (*r* = +0.13). CA3 accounted for 7.84% (Eigenvalue = 0.0020425; *P* < 0.01) of the total variation and was negatively correlated with the maximum summer temperature (*r* = −0.35) and minimum autumn temperature (*r* = −0.15). Some populations from similar environments (TS, KO, and MC) exhibited a high association in the CCA. A high association was also observed between the ZI and CR populations despite their origin environments appearing different in the PCA (Fig. 2). The association between ZI and CR was primarily a result of similarities in hair density (HD) and crown height (HF). Finally, a strong association was observed between the SM and IF populations, derived from relatively different environments (Fig. 2). In general, most of the populations (MC, TS, CG, KO, SM, and IF) tended to be located in relatively arid environments, with low rainfall and ETP, low autumn and winter temperatures, and a low number of days with temperature higher than 0 °C. The VE and IL populations were located in environments with high winter temperatures, whereas CR and ZI were in environments with relatively high rainfall and high ETP. Similar results were observed in the ordination between CA1 and CA3.

MOLECULAR ANALYSIS

Genetic diversity estimates are reported in Table 3. All of the *C. humilis* var. *humilis* populations analyzed exhibited similar levels of genetic variability (H_E and A_R) with overlapping 95% confidence intervals. The mean gene diversity (H_E) was 0.374 ± 0.011, ranging from 0.290 (TS) to 0.408 (ZI). The gene diversity corrected for the presence of null alleles [H_E(null)] exhibited the same pattern, with the ZI population being the most diverse (0.448) and the TS population
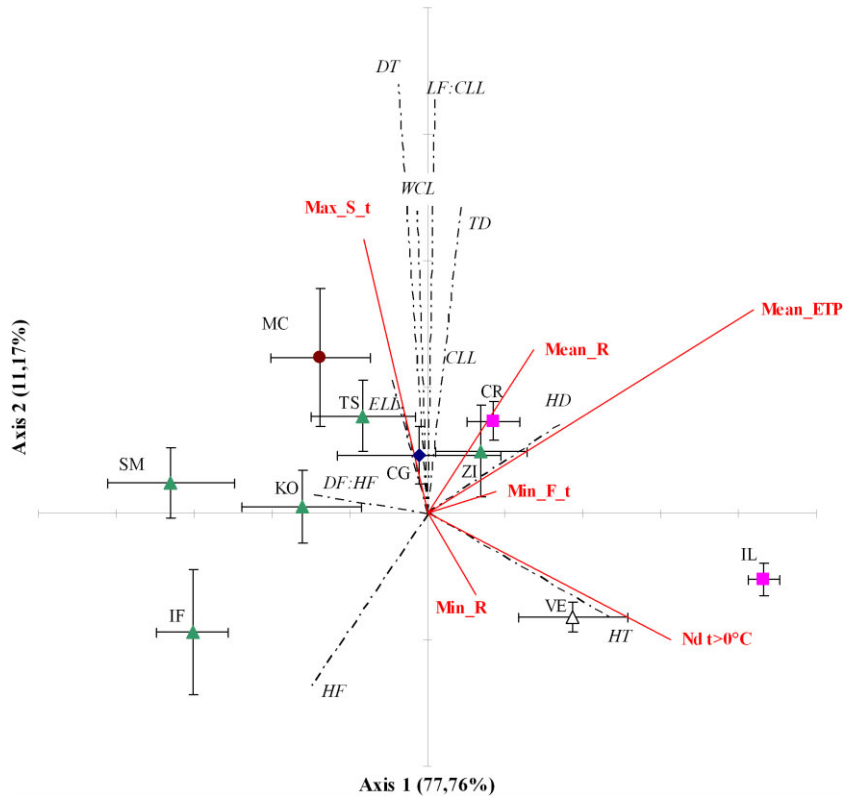
Figure 6. Ordination triplot for CA1 versus CA2 calculated based on the canonical variables calculated using the plant morphological traits (hatched-pointed black lines) weighted based on the characteristics of the selected collection sites (continuous red lines). Units of measure for Axis 1 and Axis 2 are the same (0.1). Percentage of total variance explained by each canonical axis is shown in parentheses. Percentage of variance explained by CA3 (not shown) is 7.84%, and the ordination along CA3 was similar to that along CA2. For the population acronyms, see Table 1. Populations with the same symbol are in the same cluster as defined in the PCA + CA performed based only on morphological traits. Each symbol represents the population centroid within CA1 and CA2. Bars represent the SE of the distribution of each population. Vectors ('VAR' groups, with the names in italics) intersecting at (0,0) represent the original variables. For the vector acronyms, see Fig. 3. The contribution of environmental variables ('WITH' groups, in red and bold font) to the plant morphological variability is shown: mean annual potential evapotranspiration (Mean ETP); mean (Mean R) and minimum (Min R) rainfall; maximum summer temperature (Max S t); and minimum fall temperature (Min F t). Populations that are close to each other are similar in morphological traits corrected for the effects of the environment. Populations that share a common VAR vector positively co-vary in plant morphological traits. In a similar way, similar directions of specific VAR with specific WITH vectors mean that an association occurs between plant morphological and collection site traits.

the least diverse (0.317). Nevertheless, $[H_E(\text{null})]$ values based on only three loci (GRD72UV01AQ5SS, cons56, and GRD72UV01AJTQW), displaying a low frequency of null alleles (2%–3%), were similar to those estimated using the complete set of markers (data not shown). Allelic richness (A_R) ranged from 2.47 (KO) to 4.75 (ZI) with a mean of 3.666 ± 0.013. Wright's inbreeding coefficient within populations (F_{IS}) exhibited no significant deviation from zero at any of the multilocus estimates (at $P > 0.05$; Table 3). The mean value of F_{IS} was slightly positive (0.095 ± 0.023), ranging from –0.159 (TS) to 0.192 (CG). The mean F_{ST} value at multilocus estimates was

0.181, ranging from 0.09 to 0.195. After ENA correction, the overall F_{ST} was only slightly lower $[F_{ST(ENA)} = 0.177]$ (data not shown). The comparison of the ten populations revealed significant differences in the observed proportions of heterozygotes, with populations from IL containing more heterozygotes than the other populations. By contrast, there were no significant differences in expected heterozygosity between the other populations. In addition, CG exhibited significantly higher values of inbreeding coefficients (mean $F_{IS} = 0.192$) than the other populations in the study (Table 3). The estimates of F_{ST} within the ten populations ranged from 0.39 (TS) to 0.63 (IL).

Table 3. Size (*N*); effective allele number (*N*$_E$); allelic richness calculated with the rarefaction method (*A*$_R$); expected heterozygosity (*H*$_E$); expected heterozygosity calculated on allele frequencies adjusted for null alleles [*H*$_E$(null)]; observed heterozygosity (*H*$_O$); and inbreeding coefficient (*F*$_{IS}$) of the ten *Chamaerops humilis* populations included in the present study

Accession	*N*	*N*$_E$	*A*$_R$	*H*$_E$	*H*$_E$(null)	*H*$_O$	*F*$_{IS}$
CR	65	1.869	4.25	0.373	0.328	0.363	0.094
CG	71	1.915	4.13	0.388	0.379	0.368	0.192
MC	123	1.844	3.89	0.374	0.411	0.376	0.017
KO	27	2.002	2.47	0.401	0.435	0.391	0.128
IF	51	1.831	3.13	0.366	0.396	0.318	0.187
ZI	110	2.025	4.75	0.408	0.448	0.385	0.098
TS	69	1.467	4.63	0.290	0.317	0.369	−0.159
VE	70	1.775	2.58	0.346	0.391	0.333	0.143
SM	82	2.048	3.95	0.396	0.361	0.344	0.188
IL	37	2.062	2.88	0.396	0.360	0.416	0.057
Mean	64.6	1.884	3.666	0.374	0.383	0.366	0.095
SD		0.052	0.013	0.011	0.013	0.015	0.023

Figure 7. Genetic structure of *Chamaerops humilis* populations. Mean proportion of membership of each population for the inferred number of *K* clusters = 3. For the population acronyms, see Table 1.

However, no significant differences were observed in *F*$_{ST}$ among the populations under study. The mean pairwise multilocus estimates of population differentiation were *F*$_{ST}$ = 0.257 and *R*$_{ST}$ = 0.237 among populations and *F*$_{ST}$ = 0.527 and *R*$_{ST}$ = 0.494 within populations, indicating that population differentiation was higher within than among populations. Mean pairwise *R*$_{ST}$ and *F*$_{ST}$ did not differ among the populations, whereas *F*$_{ST}$ was significantly larger than *R*$_{ST}$ among populations. In addition, the estimated *R*$_{ST}$ values among populations were larger than the *pR*$_{ST}$ (0.454) for the multilocus estimate, indicating that the stepwise mutations contributed few to genetic differentiation among the populations in the present study. The hierarchical AMOVA indicated that 3% (Φ$_{SC}$ = 0.03, *P* < 0.001) of the genetic variance was allocated among populations, indicating that most of the variation was found among individuals (52%; *P* < 0.001) or within individuals within populations (45%; *P* < 0.001). By analyzing the ten populations separately, we obtained the same patterns of variation within and among individuals for all of the collection sites on the main island; the IL population

differed from this pattern: the greatest variation was found among populations (66%) and not within populations (34%). The Mantel randomization test indicated no significant relationship between geographical and genetic distance (*r* = 0.0689, *P* = 0.406). The STRUCTURE analysis revealed the highest Δ*K* value at *K* = 3 (Fig. 7). The populations of CR, CG, and MC exhibited a higher proportion (39%) of mean membership to cluster 1, whereas most of the other populations exhibited a higher proportion (51%) of mean membership to cluster 2. The IL population exhibited an intermediate proportion (21%) of mean membership to cluster 3. The mean *F*$_{ST}$ values corresponding to the divergences of clusters 1, 2, and 3 from the hypothetical ancestral population were 0.152, 0.009, and 0.097, respectively; the population of cluster 2 showed a lower divergence from the hypothetical ancestral population, whereas the population of cluster 1 showed a higher divergence. The genetic structure analysis using SAMOVA generally confirmed the STRUCTURE results, and two major groups of populations were identified (*F*$_{CT}$ = 0.17). Results of the spatial Bayesian clustering analysis

displayed as a map of posterior probabilities indicate *K* = 3 clusters for populations of *C. humilis* (see Supporting information, Fig. S2). Therefore, in agreement with STRUCTURE, this spatial model identified three spatially distinct, genetically differentiated groups of populations of *C. humilis*.

DISCUSSION

In the present study, plant and seed morphologies of the European fan palm, *C. humilis*, were studied in relation to both its genetic variation and the climatic variability within the study area. In general, the populations in the present study were clustered similarly by the genetic and morphological analyses, with the exception of the populations from MC, CG, and CR. Other studies comparing genetic and morphological variations have reported contrasting results (Kjaer *et al*., 2004; Hatziskakis, Tsiripidis & Papageorgiou, 2011; Cecchi *et al*., 2013): in a regional analysis of sago palm (*Metroxylon sagu* Rottb.), Kjaer *et al*. (2004) found little or no relationship between genetic distance and morphology except for petiole thickness and width. However, a significant correlation was observed between genetic and geographical data and such an effect was attributed to human transportation of specimens from one collection site to another. Additionally, Hamza *et al*. (2011), who analyzed several *Phoenix dactylifera* putative subpopulations in southern Tunisia, found no correlation between morphological and molecular variations based on nine morphological traits and five molecular primer pairs, although some significant correlation was observed between fruit maturity period and consistency and between molecular and quantitative morphological traits. In the present study, specimens were collected in natural managed reserves where European fan palm populations are undisturbed, and analyses were run for a relatively high number of both morphological and genetic markers. Nonetheless, both the expected and observed heterozygosities were higher (Table 3) than those of other long-lived perennial outcrossing species (Hamrick & Godt, 1996), and the inbreeding coefficient, despite being positive, was not different than zero, suggesting that each population was at Hardy–Weinberg equilibrium. This result can imply that *C. humilis* populations in Sicily differentiated from a common ancestor and that their inter-population variations arose from secondary evolutionary processes induced by ecological adaptation, which can explain the similarity between genetic and morphological clustering. This hypothesis is consistent with the observations of higher intra-than inter-population differentiation and relatively low allelic richness in the present study. Indeed, in obligate outcrossing species, such as the European

fan palm, genetic diversity after a site colonization event proceeds from a reorganization of the genetic structure of the founder (García-Verdugo *et al*., 2009; Stuessy *et al*., 2014) through mating between neighbouring relatives, recombination (Lefèvre *et al*., 2004), nonrandom dispersal (Garant *et al*., 2005), and reproductive dominance (Luna, Epperson & Oyama, 2007). In contrast to other species (e.g. *Gypsophila struthium* L.; Martínez-Nieto *et al*., 2014), *C. humilis* is an isolated species in Sicily and thus has experienced no or few genetic introgressions from its relatives, which may imply a high environmental pressure and thus a strong relationship between genetic and morphological distances. In addition, *C. humilis* is subject to dispersal limitation, which hinders gene flow between different regions (Blach-Overgaard *et al*., 2010); this hindrance may explain the relatively low values of expected heterozygosity in the present study. From a genetic point of view, populations from MC, CG, and CR were clustered together based on both molecular (Fig. 7) and morphological analyses (Fig. 5). Two of these populations (MC and CR) appeared similar because of their high thorn density and small plant size (HF and DT) (Fig. 3), traits that were strongly and negatively correlated in the CCA. In many species, thorn density has been characterized as a defence mechanism against herbivory (Milewski, Young & Madden, 1991) or attributed to evolutionary causes, such as heat balance (Nobel, 1980) or drought resistance (Janzen, 1986). In European fan palm, thorns are larger at the petiole base than at its apex, plant size is usually < 1.5 m, and the leaflets can be eaten by mammals, which suggests that, in this species, thorns have no role against herbivory. However, considering that thorns grow especially in the petiole base and thus near the infructescence, they could still have a role in protecting fruits from animals. Indeed, in the natural reserves where the palms under study were retrieved, summer coincides with a low natural availability of nourishment for wildlife because the high aridity is a major limitation to plant development and fruiting. In these environments, *C. humilis* is one of the few plant species able to produce high-energy seeds and fruits during the summer (Giovino *et al*., 2013) and thus can play a major role in feeding wildlife. In this context, thorns could play the role of reducing ingestion by wildlife when fruits are still immature and attached to the fruiting rachis. Indeed, a significant correlation was observed between thorn density and ETP, and, on the CCA, thorn density was highly correlated with maximum summer temperatures and minimum rainfall, which suggests that in the European fan palm, thorns may play a role in genetic adaptation to arid environments. In addition, the crown height to trunk height ratio negatively correlated with ETP, and the

crown height to plant height ratio negatively correlated with both ETP and spring and summer temperatures. Considering that trunk height is somehow related to plant age, these ratios are related to the availability of nutrients, especially water and light. Reductions in crown size and the number of leaves with increasing drought have been observed in other Mediterranean species (Corcuera, Camarero & Gil-Pelegrín, 2004). Accordingly, in other palm species, trunk height has been related to drought resistance (Lakmini, Nainanayake & de Costa, 2006). This relationship could explain the negative correlation between the crown height to trunk height ratio and ETP. In addition, the European fan palm is among those palm species adapted to relatively cold conditions, which can explain its short height compared to other palm species.

In the present study, a negative correlation was also observed between winter, spring and summer temperatures and the crown height to trunk height ratio ($r < -0.64$) and between mean spring temperatures and crown diameter ($r = -0.61$) and trunk diameter to trunk height ratio ($r = -0.68$), although no correlation was observed between leaf hair density and temperature (see Supporting information, Table S2). The decrease in the crown height to trunk height ratio and crown diameter at increasingly colder conditions suggests that *C. humilis* decreases in size and assumes a more 'cushion-like' canopy shape as the mean temperature decreases, whereas the trunk diameter to trunk height ratio is an indirect measure of the availability of nutrients and the amount of GDD. Gianoli *et al.* (2004) found reductions in plant and leaf size in an Antarctic compared to an Andean *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) ecotype. Usually, morphological adaptations of a plant to environmental stresses (such as cold) involve changes in canopy shape, leaf size, and leaf hair density (Bliss, 1962). However, leaf hair density may depend upon several factors, including the genotype (Ramesar-Fortner, Dengler & Aiken, 1995) and leaf size (Roy, Stanton & Eppley, 1999). Also in the present study, hair density was significantly correlated with leaf area ($r = -0.51$) but not with the length of the leaf margin ($r = -0.28$). In many plant species, cold adaptation is controlled primarily by quantitative trait loci (QTL), for which the effects on plant phenotype are small and can be influenced further by a genotype × environment interaction (Howe *et al.*, 2003). Howe *et al.* (2003) also suggest that QTLs for cold tolerance can have pleiotropic effects on other traits, such as the timing of bud set and spring bud flush. In the present study, we did not measure any phenological traits, although the flesh thickness of the fruit (which is related to bud flush and the length of time available for fruit

growth) was negatively related to the mean and minimum temperatures and ETP ($r < -0.72$), and the pulp to fruit ratio was positively correlated with rainfall, especially winter and spring rainfall ($r > 0.87$), and the number of rainy days per year ($r = 0.90$). The flesh thickness of fruits and fruit size depends upon several environmental and growth conditions, such as GDD, water availability, and the seed-dispersing system (Mazer & Wheelwright, 1993). In the European fan palm, the fruit pulp both acts as a defence against invertebrate seed predation and inhibits seed germination (Fedriani & Delibes, 2011). In other palms, the fruit contains inhibitory compounds that can be removed by water soaking (Broschat & Donselman, 1987); thus, it is possible that the flesh thickness and the pulp to fruit ratio in the European fan palm are adaptive traits related to water availability and temperature during germination. Furthermore, in the CCA, the populations under study tended to aggregate in environments with low ETP, low autumn and winter temperatures, and a low number of days with a temperature > 0 °C, which suggests that the establishment of a *C. humilis* population depends on temperature rather than on water availability. This result agrees with the recent findings by Kissling *et al.* (2012) who showed that the distribution and latitudinal limit of palms depends on temperature seasonality and the minimum temperature in both continental and island environments.

CONCLUSIONS

A strong relationship was observed when clustering the populations based on genetic and morphological traits. Correlations between genetic variation and environmental characteristics have been observed in many plant species (Parisod & Christin, 2008; Eckert *et al.*, 2010). In addition, many morphological traits appear to be related to environmental characteristics. Despite this relation, intrapopulation variation was higher than inter-population variation, and allelic richness was low. This result could imply that the differentiation of populations of *C. humilis* in Sicily arises from secondary adaptation to the environment. Finally, population clustering in the CCA was driven by autumn and winter temperatures, which are important for seed germination, and by water-energy availability (i.e. ETP, rainfall, and the number of rainy days per year), which are parameters that impact seedling survival rather than adult plant growth and are referred to as the strongest climatic predictors of palm diversity patterns (Eiserhardt *et al.*, 2011; Kissling *et al.*, 2012). The European fan palm is among the most cold-tolerant palm species but, on the northern extreme of its natural distribution range, it only occurs near the sea; thus, it is

possible that frost damage, photosynthesis depression, and other temperature-dependent traits limit the spread of the dwarf fan palm to more northern sites. This limitation implies that the European fan palm could be used to track climatic changes in Europe. However, further studies are needed to identify the adaptations of *C. humilis* to relatively colder environments, as well as the effects of temperature and water availability on the plant's germination and competitiveness with other cold-tolerant species.

In conclusion, three main subpopulations of *C. humilis* were observed using genetic screening, whereas morphological screening suggested that two main morphotypes are present in Sicily, differentiated mainly according to autumn and winter temperatures and the number of days with a temperature > 0 °C (i.e. according to autumn and winter aridity). These findings suggest that genetic and morphological tools, in addition to statistical tools such as CCA, need to be coupled to structure an efficient conservation strategy that takes into account both the genetic variability within and among the populations targeted in the conservation programmes and the functional traits of the targeted genotypes.

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REFERENCES

Arranz SE, Avarre JC, Balasundaram C, Bouza C, Calcaterra NB, Cezilly F, Chen SL, Cipriani G, Cruz VP, D'Esposito D, Daniel C, Dejean A, Dharaneedharan S, Díaz J, Du M, Durand JD, Dziadek J, Foresti F, Peng-cheng F, Gao QB, García G, Gauffre-Autelin P, Giovino A, Goswami M, Guarino C, Guerra-Varela J, Gutiérrez V, Harris DJ, Heo MS, Khan G, Kim M, Lakra WS, Lauth J, Leclercq P, Lee J, Lee SH, Lee S, Lee T, Li YH, Liu H, Liu S, Malé PJ, Mandhan RP, Martinez P, Mayer VE, Mendel J, Mendes NJ, Mendonça FF, Minias A, Minias P, Oh KS, Oliveira C, Orivel J, Orsini L, Pardo BG, Perera A, Procaccini G, Rato C, Ríos N, Scibetta S, Sharma BS, Sierens T, Singh A, Terer T, Triest L, Urbánková S, Vera M, Villanova GV, Voglmayr H, Vyskočilová M, Wang H, Wang JL, Wattier RA, Xing R, Yadav K, Yin G, Yuan Y, Yun JC, Zhang FQ, Zhang JH, Zhuang Z. 2013. Permanent genetic resources added to molecular ecology resources database 1 December 2012–31 January 2013. *Molecular Ecology Resources* **13:** 546–549.

Blach-Overgaard A, Svenning JC, Dransfield J, Greve M, Balslev H. 2010. Determinants of palm species distributions across Africa: the relative roles of climate, non-climatic environmental factors, and spatial constraints. *Ecography* **33:** 380–391.

Bliss LC. 1962. Adaptations of arctic and alpine plants to environmental conditions. *Arctic* **15:** 117–144.

Broschat TK, Donselman H. 1987. Effects of fruit maturity, storage, presoaking, and seed cleaning on germination in three species of palms. *Journal of Environmental Horticulture* **5:** 6–9.

Cartabellotta D, Drago A, Lo Bianco B, Lombardo M. 1998. *Climatologia della Sicilia-Regione Siciliana*. Palermo: Assessorato Agricoltura e Foreste Gruppo IV-Unità di Agrometeorologia.

Cecchi L, Colzi I, Coppi A, Gonnelli C, Selvi F. 2013. Diversity and biogeography of Ni-hyperaccumulators of *Alyssum* section *Odontarrhena* (Brassicaceae) in the central western Mediterranean: evidence from karyology, morphology and DNA sequence data. *Botanical Journal of the Linnean Society* **173:** 269–289.

Chapuis MP, Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* **24:** 621–631.

Corcuera L, Camarero JJ, Gil-Pelegrín E. 2004. Effects of a severe drought on *Quercus ilex* radial growth and xylem anatomy. *Trees – Structure and Function* **18:** 83–92.

Doyle JA, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin, Botanical Society of America* **19:** 11–15.

Eckert AJ, van Heerwaarden J, Wegrzyn JL, Nelson CD, Ross-Ibarra J, González-Martínez SC, Neale DB. 2010. Patterns of population structure and environmental associations to aridity across the range of loblolly pine (*Pinus taeda* L., Pinaceae). *Genetics* **185:** 969–982.

Eiserhardt WL, Svenning JC, Kissling WD, Balslev H. 2011. Geographical ecology of the palms (Arecaceae): determinants of diversity and distributions across spatial scales. *Annals of Botany* **108:** 1391–1416.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14:** 2611–2620.

Fedriani JM, Delibes M. 2011. Dangerous liaisons disperse the Mediterranean dwarf palm: fleshy-pulp defensive role against seed predators. *Ecology* **92:** 304–315.

Garant D, Kruuk LEB, Wilkin TA, McCleery RH, Sheldon BC. 2005. Evolution driven by differential dispersal within a wild bird population. *Nature* **433:** 60–65.

García-Verdugo C, Fay MF, Granado-Yela C, DE Casas RR, Balaguer L, Besnard G, Vargas P. 2009. Genetic diversity and differentiation processes in the ploidy series of *Olea europaea* L.: a multiscale approach from subspecies to insular populations. *Molecular Ecology* **18:** 454–467.

Gianoli E, Inostroza P, Zúñiga-Feest A, Reyes-Díaz M, Cavieres LA, Bravo LA, Corcuera LJ. 2004. Ecotypic differentiation in morphology and cold resistance in populations of Colobanthus quitensis (Caryophyllaceae) from the Andes of Central Chile and the Maritime Antarctic. *Arctic, Antarctic, and Alpine Research* **36:** 484–489.

Giovino A, Marino P, Domina G, Rapisarda P, Rizza G, Saia S. 2013. Fatty acid composition of the seed lipids of *Chamaerops humilis* L. natural populations and its relation

with the environment. *Plant Biosystems*. In press. doi: 10.1080/11263504.2013.870249.

Goudet J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* **86:** 485–486.

Govaerts R, Dransfield J. 2005. *World checklist of palms*. Kew: Royal Botanic Gardens.

Guillot G, Mortier F, Estoup A. 2005. Geneland: a computer package for landscape genetics. *Molecular Ecology Notes* **5:** 712–715.

Hamrick JL, Godt MJW. 1996. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **351:** 1291–1298.

Hamza H, Elbekkay M, Ben Abederrahim MA, Ferchichi Ali A. 2011. Molecular and morphological analyses of date palm (*Phoenix dactylifera L.*) subpopulations in southern Tunisia. *Spanish Journal of Agricultural Research* **9:** 484–493.

Hardy OJ, Vekemans X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2:** 618–620.

Hatziskakis S, Tsiripidis I, Papageorgiou AC. 2011. Leaf morphological variation in beech (*Fagus sylvatica* L.) populations in Greece and its relation to their post-glacial origin. *Botanical Journal of the Linnean Society* **165:** 422–436.

Howe GT, Aitken SN, Neale DB, Jermstad KD, Wheeler NC, Chen TH. 2003. From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. *Canadian Journal of Botany* **81:** 1247–1266.

IPGRI, INRAA, INRAM, INRAT, FEM, PNUD. 2005. *Descripteurs du palmier dattier* (Phoenix dactylifera *L.*). Rome: Institut international des recoures phytogenetiques; Washington, DC: Fonds pour l' Environnement Mondial; New York, NY: Programme des Nations Unies pour le Developpement; d'Alge: Institut National de la recherche Agronomique.

Janzen DH. 1986. Chihuahuan desert nopaleras: defaunated big mammal vegetation. *Annual Review of Ecology and Systematics* **17:** 595–636.

Juan A, Fay MF, Pastor J, Juan R, Fernández I, Crespo MB. 2011. Genetic structure and phylogeography in *Juniperus oxycedrus* subsp. *macrocarpa* around the Mediterranean and Atlantic coasts of the Iberian Peninsula, based on AFLP and plastid markers. *European Journal of Forest Research* **131:** 845–856.

Kaiser HF. 1960. The application of electronic computers to factor analysis. *Educational and Psychological Measurement* **20:** 141–151.

Kissling WD, Baker WJ, Balslev H, Barfod AS, Borchsenius F, Dransfield J, Govaerts R, Svenning JC. 2012. Quaternary and pre-Quaternary historical legacies in the global distribution of a major tropical plant lineage. *Global Ecology and Biogeography* **21:** 909–921.

Kjaer A, Barfod AS, Asmussen CB, Seberg O. 2004. Investigation of genetic and morphological variation in the sago palm (*Metroxylon sagu*; Arecaceae) in Papua New Guinea. *Annals of Botany* **94:** 109–117.

Lakmini WGD, Nainanayake NPAD, de Costa WAJM. 2006. Physiological responses for moisture stress and development of an index for screening coconut (*Cocos nucifera* L.) genotypes for drought tolerance. *Tropical Agricultural Research and Extension* **9:** 17–26.

Lefèvre F, Fady B, Fallour-Rubio D, Ghosn D, Bariteau M. 2004. Impact of founder population, drift and selection on the genetic diversity of a recently translocated tree population. *Heredity* **93:** 542–550.

Luna R, Epperson BK, Oyama K. 2007. High levels of genetic variability and inbreeding in two Neotropical dioecious palms with contrasting life histories. *Heredity* **99:** 466–476.

Martínez-Nieto MI, Segarra-Moragues JG, Merlo E, Martínez-Hernández F, Mota JF. 2014. Genetic diversity, genetic structure and phylogeography of the Iberian endemic *Gypsophila struthium* (Caryophyllaceae) as revealed by AFLP and plastid DNA sequences: connecting habitat fragmentation and diversification. *Botanical Journal of the Linnean Society* **173:** 654–675.

Mazer SJ, Wheelwright NT. 1993. Fruit size and shape: allometry at different taxonomic levels in bird-dispersed plants. *Evolutionary Ecology* **7:** 556–575.

Milewski AV, Young TP, Madden D. 1991. Thorns as induced defenses: experimental evidence. *Oecologia* **86:** 70–75.

Milligan GW, Cooper MC. 1985. An examination of procedures for determining the number of clusters in a data set. *Psychometrika* **50:** 159–179.

Nobel PS. 1980. Interception of photosynthetically active radiation by cacti of different morphology. *Oecologia* **45:** 160–166.

Parisod C, Christin PA. 2008. Genome-wide association to fine-scale ecological heterogeneity within a continuous population of *Biscutella laevigata* (Brassicaceae). *New Phytologist* **178:** 436–447.

Peakall R, Smouse PE. 2006. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6:** 288–295.

Pengelly BC, Maass BL. 2001. *Lablab purpureus* (L.) Sweet – diversity, potential use and determination of a core collection of this multi-purpose tropical legume. *Genetic Resources and Crop Evolution* **48:** 261–272.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155:** 945–959.

Ramesar-Fortner NS, Dengler NG, Aiken SG. 1995. Phenotypic plasticity in leaves of four species of Arctic *Festuca* (Poaceae). *Canadian Journal of Botany* **73:** 1810–1823.

Roy BA, Stanton ML, Eppley SM. 1999. Effects of environmental stress on leaf hair density and consequences for selection. *Journal of Evolutionary Biology* **12:** 1089–1103.

SAS/STAT. 2002. *User's guide,* Version 9.1. Cary, NC: SAS Institute Inc.

Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139:** 457–462.

Stuessy TF, Takayama K, López-Sepúlveda P, Crawford DJ. 2014. Interpretation of patterns of genetic variation in endemic plant species of oceanic islands. *Botanical Journal of the Linnean Society* **174:** 276–288.

Ter Braak CJF. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology* **67:** 1167–1179.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Dendrogram classification based on the seed morphology of *Chamaerops humilis* populations clustered with the average method, truncated at the five-group level (vertical dotted line), according to pseudo F and t^2 statistics. For the population acronyms, see Table 1.

Figure S2. Genetic analysis based on microsatellite data with K fixed to three and maps of the posterior probability of individual *Chamaerops humilis*. Black dots represent the sampling sites. Probability of cluster membership increases with lighter shading.

Table S1. Sequences of 28 microsatellite loci from *Chamaerops humilis* (Arranz *et al.*, 2013) used in the present study. F, forward primer; R, reverse primer; labels used are given in bold; Ta, optimal annealing temperature. the GenBank accession number is shown.

Table S2. Linear correlation coefficients between morphological traits of the ten analyzed populations of *Chamaerops humilis* from Sicily, Italy, and climatic variables of the collection sites.