

The genetic diversity of selections and wild populations of myrtle revealed by molecular geographic contexts.

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1 **The genetic diversity of selections and wild populations of myrtle revealed by molecular geographic contexts**

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18

19 **Abstract**

20 *Myrtus communis* L. is an aromatic shrub widely diffused in the Mediterranean area. A growing interest for this species
21 is due to its pharmacological and aromatic properties. This study explored the genetic diversity and population structure
22 of 460 myrtle plants including 46 selections genotypes and 414 wild accessions from Sardinia using simple 14 SSR
23 markers. Genetic diversity parameters showed that 10 markers were sufficiently polymorphic to move forward in the
24 further analysis. The observed heterozygosity ranged from 0.21 (Porto Ferro) to 0.44 (Olia Speciosa), with an average
25 value of 0.32. This parameter significantly varied considering the genetic origin of the plant (selections and natural),
26 and the localization of the myrtle sampling sites (North, Center and South area) ($P < 0.0001$). The selections showed an
27 observed heterozygosity's lower (0.40) compared to the wild group (0.45). Based on the geographic division of
28 Sardinia, North region grouped myrtle accessions with the highest level of genetic diversity (0.45), followed by the
29 Central area (0.43) and the South, that collect the lowest value (0.42). Bayesian clustering analysis divided the myrtle
30 accessions in two principal groups, which are in overlap with the division in selections and natural populations.
31 Significant different distribution of the two clusters was found (Pearson, $P = 0.003$): one cluster was mainly distributed
32 in the South-Center of Sardinia, while the other cluster was predominant the North area. These results and the obtained
33 genetic characterization are a description of an *in situ* germplasm collection, an important step for planning future *ex*
34 *situ* collection to reinforce the genetic base of breeding programs, allowing to diversify the industrial potential of
35 products derivatives from myrtle.

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39 **Keywords** Natural populations; selections genotypes, *Myrtus communis*, genetic diversity, habitat distribution.

40

41 **1. Introduction**

42 *Myrtus communis* is a diploid ($2n = 2x = 22$) and outcrossing specie with a partial self-pollinating mating system (Mulas
43 and Fadda 2004; Lughadha and Proenca 1996) wild shrub of the Myrtaceae family that widely grows in the
44 Mediterranean Basin (Mendes et al., 2001). Myrtle is considered as one of the of the predominant species of the
45 Mediterranean shrub-land that typically characterises the specific vegetation form called “maquis” (Bruna et al., 2007;
46 Albaladejo et al., 2010). Its value as ornamental and aromatic plant is known since ancient Greeks and Romans. In the
47 last years, a renewed interest on myrtle was discovered because of it’s aromatic and medicinal uses for food and not
48 food industries (Lange 2006). Young leaves as well as mature fruits are both used for the essential oil extraction and for
49 local liquor production (Mulas and Melis 2011; Mulas 2012; Gardelli et al. 2008; Petretto et al., 2016). Both leaves and
50 berries are used as raw material for the essential oil extraction as sources of antioxidants (Tuberoso et al., 2007; Maldini
51 et al., 2016), and liquor production (Mulas and Cani 1999). The success of some food products (as liquor) and the
52 increasing request by food and not food industries of raw material is causing an intensification of the harvesting of
53 leaves and berry’s from the wild plants. The harvest of biomass, the grazing by livestock, the firewood and the
54 urbanization are the principal factors causing the reduction of natural biodiversity of the species, especially in Sardinia
55 (Italy), where the liquor represents an important consume product. The increasing reduction of myrtle populations
56 numbers and sizes, have prompted two requests. Firstly, the creation of a domestication program with candidate cultivar
57 selections for industrial purposes. Several myrtle phenotypic characters has been identified as important traits for food
58 and liquor production, that range from the plant features (big plant size; high green biomass yield; no multiple flowering
59 fluxes) to the berry morphology and production (high berry production; presence of pigmented berries; early ripening
60 time; long peduncle) (Mulas and Cani, 1999; Mulas 2012). Furtherly, the organization of a conservation program in
61 order to preserve the natural genetic diversity of myrtle and plan an efficient and rational exploitation of wild myrtle
62 biomass, without further reduction of the genetic diversity of the specie (Mulas 2012).

63 The maintaining of high level of genetic diversity provides the basis for survival of populations and species, especially
64 in environments under deep anthropic pressure and potential climatic or environmental changes (Mohammad-Panah et
65 al., 2017). Loss of genetic diversity induces decrease of probability to survive under biotic and abiotic stress condition
66 in their natural habitat with high risk of genetic erosion and species extinction (Lande 1995). Evaluation and
67 conservation genetic diversity program are essential to preserve myrtle species.

68 Genetic analysis required specific molecular markers able to give a general picture of the state of the genetic
69 diversity and to discriminate which environmental factors could influence the plant diversity distribution (Streiff
70 1998).

71 Previous studies have been conducted using ISSR and AFLP as dominant markers (Melito et al. 2013; 2014; 2017).

72 In the first case, even with a limited number of accessions, population structure analysis, highlighted two principal
73 genetic clusters corresponding to wild and selected myrtle genotypes (Melito et al 2013); however, AFLP markers
74 were used to characterize both a core collection of selected myrtle genotypes based berry production purposes
75 (Melito et al 2014), and a collection of wild myrtle accessions from Sardinia (Melito et al 2017). In these two
76 studies, altitude and geographical origin significantly affect the population genetic structure. In addition, the
77 relationship between genetic variability, morphological and ecological parameter was explored in aromatic species
78 found in the Mediterranean Basin (Rapposelli et al., 2015, Melito et al., 2013, 2014, 2016, 2017). In Sardinia,
79 where myrtle represent an important component of the flora, natural populations have been only partially explored
80 using dominant markers, such as AFLP (Agrimonti et al., 2007; Melito et al., 2014; 2017) and ISSR (Melito et al.,
81 2013a). Population genetic structure of Sardinian myrtle populations appear to be strongly influenced by several
82 parameters such as altitude, geographic location, landscape and sea proximity (Melito et al., 2013; 2014; 2017),
83 while preferred soils are sub-acids or neutrals with a low clay content (Mulas, 2012). Little information are
84 available on the genetic characterization of myrtle populations based on codominant markers isolate only few years
85 ago (Albaladejo et al., 2010). Preliminary studies conducted by Corona et al. 2015 showed that SSR marker were
86 able to differentiate myrtle in different genetic clusters. Previous research have genetically characterized wild
87 accession or myrtle selections using mainly AFLP markers (Agrimonti et al., 2007; Messaoud et al., 2011; Bruna et
88 al. 2007; Melito et al., 2014, 2016, 2017). Little is known about the genetic diversity and population structure of
89 wild and selections of myrtle genotypes from the same geographic area. Melito et al. (2013b), for the first time,
90 reported the result of ISSR characterization of selection and wild populations that included a small number of *M.*
91 *communis* accessions from Sardinia and Corsica. In this case, the authors observed a clear differentiation between
92 wild accessions and selections collected based on biomass and berry production. The evaluation of the genetic
93 diversity in wild and selected myrtle plants, is extremely important for few reasons: for the constitution of a
94 repository field, obtained from the selection of genotypes based on biomass yield that should maintain the same
95 quality of biomass collected from wild plants; the genetic diversity stored in candidate cultivar collection need to be
96 asses and eventually integrated with missing useful genotypes; finally, a rational conservation program, with the
97 constitution of repository field, needs to be associated to a genetic diversity evaluation (Pressey et al., 2007).

98 The aims of the present research are: a detailed genetic characterization of wide number of wild accessions and
99 myrtle selections collected from Sardinia using SSR markers; the exploration of the effect of important
100 environmental factors, such as geographic localization and local meteorological conditions (temperatures and rain),
101 on the genetic diversity and population structure.

102

103 2. Material and Methods

104 2.1. Germoplasm collection

105 A total of 22 *Myrtus communis* populations including 21 wild and 1 group of myrtle selections, were sampled in
106 Sardinia (Italy). About 20 plants for each natural population were collected from 10 localities (Table 1). Wild samples
107 were randomly collected: five plants were randomly sampled along every 20-meters half-alignment for a total of 20
108 samples per population in order to exclude basal resprouts of the same genotype in the same population. Differences in
109 ecological parameters among the natural sampling sites were explored. Altitude level, geographic localization and
110 climatic conditions were recorded. Meteorological data for each site were provided by Settore Idrografico della Regione
111 Sardegna. The proximal meteorological station to each sample site was used and the average annual climatic parameter
112 are shown in Tables 1. Monthly precipitation and temperature (average, maximum and minimum) of historical series
113 (1991-2011) were considered (Supporting Table 1).

114 Furthermore, 46 selections held in the same field at the Experimental Station of the University Sassari in Oristano, (39°
115 53'N, 8°37'E) were also analysed. The myrtle selections (Mulas et al., 2002) were originally
116 picked from different localities in Sardinia based on the berry, biomass production, and the positive attitude for an
117 intensive growing; the selected plants were harvested for agamic propagation and evaluated in a selection program
118 (Mulas et al 2002). The original sampling sites are reported in Table 1. Based on the locality, an identificative code was
119 assigned to wild and domesticated plant as reported in Tables 1.

120

121 2.2. DNA extraction and SSR analysis

122 Total genomic DNA of 460 myrtle samples was extracted. The young leaf tissue, collected during the summer 2015,
123 was obtained using the DNeasy® Plant kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol. DNA
124 concentration and quality was evaluated by agarose 1.0% (w/v) gel electrophoresis.

125 Fourteen SSR primers were used in this study, following the Albaladejo et al., (2010) protocol (Supporting Table 2).
126 Multiplexed PCR with fluorescent labelled primers (HEX; NED and FAM) were used for the SSR genotyping. The
127 PCR-SSR products were firstly checked in agarose gel before the sequencing at Macrogen, Inc. (Seoul, Korea).
128 Fragment analysis was carried out with Peak Scanner V1.0 (Applied Biosystems) software (Applied Biosystems).
129 Automatic allele calls were subsequently confirmed manually reviewing all electropherograms.

130

131 2.3. Genetic analysis

132 POPGENE 1.31 (Yeh et al. 1997) program was used to assess the genetic diversity, as observed and expected
133 heterozygosis observed (*Obs He*, *Exp He* respectively), the effective number of allele for each locus (N_a , N_e), haplotype
134 number and percentage of polymorphic alleles (P). Polymorphic information index was calculated using Cervus 3.0.7
135 (Kalinowski et al. 2007). Linkage disequilibrium (LD) was estimated using POPGENE (Yeh et al. 1997) by means of
136 pairwise linkage tests with 10000 permutations. Pairwise LD was evaluated for the entire myrtle genotype dataset and
137 all the SSR markers used.

138 Fixation index (F_{st}) was estimated through the Analysis of Molecular Variance using Arlequin 3.1.5 software (Excoffier
139 and Lischer 2010).

140 Population genetic structure was determined using the model-based program, STRUCTURE 2.3.3 software
141 (Pritchard et al. 2000). The number of the most likelihood populations (K) capturing the major structure in the myrtle
142 data was determined using Structure Harvester software (<http://taylor0.biology.ucla.edu/structureHarvester/>) (Earl et al.
143 2012), based on Evanno et al. (2005) method. The software was run using the admixture model adopted different K
144 values ranging from 1 to 10, and evaluated 10 replicate runs per K value. 200.000 Markov Chain Monte Carlo (MCMC)
145 interactions were evaluated. A Burn-in period of 100.000 interactions followed by an additional 500.000 interactions
146 was set. The attribution of each sample to a specific cluster was based on a coefficient of membership ($Q > 0.7$). Input
147 files for the genetic analysis software were prepared using Convert 1.31 software (Glaubitz 2004). To examine patterns
148 of fine-scale genetic structure, molecular data, were used to estimate the coancestry according to Loiselle et al. 1995.
149 For each identified Sardinia area (North, Center and South) coancestry was calculated between all possible pairs of
150 individuals within the subpopulations (max distance 30 m, as from the sampling scheme) and within the whole areas
151 (max distance different for each area according to pupations position) using SPAGeDi software (Hardy & Vekemans,
152 2002). Tests of significance for estimated coancestry values were performed by using randomization procedures to
153 generate populations under the null hypothesis of no spatial genetic structure. For a given distance class, values from
154 399 simulations were used to construct a 95% interval around the null hypothesis of no genetic structure. Coancestry
155 significant values were considered the result of IBD from non-random gene dispersal at the spatial scale examined that
156 is reasonably due to localized pollen and/or seed movement.

157 2.4. Statistical analysis

158 Correlations between site and the genetic coefficient of membership (Q) were calculated. A Pearson's χ^2 test for 2x2
159 contingency tables was performed for the categorical variables (geographic localization: North, Centre and South; and
160 assigned genetic group based on STRUCTURE analysis: CA and CB) to test correlations with the clusters identified by
161 STRUCTURE (Pritchard et al. 2000). All meteorological variables were normalized before the statistical analysis.
162 Correlations were carried out by JMP 7 software (SAS Institute, Cary, NC, USA). Climatic, geographic and genetic

163 (Fst) pairwise distance matrices were calculated for the Mantel test using XLSTAT software 2007. The climatic and
164 geographic distance matrices were calculated based on the Euclidean method, while genetic distance matrix was
165 estimated using Fst values (Excoffier and Lischer 2010).

166

167 **3. Results and Discussion**

168 *3.1. SSR analysis and genetic diversity*

169 A total of 460 individuals of myrtle from Sardinia (Italy) were studied (Tables 1). The accessions include 414 wild and
170 46 myrtle selections from the localities reported in Fig. 1.

171 Fourteen primers pairs were used (Supplementary Table 2) and 10 of them (Table 2) produced reproducible fragments
172 and were used for the further investigations. Primer *Myrcom* 2, 7, 8 and 10 did not produced a clear data and were not
173 used in the further investigations. The SSR analysis was done on all plant genotypes revealing 68 alleles with a mean of
174 6.8 allele for each marker. The marker *Myrcom* 11 was the most polymorphic (13 alleles), while *Myrcom* 13 had only
175 two alleles. The average observed heterozygosity (*Obs He*) among all the SSR marker used was 0.23, ranging from zero
176 (*Myrcom* 13) to 0.52 (*Myrcom* 6) (Table 2).

177 Within the 22 populations the average number of polymorphic loci was 5.89 ranging from 1 (Parco Sette Fratelli and
178 Paulilatino) to 9 (Santa Sofia and Fenosu). The average expected heterozygosity (*Exp He*) was 0.31 with a minimum
179 value of 0.10 (Paulilatino) and a maximum of 0.42 (Santa Sofia) (Table 3). In addition, the *Obs He* showed high
180 variability: from 0.21 (Porto Ferro) to 0.44 (Olia Speciosa), with an average value of 0.32. Myrtle genotype from
181 Paulilatino showed a very high *Obs He* (1.00). In this case, the presence of only one sample induced a high score of this
182 genetic parameter.

183 These data showed a general high level of *Obs He*, with a significant difference among the 22 myrtle populations
184 (Pearson, $P = 0.005$). Previous study conducted by Melito et al. (2017) in a wide collection of wild myrtle populations
185 showed a lower level of genetic diversity compared to the data found in this study. Differences in *Obs He* could be
186 related to different reasons: first, the different molecular marker used. Melito et al. (2017), in fact, used AFLP markers
187 while in this study the exploration of the genetic diversity was performed through SSR marker, that is a co-dominant
188 marker. Moreover, differentially to previous studies conducted on Sardinian myrtle accessions (Melito et al., 2014;
189 2017), in this research, both wild and selections genotypes were analyzed. The average *Obs He* varied considering
190 different parameters: the division in selections and wild origin, and the geographical localization of the sampling sites
191 (North, Central and South areas). Significant difference in genetic diversity was found between selections and wild
192 (Pearson, $P < 0.0001$); based on this harboring, as expected, the selections collection showed a lower *Obs He* (0.40)
193 compared to the wild group (0.45). Similar trend was found for the populations division based on the geographic origin

194 of the sampling sites (North, Central and South area). Minor differences were found among the *Obs He* of the three
195 areas. The North region grouped myrtle accessions with the highest level of genetic diversity (0.45), followed by the
196 central area (0.43) and the South, that collect the lowest *Obs He* (0.42). The average *Obs He* observed in selections and
197 wild myrtle populations is compatible with the principal self-pollination mating system. The highest values of *Obs He*
198 were found in wild myrtle populations suggesting that, despite the out-crossing is the principal mating system (Mulas
199 and Fadda 2004; González-Varo et al., 2010), a degree of cross-pollination occurs in myrtle (Barrett 2013; Cowling
200 2013).

201 High variability in haplotype number was also found considering the myrtle genotypes divided in two groups: wild and
202 selections populations. The average number of haplotypes was 32.95 with a minimum of 2 (Paulilatino) and a maximum
203 of 77 (Fenosu). The high number of haplotype found in the selections myrtle collection is the result of the strategy used
204 for the collection program. In this case, plants were selected from wild populations belonging to different Sardinia
205 locations based on plant size, vigor and berry production (Table 1), as a consequence the domesticated population
206 grouped a wide range of myrtle variability as expressed by the high value of haplotype's number.

207 The average Polymorphic information content (PIC) was 0.48, ranging from 0.12 (*Myrcom* 4) to 0.88 (*Myrcom* 9),
208 revealing the lowest and the highest polymorphic makers. Fifty percent of used SSR markers showed a PIC value higher
209 than 0.5, indicating that these markers are useful for fingerprinting studies. The average Major Allele Frequency (MAF)
210 was 0.68, ranging from 0.35 (*Myrcom* 6) to 0.93 (*Myrcom* 4). Although *Myrcom* 13 presented a MAF of 0.99,
211 considering that the two alleles present only 1bp of difference, this markers should be further investigated and explored
212 in order to acquire more support. The PIC and MAF value indicate a considerable genetic variation among all myrtle
213 collections used.

214 LD analysis on all the SSR molecular markers used, showed 45 cases of significant LD ($\alpha = 0.05$). Twenty of them
215 showed to be significant ($0.01 \leq P \leq 0.05$), and 25 resulted highly significant ($P < 0.01$). The SSR primer combinations
216 *Myrcom* 4-12/13; *Myrcom* 5-6/14 and *Myrcom* 12-13 had the highest P value.

217

218 3.2. Genetic Structure

219 Population genetic structure was explored using the model-based Bayesian clustering method implemented in
220 STRUCTURE. Evanno et al. (2005) method was applied to identify the principal genetic cluster division. The most
221 likely structure was identified at $K = 2$ (Supplementary Fig. 1). At $K = 2$ the myrtle accessions were separated in two
222 principal clusters (Cluster A and B), capturing 42% (193) and 53% (244) of the individuals, respectively. Little
223 percentage (5%) of admixed genotypes ($Q \leq 0.7$) was detected only in wild populations. Variation in the percentage of
224 individuals assigned to the two clusters was found between wild and selections populations: 52.4% (205) wild

225 accessions were assigned to cluster B, while 89% (41) was harbored to the cluster A (Pearson, $P < 0.001$). The presence
226 of admixed individual could be associated to a more frequent gene flow between the two clusters in wild populations
227 compared to the selections. As with the increasing of the K values, individuals and populations were separated in
228 smaller other genetic clusters (Supplementary Fig 2). The average coefficient of membership for each individual plant
229 and each population in each Structure group is reported in Figure 1A and 1B, respectively. In Figure 1B each population
230 is plotted in the Sardinia map. The clusters A and B were not equally detected in the island: significantly, different
231 distribution of the two genetic clusters was found in the North and South Sardinia (Pearson, $P = 0.0280$). Cluster A is in
232 fact predominant in the North, while cluster B showed has a major diffusion in the South (Fig.1 B,C). This geographical
233 oriented distribution of the clusters is even more significant (Pearson, $P = 0.003$) when a more detailed geographical
234 division of the population sampling sites were done (Fig. 1C). In this case, three geographical areas were identified:
235 North, Central and South areas (Fig. 1C). Based on this division the cluster A was mainly distributed in the South and
236 the center of the Island, while a predominant diffusion of the Cluster B was found in the North area. Overall the genetic
237 cluster identified at $K = 2$ showed that the geographical localization of the samples site had influenced the development
238 of different genetic groups. The geographic trend observed of the two clusters could in part had been influenced by the
239 shape of Sardinia that is longer than wide. Consequently, a more gene flow occurs between East to West than North to
240 South. This observation may be, at least partially, involved in the differential distribution of the cluster A and B. The
241 long distance (North - South) and the mountain localization (in the center of the island) can both have influenced the
242 distribution of the two clusters. These two factors could have act as natural barrier to the myrtle gene flux. As a
243 consequence Cluster B (North area) is isolated the cluster A distributed in center and South area. Previous studies
244 conducted on myrtle candidate cultivar from Sardinia and from Sicily, have shown that geographical distance could
245 induce a different distribution of genetic cluster (Melito et al., 2014; 2016).

246

247 3.3. Population divergence, AMOVA and coancestry analysis

248 Pairwise distance matrix of the genetic diversity, estimated through the F_{st} value was performed at different levels. At
249 the population level, the average F_{st} is 0.15 (Table 4). This value is lower to data previously found by Migliore et al.,
250 (2013) in a wild *Myrtus nivellei* collection. Despite, these two species (*M. communis* and *M. nivellei*) resulted closely
251 related, *M. novellei* have diverged from *M. communis* since its diffusion in Africa and its colonization of the of Saharan
252 mountains region (Migliore et al., 2012). These two species developed two specific pattern of evolutions from a
253 common ancestor colonizing and surviving to specific environmental conditions associated to the geographic features of
254 the land of diffusion. In addition, beside the different phylogeographic history of the two species, the apparent
255 discrepancy with *M. nivellei* could be associated several other reasons such as the sampling size of these two studies

256 and the genetic isolation that characterize the Sharan mountain region that promote a high genetic isolation with an
257 higher Fst value (Migliore et al 2013).

258 Analysis of molecular variance (AMOVA) was used to explore the sources of genetic variance among and at intra
259 population level (Table 4). Few principal parameters, considered potentially important sources of variance were
260 explored: the division in 22 populations, the partition based on STRUCTURE analysis ($K = 2$), the germplasm origin
261 (wild or selections populations) and finally the myrtle partition based on the geographic origin of each sampling sites
262 (North, Center and South).

263 AMOVA based on the 22 myrtle populations showed that most of the variance (85.21%) was found “within population”
264 while a lower contribute was given by the among population variance (14.79%). The associated Fst value is 0.15 that is
265 quite low underlining a high exchange of genetic material among the 22 populations. This value is similar to other
266 species belonging to the myrtaceae family, such as *Eucalyptus globulus* (Costa et al., 2017). As in *E. globulus* the Fst
267 value found in this research could be defined moderate, indicating that are still genetic flux among the genotypes
268 considered.

269 To evaluate the contribution of the clustering partition identified by STRUCTURE, the AMOVA was the run
270 considering the myrtle accessions divided in the two genetic groups identified at $K = 2$. As shown for the division in 22
271 populations, the AMOVA considering the $K = 2$ partition revealed a similar distribution of the genetic variance. Most of
272 the variance was associated to the “within populations” (86.34%) and a minor contribute was related to the “among
273 populations (13.66%). In this case, the Fst (0.14) is quite comparable to the Fst found for the 22 populations.

274 To complete the exploration of the genetic effect in the distribution of the molecular variance the partition in natural and
275 selections collections was performed. Considering this partition, the within populations (selections and natural) variance
276 had the highest score (96.02%), with a consequent decreasing of the among population variance contribute (3.98%). The
277 associated Fst was decreased (0.04%) comparing to the previous partition. This low Fst data suggest a limited genetic
278 differentiation between the natural and selections genotypes. The selections accessions were selected based on berry
279 production and not based on the genetic diversity. Therefore, considering the relatively recent selection of these myrtle
280 genotypes, this potential candidate cultivar collection has probably conserved a lot of the genetic material from the
281 natural population of origin.

282 Finally, the geographical area of sampling sites was considered as an environmental factor that could have influenced
283 the molecular variance. Considering the three geographic area, again the same partition of the genetic variance was
284 observed: highest variance associated to the “within geographic area” (95.83%) and lowest for among geographic areas
285 (4.17%). The Fst was found similar to the partition in selections / natural population (Fst = 0.04).

286 As expected, for all the partition considered, most of the genetic variation is associated to the “intra population level”

287 rather than among them. This data is a consequence myrtle mating system. *Mirtus communis* is mainly an autocrossing
288 specie with a partial self-pollinating mating system (Mulas and Fadda 2004; Lughadha and Proenca 1996). The pollen is
289 driven principally by small insects and coleopters (Melito et al., 2017), that might move for limited distances. This
290 vector of myrtle reproduction explained the high value of intra-population variance along all the sources of variance
291 considered. The low variance based at the “among population” level indicated a reduced population’s differentiation.
292 In Table 5 is reported the Fst distance matrix of the 22 populations tested. Telti (TEL) and Santa Sofia (SSF) showed the
293 highest genetic distance value (0.36). TEL and SSF are located respectively in North and Central areas. The
294 geographical distance as well as the presence of different climatic conditions and physical barriers could have induced
295 isolation between these two populations. The presence of the mountain range only in the central part of the Island
296 represents a natural barrier to the gene flow between these two populations.
297 Based on these findings an Fst distance matrix was run considering the partition of population in the three geographic
298 areas of Sardinia (North, Center and South). North and South regions were more divergent (Fst = 0.05) compared to the
299 Central and South region (Fst = 0.04) (data not show). This data is compatible with the predominant distribution of the
300 two genetic cluster CA and CB respectively in Center/South and in the North of the Island.
301 Analysis of fine-scale intra-population genetic structure indicates significant positive autocorrelation among individuals
302 located up to 30-m apart in the three considered areas; the only exception regarded individuals in the last distance class
303 in the South part of Sardinia island (Fig. 2, left). In the spatial window from 0 to 30 m, coancestry decrease is quite
304 clear even if it is never deep. When all the samples of each area (North, Center and South) were compared together,
305 coancestry values decreased quite fast in the first three-four spatial classes, and were not significantly different from
306 zero beyond few kilometers (Fig. 2, right). In particular, they were not significant after about 9 km in the North, 8 in the
307 Centre and 12 in the South. Some negative correlations were observed at high distances in all the studied areas.
308 Obtained results are compatible with the species pollen and seed dispersals systems. Finally, an unexpected, low
309 increase of coancestry values was observed at 22 and 37 km in Center and South area.

310

311 3.4. Correlation between genetic and local climate

312 Several studies have reported that the environmental conditions influence the pattern of genetic variation across plant
313 populations (Jump et al., 2005). Genetic variation and structure has been shown to correlate with environmental factors
314 (which included micro-climatic conditions) in several tree plant species (Savolainen et al 2007). Principal
315 meteorological data, for each collection site was given in Supplementary Table 1. Multivariate analysis was run
316 between the monthly average meteorological data (average, minimum and maximum temperatures, and rain expressed
317 as mm of water) and the coefficient of membership identified at $K = 2$. Pairwise correlation, showed a significant

318 positive association between the coefficients of membership of the Cluster A and the millimeters of rain of March.
319 Furthermore, a negative correlation was found between the same coefficient of membership and the minimum
320 temperature of the period from February to May and September, showing a possible less thermophilic character of the
321 group (Table 6).

322 The same analysis was run considering the *Obs He* of each population. The genetic diversity resulted positively
323 correlated to the mm of rain detected during the two summer months of June and July (0.160 and 0.217 respectively),
324 while is negatively correlated to the autumn rain precipitations during the months of September, October and November
325 (-0.108, -0.347 and -0.140 respectively). These results are in agreement with a wide study conducted by Jingfang et al
326 2018. In this research, the authors have reported a significant correlation between genetic diversity, expressed as *Obs*
327 *He*, and climatic factors in several woody and herbaceous species. Few climatic factors, such as mean temperatures,
328 precipitation and minimum temperatures etc., have been identified as the most significant for plant genetic diversity.
329 Climatic parameters can deeply influence the genetic diversity of plant species in three principal ways: first, reducing
330 the gene flow affecting the flowering and the pollination (Knight et al., 2005; Ortego et al., 2012; Sork et al., 2010);
331 second the climatic condition could select only the genotype that are able to survive in that specific climatic condition
332 (Davis and Shaw, 2001; Franks and Hoffmann, 2012; Hewitt, 1996); finally climate can select specific alleles, when a
333 population of a specie colonize a new habitat originated by specific climatic conditions (Excoffier and Ray, 2008). Our
334 results suggest that climatic condition have a significant effect on genetic diversity shape of myrtle populations. The
335 correlations found between myrtle *Obs He* and precipitation could be justify considering the specie phenological
336 development. The late flowering time of the species, which close the fruit set in the first weeks of July and may develop
337 fertile seeds with relative abundance when some rains occurs between July and August. On the contrary, if the summer
338 months are completely arid the young fruits drop and the plant produce news flowering fluxes according to the autumn
339 rains support. The late fruits generated by plant re-flowering showed bad maturation, low seed number and strong
340 predation by frugivorous species (Mulas and Fadda 2004).

341 All the average temperatures are negatively correlated to the *Obs He*. This data suggest that increasing the temperature
342 there is a decreasing of the genetic diversity of myrtle populations. In Table 7 are also reported the average maximum
343 and minimum temperature that are significantly negatively correlated to the *Obs He*.

344 To explore the relationship genetic divergence among population and environmental factors (geographic and climatic
345 distances matrices) the Mantel test was run (Table 7). A strong positive correlation between genetic and climatic
346 distances matrices was found ($P=0.0003$) as well as between geographic and climatic data. However, no correlation
347 was detected between genetic and geographic distance matrix. These results are compatible with the strong orographic
348 lack of homogeneity of Sardinia land. Physical barriers, such as mountains, localized in the middle of the island, might

349 create physical barriers for the gene flow, modifying the genetic exchanges between different geographic areas. The
350 spatial distances between myrtle population has a minor effect in the population genetic differentiation compared to the
351 sampling sites distances. This data is compatible with the identification of few meteorological parameter significantly
352 correlated with the genetic structure and the genetic diversity expressed as *Obs He*, shown in Table 7.

353

354 **4. Conclusions**

355 This study reported for the first time the exploration of the genetic diversity and population structure of wild and
356 selections of myrtle populations collected in Sardinia, an important island of the Mediterranean Basin. The myrtle
357 selections showed a higher value of genetic diversity in term of number of haplotype comparing to the wild plants.
358 These results give an evidence that the anthropic selection based on phenotypic, biomass and berry production of the
359 myrtle candidate selections, could have a deep impact in the genetic diversity collection. The genetic partition at $K = 2$
360 highlighted that most of the wild and the early domesticated populations belonged to the two identified clusters. In
361 addition geographic localization of the sampling sites and the climatic conditions of each area significantly affected
362 genetic cluster distribution and the heterozygosity level of myrtle. These findings will be useful to facilitating future
363 strategies of breeding programs.

364

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369

370 **Data archiving statement**

371 All primers used in this research followed the research article of Albaladejo et al. (2010).

372

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491

492 **Figure legends**

493 **Figure 1 A)** Barplots ($K = 2$) of the selections (SEL) and wild myrtle populations (see table 1 for the code); **B)** Average
494 coefficient of membership, based on STRUCTURE results at $K = 2$. For the selections (SEL) and wild populations (see
495 table 1 for the code associated to each sampling sites) is indicated the average coefficient of membership of Cluster A
496 and B. **C)** Partition of the two genetic clusters (Cluster A and B), identified by STRUCTURE, based on the geographic
497 division of Sardinia in: North and South and North, Center and South.

498

499 **Figure 2.** Correlogram of estimated coancestry for wild *M. communis* plants sampled in North (**A**), Center (**B**) and
500 South (**C**) Sardinia. Left: coancestry values calculated between all possible pairs of individuals in the subpopulations, of
501 the three areas, within 3 m distance classes. Right: coancestry values calculated between all possible pairs of individuals
502 in the three areas within ten distance classes defined in such a way that the number of pairwise comparisons within each
503 distance class was approximately constant. Dashed lines represent upper and lower 95% confidence limits around zero
504 relationship.

505

506 **Supplementary File**

507 **Supplementary Figure 1.** Identification of the most probable genetic structure, through the Delta K highest value
508 (Evanno et al., 2005).

509

510 **Supplementary Figure 2.** Barplots of the other minor picks identified by Evanno et al. 2005 ($K = 4$; $K = 6$; $K = 9$) of
511 the selections (SEL) and wild myrtle populations (see table 1 for the code).

512

513 **Supplementary Table 1.** Metereological data registrered for each sampling sites. In table are recorded monthly the
514 Average Temperature (T_m), Average maximum temperature (T_{max}), Average minimum temperature (T_{min}).

515

516 **Supplementary Table 2.** Primers used for the amplification and designed from Albaladejo et al. (2010).

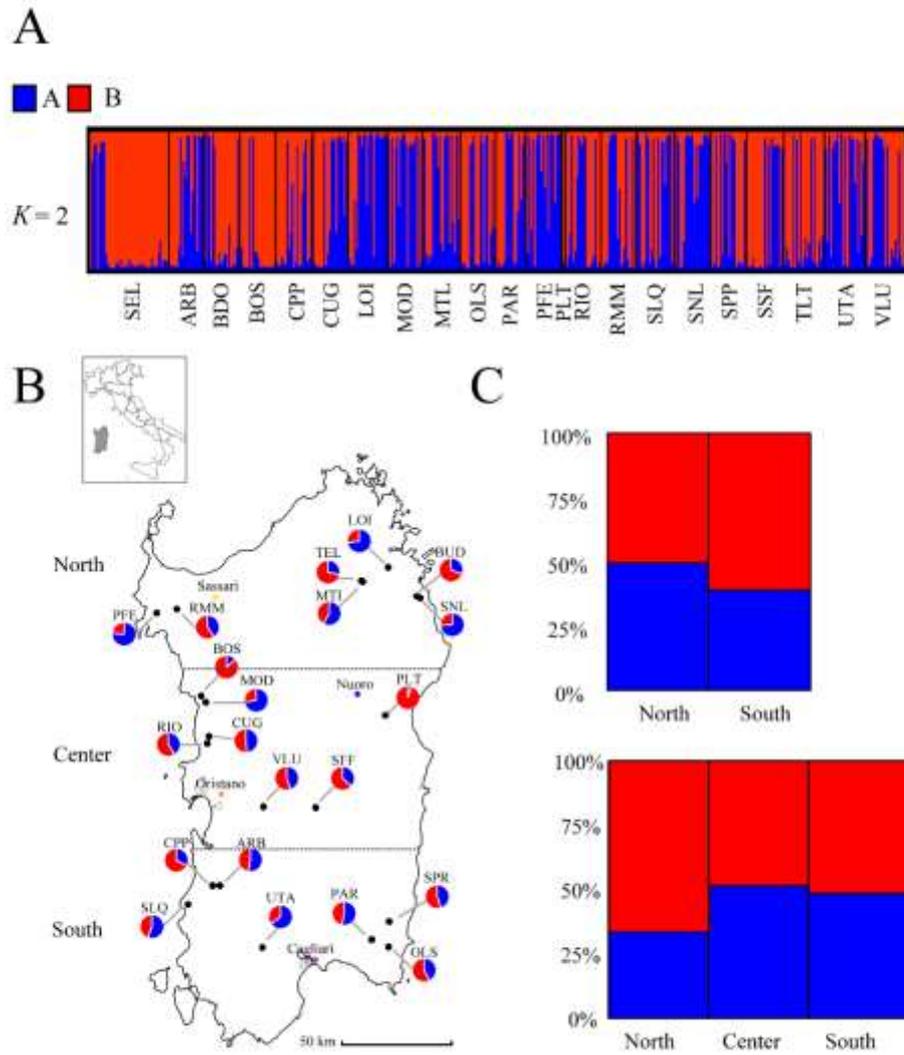


Figure 1.

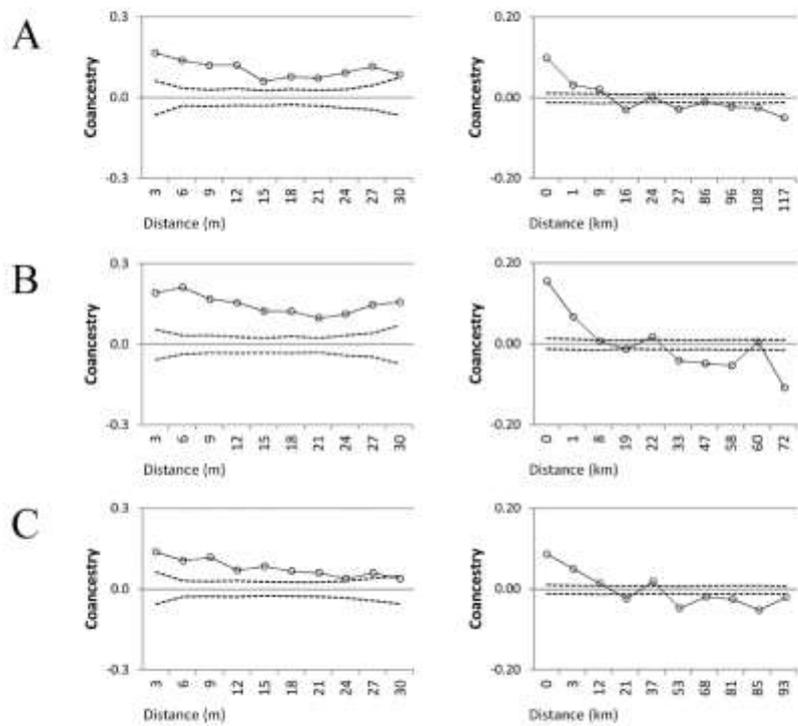


Figure 2.

Tables

Table 1

Myrtle sampling sites characteristics of wild and selected populations. In table are reported: type of plant collection (wild, W and selection, S), locality, the altitude, geographic coordinate of each sampling site (WGS84), the number of plant collected and the associated meteorological conditions for each site. Annual temperatures (°C)(average, T; average maximum, Tmax; average minimum, Tmin) and average annual rain (mm) were reported. For the selection collection the meteorological condition were registered in the experimental station of the University Sassari in Oristano, (39°53'N, 8°37'E), were the myrtles are planted.

Locality	Genotype	Code	Altitude (m.a.s.l.)	Longitude	Latitude	N of plant	T	Tmax	Tmin	Rain
Arbus	W	ARB	271	464500	4375180	20	18.12	22.53	12.61	42.75
Budoni	W	BUD	70	554899	4508931	20	17.28	22.39	12.80	56.50
Bosa	W	BOS	83	456218	4462844	20	16.99	21.33	12.67	50.42
Modolo	W	MOD	103	456218	4462848	20	18.02	22.55	11.04	57.75
Capo Pecora	W	CPP	50	461082	4375180	21	18.02	22.55	11.04	57.75
Cuglieri	W	CUG	235	459720	4444035	20	17.28	23.27	12.19	61.50
Loiri San Paolo	W	LOI	80	541729	4522211	22	15.49	20.53	12.72	65.33
Telti	W	TEL	103	541729	4522213	23	15.78	19.75	12.34	49.25
Monti	W	MTI	506	541729	4522216	21	15.78	19.75	12.34	49.25
Olea Speciosa	W	OLS	82	542915	4346888	20	14.80	20.61	8.93	58.92
Parco Sette Fratelli	W	PAR	422	542915	4346890	17	17.08	22.58	11.56	56.08
Porto Ferro	W	PFE	35	436231	4501232	20	15.19	19.67	10.94	57.92
Rumanedda	W	RMM	45	436231	4501233	20	16.98	22.30	11.67	47.58
Riola Sardo	W	RIO	68	436231	4501235	20	16.98	22.30	11.67	47.58
Siliqua	W	SLQ	68	449519	4366476	21	16.90	22.73	11.23	53.08
Siniscola	W	SNL	40	449519	4366478	21	17.46	24.80	10.13	47.75
San Priamo	W	SPR	59	449519	4366484	20	17.22	23.08	11.33	57.92
Santa Sofia	W	SSF	707	508840	4411011	21	17.91	23.48	12.26	53.92
Uta	W	UTA	151	508840	4411013	23	13.63	19.36	7.87	71.67
Villaurbana	W	VLU	155	508840	4411016	23	17.09	23.11	11.03	42.83
Paulilatino	W	PLT	265	508840	4411019	1	16.09	23.28	11.70	60.67
Asinara	S	SEL1	25	1439500	4493900	1				
Budoni	S	SEL2	70	1559000	4506850	3				
Bosa	S	SEL3	83	1459590	4456380	2				
Cuglieri	S	SEL4	235	1463250	4448970	2				
Isili	S	SEL5	523	1511345	4413070	3				
Laconi	S	SEL6	707	1511350	4413050	6				
Monti	S	SEL7	506	1527500	4517200	2				
Olea Speciosa	S	SEL8	82	1542920	4343130	2	18.12	22.53	12.61	42.75
Orosei	S	SEL9	19	1527500	4517201	4				
Parco Sette Fratelli	S	SEL10	422	1534500	4351175	2				
Rumanedda	S	SEL11	45	1439500	4493900	11				
Sassari	S	SEL12	225	1446430	4478067	1				
Siniscola	S	SEL13	40	1484550	4385190	1				
Telti	S	SEL14	103	1542780	4530430	2				
Uta	S	SEL15	151	1497500	4349950	4				

Table 2

Statistics diversity per locus: n_a = observed number of alleles; n_e = effective number of alleles; *Obs He* = Observed heterozygosity; *Exp He* = Expected heterozygosity, PIC (Polymorphic Information Content), MAF (Major Allele Frequency).

Locus name	Sample size	Alleles			<i>Obs He</i>	<i>Exp He</i>	PIC	MAF
		Size range (bp)	n_a	n_e				
<i>Myrcom 1</i>	912	210 – 213	4	3.44	0.45	0.71	0.66	0.42
<i>Myrcom 2</i>	nd	nd	nd	nd	nd	nd	nd	nd
<i>Myrcom 3</i>	908	150 – 160	6	1.80	0.36	0.44	0.40	0.71
<i>Myrcom 4</i>	902	151 – 185	7	1.15	0.12	0.13	0.12	0.93
<i>Myrcom 5</i>	894	252 – 268	8	1.80	0.36	0.44	0.39	0.72
<i>Myrcom 6</i>	912	154 – 167	11	4.25	0.52	0.77	0.73	0.35
<i>Myrcom 7</i>	nd	nd	nd	nd	nd	nd	nd	nd
<i>Myrcom 8</i>	nd	nd	nd	nd	nd	nd	nd	nd
<i>Myrcom 9</i>	900	164 – 168	6	2.50	0.04	0.60	0.88	0.59
<i>Myrcom 10</i>	nd	nd	nd	nd	nd	nd	nd	nd
<i>Myrcom 11</i>	824	209 – 224	13	3.50	0.20	0.71	0.57	0.46
<i>Myrcom 12</i>	906	146 – 148	4	1.32	0.18	0.24	0.14	0.86
<i>Myrcom 13</i>	908	188 - 189	2	1.01	0	0.01	0.22	0.99
<i>Myrcom 14</i>	888	220 – 277	7	1.70	0.07	0.40	0.70	0.75
Mean	895		6.80	2.24	0.23	0.45	0.48	0.68
St. Dev			3.29	1.13	0.18	0.26	0.27	0.22
Total			68	22.40				

Table 3

Genetic diversity parameters for the selections (S) and wild (W) myrtle population studied. In table are reported the

Population	Origin	NL	NPL	<i>Obs Het</i>	<i>Exp He</i>	NA
Selection	S	10	9	0.32	0.39	77
Arbus	W	10	8	0.31	0.34	32
Budoni	W	10	4	0.31	0.33	32
Bosa	W	10	7	0.23	0.24	23
Capo Pecora	W	10	4	0.3	0.25	30
Cuglieri	W	10	5	0.3	0.35	34
Loiri Porto San Paolo	W	10	6	0.36	0.39	37
Modolo	W	10	3	0.25	0.29	31
Monti	W	10	8	0.23	0.33	33
Olea Speciosa	W	10	6	0.44	0.29	29
Parco Sette Fratelli	W	10	1	0.23	0.37	32
Porto Ferro	W	10	8	0.19	0.27	26
Paulilatino	W	10	1	1	0.1	2
Riola Sardo	W	10	5	0.27	0.29	29
Rumandedda	W	10	7	0.38	0.38	37
Siliqua	W	10	7	0.27	0.34	36
Sanluri	W	10	5	0.26	0.36	39
San Priamo	W	10	6	0.22	0.31	31
Santa Sofia	W	10	9	0.39	0.42	37
Telti	W	10	7	0.21	0.22	26
Uta	W	10	6	0.26	0.36	42
Villaurbana	W	10	7	0.28	0.32	30
Mean		10	5.86	0.32	0.32	32.95
s.d.		0	2.23	0.16	0.07	12.59

number of loci (NL), number of polymorphic loci (NPL) the observed heterozygosity (*Obs Het*), the expected heterozygosity (*Exp Het*) and the number of haplotypes (NA).

Table 4

Partition of the genetic variance, based on AMOVA analysis. Population genetic structure at $K = 2$. the nature of the genetic material (wild or selections population's), and the geographic origin of the sampling sites were considered as different sources of variance.

Partition	Sources of variation	Df	Sum of square	% of variation	Fst
Overall (22 populations)	Among population	21	224.60	14.79	0.15
	Within population	898	1168.40	85.21	
	Total	919	1392.0		
$K = 2$	Among clusters	1	112.54	13.66	0.14
	Within clusters	918	1478.19	86.34	
	Total	919	1590.73		
Germoplasm origin (wild/selections)	Among wild and selections	1	14.09	3.98	0.04
	Within wild and selections	918	1645.71	96.02	
	Total	919	1659.80		
Geographic origin (North. Center. South)	Among geographic areas	2	50.24	4.17	0.04
	Within geographic areas	917	1609.60	95.83	
	Total	919	1659.84		

Table 5

Pairwise F_{st} value calculated among the 22 myrtle populations collected in Sardinia. All pairwise values are significant different. Selections (SEL) and wild populations (see table 1) were used to build the identity matrix below.

Table 6

Pairwise correlation between genetic parameters such as the coefficient of membership (Q) based on STRUCTURE analysis and the observed heterozygosity (*Obs He*) and meteorological data. Rain (mm), average temperature t_m , average minimum and maximum temperatures (T_{min} and T_{max}) monthly were used.

Variable	Vs Variable	Correlation	Prob. Sign
Rain March	Average Q (Cluster A)	0.1109	0.0204
Tmin February		-0.0978	0.041
Tmin March		-0.1957	<0.0001
Tmin April		-0.108	0.024
Tmin May		-0.1195	0.0124
Tmin September		-0.1041	0.0296
Rain June	<i>Obs He</i>	0.1601	0.0008
Rain July		0.217	<.0001
Rain September		-0.1081	0.0238
Rain October		-0.3465	<.0001
Rain November		-0.1404	0.0033
Tm January		-0.2074	<.0001
Tm February		-0.2697	<.0001
Tm March		-0.21	<.0001
Tm April		-0.2782	<.0001
Tm May		-0.1826	0.0001
Tm June		-0.4484	<.0001
Tm July		-0.095	0.0471
Tm May		-0.1375	0.004
Tm September		-0.2291	<.0001
Tm October		-0.2724	<.0001
Tm November		-0.2285	<.0001
Tm December		-0.2503	<.0001
Tmax January		-0.1989	<.0001
Tmax February		-0.4287	<.0001
Tmax March		-0.176	0.0002
Tmax April		-0.2507	<.0001
Tmax November		-0.195	<.0001
Tmax September		-0.2028	<.0001
Tmax December		-0.1631	0.0006
Tmin January		-0.1678	0.0004
Tmin February		-0.3031	<.0001
Tmin April		-0.2313	<.0001
Tmin August		-0.1509	0.0016
Tmin May		-0.3147	<.0001
Tmin June		-0.2432	<.0001
Tmin July		-0.1251	0.0089

Tmin September	-0.3317	<.0001
Tmin December	-0.1282	0.0073

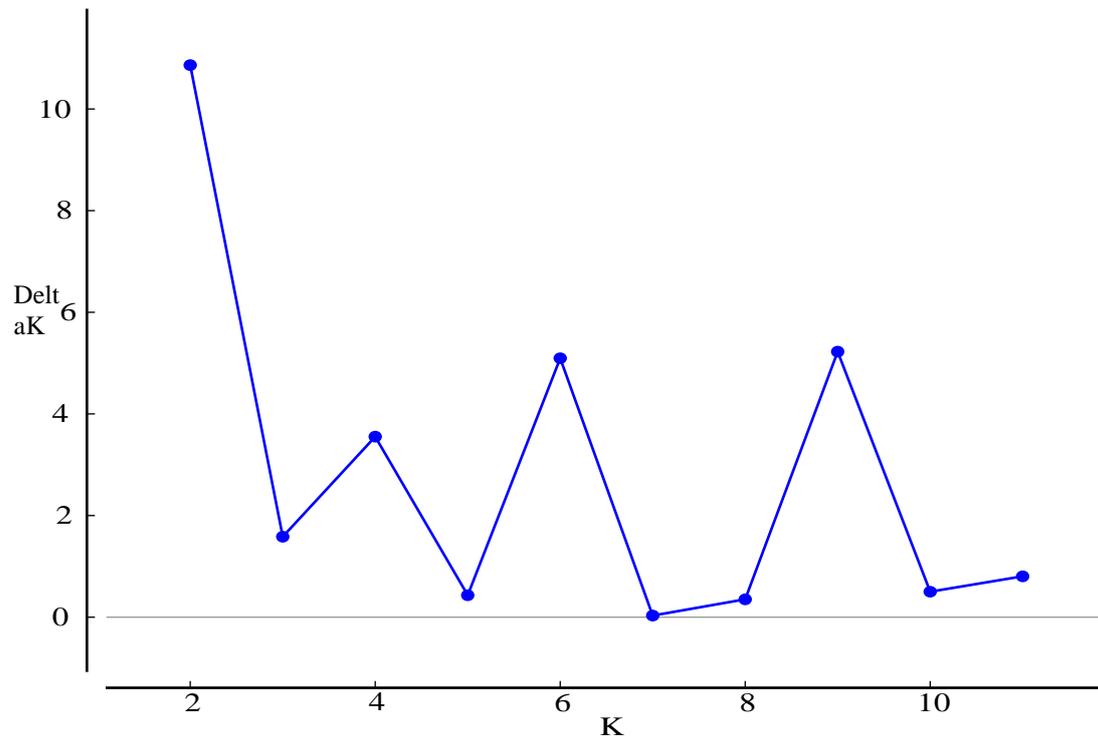
Table 7

Mantel test to evaluate the correlation between climatic, geographic and genetic distance matrices.

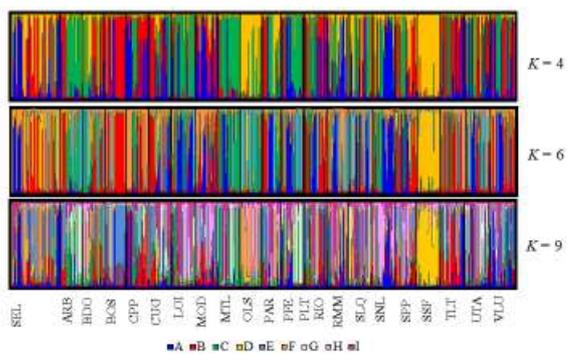
Matrices	Pearson	P value
Climatic vs Geographic	-0.149	0.021
Genetic vs Geographic	n.s.*	n.s.*
Genetic vs Climatic	0.223	0.0003

*n.s. not significant

$$\Delta K = \frac{\text{mean}(|L''(K)|)}{\text{sd}(L(K))}$$



Supplementary Figure 1 Identification of the most probable genetic structure, through the Delta K highest value (Evanno et al., 2005)



Supplementary Figure 2. 1. Barplots of the other minor picks identified by Evanno et al. 2005 ($K=4$, $K=6$, $K=9$) of the selections (SEL) and wild myrtle populations (see table 1 for the code).

