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Original scientific article

GENETIC DIVERSITY AND SPATIAL STRUCTURE OF THE IMPERILED EUROPEAN POPULATION OF *Malus trilobata* IN GREECE

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Malus trilobata, is a rare tree species occurring in several small and disjunct populations in the eastern part of the Mediterranean basin. The main European population is found in the region of Evros (NE Greece) and is divided in five distinct subpopulations following the species geographical pattern. The genetic diversity of approximately the entire population (69 trees) was analyzed using nuclear microsatellite and random genomic markers. Polymorphism was discovered in 29 out of 45 genomic marker loci (64.44%), while for nuclear microsatellite markers, all three loci were polymorphic with an average of 3.75 alleles per locus. Our results unraveled a specific grouping pattern for both markers. Both genetic markers exhibited relatively low genetic diversity which is in accordance with the prevalent perception that species with fragmented distributions tend to have low genetic diversity, while the differentiation among individuals, revealed a patchy pattern among small groups of trees separated by roads, firebreaks or distance. These results indicate a high genetic fragmentation level for the main European

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population of *M. trilobata*, while the presence of roads, firebreaks, conifer plantations and agricultural land, seem to function as potential barriers to gene flow. Consequently, and since it is well-documented that bees hesitate to change foraging patches, as long as their food is abundant, the observed genetic differentiation patterns could be partially attributed to the foraging and flight behavior of bees, which are the main pollinators of the species. The low levels of available genetic diversity combined with the small overall population and repeated events of forest fires inside the *M. trilobata* distribution, perils the survival of the species and imposes the necessity for a thoroughly organized conservation strategy.

Keywords: *Malus trilobata*, rare species, fragmentation, pollination, conservation

INTRODUCTION

Malus trilobata (Poiret) C.K. Schneider (Rosaceae), a wild apple tree, is one of the rarest tree species in the eastern part of the Mediterranean basin. It appears in several isolated populations in northern Israel, Lebanon, south and west Anatolia in Turkey (BROWICZ, 1972). In Europe, *M. trilobata* reaches its northern as well as westernmost limit in Greek Thrace and SE Bulgaria (BROWICZ, 1972; VALEV, 1973; BORATYŃSKI *et al*., 1992; KORAKIS *et al*., 2006).

M. trilobata is an unarmed, relatively small tree or shrub, differentiated from the rest apple tree species by the deeply three-lobed leaves, while it is easily distinguished from other shrubs/trees due to its rather early and abundant flowering, which occurs from mid-May to early June. *M. trilobata* has been included in genus *Malus* according to the basic floras of the area (TERPÓ, 1968; DIMOPOULOS *et al*., 2013). Moreover, *M. trilobata*, along with *M. florentina*, has been assigned to section *Eriolobus* which includes the *Malus* species with lobed leaves and stone-celled containing fruits (TERPÓ, 1968; PHIPPS *et al*., 1991). However, other authors suggest placing *M. trilobata* in the monotypic genus *Eriolobus* as *E. trilobatus*, based on morphological traits (LUBY, 2003; CAMPBELL *et al*., 2007; ROBERTSON *et al*., 1991) and cytogenetic observations (POTTER *et al*., 2007). FORTE *et al*., (2002) who studied the phylogenetic relationships among *Malus* s.l. species report that *M. trilobata* consists a relict species closely related to the American species *M. angustifolia*, *M. coronaria*, and *M. ioensis*.

The first European recording of *M. trilobata* was made in 1876 by DINGLER (1883) in Evros district in Greek Thrace. Detailed recordings of the European populations were provided by BROWICZ (1982) and KORAKIS *et al*., (2006), reporting a total of 94 locations of at least one individual in Evros district. A Bulgarian population was reported in the southern part of the country (STOJANOV *et al*., 1955), yet the indigenous status of these particular individuals has been under question (TERPÓ, 1968). The distribution of the Evros population follows the 150-350m altitudinal zone, while the individuals are found solitarily or dispersed in sparse small groups in thickets, open woodlands and forest ecotones of maquis, deciduous shrub, oak and pine forest habitats.

M. trilobata is considered as rare species both in Asia and Europe (SHMIDA *et al*., 2002). According to the IUCN criteria, it has been previously regarded as "Vulnerable" in Greece (CHRISTENSEN, 1995) and "Critically Endangered" in Bulgaria (VELCHEV, 1984; PETROVA, 2004; PETROVA and VLADIMIROV, 2009), while at a global scale, it is now considered "near threatened" (WILSON and STEPHAN, 2018). Although it has been traditionally protected from logging due to its edible fruits, *M. trilobata* suffers from population depletion in recent decades, mainly due to

extended wildfires that occurred during the summers of 2007, 2009 and 2011 that devastated approximately 40% of the species geographical distribution (unpublished data).

Rare plant species, having limited geographic expansion and existing in small isolated populations are generally considered more vulnerable to external environmental perturbations and occasional demographic fluctuations caused by changes in rates of survival and fecundity (DAVIES *et al*., 2000). Most threats to rare plant species are directly or indirectly human-induced and are frequently connected to stochastic or random processes able to cause increasing instability, decline and eventually extinction (LACY, 2000). Habitat fragmentation is one of the major threats to rare outcrossing plant species, due to possible insufficient pollination (KERY *et al*., 2000; AGUILAR *et al*., 2006) and/or altered abiotic conditions associated with edge effects (BRUNA, 2002; TOMIMATSU and OHARA, 2006). In addition to these threats, habitat degradation, pollution and deforestation combined with land conversion to agricultural land (JACQUEMYN *et al*., 2005) and increased urbanization and road construction (LAVERGNE *et al*., 2005), may cause shifts in the amount and spatial distribution of genetic variation (BAUCOM *et al*., 2005).

This study aims to assess the level of genetic diversity of the main natural European population of *M. trilobata* in N.E. Greece and to describe the spatial pattern of genetic differentiation among the patches of the species distribution. The study will further attempt to define potential factors shaping these genetic patterns and to suggest conservation measures for the genetic resources of this rare plant species.

MATERIALS AND METHODS

Plant material

All known localities of the main *M. trilobata* expansion in Greece were visited and all major trees were sampled. Based on previous recordings in the region (KORAKIS *et al*., 2006), a total of 69 trees growing at the southern edges of the Dadia forest and near the villages of Pessani and Nipsa, were sampled. Individual trees were categorized in five different geographic groups, representing patches of the area covered by the population sampled (Figure 1).

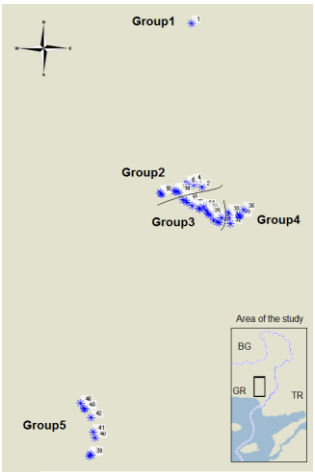

Figure 1. Geographical location of the sampled trees and tree groups.

Group1 (Lat: 41.0284, Long: 26,05254, Alt: 370m) contained only one isolated tree in the northern part of the study area. The main population in Pessani was divided in three groups, Group2 (Lat: 40.9949, Long: 26,05172, Alt: 345m), Group3 (Lat: 40.9925, Long: 26,04282, Alt: 312m) and Group 4 (Lat: 40.9930, Long: 26,04714, Alt: 330m). Trees growing near the village of Nipsa belonged to Group5 (Lat: 40.9377, Long: 26,0214, Alt: 203m). Approximately five young leaves were randomly collected from each tree, afterwards stored at -20°C until required for DNA extraction.

DNA isolation and PCR

Genomic DNA was extracted from fresh leaves using the DNeasy Plant Kit (Qiagen®) following the manufacturer's instructions. The quality and quantity of the isolated DNA were assessed spectrophotometrically and by agarose gel electrophoresis. Genetic analysis was carried out using random genomic (RAPD) and nuclear microsatellite (SSR) markers. For RAPDs, ten decamer primers were tested and the six most stable and diverse were finally selected (primer kits A to Z; Operon Technology Inc., Alameda, California, USA). The PCR reactions were performed in a total volume of 15 μl containing 50ng DNA template, 2 mM MgCl2, 0.1 mMdNTPs, 0.3 μM primer, Q solution and 1U HotStartTaq Polymerase (Qiagen ®). The amplification conditions for RAPD were 94°C for 15 minutes, followed by 45 cycles of 94°C for 1 minute, 36°C for 1 minute and 72°C for 1 minute and a final elongation step at 72°C for 10 minutes. For SSRs, three pairs of nuclear microsatellite primers, tested for *Malus* species by LIEBHARD *et al*., (2002), were selected (Table 1). Forward primers were labeled with FAM fluorescent dye. PCR amplification was performed in a total volume of 20μl, containing 50ng DNA template, 2 mM MgCl2, 0.1 mMdNTPs, 0.3 μM of each primer, Q solution and 1.2 U HotStartTaq Polymerase (Qiagen®). The cycling profile started with an initial denaturation step of 2 min followed by 35 cycles of 20 seconds at 94°C, 30 seconds at 56°C and 60 seconds at 72°C and a final elongation for 5 minutes at 72°C, according to LIEBHARD *et al*. (2002).

Table 1. Nuclear microsatellite primer pairs used in this study

Name	Forward primer sequence	Reverse primer sequence
CH02c02a	ctt caa gtt cag cat caa gac aa	tag ggc aca ctt gct ggt c
CH02h11a	cgt ggc atg cct atc att tg	ctg ttt gaa ccg ctt cct tc
CH03d12	gcc cag aag caa taa gta aac c	att gct cca tgc ata aag gg

All PCR reactions were carried out in an Applied Biosystems® Veriti® 96 well thermal cycler. For RAPD analysis, the PCR products were visualized under UV light after electrophoresis on 1.2% agarose gel stained with ethidium bromide. For SSR analysis, the PCR products, after denaturation at 75ºC for 5 minutes were analyzed by electrophoresis in 6% denaturing polyacrylamide gels. Following electrophoresis, the polyacrylamide gels were scanned with a fluorescent image analyzer (Molecular Imager Fx, Bio-Rad®). For RAPDs, PCR amplifications were performed twice and only the reproducible bands were scored as present (1) or absent (0) in each zone and a binary data matrix was prepared. For SSRs, single bands were

identified as homozygotes and double bands as heterozygotes for each specific marker and the genotypes were recorded.

Analysis of genetic data

For RAPDs, HW equilibrium was assumed and allele frequencies were calculated using the square root method. Using these frequencies, the genetic diversity within and among groups was partitioned, with a hierarchical analysis of molecular variance (AMOVA) and 9999 permutations, using Genalex 6.5 (PEAKALL and SMOUSE, 2012), following the approach developed for dominant markers (HUFF *et al*., 1993; PEAKALL *et al*., 1995). The percentage of polymorphic zones (P%), the effective number of alleles (Ne) and the average gene diversity (He) (NEI, 1973) were used to describe the diversity within subpopulations. The same software was used to perform AMOVA on the genotype and allele frequencies of the loci corresponding to the three SSR markers. Besides the effective number of alleles (Ne) and the average gene diversity (He), the heterozygosity observed (Ho) in the population and the inbreeding coefficient F were estimated. For both markers, the unbiased genetic distances among subpopulations (NEI, 1987) were used to describe differentiation and produce UPGMA dendrograms visualized with TreeView (PAGE, 1996). The R package adegenet (JOMBART, 2008) was used to perform PCA and discriminant analysis of principle components (DAPC), for both markers separately, in order to cluster the individual trees and to compare cluster membership of trees among subpopulations (JOMBART *et al*., 2010). For the pairwise comparison of the geographical distances among individuals and the relevant genetic dissimilarity tables of both markers, a Mantel test (MANTEL, 1967) was performed with the zt software (BONNET and VAN DE PEER, 2002). Using the data from both markers and the geographic coordinates of the individuals simultaneously, a MCMC simulation was carried out to infer the number of panmictic groups, to assign each individual tree in one of these groups (clusters) and to present the membership of each tree spatially. The R package Geneland (GUILLOT and SANTOS, 2010) was used for this analysis, with 100,000 iterations for each simulation and keeping the simulation with the best mean posterior density after ten runs. The patterns of large-scale spatial genetic structure were investigated using kinship coefficients (Fij) which were assessed between all pairs of trees using the data for RADPs, according to HARDY (2003). The regression slope (bF) of kinship coefficients on log-transformed distance was computed based on the relationship between genetic similarity and geographical distance between individuals. For both analyses the statistical significance was determined using the 99% confidence interval while the Fij was defined after 10,000 permutations; both measures were calculated using SPAGeDi 1.4 (HARDY and VEKEMANS, 2002).

RESULTS

Polymorphism was found in 29 out of 45 RAPD zones (64.44%), hereafter described as "loci". The mean number of alleles / locus was 1.64, the mean effective number of alleles 1.35 and the mean expected heterozygosity 0.20. The AMOVA placed most of diversity within groups (84%). The diversity within groups was lower in Group5 which was represented by less individuals (Table 2). For nuclear SSR markers, all three loci were polymorphic with an average of 3.75 alleles per locus. The mean effective number of alleles was 2.79, while the mean

observed and expected heterozygosity were 0.60 and 0.55 respectively. As a result, the average inbreeding coefficient F was slightly negative (F = -0.076). For SSR markers, the AMOVA showed that diversity was found mostly within groups (96%) rather than among them (4%). Allele richness and the effective number of alleles were lower in Group5 and higher in Group3. While expected heterozygosity did not differ much among groups, there were large differences for the observed heterozygosity, with Group3 exhibiting the largest. For this reason, Group3 demonstrated the most negative inbreeding coefficient (F = -0.197). The only group with a heterozygote deficit (F = 0.108) was Group5 (Table 3).

Table 2. Genetic diversity within subpopulations using RAPDs. N: number of individuals, n: mean number of alleles, n(e): mean effective number of alleles, He: mean expected heterozygosity and P%: percentage of polymorphic zones

Group	N	n	n(e)	He	P%
Group2	20	1.47	1.31	0.18	51.11
Group3	21	1.49	1.32	0.19	53.33
Group4	16	1.49	1.29	0.17	51.11
Group5	8	1.40	1.25	0.15	46.67

Table 3. Genetic diversity within subpopulations using nuclear SSR markers. N: mean number of individuals, n: mean number of alleles, n(e): mean effective number of alleles, Ho: mean observed hereozygosity, He: mean expected heterozygosity, F: inbreeding coefficient

Group	N	n	n(e)	Ho	He	F
Group2	18.00	4.00	2.54	0.56	0.53	-0.095
Group3	19.67	4.33	3.19	0.70	0.58	-0.197
Group4	15.67	4.00	3.05	0.63	0.56	-0.122
Group5	06.67	2.67	2.37	0.50	0.54	0.108

Since diversity for both types of markers was mainly found within groups, differentiation was relatively low. RAPD markers showed higher levels of differentiation among groups, with a highly significant Φpt = 0.161, while SSR markers produced a significant but lower Φpt = 0.044. Genetic distances between groups were generally larger for the SSR markers and smaller for RAPDs, but clustering was clearer in the case of RAPD markers. Both markers produced a similar differentiation pattern among groups. Group5, representing the geographically distant group of Nipsa, was genetically more distant than the three groups of Pessani. Within Pessani, Group3 and Group4 were genetically closer to each other than Group2 (Figure 2). A similar grouping pattern was observed when differentiation was described at the individual level. Both for RAPD and SSR markers, individual trees belonging to Group5 clustered separately in the

DAPC plot of the two first axes (Figures 3 and 4). For RAPDs, Group2 also clustered separately in the same analysis. This is more evident when the individual probability of the DAPC discriminant clusters were considered, where Group2 and Group5 appeared clearly different than the other groups (Figure 5). A patchy grouping pattern was further observed for RAPDs within groups, as neighboring individuals clustered together.

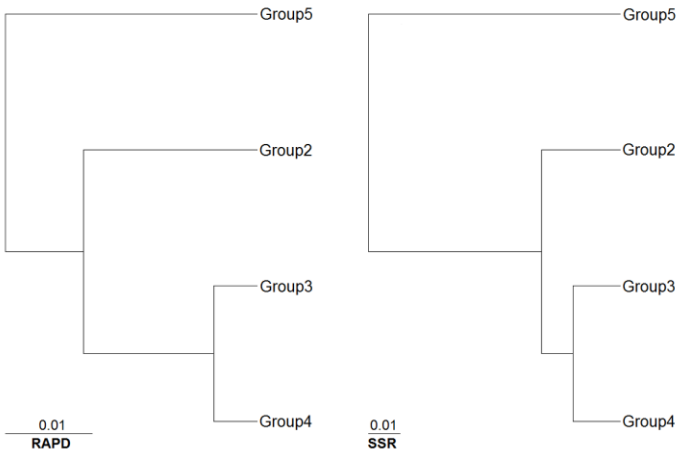

Figure 2. UPGMA dendrograms based on genetic distances for RAPD (left) and SSR (right) markers.

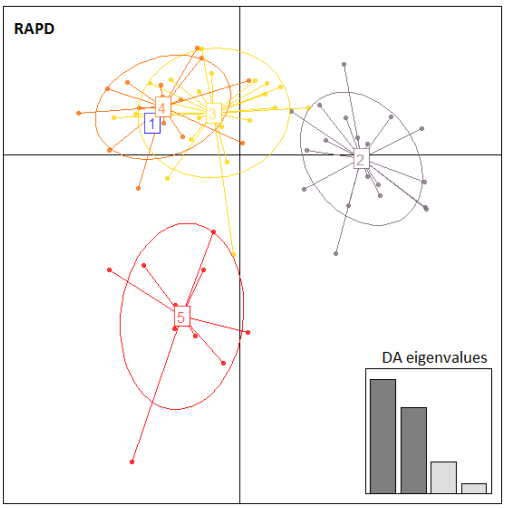

Figure 3. DAPC plot of individuals based on RAPD data for the two first PC axes. Numbers correspond to the relevant geographical groups.

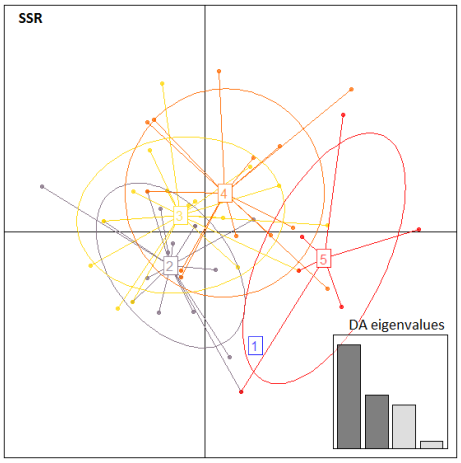

Figure 4. DAPC plot of individuals based on SSR data for the two first PC axes. Numbers correspond to the relevant geographical groups.

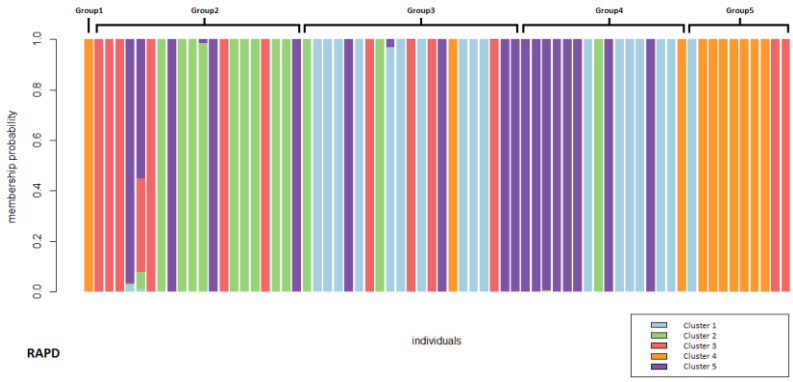

Figure 5. Plot of membership probability in the DAPC clusters for each individual.

The matrix of geographical distances between individuals was compared with the respective matrixes of genetic distances for RAPDs and SSRs using a Mantel test and the results showed for both markets a highly significant correlation (*P=0.0001*). The correlation coefficient between genetic distances and geographical distances for RAPDs was lower than the one for SSRs (*r=0.484* and *r=0.747* respectively). The MCMC simulation considering the genetic diversity of both markers and the geographical positions of the trees produced four clusters (Figure 6) following the above-mentioned differentiation trend. In particular, Group5 and Group2 fell into different clusters than Group3 and Group4 that were linked together. In two cases, individual trees that were geographically separated from their groups by roads or firebreaks, clustered separately from the trees of their group.

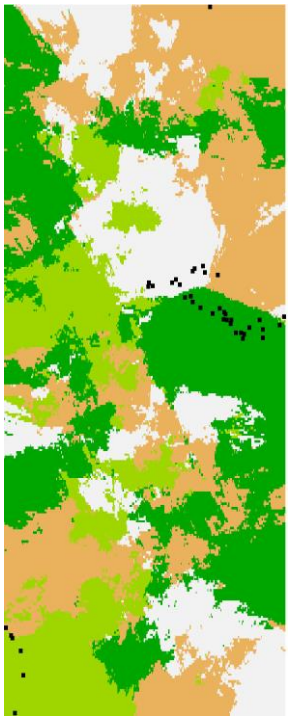

Figure 6. Map of estimated population membership for each individual after a MCMC simulation, using RAPD and nuclear SSR data. Each color corresponds to a different panmictic population.

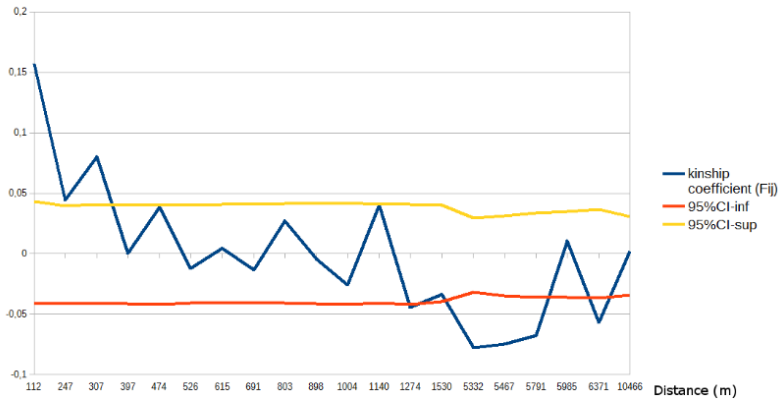

Figure 7. Large-scale spatial genetic structure autocorrelogramme, using the kinship coefficients (Fij) for RAPDs.

The large-scale spatial genetic structure analysis, using the kinship coefficients (Fij) for RAPDs, showed a continuously negative slope over space in the autocorrelogram (Figure 7). For this analysis, 20 distance classes were selected in order to display an equal number of pairs of individuals in each class. A high and significant mean value of Fij was detected in the first distance (Fij = 0.157), where a steeper decrease of Fij was also detected in this class. Similar patterns but with lower signal and values of Fij were also observed for SSR markers. The study area showed a significant Fij up to 360 m for RAPDs, while significant negative values of Fij were detected in larger geographical distances (1529 – 5790 m).

DISCUSSION

For both RAPD and nuclear SSR markers, diversity and especially differentiation among groups were found relatively low. Perennial and outcrossing plant species with similar geographical expansion usually exhibit higher levels of diversity within populations, when examined with the same type of genetic markers (e.g. ADAMIDIS *et al*., 2014; NYBOM, 2004). However, peripheral populations of plant species with a patchy distribution type in a fragmented landscape often have low genetic diversity (e.g. MONTGOMERY *et al*., 2000; HEDRICK, 2005), as observed in this study. Furthermore, diversity within groups was probably related with the number of individuals existing in each group. Group5 was less diverse for both markers, with lower observed heterozygosity and higher inbreeding coefficient for SSRs. Group5 was geographically isolated and included fewer individuals than the other groups. A significant correlation between genetic diversity and population size has been previously reported for various plant species (e.g. LEIMU *et al*., 2006).

Our results indicated a specific grouping pattern for both markers, with RAPDs showing the strongest differentiation. According to this grouping pattern, Group5 was genetically distant from all other groups. This was rather expected since Group5 was a geographically isolated and small group of trees in Nipsa. Within the largest set of individuals in Pessani, Group3 and Group4 were closely linked for both markers, while Group2 clustered separately. These three groups represented trees growing on roadsides. Group3 and Group4 were sets of trees growing on the same road, while Group2 was separated from the other two by a large firebreak.

The differentiation among individuals, described by both markers, revealed a patchy pattern among small groups of trees separated by roads, firebreaks and/or distance. This patchy differentiation was observed within groups as well, especially in Group2 and Group4. This was confirmed by the autocorrelation analysis that described a significant large-scale spatial genetic structure, probably revealing ineffective gene flow within the study area. The continuously negative slope of the autocorrelogram and the negative significant regression slope implied a structure across space (e.g. DINIZ-FILHO and TELLES, 2002; HEUERTZ *et al*., 2003). The Mantel test further revealed a significant correlation between genetic and geographic distances between individual trees and this positive correlation was stronger for the SSR markers. These findings suggest a high genetic fragmentation level for the European population of *M. trilobata*, typical for a peripheral and marginal plant population. It seems that in our study, RAPDs better described the differentiation caused by gene flow barriers, such as roads, firebreaks and streams, creating patches and spatial discontinuity, while SSRs seemed to describe more efficiently differentiation caused by distance in a geographically continuous set of individuals (within

patches). This presumably lies in the different attributes these two distinct markers have: Random genomic markers such as RAPDs probably represent better the whole genome, since they usually include several zones. SSRs are represented by much fewer loci, but they are codominant and much more diverse and thus they usually reflect the role of mating more efficiently and produce smoother differentiation patterns (eg. NYBOM, 2004; WAN *et al*., 2004; AI, 2014).

M. trilobata is an entomophilous and zoochorous species. In this type of plants, the movement of animals is crucial for plant mating events, effective pollen gene flow and the maintenance of sufficient levels of genetic diversity for the survival of the species under changing environmental conditions (ELLSTRAND, 2014; LIU *et al*., 2015), especially since it can bridge the distance among patches within a fragmented landscape (PETIT *et al*., 2005).

Humans induce habitat fragmentation through the establishment of roads, fields, plantations, buildings and infrastructure that represent barriers to animal movement (DIDHAM *et al*., 1996; FORMAN and ALEXANDER, 1998). Failing to cross anthropogenic barriers decreases food availability and ultimately might lead to increased mortality (FAHRIG, 2007). While flying insects are able to cover larger distances, several studies show that they seem to remain within their own forage patches in many cases, regardless of the distance among them (e.g. LEVIN and KERSTER, 1974; COMBA, 1999; BHATTACHARYA *et al*., 2003; FERNÁNDEZ *et al*., 2019).

Plants that belong to the genus *Malus* are reported to be mostly pollinated by honeybees and bumblebees (DENNIS, 2003). Although there are no studies on flowering and pollination of *M. trilobata*, it probably depends on bees for pollen transfer, as reported for most crabapple and apple species and their cultivars (e.g. MAYER *et al*., 1989). Bees usually demonstrate limited movement across foraging patches (RASMUSSEN and BRØDSGAARD, 1992; OSBORNE and WILLIAMS, 2001; BHATTACHARYA *et al*., 2003) and they usually do not fly across patches, even when these patches are close to each other and are separated only by small roads (BHATTACHARYA *et al*., 2003). Bees usually visit closest neighboring flowering plants and remain site constant within patches (RASMUSSEN and BRØDSGAARD, 1992; COMBA, 1999). This spatial pattern of bee movement lies probably in their preference to spend less energy by visiting nearby and familiar flowers, compared to taking the risk of searching for new sites with new flowers and learning to use them (BHATTACHARYA *et al*., 2003). Especially in highly fragmented landscapes, the higher mortality rate of using the surrounding matrix (FAHRIG, 2001; 2007) often leads to lower dispersal rates between patches (SCHTICKZELLE *et al*., 2006).

Our results indicate that the population of the present study is fragmented and that roads, firebreaks, areas with plantations and agriculture, all function as potential barriers to gene flow. Considering that bee hives are frequent in the sites where *M. trilobata* grows, we suggest that the genetic differentiation pattern described in this study was mainly caused by the foraging and movement behavior of the main pollinators. In our results, Group3 and Group4, growing on the same side of the road, were genetically linked, while Group2 that was separated from Group3 by a firebreak, was found genetically distanced. Trees growing in the same geographical group belonged to the same genetic cluster, with trees separated by roads being the only exception (Figure 6). We therefore assume that short distances that separate roadsides, firebreaks, plantations and fields can become gene flow barriers for *M. trilobata* due to the fact that pollinating bees tend to remain on one side of a barrier and do not prefer to cross that barrier

unless their food supply declines (BHATTACHARYA *et al*., 2003). Several studies report that habitat fragmentation can have a possible impact on plant–pollinator interactions (AIZEN and FEINSINGER, 1994; STEFFAN-DEWENTER and TSCHARNTKE, 1999), while LIU *et al*., (2015) suggest that correspondence of spatial genetic patterns between plants and insects does not necessarily occur, due to the complexity of the mating mechanism of both organisms.

Besides gene flow, the peripheral nature of the European population of *M. trilobata* studied here may have influenced the profile of genetic diversity observed, assuming that this population grows in ecological marginal sites, under intense selection pressure that may have reduced its effective population size. Genetic diversity in such populations is frequently low and unevenly distributed in space, due to fragmentation and ecological differentiation at the small geographical scale (e.g. ELIADES *et al*., 2019).

CONCLUSIONS AND CONSERVATION IMPLICATIONS

The European peripheral population of *M. trilobata* is small, isolated and spatially fragmented (KORAKIS *et al*., 2006). Its patchy distribution, separated geographically by barriers such as fire brakes, roads, pine plantations, natural habitats of other species and agricultural fields, has led to a decline of genetic diversity. This population is endangered by accidental habitat destruction and an increasing habitat fragmentation by human activities, such as agriculture, road construction, forestry operations and mining, among others.

According to our results, pollen flow between patches and small groups of trees is probably restricted. This fact indicates that the European population of *M. trilobata* would be still endangered by genetic erosion and extinction, even if its current habitat would remain intact. Division of plant patches can result to a further reduction of the already low frequency of bee movement, leading to lower rates of visitation in small isolated parts of the population (BHATTACHARYA *et al*., 2003). Although most roads and other human constructions exist only for a few generations, several studies have reported the negative effects of roads on genetic diversity and genetic differentiation in animal species, including pollinating insects (HOLDEREGGER and DI GIULIO, 2010). Along with measures to prevent habitat loss, when preparing conservation strategies and plans, the maintenance of inter-population pollen transfer in fragmented habitats should be considered (HADLEY and BETTS, 2012).

A conservation strategy for the studied population should include *in situ* measures to maintain the existing patches of the population by preventing habitat loss via targeted or accidental human interference. These measures should be designed and implemented in short time and may include fire protection plans and actions, vegetation management and consideration of the tree locations in all future development plans in the region. The regeneration via seedlings and sprouting in the natural population should be secured and promoted. Habitat continuity, especially as far as the movement of bees is concerned, should be restored and maintained. Furthermore, *ex situ* measures should be planned for the near future, including the creation of backup assessments and seed orchards on selected sites, close to the original population patches, using vegetative propagation of the existing trees and seedlings. Artificial plant establishment could then enrich the gene pool of the current population patches and promote gene transfer across them. It is important to note that sampling for *ex situ* conservation purposes should be designed in a representative way according to the different patches of the

population distribution, in order to capture possible adaptive genetic variants that follow small scale differentiation patterns related to ecological conditions.

More research on the reproductive biology of *M. trilobata*, the vegetative propagation of this species and the ecological processes occurring in its habitat is needed in order to design an effective conservation strategy.

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GENETIČKA RAZNOLIKOST I PROSTORNA STRUKTURA UGROŽENIH EVROPSKIH POPULACIJA Malus *trilobata* U GRČKOJ

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Izvod

Malus trilobata je retka vrsta drveća koja se javlja u nekoliko malih i razdvojenih populacija u istočnom delu mediteranskog basena. Glavna evropska populacija nalazi se u regionu Evros (SI Grčka) i podeljena je u pet različitih podpopulacija prateći geografski obrazac vrsta. Genetska raznolikost približno cele populacije (69 stabala) analizirana je korišćenjem nuklearnih mikrosatelita i slučajnih genomskih markera. Polimorfizam je otkriven u 29 od 45 lokusa genomskih markera (64,44%), dok su za nuklearne mikrosatelitske markere sva tri lokusa bila polimorfna sa prosečno 3,75 alela po lokusu. Naši rezultati su otkrili određeni obrazac grupisanja za oba markera. Oba genetska markera pokazivala su relativno nisku genetsku raznolikost, što je u skladu sa prevladavajućom percepcijom da vrste sa fragmentovanim rasprostranjenjem imaju nisku genetsku raznolikost, dok je diferencijacija među jedinkama otkrila neravnomerni obrazac među malim grupama drveća razdvojenih putevima, protivpožarnim zaštitama ili udaljenostima . Ovi rezultati ukazuju na visok nivo genetske fragmentacije za glavnu evropsku populaciju *M. trilobata*, dok prisustvo puteva, protivpožarnih pregrada, plantaža četinara i poljoprivrednog zemljišta izgleda da funkcioniše kao potencijalne prepreke protoku gena. Shodno tome, i pošto je dobro dokumentovano da pčele izbegavaju da menjaju mesta za ishranu, sve dok je njihove hrane u izobilju, uočeni obrasci genetske diferencijacije mogli bi se delimično pripisati ishrani i letačkom ponašanju pčela, koje su glavni oprašivači ovih vrsta. Niski nivoi dostupne genetske raznolikosti u kombinaciji sa malom ukupnom populacijom i ponovljenim događajima šumskih požara unutar rasprostranjenosti *M. trilobata*, ugrožavaju opstanak vrste i nameću potrebu za temeljno organizovanom strategijom očuvanja.

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