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RESEARCH ARTICLE

Genetic consequences of landscape features in two rear edge, highly fragmented metapopulations of a mediterranean conifer

Camilla Avanzi · Alessandro Vitali · Paolo Piovani · Ilaria Spanu · Carlo Urbinati · Giovanni Giuseppe Vendramin · Matteo Garbarino · Andrea Piotti

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Abstract

Context Habitat fragmentation is expected to erode genetic diversity, which instead needs to be preserved for promoting species adaptation to a changing climate. As this expectation has found mixed support in forest trees, consistent results on the genetic consequences of fragmentation requires adequately replicated experimental designs, as well as an explicit assessment of which landscape features, if any, could mitigate its detrimental effects.

Objective Evaluating the role of several landscape attributes in buffering the detrimental effects of fragmentation in two metapopulations of silver fir.

Methods We genotyped 904 silver fir (*Abies alba* Mill.) trees from 18 local populations forming two metapopulations comparable for size and extension in the Apennines, a Mediterranean mountain range. We identified the signatures left by the fragmentation process on the genetic features of silver fir local populations. After removing potentially confounding effects due to different evolutionary histories, we used a multivariate approach for testing the relative effect of demographic, geographic, environmental and topographic factors on genetic features of both metapopulations.

Results We found comparable signals of the habitat fragmentation impact on the genetic diversity and structure of both investigated metapopulations. Fragmentation effects were less pronounced in the largest local populations (but not the least isolated), located on gentler slopes with higher soil water availability and lower heat exposure.

Conclusions Our results suggest the existence of a set of demographic and environmental factors that could have coherently buffered the detrimental genetic effects of fragmentation in both metapopulations. These findings could be useful to plan landscape restoration for the evolutionary rescue of mixed forests that once characterized Mediterranean mountain ecosystems.

Keywords Habitat fragmentation · Genetic diversity · Landscape genetics · Marginal populations · Metapopulation ecology · *Abies alba*

C. Avanzi · I. Spanu · G. G. Vendramin · A. Piotti
Institute of Biosciences and Bioresources, National Research Council, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Florence, Italy

A. Vitali (✉) · C. Urbinati
Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche 10, 60131 Ancona, Italy
e-mail: alessandro.vitali@univpm.it

P. Piovani
Freelance biologist, Langhirano, Italy

M. Garbarino
Department DISAFA, Università degli Studi di Torino, Largo Braccini 2, 10095 Grugliasco, Italy

Introduction

Forest ecosystems are seriously threatened by climate change (Allen et al. 2010; Seidl et al. 2017) as well as by other human-driven disturbances, such as habitat fragmentation (Wilson et al. 2016). The impact of such threats on forests largely depends on the responses of trees, which are forests' pillars. Tree species can cope with global changes by tracking the shift of their ecological niche through migration, as well as by phenotypic plasticity and adaptation, or by combining these responses (Aitken et al. 2008). Genetic diversity underpins adaptive processes guaranteeing local persistence despite changing environmental conditions (Hampe and Petit 2005). Trees developed several strategies to maintain high levels of genetic diversity, such as flexible mating systems, high outcrossing rates and effective long-distance dispersal (Petit and Hampe 2006). Developing a dynamic approach for the conservation of such genetic diversity is vital to maintain the evolutionary potential of tree populations (Alfaro et al. 2014; Fady et al. 2016a), thus fostering forest resilience (Vinceti et al. 2020). The ultimate objective of genetic conservation and management of forest trees should be the maintenance of evolutionary processes acting across a mosaic of diverse populations and environmental gradients, rather than the mere preservation of alleles and genotypes (Koskela et al. 2013). In this perspective, the conservation of peripheral tree populations at the rear edge of species distribution ranges is particularly relevant as they are located where the most significant environmental and evolutionary changes will likely occur (Fady et al. 2016b). Besides preserving the genetic diversity heritage of glacial refugia, rear edge populations also represent reservoirs of potentially pre-adapted genetic variants for reinforcing the adaptive potential of more northern populations (Aitken and Bemmels 2016) and for producing resistant planting material (Konnert et al. 2015).

Habitat fragmentation is a landscape-scale process whose outcomes are the decrease in number and size of populations and the increase of average inter-population distances (Fahrig 2003). It is considered as one of the most serious threats to the persistence of populations and species (Wilson et al. 2016) as it exposes increasingly small and isolated populations to risks related to demographic, environmental and genetic stochasticity (Ouborg et al. 2006). Although fragmented populations are expected to suffer from genetic depletion and to exhibit a larger degree of genetic divergence due to increased genetic drift, elevated inbreeding and reduced gene flow (Young et al. 1996), such theoretical predictions have found mixed support in empirical studies on forest trees leading to what is known as 'the paradox of forest fragmentation genetics' (Aguilar et al. 2008; Kramer et al. 2008; Lowe et al. 2015). Besides paradoxical results, studies on the impact of forest fragmentation—highly heterogeneous in terms of fragmentation levels and dispersal syndromes investigated (Lowe et al. 2005; Ottewell et al. 2009)—are often lacking suitable experimental designs, hindering consistent conclusions at the species level (Jump and Peñuelas 2006; Bacles and Jump 2011). This is partially linked to the intrinsic nature of the fragmentation process which, by determining highly diverse and stochastic outcomes, often forces researchers to apply suboptimal, largely descriptive and idiosyncratic sampling designs (Bacles and Jump 2011). Beyond the basic comparison between fragmented and continuous populations, any attempt to reach consistent conclusions about the genetic consequences of fragmentation on forest trees would benefit both from replicating such investigation in more than one metapopulation (i.e., 'a set of local populations which interact via individuals moving among populations', sensu Hanski and Gilpin 1991), as well as from an explicit assessment of which and how landscape attributes impact the genetic diversity and structure of tree populations (Manel et al. 2003; Sork and Smouse 2006; McRae and Beier 2007). The combined analysis of genetic and environmental data could help to detect environmental critical thresholds to guarantee sufficient levels of genetic diversity (Bacles and Jump 2011). Nonetheless, very few studies on the genetic consequences of fragmentation have explored the effects of landscape variables besides the standard metapopulation ecology indices (e.g., size, isolation and density of remnant forest patches) (da Silva Carvalho et al. 2015; DeSilva and Dodd 2020; Delnevo et al. 2021) but none applied a suitably replicated experimental design.

Silver fir (*Abies alba* Mill.) is one of the most relevant conifer species in the montane belts of Mediterranean Europe. With the current climatic change, this species has gained renewed interest due to its unexpected drought tolerance in central and southern Europe (Carrer et al. 2010; Tinner et al. 2013; Vitali et al. 2017; Vitasse et al. 2019). In the Apennines, at one of the southern margins of the species distribution, silver fir features a complex genetic structure (Piotti et al. 2017) and high levels of fragmentation (Rovelli 1995), determined by a controversial combination of past climatic oscillations and relatively recent anthropogenic impacts, that have reduced its role of dominant forest species over the last thousands of years (Di Pasquale et al. 2014; Magri et al. 2015; Piotti et al. 2017; Morales-Molino et al. 2021).

Within these prolonged and widespread conditions of fragmentation, we investigated the genetic consequences of landscape features in two similarly sized silver fir metapopulations, located respectively in the northern and in the central Apennines. We characterized all the existing natural local populations occurring within each metapopulation with a set of microsatellite genetic markers to identify the genetic footprints left by the fragmentation process on the levels of genetic diversity and differentiation, inbreeding, effective population size and spatial genetic structure. Then, after standardizing all parameters at the metapopulation level to remove potential confounding effects due to different evolutionary histories, we used a multivariate approach to investigate the relationships among genetic features and a set of landscape attributes calculated at the local population-level in a GIS environment. Our main goal was testing the relative effect of demographic (i.e., population size), geographic (i.e., degree of isolation), and a set of environmental and topographic factors on altering the genetic layout of fragmented silver fir local populations. The specific experimental design and data treatment applied in this study maximized the chance to isolate the landscape attributes most likely involved in buffering the negative fragmentation effects. We finally discussed the implications of unravelling these synergic factors for the development of genetic-based strategies for the conservation and appropriate management of fragmented forest populations.

Materials and methods

Study areas and sampling activities

A total of 904 silver fir trees from 18 natural local populations were sampled within two metapopulations (Fig. 1 and Table 1). The first one is located in the northern Apennines, within the Tuscan-Emilian Apennine (TEA) National Park and the second one in the central Apennines, within the Gran Sasso–Monti della Laga (GSL) National Park. In both TEA and GLS metapopulations, silver fir has a highly fragmented distribution, made of small and isolated local populations (Fig. 2) frequently surrounded by plantations of unknown origin (Rovelli 1995; Gallucci and Urbinati 2009; Urbinati 2014; Santini et al. 2018). A previous genetic survey showed that northern and central Apennine silver fir populations belong to different gene pools which separated shortly before the Last Glacial Maximum (Piotti et al. 2017). Whether such separation and more recent demographic dynamics were driven by long-term climatic patterns, neolithic fires and other anthropogenic factors, or a combination of them, it is still debated (Tinner et al. 2013; Magri et al. 2015; Piotti et al. 2017; Morales-Molino et al. 2021). However, the scarcity of palaeobotanical data from the eastern slope of central Apennines prevents developing sound hypotheses on the relative importance of such factors in this geographic area.

All natural local populations occurring in both metapopulations were sampled (8 in TEA and 10 in GSL, Table 1 and Fig. 1), collecting approximately 50 trees in each local population, respecting a minimum distance of 20 m among each other. For each sampled tree, fresh needles were collected for genetic analyses and spatial coordinates were recorded with a metric handheld GNSS device (Garmin Ltd., USA).

Genotyping

DNA was extracted from around 50 mg of frozen needles using the DNeasy 96 Plant Kit (Qiagen, Germany) following manufacturer's instructions. All individuals were genotyped at 16 unlinked nuclear microsatellite markers (nSSRs) (NFF7: Hansen et al. 2005; Aag01, Aat01, Aat02, Aat03, Aat04, Aat05, Aat06, Aat08, Aat09, Aat10, Aat11, Aat13, Aat14, Aat15 and Aat16: Postolache et al. 2014) and three chloroplast microsatellite markers (cpSSRs) (Pt30141 and Pt30249: Liepelt et al. 2001; Pt71936: Vendramin et al. 1996). The procedures for multiplexing and PCR amplification are reported in Postolache et al. (2014) for nSSRs and in Leonarduzzi et al. (2016a) for cpSSRs. All PCR reactions were performed on

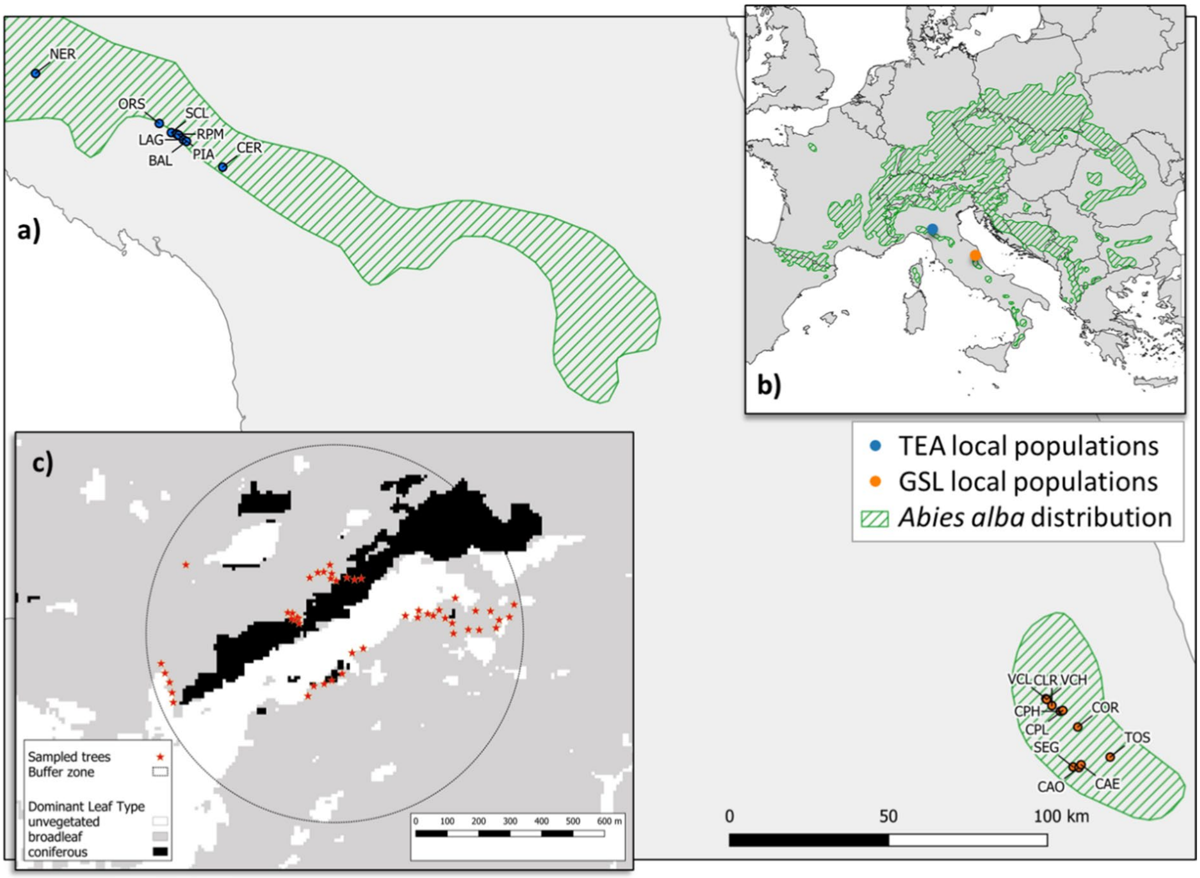

Fig. 1 Spatial distribution of silver fir local populations sampled (**a**) within the Tuscan-Emilian Apennine National Park (TEA, blue dots) and within the Gran Sasso–Monti della Laga National Park (GSL, orange dots). See Table 1 for exact local population names and labels. The location of the two metapopulations with respect to the species distribution is reported in box (**b**). Although highly imprecise regarding natural local populations in these areas, the species distribution according to Caudullo et al. (2017) is overlapped with green lines. Example of the sampling scheme applied (**c**), where red stars represent the trees sampled for genetic analyses, and the circle the 600-m radius buffer zone around each local population centroid that was used to extract landscape attributes. Dominant Leaf Type data are shown as under-layers, representing non-forested areas (white), broadleaved forests (grey) and coniferous stands (black)

a GeneAmp PCR System 9700 thermal cycler (Perkin Elmer). PCR products were run on a AB 3500 sequencer (Applied Biosystems, USA), with LIZ-500 as internal size standard. The resulting profiles were sized using GeneMarker (SoftGenetics).

Landscape attribute assessment

Landscape attributes describing local population size, land cover, climate, topography and human pressure data proxies were extracted by public datasets using QGIS software (Table 1). The borders of each local population were drawn using both tree positions collected with a GNSS device during field activities, and Google satellite images for a posteriori check assessment. The resulting convex hull polygons were used to compute the surface area (AREA) of each local population. The attributes available as raster layers were extracted using a 600 m fixed buffer drawn around each local population centroid (Fig. 1c) and computing the median value of all pixels included in each buffer. The size of the adopted buffer was based on the minimum circumscribed circle that includes all sampled trees of the largest local population.

Information on broadleaved (BROAD), coniferous (CONIF) and treeless (TRLES) proportional

Table 1 Geographic coordinates, number of sampled trees and landscape attributes of each local population of northern (TEA) and central (GSL) Apennine metapopulations

Metapopulation	Local population	Site	Lat (°N)	Long (°E)	Sampled trees (N)	AREA (ha)	BROAD (%)	CONIF (%)	TRLES (%)	AMT (°C)	MTWQ (°C)	AP (mm)	PWQ (mm)	ELEV (m)	SLO (°)	HLI	CTI	ROU	WIND (m/s)	ISO	ROAD (m)
TEA	NER	Monte Nero	44.559	9.506	48	84	66.8	13.0	20.2	4.7	14.1	1399	218	1648	17	0.73	6.35	1.57	6.3	0.905	962
	ORS	Monte Orsaro	44.415	9.993	48	4	71.5	0.5	28.0	4.4	13.9	1467	221	1579	30	0.62	5.69	2.20	7.8	0.180	1791
	SCL	Monte Scala	44.389	10.041	48	2	87.2	0.6	12.2	5.7	15.2	1380	211	1402	20	0.60	6.20	1.72	5.6	0.026	4567
	RPM	Rocca Pumacciola	44.381	10.069	50	2	85.5	0.0	14.5	5.7	15.3	1343	206	1470	26	0.64	5.64	2.00	5.2	0.004	2924
	PIA	Rocca Pianaccia	44.385	10.063	48	4	90.1	0.1	9.8	5.8	15.3	1357	207	1375	24	0.57	5.94	1.92	5.2	0.001	2442
	LAG	Lago Verde	44.369	10.086	50	6	89.2	0.3	10.5	4.9	14.3	1381	214	1485	23	0.49	6.10	1.88	5.8	0.011	2466
	BAL	Lago Ballano	44.363	10.099	48	1	77.3	0.2	22.5	5.1	14.6	1365	211	1494	27	0.60	6.08	2.05	6.2	0.027	2622
	CER	Cerreto	44.289	10.241	50	18	66.9	0.1	33.0	4.3	13.7	1222	208	1615	21	0.54	6.03	1.78	6.8	0.586	2197
GSL	VCH	Valle Corte High	42.703	13.377	50	3	99.3	0.0	0.7	6.9	16.2	933	248	1540	34	0.74	5.90	2.37	4.4	0.310	4496
	VCL	Valle Corte Low	42.705	13.374	50	2	98.2	0.3	1.5	8.5	17.9	891	232	1369	36	0.70	5.75	2.45	3.6	0.331	4438
	CLR	Colle Romicito	42.685	13.397	48	27	99.1	0.1	0.8	7.2	16.5	883	227	1355	34	0.61	5.86	2.33	3.7	0.161	4499
	CPH	Ceppo High	42.667	13.427	50	4	99.1	0.4	0.5	6.0	15.3	909	235	1520	27	0.54	6.17	2.05	4.9	0.054	3028
	CPL	Ceppo Low	42.669	13.436	71	9	99.5	0.4	0.1	7.1	16.4	907	229	1347	30	0.53	5.84	2.18	3.1	0.063	2206
	COR	Cortino	42.621	13.489	50	16	89.1	4.9	6.0	8.8	18.1	885	207	1296	26	0.63	5.94	2.00	4.7	0.090	680
	SEG	Sega D'Acqua	42.509	13.464	50	4	99.8	0.1	0.1	7.9	17.1	922	234	1370	32	0.68	5.85	2.28	4.7	0.484	2745
	CAO	Colle Abete West	42.505	13.486	48	3	99.2	0.1	0.7	6.0	15.2	980	265	1575	25	0.80	6.20	1.95	4.3	0.488	4171
	CAE	Colle Abete East	42.514	13.495	48	4	80.8	0.0	19.2	6.9	16.1	961	256	1537	17	0.69	6.61	1.57	5.2	0.438	3462
	TOS	Tossicia	42.530	13.608	49	22	95.1	4.9	0.0	8.7	17.9	924	229	1140	23	0.51	6.87	1.88	2.5	0.824	1819

AREA population surface area, *BROAD, CONIF and TRLES* land cover values expressed as percentages of broadleaved, conifer and non-forested areas, respectively, *AMT* annual mean temperature, *MTWQ* mean temperature of the warmest quarter, *AP* cumulative annual precipitation, *PWQ* cumulative precipitation of the warmest quarter, *ELEV* elevation, *SLO* slope, *HLI* Heat Load Index, *CTI* Compound Topographic Index, *ROU* Roughness Index, *WIND* mean wind speed, *ISO* degree of isolation, *ROAD* proximity to the closest road as a proxy of human pressure. Labels: see Table S1

Fig. 2 Aerial images of two silver fir local populations in the Tuscan-Emilian Apennine (TEA) National Park at **a** Lago Ballano (BAL) and **b** Lago Verde (LAG) sites. Images were taken in winter to enhance the contrast with the broad-leaved forest

cover was extracted from the Dominant Leaf Type (DLT) product, available on the Copernicus Land Monitoring Service for the 2018 as reference year (https://land.copernicus.eu/pan-european/high-resolution-layers; last access: March 16, 2023). The main bioclimatic features were extracted from CHELSA database (Karger et al. 2017, 2018), a high resolution (30 arc sec) climate data set currently hosted by the Swiss Federal Institute for Forest, Snow and Landscape Research WSL. In particular, Annual Mean Temperature (AMT), Mean Temperature of Warmest Quarter (MTWQ), Annual Precipitation (AP) and Precipitation of Warmest Quarter (PWQ) were used in the analyses. Topographic features were obtained from the TINITALY DEM (Digital Elevation Model with 10 m resolution, Tarquini et al. 2007): elevation (ELEV), slope (SLO), Heat Load Index (HLI), Compound Topographic Index (CTI) and Roughness Index (ROU). HLI expresses the temperature of a surface by considering the solar radiation, the aspect and slope of that surface (McCune and Keon 2002) and it represents a measure of heat exposure. CTI is a measure of wetness based on the ratio of the catchment area of a given pixel and its slope (Burrough and McDonnell 1998). ROU is a measure of terrain heterogeneity, and it is calculated for each location by summarizing the change in elevation within a 3×3 pixel grid (Riley et al. 1999). The mean wind speed (WIND) at 50 m above the ground was obtained by the national grid (1 km-resolution) of the Italian aeolian atlas (http://atlanteeolico.rse-web.it/; last access: October 10, 2022). Isolation value (ISO) was computed averaging all the Euclidean distances between the centroid of single local populations and the others. Finally, proximity of each local population centroid to the closest road (ROAD) was extracted using the national and provincial road network as a proxy of the past and ongoing anthropogenic impact (Dainese et al. 2017; Vitali et al. 2018; Garbarino et al. 2020).

Data analysis

The genetic structure of the 18 local populations was investigated by Principal Component Analysis (PCA) on the matrix of both allele and haplotype counts per population, using the *dudi.pca* function of the R package *adegenet* (Jombart and Ahmed 2011).

To explore how different genetic features of silver fir local populations were affected by the fragmentation process, 14 genetic parameters were calculated at the local population level. Within-population genetic diversity was measured by calculating allelic richness (Ar) and expected heterozygosity (H_E) on nSSRs, and haplotypic richness (Pb) and diversity (H_k) on cpSSRs. Ar was calculated using HP-RARE expected (Kalinowski 2005), H_E using GenAlEx v6.5 (Peakall and Smouse 2012) while Pb and H_k using Contrib (Petit et al. 1998). Both Ar and Pb were calculated using a rarefaction method and a minimum sample size of 42 individuals. Within-population genetic differentiation was measured on nSSRs by calculating the average genetic distance among individuals (GD) and its standard deviation (GDsd) using GenAlEx. The average genetic distance among individuals was also calculated on cpSSRs (D^2_{sh}), applying the Goldstein et al. (1995)'s distance as implemented in Robledo-Arnuncio et al. (2005). Among-population genetic differentiation was expressed by G_{ST} (Nei 1973) and D (Jost 2008), calculated as the average pairwise differentiation of each local population from the others of the same metapopulation. Both G_{ST} and D were calculated on nSSRs using the *mmod* R package (Winter 2012). G_{ST} and D range from zero (when two populations are genetically identical) to one (when they are completely fixed for different alleles), but they differ in their sensitivity to the biological processes that drive differentiation and fixation (Jost et al. 2018). The average level of inbreeding characterizing each local population (F_{IS}) was estimated on nSSRs using GenAlEx. The effective population size (*Ne*), that is the actual number of reproductive trees, was used as a proxy of the intensity of genetic drift, and it was calculated on nSSRs using NeEstimator (Do et al. 2014). Finally, spatial genetic structure (SGS) was assessed with spatial correlograms. Analyses were performed on nSSR data with INEST v2.2, using Nason's F_{ij} kinship coefficients (Loiselle et al. 1995) and even distance classes (30 m). SGS of each local population was summarized in terms of average kinship coefficient among individuals in the first distance class (F_1), slope of the log-linear regression of F_{ij} against spatial distances (b-log) and intensity of SGS (*Sp*) (Vekemans and Hardy 2004).

The possible occurrence of a bottleneck within each local population was assessed by applying both heterozygosity (*H*) excess and M-ratio (MR) deficiency tests (Cornuet and Luikart 1996; Garza and Williamson 2001), as implemented in INEST v2.2 (Chybicki and Burczyk 2009). In the calculation, the proportion (p_g) and average size (δ_g) of multi-step mutations were set to 0.22 and 3.1, respectively, as recommended by Peery et al. (2012). The statistical significance of the test was evaluated using the one-tailed Wilcoxon test with 1×10^6 permutations. The bottleneck intensity was assessed by calculating ΔH and ΔMR, which were, respectively, the difference between *H* and MR values expected at the equilibrium (H_{eq} and MR_{eq}) and the observed *H* and MR values. As *H* is expected to be higher than H_{eq}, and MR to be lower than MR_{eq} under bottleneck, the smaller the ΔH (and the larger the ΔMR), the higher the intensity of the bottleneck. The heterogeneity in the bottleneck intensity among local populations was evaluated by Friedman tests, with ΔH and ΔMR values across loci used as an independent blocking variable and local populations used as the independent grouping factor.

To investigate the relationships among genetic and landscape attributes, all genetic parameters were standardized at the metapopulation level to account for the potential differences due to past demographic divergence. A PCA was then carried out on the main matrix of 14 standardized genetic parameters with the *prcomp* function of the R package *stats* and using the set of landscape attributes (Table 1) as secondary matrix. The graphical output of the analysis was a 'triplot' where landscape attributes were displayed as secondary vectors whose direction and length represented their correlation with the first and second principal components of the PCA on genetic parameters.

Results

The PCA applied to nuclear and chloroplast markers showed that silver fir local populations from northern and central Apennines were clearly genetically separated along both first principal components, which respectively explained about 21% (nSSRs) and 28% (cpSSRs) of the total variance (Fig. 3).

Allelic richness (*Ar*) ranged from 3.41 (CAO) to 4.71 (NER), which means that the most genetically diverse local population had ~ 38% alleles per locus more than the lowest genetically diverse one (Table 2). NER also exhibited the largest values of expected heterozygosity ($H_E = 0.46$), haplotypic richness ($Pb = 25.26$) and haplotypic diversity ($H_k = 0.97$), while the lowest values were shown by the locally peripheral populations ORS ($H_E = 0.36$), CAO ($Pb = 25.26$) and VCL ($H_k = 0.81$). Among these four measures of within-population genetic diversity, only H_E was, on average, significantly different between the two metapopulations, with TEA local populations characterized by larger H_E values ($H_{E\text{-}TEA} = 0.43$) than GSL local populations ($H_{E\text{-}GSL} = 0.39$) ($t = -2.64$, $df = 11.54$, $P = 0.022$). Within-population genetic distances were significantly different between the two metapopulations both in their average (GD: $t = -3.45$, $df = 11.55$, $P = 0.005$) and standard deviation values (GDsd: $t = -2.91$, $df = 15.55$, $P = 0.011$). GD values of TEA local populations ranged from 15.89 (NER) to 12.31 (ORS), with an average of 14.29, while GD values of GSL local populations ranged from 13.63 (CAE) to 11.85 (VCL), with an average of 12.83. Genetic differentiation among local populations of the same metapopulation ranged from 0.014 to 0.035 for *D* values, and from 0.009 to 0.026 for G_{ST} values. For both parameters, ORS was the most genetically differentiated local population. The fixation index (F_{IS}) ranged from − 0.04 (COR) to 0.06 (ORS), but it was significantly larger than zero only in the CPL

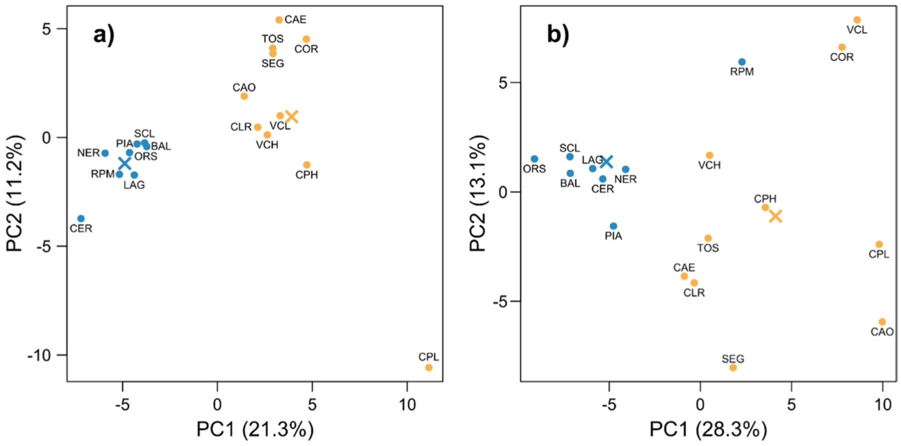

Fig. 3 Biplot from Principal Component Analysis (PCA) on data from **a** nuclear and **b** chloroplast microsatellite markers. Blue dots represent local populations from the Tuscan-Emilian Apennine (TEA) National Park in northern Apennines, while orange dots represent local populations from the Gran Sasso–Monti della Laga (GSL) National Park in central Apennines. The '×' symbols represent the metapopulation centroids. Labels: see Table 1

local population ($F_{IS}=0.06\pm0.027$). The effective population size (*Ne*) ranged from 10 (CAO) to 4251 (CER), meaning that the largest local population was characterized by ~ 400 times the number of reproducing individuals with respect to the smallest one. Eight local populations showed *Ne* values <50 (ORS, SCL, PIA, BAL, VCL, SEG, CAO, CAE), while only three local populations had a $Ne>500$ (CER, CPL and TOS). Finally, the intensity of spatial genetic structure (*Sp*) ranged from − 0.002 (TOS) to 0.040 (ORS). Beyond H_E, GD and GDsd, no other genetic parameter was significantly different between the two metapopulations.

In almost all local populations, a significant signature of genetic bottleneck was found according to the test for deficiency in M-ratio but not to the test for heterozygosity excess (Table 3). The only local population showing a significant heterozygosity excess was PIA. The Friedman tests revealed that local populations were not heterogeneous with respect to bottleneck intensity (ΔH: Friedman chi-squared = 13.363, df = 16, $P=0.646$; ΔMR: Friedman chi-squared = 13.255, df = 16, $P=0.654$).

The first two principal components (PC1 and PC2) of the multivariate analysis on standardized genetic parameters explained 62% of the total variance. With reference to the Euclidean space of Fig. 4, nuclear allelic richness (*Ar*), expected heterozygosity (H_E) and average within-population genetic distances (GD) had negative loadings on PC1 while genetic differentiation (G_{ST} and *D*) and parameters describing spatial genetic structure (*Sp* and F_1) had positive loadings. Thus, PC1 can be interpreted as a 'diversity vs differentiation' spectrum which runs from highly diverse and scarcely differentiated/spatially structured local populations to poorly diverse and highly differentiated/structured ones. Instead, haplotypic diversity (H_k, *Pb* and D^2_{sh}) and inbreeding (F_{IS}) had strong negative loading values on PC2.

The scores of PC1 were negatively correlated with AREA ($\rho=-0.450$, $P=0.061$) and CTI ($\rho=-0.447$, $P=0.063$), while positively correlated with HLI ($\rho=0.43$, $P=0.075$), ROU ($\rho=0.425$, $P=0.079$) and SLOPE ($\rho=0.416$, $P=0.086$) (Fig. 4). This means, for instance, that CAO and ORS, which mostly suffered the effects of fragmentation (i.e., lowest genetic diversity, highest differentiation and spatial structuring), were the smallest silver fir stands in the two metapopulations, located on the steepest and roughest slopes, with lower soil water availability and higher heat exposure. In contrast, NER and TOS, which minimally suffered the fragmentation effects (i.e., highest genetic diversity, and lowest differentiation from surrounding populations) were the largest silver fir stands, located on the less steep slopes, with higher soil water availability and lower heat exposure. Dividing the set of local populations in two groups (above and below the median value of the distribution

Table 2 Genetic parameters of each local population of northern (TEA) and central (GSL) Apennine metapopulations

Type of markers		*nSSRs*											*cpSSRs*		
Metapopulation	Local population	*Ar*	H_E	GD	GDsd	*D*	G_{ST}	F_{IS}	*Ne*	F_1	b-log	*Sp*	*Pb*	H_K	D^2_{sh}
TEA	NER	4.71	0.46	15.89	3.87	0.0169	0.0107	0.05	474	0.0153	– 0.0072	0.0073	25.26	0.97	22.73
	ORS	3.53	0.36	12.31	4.50	0.0354	0.0258	0.06	20	0.0519	– 0.0376	0.0397	12.34	0.90	20.59
	SCL	3.79	0.44	14.38	4.06	0.0190	0.0123	-0.01	27	0.0086	– 0.0090	0.0091	15.85	0.91	20.61
	RPM	4.02	0.43	14.21	3.84	0.0143	0.0094	0.00	73	0.0266	– 0.0149	0.0153	10.33	0.87	26.26
	PIA	3.79	0.43	13.96	3.88	0.0210	0.0137	-0.03	37	0.0352	– 0.0229	0.0237	14.57	0.94	40.73
	LAG	4.32	0.43	15.13	3.66	0.0167	0.0113	0.03	406	0.0216	– 0.0193	0.0198	17.75	0.95	32.93
	BAL	3.66	0.41	13.92	4.41	0.0320	0.0217	0.05	24	0.0139	– 0.0129	0.0131	15.44	0.95	32.25
	CER	4.26	0.45	14.51	3.92	0.0211	0.0136	-0.03	4251	0.0202	– 0.0049	0.0049	18.81	0.96	24.13
GSL	VCH	3.57	0.37	12.21	3.55	0.0173	0.0134	0.05	109	0.0167	– 0.0131	0.0133	19.88	0.96	24.46
	VCL	3.95	0.37	11.85	3.22	0.0144	0.0113	-0.01	47	0.006	– 0.0059	0.0059	12.00	0.81	14.50
	CLR	4.09	0.40	13.37	3.66	0.0190	0.0142	0.06	104	0.0562	– 0.0139	0.0148	17.10	0.94	28.90
	CPH	4.05	0.38	12.33	3.15	0.0176	0.0136	-0.02	114	0.0047	– 0.0020	0.0020	16.35	0.93	25.76
	CPL	4.10	0.40	13.47	3.44	0.0195	0.0144	0.06	1310	– 0.0026	– 0.0005	0.0005	15.83	0.92	20.00
	COR	3.89	0.43	13.13	3.59	0.0279	0.0196	– 0.04	156	– 0.0048	– 0.0006	0.0006	10.57	0.86	15.30
	SEG	4.06	0.40	12.84	3.83	0.0181	0.0134	0.02	21	0.0296	– 0.0243	0.0250	13.15	0.90	26.80
	CAO	3.41	0.37	12.00	4.17	0.0258	0.0200	0.02	10	0.0627	– 0.0296	0.0316	10.24	0.82	16.56
	CAE	4.03	0.41	13.63	3.93	0.0142	0.0104	0.04	43	0.0110	– 0.0033	0.0033	18.69	0.94	27.39
	TOS	4.38	0.40	13.46	3.48	0.0196	0.0145	0.00	709	– 0.0017	0.0016	– 0.0016	19.10	0.95	25.39
P-values		0.736	**0.022**	**0.005**	**0.011**	0.390	0.886	0.863	0.465	0.494	0.191	0.207	0.618	0.199	0.124

Ar: allelic richness; H_E: expected heterozygosity; GD and GDsd: average genetic distance among individuals of the same local population, and its standard deviation, calculated on nuclear microsatellites (nSSRs); *D* and G_{ST}: pairwise Jost's *D* and Nei's G_{ST} values, averaged at the metapopulation level; F_{IS}: fixation index; *Ne*: effective population size; F_1: average pairwise kinship coefficient among individuals in the first distance class (0–30 m); b-log: slope of the log-linear regression of kinship coefficients against distances; *Sp*: intensity of spatial genetic structure; *Pb*: haplotypic richness; H_k: haplotypic diversity; D^2_{sh}: average Goldstein et al. (1995)'s distance calculated on chloroplast microsatellites (cpSSRs). The statistical support of the comparisons of each genetic parameter between metapopulations is reported in the last row of the table. Significant *P*-values ($\alpha < 0.05$) are in bold. Labels: see Table 1

Table 3 Results of the bottleneck detection analysis

		Heterozygosity excess test				M-ratio deficiency test			
Metapopulation	Local population	*H*	H_{eq}	Z score	*P*	MR	MR_{eq}	Z score	*P*
TEA	NER	0.462	0.4692	− 0.2585	0.6092	0.706	0.8316	− 1.9132	0.0256
	ORS	0.3865	0.3959	0.7150	0.7188	0.7497	0.8511	− 1.3631	0.0869
	SCL	0.4764	0.4407	1.1927	0.1268	0.6591	0.8364	− 2.5558	0.0034
	RPM	0.4389	0.4226	0.2040	0.2163	0.7634	0.8417	− 0.9308	0.1775
	PIA	0.4979	0.4398	1.8519	0.0338	0.7236	0.836	− 1.6008	0.0535
	LAG	0.4315	0.4378	0.0000	0.5103	0.6927	0.8384	− 2.0684	0.0174
	BAL	0.4424	0.422	0.9655	0.1795	0.6471	0.84	− 2.499	0.0044
	CER	0.4542	0.453	0.0000	0.5103	0.6556	0.8336	− 2.5854	0.0032
GSL	VCH	0.3981	0.4058	− 0.0568	0.5324	0.7547	0.848	− 1.5903	0.0554
	VCL	0.3969	0.4337	− 0.9087	0.82	0.7527	0.8391	− 1.6471	0.0487
	CLR	0.4266	0.4494	− 0.7384	0.7734	0.7311	0.8354	− 1.7039	0.0421
	CPH	0.3815	0.4182	− 1.0342	0.8512	0.6913	0.842	− 2.1718	0.0129
	CPL	0.4023	0.4239	− 0.4654	0.6831	0.6616	0.8425	− 2.5854	0.0032
	COR	0.4315	0.4123	0.6722	0.2636	0.6934	0.8471	− 2.0166	0.0199
	SEG	0.4347	0.4289	0.1704	0.4456	0.735	0.8419	− 1.6471	0.0486
	CAE	0.4102	0.4112	− 0.0517	0.53	0.7257	0.8463	− 1.7581	0.038
	CAO	0.4047	0.3997	0.3248	0.3392	0.7887	0.8489	− 1.0223	0.1558
	TOS	0.3774	0.4242	− 0.9308	0.8257	0.7428	0.8407	− 1.6547	0.0482

H and MR are, respectively, the observed values of expected heterozygosity and M-ratio, while H_{eq} and MR_{eq} the values expected at the equilibrium. *P* is the *P*-value of the Wilcoxon signed-rank test used to assess the statistical significance of observed vs expected differences. Labels: see Table 1

of PC1 scores), local populations that retained larger genetic diversity (negative scores in Fig. 4) were, on average, 2.82 times larger than local populations with lower genetic diversity. Accordingly, their Compound Topographic Index was 1.07 times larger, while slope, Roughness Index, and Heat Load Index were, respectively, 1.32, 1.18, 1.14 smaller than local populations with genetic diversity below the median value. Finally, the scores of PC2 were not significantly correlated with any landscape attributes.

Discussion

In this study, we dug into genetic data from two rear edge, highly fragmented silver fir metapopulations testing for a possible mitigating role of landscape features on the genetic consequences of fragmentation. We found that past bottlenecks have left a coherent genetic footprint in the two metapopulations. After removing variance between metapopulations to properly focus on non-idiosyncratic effects of fragmentation, we found a gradient in its impacts on genetic features. Some silver fir local populations went through pronounced effects (i.e., depletion of genetic diversity and increase of genetic divergence) whilst others displayed more resistance in both metapopulations. The latter were the largest local populations (but not the least isolated), located on less steep slopes, with higher soil water availability and lower heat exposure. This suggested the existence of a set of demographic and environmental factors that could have coherently buffered the detrimental effects of fragmentation in both metapopulations. These results could be used in planning landscape restoration for the evolutionary rescue of the potential Apennine montane belt vegetation composed by mixed *Fagus-Abies* forests.

Genetic consequences of silver fir habitat fragmentation

The two metapopulations (TEA and GSL), within a surface area of a few squared kilometres, include

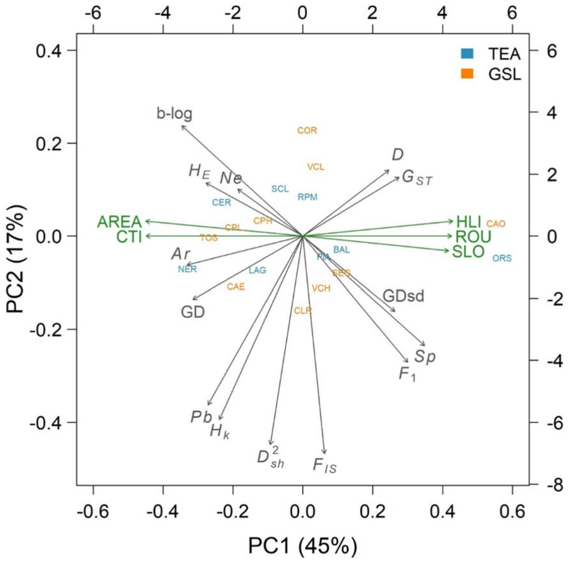

Fig. 4 Triplot from the Principal Component Analysis (PCA) on genetic parameters standardized at the metapopulation level. PC1 is mainly related to nuclear genetic variation, while PC2 to haplotypic diversity and overall inbreeding. Local population labels (see Table 1) were coloured according to the metapopulation (blue = Tuscan-Emilian Apennine National Park—TEA; orange = Gran Sasso–Monti della Laga National Park—GSL). Grey arrows are the loadings of each genetic parameter (see Table 2), whereas green arrows indicate the significant correlations of PC1 scores with the landscape attributes (see Table 1)

several small silver fir local populations with similar census size ranges (dozens to few thousands of individuals) and levels of isolation (average pairwise distance among local populations of 18 and 13.5 km in TEA and GLS, respectively). Similar levels of fragmentation—which allowed to set up an ideally replicated experimental design for studying the genetic consequences of the process—have apparently left a coherent genetic footprint. Firstly, almost all studied local populations showed consistent signals of past demographic changes. Although both the tests on the deficiency of M-ratio and excess of heterozygosity (H_k) are effective for identifying demographic reductions given a proper sample size in terms of individuals and loci (Peery et al. 2012), they tend to intercept different types of bottlenecks. M-ratio, that is the range in allele size conditioned on the number of alleles, is suitable for identifying more ancient and prolonged bottlenecks; on the contrary, H_k, that is the heterozygosity given the number of alleles, is effective to identify more recent bottlenecks (Williamson-Natesan 2005). Although they could be refined by applying genomic marker set with higher resolution, our results seem to tell that current silver fir local populations are remnants of larger populations that underwent ancient, severe bottlenecks which, according to reconstructions based on different data sources, dated back from 70 to 5k yrs BP (Follieri et al. 1998; Magri et al. 2015; Piotti et al. 2017). The results of such past demographic dynamics and more recent human pressure have determined a generally low effective population size (*Ne*). In most cases (15 out of 18 populations), *Ne* estimates were below the critical threshold of 500. It is still debated how to interpret the 50/500 rule of thumb proposed by Franklin (1980) and the reliability of *Ne* as a suitable indicator of genetic diversity for forest trees, particularly when populations are large and continuously distributed (Santos-del-Blanco et al. 2022). Generally, *Ne* value > 500 is considered the minimum size for ensuring the viability of populations in the long run (Jamieson and Allendorf 2012).

Although the bottleneck analysis showed a coherent genetic footprint on allele frequencies left by past size reductions, we found that other genetic features were quite heterogeneous among local populations within each metapopulation. For instance, both genetic diversity and differentiation measures showed similar ranges within the two metapopulations. Least diverse and most differentiated local populations exhibited a decrease in allelic richness of 25% (in TEA) and 22% (in GLS) as well as an increase in genetic differentiation of 174% (in TEA) and 92% (in GLS) with respect to most diverse and less differentiated ones. This shows a strong and similar impact of fragmentation on the genetic structure of the two metapopulations, in sharp contrast with the general lack of signals at the basis of the so-called ‘paradox of forest fragmentation genetics’ (Kramer et al. 2008). These differences matched with those obtained in the few studies showing detectable genetic signals of fragmentation in *Fagus sylvatica*, a wind pollinated species often co-occurring with silver fir in southern European mountain ranges, when comparing continuous and fragmented populations (Jump and Peñuelas 2006; Leonardi et al. 2012). However, such comparison is impossible when studying silver fir in the Apennines due to the lack of continuous populations, and this is the reason why we focused on targeting results on local dynamics in two comparable metapopulations.

We applied a multivariate approach to a set of genetic parameters to find the main axes summarizing the metapopulations' genetic structure after standardizing the parameters at the metapopulation level to remove potential confounding effects due to different evolutionary histories. There is clear evidence that silver fir populations of northern and central Apennines belong to different genetic lineages that started diverging around 66k yrs ago (Piotti et al. 2017). The first axis of the resulting PCA depicted a 'diversity vs differentiation' spectrum, defining a continuum from local populations that were more genetically diverse and less differentiated (i.e., NER and TOS) to those that were more genetically differentiated and less diverse (i.e., CAO and ORS). Since fragmentation is expected to deplete genetic variation and increase genetic divergence, this 'diversity vs differentiation' spectrum could be interpreted as a severity gradient of the fragmentation effects. The same gradient was found in both metapopulations, as TEA and GSL local populations were similarly distributed on the first PCA axis. The inherently limited number of local populations within each metapopulation (eight and ten for TEA and GSL, respectively) precluded a separate statistic treatment for each metapopulation to test the consistency between metapopulations, only allowing such a qualitative evaluation. Nonetheless, even when genetic parameters were not standardized within metapopulations, the same gradient emerged along the first PCA axis, with the two metapopulations separated on the second axis (results not shown). Overall, these results point towards the existence of landscape features that enhanced or buffered the genetic consequences of fragmentation at the local scale, leaving a genetic footprint that prevailed over the one left by regional-scale dynamics. The standardization procedure indeed enabled the comparison of the genetic signals between metapopulations. However, in the analysis we could not thoroughly test the effect of possibly different demographic histories within metapopulations, as we have little information about them. There are no historical records available to reconstruct recent demographic dynamics in the two investigated areas. Instead, paleobotanical records indicate that, until 5k years ago, *Abies alba* was far more locally abundant in northern Apennines, both at high and low elevations, even in sites where today is absent (Follieri et al. 1998; Bertoldi et al. 2004; Vescovi et al. 2010; Magri et al. 2015; Morales-Molino et al. 2021). Unfortunately, the lack of paleobotanical records from the eastern slope of the central Apennines hindered possible comparison based on this layer of information. However, the demographic reconstruction based on genetic data about these two areas by Piotti et al. (2017) showed that the species had similar demographic trajectories in the two investigated areas since the two genetic lineages diverged before the Last Glacial Maximum. Although detailed demographic reconstructions were not performed at the local population level, available evidence suggests that the two metapopulations were two single, large populations that have been shrinking over the last millennia. This is in line with the bottleneck analysis showing homogeneity in the bottleneck intensity among local populations and, overall, provides support to the robustness of our results when based on the comparability between metapopulations. Of course, as pointed out before, deeper genomic investigations will provide more precise estimates of demographic processes at a higher temporal resolution.

Population size and soil water availability mitigate the detrimental effects of fragmentation

We found that local populations least affected by fragmentation were the largest ones, even when large local populations were highly isolated (e.g., NER). Population size is positively correlated with levels of genetic variation in many plant and animal species (Frankham 1996). When fragmentation effects were detected, larger tree populations often exhibited the highest values of genetic diversity as well as the lowest values of differentiation (Prober and Brown 1994; Hall et al. 1996; Petit et al. 2002; Vakkari et al. 2006). Fragmentation acts not only by decreasing the average number and size of populations, but also increasing the average distance among remaining ones (Fahrig 2003). Isolated populations should undergo reduced gene flow and, therefore, face a greater risk of entering trajectories of increasing genetic differentiation and pauperization. However, we found that isolation was not significantly associated with the genetic layout of silver fir local populations. This means that the average distance among remnant fir patches neither prompted nor hindered local genetic dynamics and that levels of gene flow among silver fir remnants were still sufficiently high for counteracting the effects of genetic drift (Hamrick 2004). When studies on the genetic consequences of fragmentation were set up in order to disentangle the relative role of population size and isolation in speeding up genetic drift, they found that highly outcrossing species were more sensitive to demographic reductions (Leonardi et al. 2012). Among conifers, silver fir pollen and seeds are relatively heavy-weighted (Eisenhut 1961), but considerable rates of pollen (62–83%) and seed (48–79%) gene flow were detected by a previous study carried out in the central Apennines within two of our study populations (CPL and CPH) (Leonarduzzi et al. 2016b). Reconstructing the parentage of seedlings and trapped seeds also showed that seed dispersal can occasionally reach up to 200 m (Cremer et al. 2012; Leonarduzzi et al. 2016b; Major et al. 2021). Pollen dispersal can even reach farther distances, and there is substantial evidence on its effectiveness in maintaining genetic connectivity among fragmented populations of wind-pollinated trees (e.g., Bacles et al. 2005; Kamm et al. 2009; Aleksic et al. 2022), with surprisingly high levels of pollen immigration documented also in highly isolated stands of *Quercus robur* and *Pinus sylvestris* (Robledo-Arnuncio and Gil 2005; Buschbom et al. 2011). The low correlation between parameters describing chloroplast and nuclear diversity as well as the smaller variance among local populations intercepted by PCA in parameters describing chloroplast diversity point to the non-interruption of gene flow by pollen in the two metapopulations, even in small local populations and at distances up to 40 km (NER-ORS). In addition, it is worth bearing in mind that, in both geographic areas, there are silver fir plantations that could have acted as additional (and unconsidered) pollen sources at different time scales. Nonetheless, if we consider the coniferous forest cover (CONIF) as a proxy of the presence of any plantations in the buffer area of each local population, we found that this was not correlated with genetic diversity and differentiation patterns. Since most of these stands were planted over the last century with material of unknown origin (Urbinati 2014; Santini et al. 2018), characterizing their genetic make-up and monitoring gene flow in natural silver fir regeneration will be pivotal for assessing whether they represent sources of maladapted and/or genetically depleted material (Steinitz et al. 2012; Unger et al. 2016) or instead help to maintain the genetic connection among natural local populations. Besides the potential effects of outbreeding depression, dispersal dynamics may also be affected by genetic erosion itself in the long run (Ouborg et al. 2006). Inbreeding depression effects on plant fecundity, seed germination and seed dispersal capabilities were indeed observed in plants (Mix et al. 2006; Leimu et al. 2010). Unfortunately, no data on the reproductive dynamics of the studied local populations (e.g., average number of cones and seeds produced) are available to be correlated against genetic metrics estimated in the present study.

Besides their large size, local populations that maintained high levels of genetic diversity also exhibited a combination of specific topographic features: they were located on the roughest and steepest slopes, characterized by the lowest values of Heat Load Index (HLI) and the highest values of Compound Topographic Index (CTI). HLI represents a measure of soil heat exposure, while CTI is a measure of soil wetness. Taken together, these variables can be considered a proxy for describing soil water availability. Soil water availability is a key factor for silver fir growth and survival at different life stages (e.g., Nourtier et al. 2014; Sánchez-Salguero et al. 2017; Walder et al. 2021). For instance, Piedallu et al. (2023) found ten times higher mortality rates in silver fir forests with a high level of vulnerability directly related to water stress. Cailleret et al. (2014) found that, at regional scale, more xeric conditions (i.e., south facing, high slopes) can increase silver fir mortality. Although silver fir has a deep taproot system and can use water from relatively deep soil during drought periods (Magh et al. 2019), shallow soils, or any combination of topographic features determining low soil water availability, had an impact on its resistance to drought. In this regard, our results provide evidence that topographic features determining limited soil water availability influenced not only the probability of dieback but also to the capacity of a forest tree population to retain genetic diversity.

Human influence could be a confounding factor when linking genetic diversity and environmental features. However, the role of human activities was accounted by including the proximity of each local population to the closest road (ROAD) in the analysis (Dainese et al. 2017; Vitali et al. 2018; Garbarino et al. 2020) and it was seemingly unrelated to genetic features. Unravelling the reliance of genetic diversity on environmental variables is crucial to model future habitat suitability at a fine scale (Kittlein et al. 2022) and to support actions for management and conservation of forest tree species in a changing environment (Hoban et al. 2021; Vajana et al. 2022), as it is discussed in the following paragraph.

Linking genetic diversity and environmental variables to inform management and conservation actions

A higher number of tree species tends to increase the productivity of forest ecosystems (Vilà et al. 2013; Liang et al. 2016), and their resistance/resilience to biotic and abiotic stresses (Jactel and Brockerhoff 2007; Seidl et al. 2016) as well as to enlarge the range of ecosystem services they can provide compared to monospecific stands (Gamfeldt et al. 2013; Rudow et al. 2020). Besides some still controversial aspects, there is increasing evidence that such benefits occur also in the *Fagus-Abies* forests (Versace et al. 2021), and silver fir is considered, in some European countries, as the ideal species to steer forests towards greater production and resistance to climate change (Tinner et al. 2013; Vitasse et al. 2019). Despite their ecological relevance, Mediterranean mixed *Fagus-Abies* forests are on a long-term trajectory of demographic decline (e.g., Magri et al. 2015), in an area where the consequences of the climatic crisis are expected to hit hardest. Therefore, it is critical to take effective actions on extant natural populations of silver fir to enhance their regeneration in areas of potential expansion. Nature-based sylvicultural treatments such as selective thinning to release regeneration core areas (with tree age up to 70 years), gap opening to simulate the natural windthrow, or girdling of a limited number of dominant or concurrent beech trees can progressively improve the conditions for silver fir to establish and grow (Urbinati 2014). Beyond any type of sylvicultural treatment, recent literature calls for management programs to be fine-tuned based on a careful characterization of the ecological requirements of the species (Kittlein et al. 2022; Vejpustková et al. 2023). This study is precisely going in this direction by exploring the dependency of genetic diversity on landscape features and providing an additional, crucial information to the management and conservation of fragmented silver fir local populations. Genetic information is considered essential to assess the conservation status of populations (Laikre et al. 2020) and to design suitable strategies for the management, translocation and conservation of genetic resources (Aitken and Whitlock 2013; Fady et al. 2016a; Andrello et al. 2022). Although our study was based on neutral genetic markers, which are not informative regarding adaptive dynamics (Holderegger et al. 2006), our findings can nonetheless be distilled in few, clear indications for the conservation of shrinking silver fir Apennine forests. Firstly, our results tell which local populations, given their levels of genetic diversity and distinctiveness, should be prioritized for conservation actions. Secondly, they give new insights on which combination of environmental and topographic variables is buffering the detrimental effects of fragmentation. In particular, our findings point to the fact that sylvicultural treatments aimed at making local populations larger, rather than less isolated, could be more beneficial in counteracting the negative effects of genetic drift. They also provided a quantitative link between levels of genetic diversity and a multidimensional topographic space, which could be applied to enhance the chance of silver fir local populations to thrive and, thus, resist the genetic consequences of fragmentation. In fact, mapping this favourable multidimensional space can provide forest managers a tool to define areas where to intensify ad hoc reforestation efforts, obviously keeping in mind that our results can not be extended beyond the space of environmental and topographic parameters explored in the two metapopulations.

Conclusions

Studies on the genetic consequences of habitat fragmentation have often relied on suboptimal and largely descriptive sampling designs. To draw robust conclusions on silver fir rear edge populations, we *i*) selected two highly fragmented silver fir metapopulations featuring similar geographical extension and *ii*) set up data analysis to remove variance in genetic data possibly due to different demographic histories. We could then isolate genetic signals which point to population size and soil water availability as the driving factors that mitigated the detrimental effects of fragmentation. As drought is becoming more intense and prolonged in Mediterranean areas, the relationship between soil water availability and genetic diversity is particularly relevant when put in the context of climatic changes predicted at the southern edge of silver fir distribution.

Hopefully, our study would trigger new research on detecting consistent genetic consequences determined by habitat fragmentation, to increase our knowledge on the impact of this process on forest ecosystems. There are several ways to improve the data collection needed for that. First, the use of unmanned aerial vehicles will provide higher resolution spatial data, enhancing the recognition of small patches of adult trees and regeneration and a more accurate characterization of the topographic space where trees grow and regenerate. Including trees in their regeneration stages (seedlings and saplings) would be particularly beneficial to future studies. Since fragmentation is relatively recent in many forest ecosystems, shifting the research focus from adult trees to regeneration would allow a better detection of early genetic consequences of fragmentation in the short term (Lowe et al. 2015).

In addition, next generation sequencing genomic resources will permit a much deeper investigation of the evolutionary processes that produced those signals highlighted in our study. In particular, the recent release of the first version of silver fir genome (Mosca et al. 2019) and the increasing knowledge of the evolutionary significance of specific candidate genes and stress responses (Cailleret et al. 2014; Brousseau et al. 2016; Csilléry et al. 2020) could encourage the transition from landscape genetics to landscape genomics approaches. Landscape genomics investigations would improve the understanding of the functional link between genetic diversity and environmental features, useful grounds for developing genomic-informed conservation actions that could guide afforestation and reforestation initiatives to counteract the negative impact of habitat fragmentation on forest ecosystems.

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Data availability The datasets analysed during the current study will be made available from the corresponding author under request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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