Unexpected scenarios from Mediterranean refugial areas: disentangling complex demographic dynamics along the Apennine distribution of silver fir

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Title: Unexpected scenarios from Mediterranean refugial areas: disentangling complex demographic dynamics along the Apennine distribution of silver fir

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Running head: Demographic history of *Abies alba* in the Apennines

ABSTRACT

Aim Mediterranean refugial areas are generally underrepresented in large-scale genetic surveys of forest trees. In the case of silver fir (*Abies alba* Mill.), this has led to divergent hypotheses about the exact location of glacial refugia and the trajectory of recolonization routes. Based on comprehensive sampling of Apennine populations, we aimed to reconcile discrepancies about the number and location of refugia for silver fir in the Apennines and test alternative demographic scenarios developed from palaeobotanical and genetic data.

Location Mediterranean Basin; the Apennines and surrounding areas.

Methods 1167 individuals from 16 Apennine populations, extensively covering the species' distribution along the Italian Peninsula, and eight populations from the Alps and Eastern Europe were genotyped at 16 nuclear and three chloroplast microsatellite markers. The geographical distribution of genetic variation was explored using Bayesian clustering and multivariate methods. Based on inferred genetic structure, the demographic history of *A. alba* was assessed by approximate Bayesian computation (ABC) analysis.

Results Two unexpected characteristics of genetic structure emerged: a sharp genetic boundary in the central Apennines and a tight genetic connection between southern Apennine and Eastern European gene pools. Two Apennine areas, corresponding precisely with refugial areas hypothesized in most recent palaeobotanical syntheses, have high genetic diversity on a par with Eastern European populations. ABC analysis showed an ancient separation between Apennine and Eastern European gene pools followed by an admixture event that, mainly through directional gene flow via pollen, might have established the genetic similarity between southern Apennine and Eastern European populations. In addition, there was evidence that the central Apennines acted as a small-scale, isolated

refugium during the Last Glacial Maximum.

Main conclusions Silver fir rear edge populations have experienced a complex demographic history across several glacial-interglacial cycles, leading to unexpected genetic structure. Our study provides new insights into forest tree dynamics in the Mediterranean, showing the putative presence of multiple refugia for silver fir in the Apennines and a trans-Adriatic connection between silver fir populations in southern Italy and the Balkans.

Keywords *Abies alba*; approximate Bayesian computation; glacial refugia; Mediterranean basin; phylogeography; Pleistocene; post-glacial recolonization; nuclear and chloroplast microsatellites; rear edge populations; trans-Adriatic gene flow

INTRODUCTION

There is continuing debate about the location of glacial refugia. Such interest is justified by the importance of correctly placing glacial refugia and recolonization routes when interpreting current species' distributions, estimating species' migration potential and foreseeing possible range shifts (Petit *et al.*, 2008). Coupling genetic and palaeobotanical data is considered the most effective approach to infer past retraction-colonization dynamics in plants (Hu *et al.*, 2009). For a very small number of tree species, genetic and palaeobotanical records covering both the distribution core and edges are available, and well-grounded hypotheses on the topography of glacial refugia and post-glacial recolonization routes have been developed. Inevitably, such inference on past demography has also been tried for species with much less complete data available. In particular, biogeographic literature highlights that northern Mediterranean refugial areas, such as the Italian and Balkan Peninsulas, are generally underrepresented in genetic and palaeobotanical large-scale surveys on forest trees (Hampe & Petit, 2005; Liepelt *et al.*, 2009; Linares, 2011). Trees, and plants in general, have experienced complex dynamics due to environmental heterogeneity and palaeoclimatic events in these areas, leading to high phylogeographic complexity and idiosyncratic patterns (Nieto Feliner, 2014). The increasing availability of molecular data and refined statistical approaches are providing unprecedented power to unravel complex demographic histories, but to be effective these tools must be applied to appropriate sampling of the focal species.

Silver fir (*Abies alba* Mill.) is one of the most important forest tree species in Europe and results from available palaeobotanical and genetic studies (e.g. Liepelt *et al.*, 2002, 2009; Linares, 2011; Cheddadi *et al.*, 2014) have generated contrasting hypotheses about its Quaternary history. In particular, important but unsolved points are the location of isolated refugia (i.e. refugial populations that did not expand after the Ice Ages) and effective refugia (i.e. refugial populations that contributed to recolonization) and the phylogenetic relationships among populations from refugial areas. There is general agreement about the existence of at least two effective refugia in the Apennines and southern

Balkans, but so far only inconsistent speculations on their exact location and on recolonization routes have been proposed (Liepelt *et al.*, 2009; Linares, 2011; Cheddadi *et al.*, 2014). An attempt to synthesise different interpretations of genetic and palaeobotanical data is difficult since both disciplines suffer low availability of data for refugial areas (Liepelt *et al*., 2009; Linares, 2011; Tinner *et al*., 2013).

In the Apennines, the presence of *A. alba* during the late-glacial period has been demonstrated by palynological surveys, mainly from the northern Apennines and the Tyrrhenian side of the central Apennines (e.g. Follieri *et al.*, 1998; Vescovi *et al.*, 2010; Magri *et al*., 2015). Previous genetic work, based on a limited number of populations located mostly in Calabria and the northern Apennines, inferred the presence of at least two genetic clusters (Konnert & Bergmann, 1995; Liepelt *et al.*, 2009). This led to the hypothesis of an isolated refugium in the southern Apennines and an effective refugium, located either in the central (e.g. Konnert & Bergmann, 1995) or in the north-western Apennines (e.g. Cheddadi *et al.*, 2014), from which the recolonization of the Alps and central Europe may have started. In contrast, following early hypotheses about southern Italy as the starting point for the recolonization of Central Europe, a possible genetic continuity along the entire Apennine chain has been hypothesized several times (Scaltsoyiannes *et al.*, 1999; Linares, 2011; Camerano *et al.*, 2012). Some genetic studies even considered central Italy as a possible melting pot of recolonization routes from the Balkans and southern Apennines (Parducci *et al.*, 1996) or from the northern and southern Apennines (Larsen & Mekic, 1991). However, all of these previous studies have been based on limited sampling and critical questions remain regarding the genetic relationship between populations from the northern and southern Apennines and the origin of populations from the central Apennines. Establishing the evolutionary history of these fragmented Apennine silver fir populations is particularly urgent because they have high evolutionary and conservation value due to their unique genetic and eco-physiological features (Hansen & Larsen, 2004; Carrer *et al.*, 2010; Cheddadi *et al.*, 2014; Brousseau *et al.*, 2016). An intensive genetic survey covering the entire Italian Peninsula will allow robust testing of different phylogeographic hypotheses developed from palaeobotanical and

117 genetic data and resolution of these questions.

118 The main objective of this work was to investigate the genetic structure of Apennine silver fir
119 populations and reconstruct past demographic and recolonization dynamics affecting Apennine
120 genetic clusters. We intensively sampled 16 populations along the entire Apennine range, with a
121 particular focus on previously unsampled areas and adding several populations from surrounding
122 regions (i.e. the Alps and Eastern Europe). Overall, 1167 individuals were genotyped with
123 biparentally inherited nuclear and paternally inherited chloroplast microsatellite markers (hereafter
124 nSSRs and cpSSRs, respectively). The intensive sampling strategy and the large marker set used
125 allowed us to investigate: *i*) the genetic relationship between populations from the northern and
126 southern Apennines and the origin of central Apennine populations, *ii*) the possible presence of
127 genetic discontinuities and/or contact zones between different genetic clusters along the Apennines,
128 and *iii*) the genetic relationship between Apennine gene pools and those from surrounding areas.
129 Based on the genetic structure emerging from analyses of our data, we assessed the support for
130 alternative hypotheses about Pleistocene dynamics of silver fir populations in the Apennines
131 developed from palaeobotanical and genetic data through approximate Bayesian computation (ABC)
132 analyses.

133

134 **MATERIALS AND METHODS**

135

136 **Sample collection and genotyping**

137

138 Sixteen putatively autochthonous populations were sampled along the Apennine chain (five in the
139 northern, five in the central, six in the southern Apennines, Fig. 1 and Table S1.1) according to two
140 general criteria: *i*) extensively covering the species' distribution in this area, and *ii*) increasing the
141 sampling effort in terms of number of populations in areas sparsely covered by previous studies (i.e.
142 the central Apennines). We included five populations from the Alps and three populations from

143 Eastern Europe to investigate the genetic relationship of Apennine populations with those from
144 surrounding areas. From each population, needle tissues were collected from c. 50 adult individuals
145 at least 20 metres apart in order to adequately cover a large area (c. 3 ha) within each stand.
146 All sampled individuals were genotyped at 16 unlinked nSSRs (Aag01, Aat01, Aat02, Aat03, Aat04,
147 Aat05, Aat06, Aat08, Aat09, Aat10, Aat11, Aat13, Aat14, Aat15 and Aat16, Postolache *et al*., 2014;
148 NFF7, Hansen *et al.*, 2005) and three cpSSRs (Pt71936, Vendramin *et al.*, 1996; Pt30141 and
149 Pt30249, Liepelt *et al.*, 2001). The multiplexing and amplification procedures for nSSRs are reported
150 in Postolache *et al*. (2014). CpSSRs were multiplexed using the Type-it Microsatellite PCR kit
151 (Qiagen, Germany) with primer concentrations 0.1µM (Pt71936), 1µM (Pt30141) and 1.5µM
152 (Pt30249). PCR products were run on AB 3500 (Applied Biosystems, USA), with LIZ-500 as the
153 internal size standard. The resulting profiles were sized using GeneMarker (SoftGenetics).
154
155 **Genetic diversity and population structure**
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157 Standard genetic parameters describing within-population genetic variation and genetic
158 differentiation were estimated by GENALEX (Peakall & Smouse, 2012) and HP-RARE (Kalinowski,
159 2005) for nSSRs, and CONTRIB (Petit *et al*., 1998) for cpSSRs.
160 The presence of a genetic structure among sampled populations and the putative number of different
161 genetic clusters were evaluated using the model-based Bayesian clustering algorithm implemented in
162 STRUCTURE 2.3 (Pritchard *et al.*, 2000) and the empirical statistic ΔK (Evanno *et al.*, 2005; Earl &
163 von Holdt, 2012) on nSSR data. STRUCTURE was run using default settings and parameter values, and
164 varying K from one to 10. Each run consisted of 1×10^5 burn-in iterations and 5×10^5 data collection
165 iterations, and was replicated 10 times. After checking for convergence of diagnostic statistics,
166 different runs for the same K were averaged using the software CLUMPP (Jakobsson & Rosenberg,
167 2007). Bayesian analysis of population structure was run on the cpSSR dataset using BAPS (Corander
168 *et al.*, 2008) based on a non-spatial genetic mixture analysis for linked loci, with K varying from one

to 10. The best partition of populations into K clusters with the highest marginal log-likelihood after 10 replicates was chosen as the most representative one. Principal component analysis (PCA) was also performed on the arcsine square root transformed population allele and haplotype frequencies in R 3.2.4 (R Core Team, 2015) to evaluate the main features of the genetic structure obtained through Bayesian clustering with an independent approach.

The hierarchical partitioning of total molecular variance due to the genetic structure emerging from Bayesian clustering analyses was estimated through AMOVA (Excoffier *et al.*, 1992). Statistical significance of each hierarchical level (among K genetic clusters, among populations within genetic clusters, and within populations) was evaluated by 1×10^4 nonparametric permutations using GENALEX.

Approximate Bayesian computation to infer demographic history

To trace the demographic history of *A. alba*, the ABC procedure (Beaumont *et al.*, 2002) implemented in DIYABC 2.1 (Cornuet *et al.*, 2014) was performed based on the nSSR dataset. To simplify the analysis and limit the number of scenarios tested, we relied on the results of Bayesian and multivariate clustering analyses on our datasets (see Results) and on previously available palaeobotanical and genetic information. Therefore, ABC analyses were carried out on four groups of populations, hereafter referred to as NAPP (northern Apennines and the Alps), CAPP (central Apennines), SAPP (southern Apennines), and EAST (Eastern Europe), and scenarios were designed to cover plausible phylogenetic relationships among such genetic clusters.

The analysis of different demographic models (see Appendix S2) was performed following a two-step approach. In the first step, we compared six scenarios in which, for simplicity, all populations were assumed to have an identical prior distribution (Uniform distribution: 10-500000, Table S2.5) for the effective population size (*Ne*). In the second step, the whole dataset was used to compare the two scenarios that showed comparably high posterior probability in the previous analysis (Fig. 2). In

this final analysis, the prior distributions of effective population size were set according to results from the first step analysis (Table S2.6). To underline this main difference with respect to scenarios from the first step analysis, the two retained scenarios were then referred to as scenario A and B (Fig. 2).

The main characteristics and peculiarities of the two scenarios compared in the second step of the DIYABC analysis were as follows:

Scenario A is a hierarchical split scenario directly following STRUCTURE results (see Results), in which NAPP separated from EAST before generating, respectively, CAPP and SAPP.

Scenario B takes into account *i*) that silver fir populations from the Italian Peninsula and Eastern Europe are characterized by different and almost fixed mitochondrial variants (Liepelt *et al.*, 2002), and *ii*) the genetic structure emerging from STRUCTURE results. Therefore, considering that SAPP shares the same mitotype with NAPP and that SAPP and EAST belong to the same STRUCTURE cluster, the scenario was designed hypothesizing that, after an initial split between NAPP and EAST, SAPP was generated by admixture of EAST and an unsampled ghost population, which merged with NAPP. CAPP merged relatively recently with NAPP considering the likely post-glacial connection between these two groups of populations (Magri *et al.*, 2015). The admixture rates *ra* and 1-*ra* are the genetic contribution of each of the source populations to the origin of SAPP.

In both scenarios, $t_{\#}$ represents the time of occurrence of an event (expressed in number of generations) and $N_{\#}$ refers to the effective population size of the corresponding populations (N_{NAPP}, N_{CAPP}, N_{SAPP}, and N_{EAST} for the four clusters described above, N_{GHOST} for an unsampled ghost population, and Na for the ancestral population 'a') during each time period (e.g. $0 - t_1$ or $t_2 - t_3$) (Fig. 2).

Details of each competing scenario in the first step analysis, mutation rate, summary statistics, and model checking are provided in Appendix S2, Fig. S2.5 and Table S2.5.

RESULTS

221

222 **Genetic diversity and population structure**

223

224 The 16 nSSRs showed a total and mean number of alleles of 151 and 9.43 (from two alleles at Aat16
225 to 34 at NFF7), respectively. No evidence was found for the presence of null alleles or significant
226 genotypic disequilibrium among locus pairs. In addition, no evidence for selection was found by
227 outlier detection tests (FDIST and BAYESCAN, see Appendix S3 and Fig. S3.6), indicating that the
228 analyzed nSSRs were likely to be neutral, as suggested by Postolache *et al.* (2014). The allelic
229 richness (Ar_{84}) and expected heterozygosity (H_E) were above average in southern Italy, Eastern
230 Europe and the NER population in the northern Apennines (Fig. 3a, Table S1.1). Differentiation
231 indices showed a globally moderate differentiation among populations, with F_{ST}=0.097 and Hedrick's
232 G'_{ST}=0.155.
233 CpSSRs displayed 12 (Pt71936), 17 (Pt30141), and 6 (Pt30249) size variants, which combined into
234 164 haplotypes and a mean haplotypic diversity over populations of h=0.935. The haplotype richness
235 per population (Hr_{37}) ranged from 10 (COR) to 28 (ROM) (Fig. 3b, Table S1.1). Genetic
236 differentiation was in line with that found at nSSRs (G_{ST}=0.045).
237 STRUCTURE analysis revealed an optimal grouping at K=2 (Fig. S1.1) clearly distinguishing, along
238 the Apennines, populations located north and south of the Gran Sasso and Majella massifs (blue and
239 red, respectively, in Fig. 3c). Populations from the southern Apennines clustered with those from
240 Eastern Europe. AMOVA analysis showed that the proportion of total genetic variation explained by
241 differences between these two main genetic clusters was 7% (P<0.001). The next strongest level of
242 structuring was K=4, which grouped populations from the central Apennines separately from those
243 in the northern Apennines and the Alps, and populations from the southern Apennines were separated
244 from those in Eastern Europe (Fig. 3d). The main characteristics of the genetic structure emerging
245 from STRUCTURE analysis were confirmed by BAPS analysis on cpSSRs (Fig. 3e), PCA on both
246 marker types (Fig. S1.2), and pair-wise differentiation indices (Tables S1.2 and S1.3). In particular,

PCA analyses of both marker types showed a main separation resembling STRUCTURE results at K=2 along the first principal component (i.e. southern Apennine and Eastern European populations differentiated from northern Apennine and Alpine populations), whereas the second principal component highlighted the differentiation between southern Apennine and Eastern European populations.

Approximate Bayesian computation to infer demographic history

In the first step of DIYABC analysis, it was not possible to distinguish the most-likely scenario because similarly high posterior probabilities were found for scenarios 3 and 4 (respective probabilities 0.49 and 0.42, with largely overlapping 95% CIs; Table 1). Therefore, we decided to compare the two scenarios in a final analysis, in which scenario B showed a posterior probability significantly higher than scenario A (Table 1). The observed summary statistics and PCA results (Table S1.4 and Fig. S1.3) confirmed the good fit of scenario B to the data. The type I error rate was 0.314, and the average type II error rate was 0.257.

Under scenario B, SAPP originated at t_2 by an admixture event between EAST and a ghost population. The ghost population merged with NAPP at t_3 and gave the largest contribution (*ra*=0.780) to the formation of SAPP. The median values of the effective population sizes were 41800, 15500, 97900, 93500, 51300, and 2870 for N_{NAPP}, N_{CAPP}, N_{SAPP}, N_{EAST}, N_{GHOST}, and Na, respectively (Table 2 and Fig. S1.4). The posterior parameters showed that the effective population size of the ancestral population was estimated to be 14.5 and 32.5 times lower than those of NAPP and EAST, respectively, suggesting an expansion event at t_4. The results also indicated that the demographic expansion continued in the following events, which led to the formation of SAPP at t_2. More recently, a bottleneck is likely to have given rise to the formation of CAPP at t_1.

The median values of the divergence times t_1 (for CAPP and NAPP), t_2 (for the appearance of SAPP from the admixture between EAST and a ghost population), t_3 (for the ghost population and NAPP),

and t_4 (for EAST and NAPP) were 1320 (95%CI: 300-3110), 4750 (95%CI: 1800-6850), 7790 (95%CI: 4230-9860), 12000 (95%CI: 6950-14800) generations ago, respectively (Table 2 and Fig. S1.4). Assuming a generation time of 50 years (Liepelt *et al.*, 2002; Dering *et al.*, 2014; Ruosch *et al.*, 2016), these values can be translated into 66000 (95% CI: 15500-155500), 237500 (95% CI: 90000-342500), 389500 (95% CI: 211500-493000), 600000 yrs BP (95% CI: 347500-740000) for t_1, t_2, t_3, and t_4 respectively.

DISCUSSION

Spatial distribution of genetic diversity

Along the Apennine chain there are only two areas where genetic diversity is large and comparable with populations from Eastern Europe. They correspond strictly to refugial areas hypothesized according to the most recent and detailed palaeobotanical syntheses (Magri *et al.*, 2015).

The first area is located in the southern Apennines (Fig. 1), from the southernmost population (GAM) to the latitude of CIL and LAU, with the highest haplotype and allelic richness recorded for the TDP population. The only Apennine silver fir populations regularly included in genetic surveys at the biogeographical scale were those from Calabria, which often showed higher genetic variation than northern ones (Bergmann *et al.*, 1990; Vicario *et al*., 1995; Liepelt *et al.*, 2002; Longauer *et al.*, 2003; Liepelt *et al.*, 2009). Such high diversity, together with the high vitality and growth vigour seen in provenance trials (e.g. Larsen & Mekic 1991; Kerr *et al.*, 2015), has led many authors to consider Calabrian populations as part of a long-lasting but isolated refugial area (Liepelt *et al.*, 2009; Cheddadi *et al.*, 2014). Our results strongly support the hypothesis of an isolated refugium in the southern Apennines and indicate that the upper latitudinal limit of this refugial area was at ~40.5° N, i.e. the latitude of CIL and LAU. These two populations have retained high genetic diversity during the postglacial period despite erosion to their current small, highly-isolated state. In particular, CIL is

299 composed of few hundred individuals and located ~40 km from the nearest stand (Rovelli, 1995; Di
300 Pietro & Fascetti, 2005). Nevertheless, CIL still maintains levels of genetic diversity comparable to
301 much larger stands (e.g. TDP, the largest stand in southern Italy; Schettino & Travaglio, 2015).
302 The second area is located in northern Apennines, a region for which several palaeobotanical surveys
303 are available but whose silver fir populations have rarely been included in large-scale genetic studies.
304 Among the three populations sampled, it is surprising that NER is the only one showing high genetic
305 diversity because it is the northernmost population along the Apennines, and highly isolated and
306 extremely small (c. 500 individuals, Piovani *et al*., 2010). From NER to the central Apennines, genetic
307 diversity gradually decreases to populations north of the Gran Sasso massif which, along with Alpine
308 populations, are the least genetically diverse in our survey. NER has an allelic and haplotype richness
309 16% and 54% higher, on average, than much larger and less isolated Alpine populations. This suggests
310 that the northernmost part of the Apennine distribution is the most likely refugial area or, at least, the
311 area where silver fir was most abundant during the Last Glacial Maximum (LGM): a hypothesis that
312 fits well with palaeobotanical data (Vescovi *et al.*, 2010; Magri *et al.*, 2015). Besides its conservation
313 relevance, the correct location of refugial populations can help solve the long-lasting problem of
314 accurately calculating historical migration rates (Cheddadi *et al.*, 2014). This might be particularly
315 important for silver fir, whose northern Apennine effective refugium has often been hypothesised in
316 different locations, from the north-western (e.g. Cheddadi *et al.*, 2014) to central Apennines (e.g.
317 Konnert & Bergmann, 1995). The high genetic similarity between populations from the northern
318 Apennines and eastern Alps, with the former being much more diverse despite the much lower census
319 size, seems to confirm the hypothesised post-glacial migration route from the northern Apennines to
320 central Europe (e.g. Konnert & Bergmann 1995; Liepelt *et al.*, 2009; Cheddadi *et al.*, 2014).
321 A third area might also have acted as a refugium in the Italian Peninsula. Bayesian clustering results,
322 genetic differentiation from surrounding populations, and DIYABC estimate that CAPP diverged from
323 NAPP ~66,000 yrs BP, all point towards the local persistence of silver fir during the last glacial period
324 in the central Apennines. The existence of a glacial refugium in this area has been postulated several

325 times, but always as an alternative to the northern Apennine one (e.g. Konnert & Bergmann, 1995).
326 On the contrary, our analyses suggest the existence of two separate genetic clusters during the LGM
327 north of the Gran Sasso massif, one in the northern and one in the central Apennines. The latter was
328 characterized by a lower effective population size than other Apennine refugia, in accordance with
329 palaeobotanical records showing a marked demographic reduction of silver fir since 70,000 yrs BP
330 in central Italy (Follieri *et al.*, 1998). Although northern and central Apennine populations are
331 generally small and fragmented, and considered as highly impacted by human intervention in the last
332 millennia (Piovani *et al.*, 2010; Urbinati & Romano, 2012; Tinner *et al*. 2013; Leonarduzzi *et al.*,
333 2016), no clear signals of translocation and/or mixing of different provenances were found, contrary
334 to what was observed in other European conifers (e.g. Wagner *et al.*, 2015), and the spatial distribution
335 of genetic diversity seems mainly related to events dating to before the Neolithic age.
336
337 **Genetic structure and demographic history**
338
339 Two unexpected characteristics of the genetic structure of silver fir in the Apennines emerged from
340 our analyses: the existence of a sharp genetic boundary separating populations north and south of the
341 Gran Sasso and Majella massifs in the central Apennines, and the tight genetic connection between
342 southern Apennine and Eastern European gene pools.
343 In the central Apennines, the four populations within the Gran Sasso e Monti della Laga National
344 Park (VDC, CEP, COR and TOS) and ABS are separated by only 90 km, an area comprising the Gran
345 Sasso and Majella massifs where no natural populations of silver fir are present. Despite their
346 geographic proximity, they showed pair-wise genetic differentiation values among the highest
347 detected (G'_{ST} = 0.22, 0.21, 0.25 and 0.15, respectively, Table S1.3). According to DIYABC estimates,
348 the Apennine populations from the two main gene pools detected by STRUCTURE analysis have had
349 separate dynamics during the last 400,000 yrs. Therefore, hypotheses based on a recent genetic
350 continuity along the entire Apennine chain and about the central Apennines as a melting pot between

different recolonization routes (Larsen & Mekic, 1991; Scaltsoyiannes *et al.*, 1999; Linares, 2011; Camerano *et al.*, 2012) seem highly unlikely. The processes underlying the origin and persistence of a long-lasting genetic boundary in central Italy are not clear and any hypotheses will require a multidisciplinary approach considering a longer period, from the Neogene Apennine orogeny to climatic dynamics during several Pleistocene glacial cycles. However, it is worth noting that this area represents a steep discontinuity at different time scales. For example, it is considered as the tectonic separation between the northern Apennines Arc and the southern Apennines-Calabrian Arc (Satolli & Calamita, 2008), and an ecotone between different precipitation patterns that have had a large, long-lasting influence on vegetation dynamics in the Italian Peninsula (Comborieu-Nebout *et al*., 2015). Populations along the rear edge of silver fir distribution are indeed separated by neat genetic boundaries, as previously hypothesized, but the present study demonstrates their location is not where they were previously thought. In particular, we found a genetic similarity between populations from Eastern Europe and southern Italy that are almost fixed for different mitotypes (Liepelt *et al.*, 2002). A genetic similarity at isozymes and chloroplast markers between these two areas was reported by Liepelt *et al*. (2002) and Longauer *et al.* (2003). The former hypothesized a possibly extensive trans-Adriatic gene flow via pollen homogenizing chloroplast haplotype frequencies over long distance. Our data support this hypothesis as the most likely scenario, considering that SAPP was generated by the admixture of EAST and a population originating from NAPP. These ancient gene pools would have diverged long before the last glacial period, corroborating the hypothesis of a separation lasting for several Quaternary glacial cycles (Liepelt *et al.*, 2009). The median admixture rate estimated (78% from NAPP *vs*. 22% from EAST) is compatible with an introgression via-pollen from the Balkan into the northern Apennine gene pool having shaped, together with the isolation between populations at the two extremes of the Apennine chain, the genetic layout of southern Apennine populations between 340,000 and 90,000 yrs BP. Geological studies demonstrated that the Apulian platform connected several times to the Balkans by a trans-Adriatic land bridge (Patacca *et al.*, 2008), and the presence of shared haplotypes in the Balkan and Italian Peninsulas for other tree species (e.g. Bagnoli *et al.*,

2016) supports the idea of effective gene flow being not rare across what is nowadays considered as a geographic barrier.

The scenario depicted by our intensive genetic survey of Apennine populations provides new insights into the Quaternary history of silver fir. Among them, two seem particularly relevant for studying adaptive responses of rear edge silver fir populations. First, a solid knowledge about past demographic patterns can improve the study of local adaptation. So far, it has been based on considering a main separation between Alpine and Apennine populations in silver fir (Mosca *et al*., 2014), which seems quite unlikely from our results. Incorrectly considering demographic scenarios can hinder the study of local adaptation by introducing biases in selection tests (Nielsen, 2005). In addition, silver fir tree-ring growth series in Italy show three differentiated groups of populations: 1) the western Alps, 2) the northern Apennines and the eastern Alps, and 3) the central and southern Apennines (Carrer *et al.*, 2010). This subdivision partially corresponds to the genetic structure found in our survey, with some exceptions. In fact, central Apennine populations are genetically similar to northern populations but show growth responses comparable to the ones from southern populations, raising several questions about the relative strength of past migration dynamics *vs.* adaptation to climate in highly heterogeneous regions.

Second, the existence of a sharp genetic boundary in the central Apennines poses the basis for studying possible south-to-north adaptive gene flow in this area. With ongoing climate warming, genotypes able to perform well in warmer and drier conditions are expected to be selectively advantaged both in local persistence and during latitudinal and altitudinal migrations, in particular at Mediterranean latitudes (Fady *et al.*, 2016). In this context, the genetic boundary found in central Italy, corresponding to an area where ecological conditions markedly change (Brunetti *et al.*, 2004; Comborieu-Nebout *et al*., 2015), might represent an ideal location for studying the role of gene flow in promoting adaptive responses to climate change (Kremer *et al.*, 2012). In such a study, it should be noted that, although Mediterranean silver fir provenances are often considered less sensitive to drought (Aussenac, 2002; Carrer *et al.*, 2010, but see Gazol *et al.*, 2015), fossil data from the last few

403 millennia, which were characterized by increasing aridity, show that silver fir populations have
404 declined similarly throughout the Italian Peninsula (Magri *et al*., 2015).
405 Our study has revealed that unexpected processes have shaped the spatial distribution of silver fir
406 genetic diversity in the environmentally heterogeneous Apennine area through several Quaternary
407 glacial cycles. These findings, besides helping to resolve questions about forest tree dynamics at the
408 distributional edge and raising new testable hypotheses, emphasise the importance of robust sampling
409 within refugial areas to quantitatively describe genetic structure for the conservation of extant
410 diversity. The availability of suitable genetic data is essential for effective protection of forest genetic
411 resources (de Vries *et al.*, 2015). Although rear edge silver fir populations have been heavily impacted
412 by human activities for centuries, recently they have also displayed a marked growth decline with
413 changing environmental conditions (Gazol *et al.*, 2015) and their adaptive potential might be
414 constrained by their small size and high geographic marginality. A conservation plan is urgently
415 needed and our data can play a key role in getting it established.
416

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418

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REFERENCES

Aussenac, G. (2002) Ecology and ecophysiology of circum-Mediterranean firs in the context of climate change. *Annals of Forest Science*, **59**, 823-832.

Bagnoli, F., Tsuda, Y., Fineschi, S., Bruschi, P., Magri, D., Zhelev, P., Paule, L., Simeone, M.C., González-Martínez, S.C. & Vendramin, G.G. (2016) Combining molecular and fossil data to infer demographic history of *Quercus cerris*: insights on European eastern glacial refugia. *Journal of Biogeography*, **43**, 679-690.

Beaumont, M.A., Zhang, W. & Balding, D.J. (2002) Approximate Bayesian computation in population genetics. *Genetics*, **162**, 2025-2035.

Bergmann, F., Gregorius, H.-R. & Larsen, J.B. (1990) Levels of genetic variation in European silver fir (*Abies alba*). *Genetica*, **82**, 1-10.

Brousseau, L., Postolache, D., Lascoux, M., Drouzas, A.D., Källman, T., Leonarduzzi, C., Liepelt, S., Piotti, A., Popescu, F., Roschanski, A.M., Zhelev, P., Fady, B. & Vendramin, G.G. (2016) Local adaptation in European firs assessed through extensive sampling across altitudinal gradients in Southern Europe. *PLoS ONE*, **11**, e0158216.

450 Brunetti, M., Maugeri, M., Monti, F. & Nanni, T. (2004) Changes in daily precipitation frequency
451 and distribution in Italy over the last 120 years. *Journal of Geophysical Research*, **109**, D05102.

452 Camerano, P., Ferrazzini, D., Ducci, F. & Belletti, P. (2012) Regioni di provenienza per l'abete
453 bianco. *Sherwood*, **17**, 35-40.

454 Carrer, M., Nola, P., Motta, R. & Urbinati, C. (2010) Contrasting tree-ring growth to climate
455 responses of *Abies alba* toward the southern limit of its distribution area. *Oikos*, **119**, 1515-1525.

456 Cheddadi, R., Birks, H.J.B., Tarroso, P., Liepelt, S., Gömöry, D., Dullinger, S., Meier, E.S., Hülber,
457 K., Maiorano, L. & Laborde, H. (2014) Revisiting tree-migration rates: *Abies alba* (Mill.), a case
458 study. *Vegetation History and Archaeobotany*, **23**, 113-122.

459 Combourieu-Nebout, N., Bertini, A., Russo-Ermolli, E., Peyron, O., Klotz, S., Montade, V.,
460 Fauquette, S., Allen, J., Fusco, F., Goring, S., Huntley, B., Joannin, S., Lebreton, V., Magri, D.,
461 Martinetto, E., Orain, R. & Sadori, L. (2015) Climate changes in the central Mediterranean and
462 Italian vegetation dynamics since the Pliocene. *Review of Palaeobotany and Palynology*, **218**,
463 127-147.

464 Corander, J., Marttinen, P., Sirén, J. & Tang, J. (2008) Enhanced Bayesian modelling in BAPS
465 software for learning genetic structures of populations. *BMC Bioinformatics*, **9**,539.

466 Cornuet, J.M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., Marin, J.M. &
467 Estoup, A. (2014) DIYABC v2.0: a software to make approximate Bayesian computation
468 inferences about population history using single nucleotide polymorphism, DNA sequence and
469 microsatellite data. *Bioinformatics*, **30**, 1187-1189.

Dering, M., Sękiewicz, K., Boratyńska, K., Litkowiec, M., Iszkuło, G., Romo, A. & Boratyński, A. (2014) Genetic diversity and inter-specific relations of western Mediterranean relic *Abies* taxa as compared to the Iberian *A. alba*. *Flora*, **209**, 367-374.

de Vries, S.M.G., Alan, M., Bozzano, M., Burianek, V., Collin, E., Cottrell, J., Ivankovic, M., Kelleher, C.T., Koskela, J., Rotach, P., Vietto, L. & Yrjänä, L. (2015) Pan-European strategy for genetic conservation of forest trees and establishment of a core network of dynamic conservation units. European Forest Genetic Resources Programme (EUFORGEN), Bioversity International, Rome, Italy.

Di Pietro, R. & Fascetti, S. (2005) A contribution to the knowledge of *Abies alba* woodlands in the Campania and Basilicata regions (southern Italy). *Fitosociologia*, **42**, 71-95.

Earl, D.A. & von Holdt, B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetic Resources*, **4**, 359-361.

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

Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **491**, 479-491.

Fady, B., Cottrell, J., Ackzell, L., Alia, R., Muys, B., Prada, A. & Gonzalez-Martinez, S.C. (2016) Forests and global change: what can genetics contribute to the major forest management and policy challenges of the twenty-first century? *Regional Environmental Change*, **16**, 927-939.

491 Follieri, M., Giardini, M., Magri, D. & Sadori, L. (1998) Palynostratigraphy of the last glacial period
492 in the volcanic region of central Italy. *Quaternary International*, **47/48**, 3-20.

493 Gazol, A., Camarero, J.J., Gutiérrez, E., Popa, I., Andreu-Hayles, L., Motta, R., Nola, P., Ribas, M.,
494 Sangüesa-Barreda, G., Urbinati, C. & Carrer, M. (2015) Distinct effects of climate warming on
495 populations of silver fir (*Abies alba*) across Europe. *Journal of Biogeography*, **42**, 1150-1162.

496 Hampe, A. & Petit, R.J. (2005) Conserving biodiversity under climate change: the rear edge matters.
497 *Ecology Letters*, **8**, 461-467.

498 Hansen, J.K. & Larsen, J.B. (2004) European silver fir (*Abies alba* Mill.) provenances from Calabria,
499 southern Italy: 15-year results from Danish provenance field trials. *European Journal of Forest*
500 *Research*, **123**, 127-138.

501 Hansen, O.K., Vendramin, G.G., Sebastiani, F. & Edwards, K.J. (2005) Development of
502 microsatellite markers in *Abies nordmanniana* (Stev.) Spach and cross-species amplification in
503 the *Abies* genus. *Molecular Ecology Notes*, **5**, 784-787.

504 Hu, F.S., Hampe, A. & Petit, R.J. (2009) Paleoecology meets genetics: deciphering past vegetational
505 dynamics. *Frontiers in Ecology and the Environment*, **7**, 371-379.

506 Jakobsson, M. & Rosenberg, N.A. (2007) CLUMPP: a cluster matching and permutation program for
507 dealing with label switching and multimodality in analysis of population structure.
508 *Bioinformatics*, **23**, 1801-1806.

509 Kalinowski, S.T. (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures
510 of allelic richness. *Molecular Ecology Notes*, **5**, 187-189.

511 Kerr, G., Stokes, V., Peace, A. & Jinks, R. (2015) Effects of provenance on the survival, growth and
512 stem form of European silver fir (*Abies alba* Mill.) in Britain. *European Journal of Forest*
513 *Research*, **134**, 349-363.

514 Konnert, M. & Bergmann, F. (1995) The geographical distribution of genetic variation of silver fir
515 (*Abies alba*, Pinaceae) in relation to its migration history. *Plant Systematics and Evolution*, **196**,
516 19-30.

517 Kremer, A., Ronce, O., Robledo-Arnuncio, J.J., Guillaume, F., Bohrer, G., Nathan, R., Bridle, J.R.,
518 Gomulkiewicz, R., Klein, E.K., Ritland, K., Kuparinen, A., Gerber, S. & Schueler, S. (2012)
519 Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology Letters*,
520 **15**, 378-392.

521 Larsen, J.B. & Mekic, F. (1991) The geographic variation in European silver fir (*Abies alba* Mill.).
522 *Silvae Genetica*, **40**, 188-198.

523 Leonarduzzi, C., Piotti, A., Spanu, I. & Vendramin, G.G. (2016) Effective gene flow in a historically
524 fragmented area at the southern edge of silver fir (*Abies alba* Mill.) distribution. *Tree Genetics*
525 *and Genomes*, **12**, 95.

526 Liepelt, S., Kuhlenkamp, V., Anzidei, M., Vendramin, G.G. & Ziegenhagen, B. (2001) Pitfalls in
527 determining size homoplasy of microsatellite loci. *Molecular Ecology Notes*, **1**, 332-335.

528 Liepelt, S., Bialozyt, R. & Ziegenhagen B. (2002) Wind-dispersed pollen mediates postglacial gene
529 flow among refugia. *Proceedings of the National Academy of Sciences*, **99**, 14590-14594.

530 Liepelt, S., Cheddadi, R., de Beaulieu, J.-L., Fady, B., Gomory, D., Hussendorfer, E., Konnert, M.,
531 Litt, T., Longauer, R., Terhurne-Berson, R. & Ziegenhagen, B. (2009) Postglacial range

532 expansion and its genetic imprints in *Abies alba* (Mill.) — A synthesis from palaeobotanic and
533 genetic data. *Review of Palaeobotany and Palynology*, **153**, 139-149.

534 Linares, J.C. (2011) Biogeography and evolution of *Abies* (Pinaceae) in the Mediterranean Basin: the
535 roles of long-term climatic change and glacial refugia. *Journal of Biogeography*, **38**, 619-630.

536 Longauer, R., Paule, L. & Andonoski, A. (2003) Genetic diversity of southern populations of *Abies*
537 *alba* Mill. *Forest Genetics*, **10**, 1-9.

538 Magri, D., Agrillo, E., Di Rita, F., Furlanetto, G., Pini, R., Ravazzi, C. & Spada, F. (2015) Holocene
539 dynamics of tree taxa populations in Italy. *Review of Palaeobotany and Palynology*, **218**, 267-
540 284.

541 Mosca, E., González-Martínez, S.C. & Neale, D.B. (2014) Environmental versus geographical
542 determinants of genetic structure in two subalpine conifers. *New Phytologist*, **201**, 180-192.

543 Nielsen, R. (2005) Molecular signatures of natural selection. *Annual Review of Genetics*, **39**, 197-
544 218.

545 Nieto Feliner, G. (2014) Patterns and processes in plant phylogeography in the Mediterranean Basin.
546 A review. *Perspectives in Plant Ecology, Evolution and Systematics*, **16**, 265-278.

547 Parducci, L., Szmidt, A.E., Villani, F., Wang, X.R. & Cherubini, M. (1996) Genetic variation of *Abies*
548 *alba* in Italy. *Hereditas*, **125**, 11-18.

549 Patacca, E., Scandone, P. & Mazza, P. (2008) Oligocene migration path for Apulia macromammals,
550 the Central-Adriatic bridge. *Bollettino della Società Geologica Italiana*, **127**, 337-355.

551 Peakall, R. & Smouse, P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic
552 software for teaching and research-an update. *Bioinformatics*, **28**, 2537-2539.

553 Petit, R.J., El Mousadik, A. & Pons, O. (1998) Identifying populations for conservation on the basis
554 of genetic markers. *Conservation Biology*, **12**, 844-855.

555 Petit, R.J., Hu, F.S. & Dick, C.W. (2008) Forests of the past: a window to future changes. *Science*,
556 **320**, 1450-1452.

557 Piovani, P., Leonardi, S., Piotti, A. & Menozzi, P. (2010) Conservation genetics of small relic
558 populations of Silver fir (*Abies alba* Mill.) in northern Apennines. *Plant Biosystems*, **144**, 683-
559 691.

560 Postolache, D., Leonarduzzi, C., Piotti, A., Spanu, I., Roig, A., Fady, B., Roschanski, A., Liepelt, S.
561 & Vendramin, G.G. (2014) Transcriptome versus genomic microsatellite markers: Highly
562 informative multiplexes for genotyping *Abies alba* Mill. and congeneric species. *Plant*
563 *Molecular Biology Reporter*, **32**, 750-760.

564 Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using
565 multilocus genotype data. *Genetics*, **155**, 945-959.

566 R Core Team (2015). R: A language and environment for statistical computing. R Foundation for
567 Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

568 Rovelli, E. (1995) La distribuzione dell'abete (*Abies alba* Mill.) sull'Appennino. *Monti e Boschi*, **6**,
569 5-13.

570 Ruosch, M., Spahni, R., Joos, F., Henne, P.D., van der Knaap, W.O. & Tinner, W. (2016) Past and
571 future evolution of *Abies alba* forests in Europe - comparison of a dynamic vegetation model
572 with palaeo data and observations. *Global Change Biology*, **22**, 727-740.

573 Satolli, S. & Calamita, F. (2008) Differences and similarities between the central and the southern
574 Apennines (Italy): Examining the Gran Sasso versus the Matese-Frosolone salients using
575 paleomagnetic, geological, and structural data. *Journal of Geophysical Research*, **113**, B10101.

576 Scaltsoyiannes, A., Tsaktsira, M. & Drouzas, A.D. (1999) Allozyme differentiation in the
577 Mediterranean firs (*Abies*, Pinaceae). A first comparative study with phylogenetic implications.
578 *Plant Systematics and Evolution*, **216**, 289-307.

579 Schettino, A. & Travaglio, G. (2015) *Alberi monumentali del Parco Nazionale del Pollino*. Ente Parco
580 Nazionale del Pollino, Rotonda.

581 Tinner, W., Colombaroli, D., Heiri, O., Henne, P.D., Steinacher, M., Untenecker, J., Vescovi, E.,
582 Allen, J.R.M., Carraro, G., Conedera, M., Joos, F., Lotter, A.F., Luterbacher, J., Samartin, S. &
583 Valsecchi, V. (2013) The past ecology of *Abies alba* provides new perspectives on future
584 responses of silver fir forests to global warming. *Ecological Monographs*, **83**, 419-439.

585 Urbinati, C. & Romano, R. (2012) *Foresta e monaci di Camaldoli: un rapporto millenario tra*
586 *gestione e conservazione*. INEA, Rome.

587 Vendramin, G.G., Lelli, L., Rossi, P. & Morgante, M. (1996) A set of primers for the amplification
588 of 20 chloroplast microsatellites in *Pinaceae*. *Molecular Ecology*, **5**, 595-598.

589 Vescovi, E., Ammann, B., Ravazzi, C. & Tinner, W. (2010) A new Late-glacial and Holocene record
590 of vegetation and fire history from Lago del Greppo, northern Apennines, Italy. *Vegetation*
591 *History and Archaeobotany*, **19**, 219-233.

592 Vicario, F., Vendramin, G.G., Rossi, P., Liò, P. & Giannini, R. (1995) Allozyme, chloroplast DNA
593 and RAPD markers for determining genetic relationships between *Abies alba* and the relic
594 population of *Abies nebrodensis*. *Theoretical and Applied Genetics*, **90**, 1012-1018.

595 Wagner, S., Liepelt, S., Gerber, S. & Petit, R.J. (2015) Within-range translocations and their
596 consequences in European larch. *PLoS ONE*, **10**, e0127516.

597

598 **Supporting Information**

599

600 Additional Supporting Information may be found in the online version of this article:

601 **Appendix S1** Supplementary tables and figures

602 **Appendix S2** DIYABC materials and methods details

603 **Appendix S3** Selection tests

604

605 **DATA ACCESSIBILITY**

606 Microsatellite data with geographic coordinates are available on Figshare at
607 https://doi.org/10.6084/m9.figshare.4704748.v1

608

609 **BIOSKETCH**

610

611 The authors belong to a research consortium dealing with population genetics of forest tree species.
612 Their research is particularly focused on the analysis of the distribution of extant genetic variation of
613 forest tree species using genetic markers to dissect the role of demography and selection in shaping
614 genetic diversity at different temporal and spatial scales. They have been involved in projects studying
615 the range-wide phylogeography of several forest tree species in Europe and the Mediterranean Basin.

616

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Author contributions: A.P., C.L. and G.G.V. designed the study; A.P., C.L., D.P., C.U. and S.L. organized and performed the sampling; C.L., I.S., D.P. and G.G.V. arranged the molecular laboratory analyses; A.P., C.L., F.B., L.B., S.L. carried out the data analysis; A.P. and C.L. led the writing with contributions from all authors, who revised and approved the manuscript.

624 **TABLES**

625

626 **Table 1** Posterior probability of each tested demographic scenario and its 95% confidence interval
627 based on the logistic estimate according to DIYABC. In Step 1 analysis, scenarios 1, 2, and 6 are based
628 on an ancient separation between populations from the Apennines and Eastern Europe, scenario 3
629 strictly represents STRUCTURE results (Fig. 3c,d), and scenarios 4 and 5 consider an admixed origin
630 of southern Apennine populations (see details in the Appendix S2). Scenarios from Step 2 analysis
631 are summarized in Fig. 2.

632

Scenario	Posterior probability	95% CI (lower - upper)
Step 1 - Preliminary test with 100 individuals per population		
1	0.0666	0.0266-0.1066
2	0.0021	0.0000-0.0478
3	0.4923	0.4315-0.5531
4	0.4200	0.3582-0.4818
5	0.0172	0.0000-0.0611
6	0.0018	0.0000-0.0476
Step 2 - Analysis with the whole dataset		
A	0.2680	0.2252-0.3109
B	0.7320	0.6891-0.7748

633

634

635

636 **Table 2** Parameter estimates for the best demographic scenario based on approximate Bayesian
637 computation.
638

				Quantiles			
Parameter	Mean	Median	Mode	2.5%	5%	95%	97.5%
N_{NAPP}	4.25×10^{4}	4.18×10^{4}	4.29×10^{4}	2.00×10^{4}	2.31×10^{4}	6.57×10^{4}	7.13×10^{4}
N_{CAPP}	1.94×10^{4}	1.55×10^{4}	1.19×10^{4}	3.90×10^{3}	5.02×10^{3}	4.98×10^{4}	6.11×10^{4}
N_{SAPP}	1.02×10^{5}	9.79×10^{4}	8.56×10^{4}	4.25×10^{4}	5.03×10^{4}	1.72×10^{5}	1.84×10^{5}
N_{EAST}	9.65×10^{4}	9.35×10^{4}	8.40×10^{4}	4.55×10^{4}	5.29×10^{4}	1.53×10^{5}	1.70×10^{5}
N_{GHOST}	6.72×10^{4}	5.13×10^{4}	1.11×10^{4}	2.90×10^{3}	4.96×10^{3}	1.76×10^{5}	1.89×10^{5}
Na	3.50×10^{3}	2.87×10^{3}	9.10×10^{2}	1.88×10^{2}	3.44×10^{2}	8.57×10^{3}	9.22×10^{3}
t_1	1.41×10^{3}	1.32×10^{3}	1.16×10^{3}	3.00×10^{2}	4.26×10^{2}	2.68×10^{3}	3.11×10^{3}
t_2	4.65×10^{3}	4.75×10^{3}	4.78×10^{3}	1.80×10^{3}	2.23×10^{3}	6.69×10^{3}	6.85×10^{3}
t_3	7.61×10^{3}	7.79×10^{3}	8.06×10^{3}	4.23×10^{3}	4.73×10^{3}	9.72×10^{3}	9.86×10^{3}
t_4	1.17×10^{4}	1.20×10^{4}	1.25×10^{4}	6.95×10^{3}	7.78×10^{3}	1.46×10^{4}	1.48×10^{4}
ra	7.23×10^{-1}	7.80×10^{-1}	9.05×10^{-1}	1.48×10^{-1}	2.64×10^{-1}	9.71×10^{-1}	9.84×10^{-1}
μmic	2.64×10^{-5}	2.46×10^{-5}	2.12×10^{-5}	1.03×10^{-5}	1.22×10^{-5}	4.64×10^{-5}	5.32×10^{-5}
pmic	2.28×10^{-1}	2.37×10^{-1}	3.00×10^{-1}	1.19×10^{-1}	1.30×10^{-1}	2.97×10^{-1}	3.00×10^{-1}
snimic	1.62×10^{-6}	6.89×10^{-7}	1.78×10^{-8}	1.72×10^{-8}	2.50×10^{-8}	7.05×10^{-6}	8.63×10^{-6}

639

640 N_{NAPP} = NAPP effective population size; N_{CAPP} = CAPP effective population size; N_{SAPP} = SAPP
641 effective population size; N_{EAST} = EAST effective population size; N_{GHOST} = unsampled ghost
642 population effective population size; Na = effective population size of the ancestral population; times
643 are considered from present (0) backwards in time, t_1=divergence of CAPP from NAPP; t_2=
644 generation of SAPP by admixture of EAST and an unsampled ghost population; t_3 = divergence of
645 the ghost population from NAPP; t_4 = divergence of NAPP and EAST; *ra* = admixture rate; μmic =
646 mean mutation rate of microsatellites; pmic = mean parameter of geometric distribution (GSM,
647 Generalized Stepwise Mutation Model); snimic = individual locus SNI (Single Nucleotide
648 Insertion/deletion) rate.
649

650 **FIGURE LEGENDS**

651

652 Fig. 1 Location of sampled populations, with the distribution of *Abies alba* in green (Source:
653 EUFORGEN, http://www.euforgen.org/distribution-maps/).

654

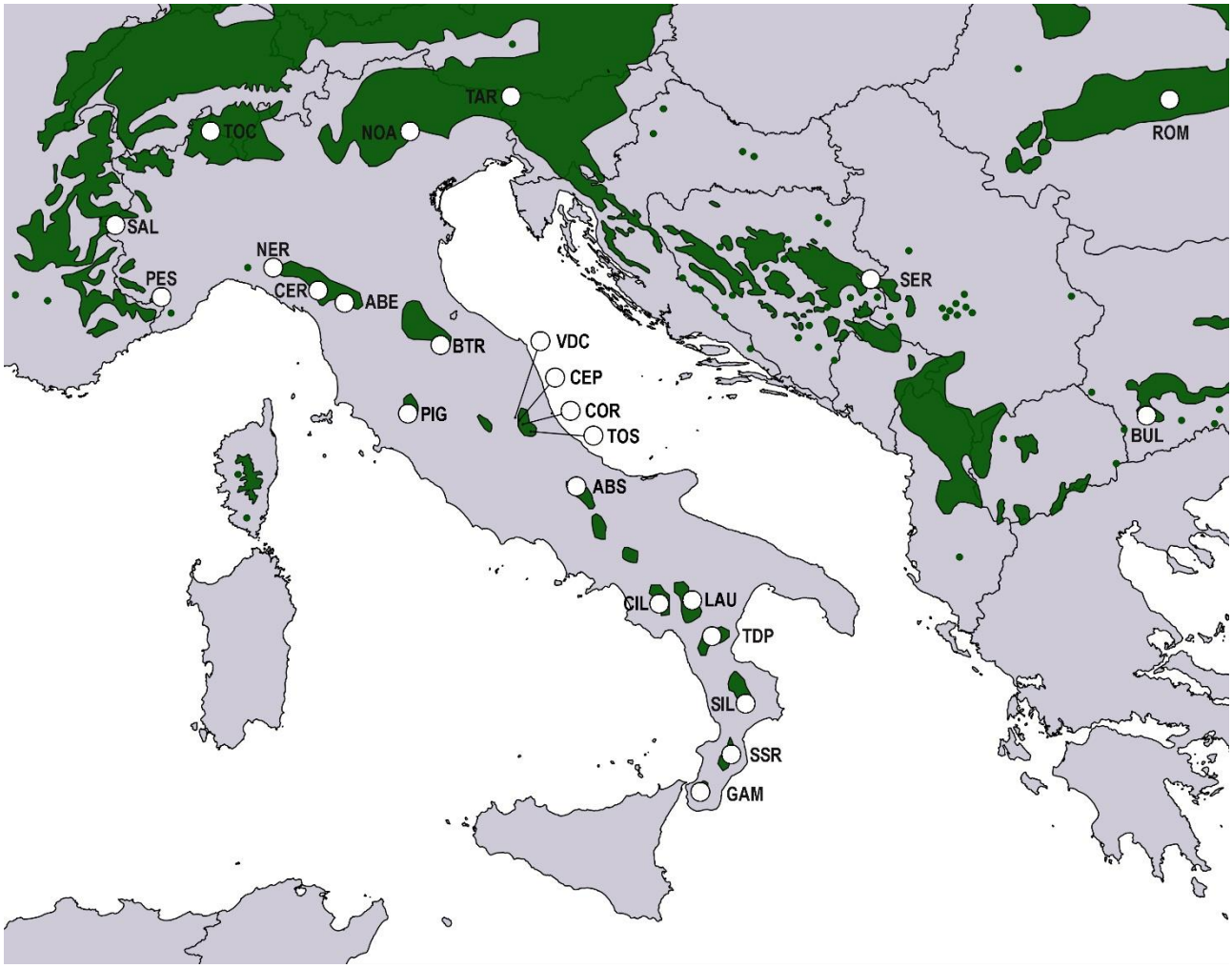
655 Fig. 2 Demographic models tested in the second step of DIYABC analysis. In both scenarios, $t_{\#}$
656 represents the time of occurrence of an event (expressed in number of generations) and $N_{\#}$ is the
657 effective population size of the corresponding populations during each time period (see the Materials
658 and Methods for abbreviations). In scenario B, the admixture rates *ra* and 1-*ra* are the genetic
659 contribution of each of the source populations to the origin of SAPP.

660

661 Fig. 3 Geographical distribution of allelic (a) and haplotype (b) richness, and STRUCTURE (c,d) and
662 BAPS (e) results. Locations of populations (f) are shown for reference.

663

664



665

666 Figure 1

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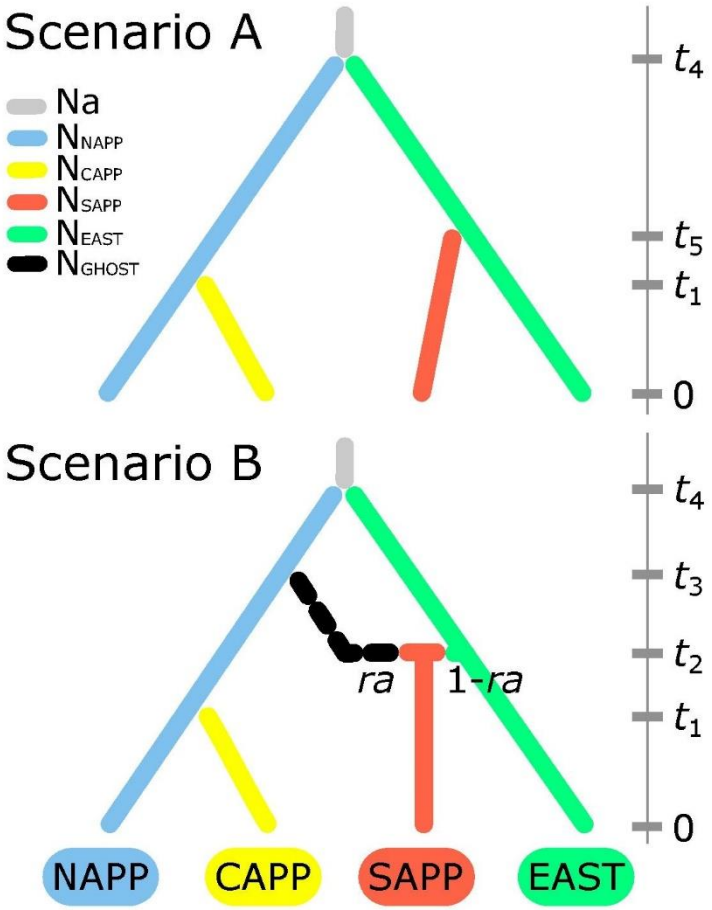

670

671 Figure 2

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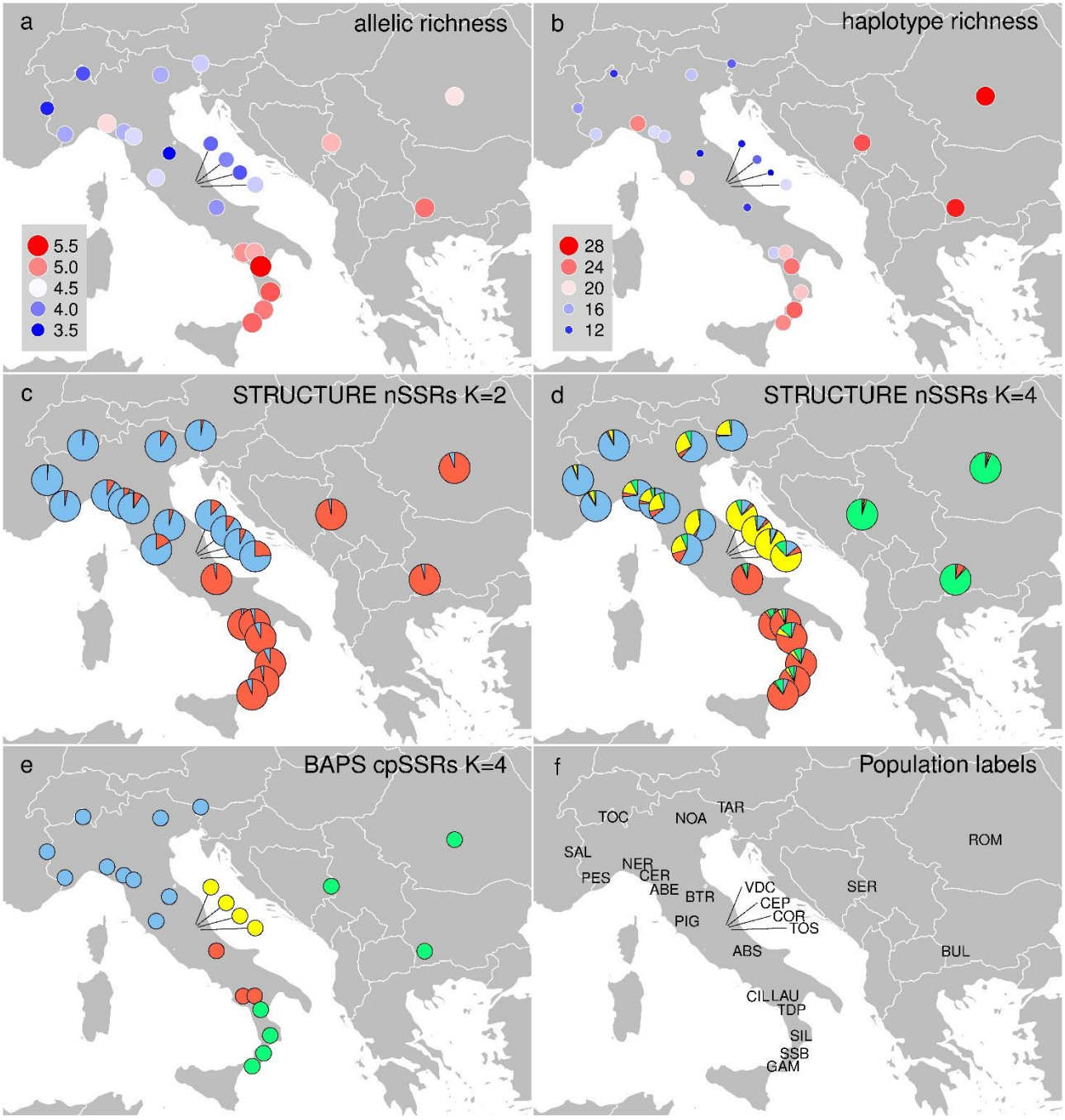
673



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675 Figure 3