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

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24	Running head: Demographic history of Abies alba in the Apennines
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26 ABSTRACT

27

Aim Mediterranean refugial areas are generally underrepresented in large-scale genetic surveys of 28 29 forest trees. In the case of silver fir (Abies alba Mill.), this has led to divergent hypotheses about the 30 exact location of glacial refugia and the trajectory of recolonization routes. Based on comprehensive 31 sampling of Apennine populations, we aimed to reconcile discrepancies about the number and 32 location of refugia for silver fir in the Apennines and test alternative demographic scenarios 33 developed from palaeobotanical and genetic data. 34 35 Location Mediterranean Basin; the Apennines and surrounding areas. 36 37 Methods 1167 individuals from 16 Apennine populations, extensively covering the species' 38 distribution along the Italian Peninsula, and eight populations from the Alps and Eastern Europe were 39 genotyped at 16 nuclear and three chloroplast microsatellite markers. The geographical distribution 40 of genetic variation was explored using Bayesian clustering and multivariate methods. Based on 41 inferred genetic structure, the demographic history of A. alba was assessed by approximate Bayesian 42 computation (ABC) analysis.

43

44 **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 45 46 gene pools. Two Apennine areas, corresponding precisely with refugial areas hypothesized in most 47 recent palaeobotanical syntheses, have high genetic diversity on a par with Eastern European 48 populations. ABC analysis showed an ancient separation between Apennine and Eastern European 49 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 50 51 populations. In addition, there was evidence that the central Apennines acted as a small-scale, isolated 52 refugium during the Last Glacial Maximum.

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phylogeography; Pleistocene; post-glacial recolonization; nuclear and chloroplast microsatellites;
rear edge populations; trans-Adriatic gene flow

65 INTRODUCTION

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67 There is continuing debate about the location of glacial refugia. Such interest is justified by the 68 importance of correctly placing glacial refugia and recolonization routes when interpreting current species' distributions, estimating species' migration potential and foreseeing possible range shifts 69 70 (Petit et al., 2008). Coupling genetic and palaeobotanical data is considered the most effective 71 approach to infer past retraction-colonization dynamics in plants (Hu et al., 2009). For a very small 72 number of tree species, genetic and palaeobotanical records covering both the distribution core and 73 edges are available, and well-grounded hypotheses on the topography of glacial refugia and post-74 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 75 76 literature highlights that northern Mediterranean refugial areas, such as the Italian and Balkan 77 Peninsulas, are generally underrepresented in genetic and palaeobotanical large-scale surveys on 78 forest trees (Hampe & Petit, 2005; Liepelt et al., 2009; Linares, 2011). Trees, and plants in general, 79 have experienced complex dynamics due to environmental heterogeneity and palaeoclimatic events 80 in these areas, leading to high phylogeographic complexity and idiosyncratic patterns (Nieto Feliner, 81 2014). The increasing availability of molecular data and refined statistical approaches are providing 82 unprecedented power to unravel complex demographic histories, but to be effective these tools must 83 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 91 Balkans, but so far only inconsistent speculations on their exact location and on recolonization routes 92 have been proposed (Liepelt *et al.*, 2009; Linares, 2011; Cheddadi *et al.*, 2014). An attempt to 93 synthesise different interpretations of genetic and palaeobotanical data is difficult since both 94 disciplines suffer low availability of data for refugial areas (Liepelt *et al.*, 2009; Linares, 2011; Tinner 95 *et al.*, 2013).

96 In the Apennines, the presence of A. alba during the late-glacial period has been demonstrated by 97 palynological surveys, mainly from the northern Apennines and the Tyrrhenian side of the central 98 Apennines (e.g. Follieri et al., 1998; Vescovi et al., 2010; Magri et al., 2015). Previous genetic work, 99 based on a limited number of populations located mostly in Calabria and the northern Apennines, 100 inferred the presence of at least two genetic clusters (Konnert & Bergmann, 1995; Liepelt et al., 101 2009). This led to the hypothesis of an isolated refugium in the southern Apennines and an effective 102 refugium, located either in the central (e.g. Konnert & Bergmann, 1995) or in the north-western 103 Apennines (e.g. Cheddadi et al., 2014), from which the recolonization of the Alps and central Europe 104 may have started. In contrast, following early hypotheses about southern Italy as the starting point for 105 the recolonization of Central Europe, a possible genetic continuity along the entire Apennine chain 106 has been hypothesized several times (Scaltsoyiannes et al., 1999; Linares, 2011; Camerano et al., 107 2012). Some genetic studies even considered central Italy as a possible melting pot of recolonization 108 routes from the Balkans and southern Apennines (Parducci et al., 1996) or from the northern and 109 southern Apennines (Larsen & Mekic, 1991). However, all of these previous studies have been based 110 on limited sampling and critical questions remain regarding the genetic relationship between 111 populations from the northern and southern Apennines and the origin of populations from the central 112 Apennines. Establishing the evolutionary history of these fragmented Apennine silver fir populations 113 is particularly urgent because they have high evolutionary and conservation value due to their unique 114 genetic and eco-physiological features (Hansen & Larsen, 2004; Carrer et al., 2010; Cheddadi et al., 115 2014; Brousseau et al., 2016). An intensive genetic survey covering the entire Italian Peninsula will 116 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 particular focus on previously unsampled areas and adding several populations from surrounding 121 122 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.

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134 MATERIALS AND METHODS

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136 Sample collection and genotyping

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Sixteen putatively autochthonous populations were sampled along the Apennine chain (five in the northern, five in the central, six in the southern Apennines, Fig. 1 and Table S1.1) according to two general criteria: *i*) extensively covering the species' distribution in this area, and *ii*) increasing the sampling effort in terms of number of populations in areas sparsely covered by previous studies (i.e. the central Apennines). We included five populations from the Alps and three populations from Eastern Europe to investigate the genetic relationship of Apennine populations with those from surrounding areas. From each population, needle tissues were collected from c. 50 adult individuals 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

150 in Postolache *et al.* (2014). CpSSRs were multiplexed using the Type-it Microsatellite PCR kit

Pt30249, Liepelt et al., 2001). The multiplexing and amplification procedures for nSSRs are reported

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).

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155 Genetic diversity and population structure

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Standard genetic parameters describing within-population genetic variation and genetic
differentiation were estimated by GENALEX (Peakall & Smouse, 2012) and HP-RARE (Kalinowski,
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 STRUCTURE 2.3 (Pritchard *et al.*, 2000) and the empirical statistic ΔK (Evanno *et al.*, 2005; Earl & 162 163 von Holdt, 2012) on nSSR data. STRUCTURE was run using default settings and parameter values, and varying K from one to 10. Each run consisted of 1×10^5 burn-in iterations and 5×10^5 data collection 164 iterations, and was replicated 10 times. After checking for convergence of diagnostic statistics, 165 166 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.

179

180 Approximate Bayesian computation to infer demographic history

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182 To trace the demographic history of A. alba, the ABC procedure (Beaumont et al., 2002) implemented 183 in DIYABC 2.1 (Cornuet et al., 2014) was performed based on the nSSR dataset. To simplify the 184 analysis and limit the number of scenarios tested, we relied on the results of Bayesian and multivariate 185 clustering analyses on our datasets (see Results) and on previously available palaeobotanical and 186 genetic information. Therefore, ABC analyses were carried out on four groups of populations, 187 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 188 189 phylogenetic relationships among such genetic clusters.

The analysis of different demographic models (see Appendix S2) was performed following a twostep 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 theDIYABC analysis were as follows:

201 *Scenario A* is a hierarchical split scenario directly following STRUCTURE results (see Results), in 202 which NAPP separated from EAST before generating, respectively, CAPP and SAPP.

203 Scenario B takes into account i) that silver fir populations from the Italian Peninsula and Eastern 204 Europe are characterized by different and almost fixed mitochondrial variants (Liepelt et al., 2002), 205 and *ii*) the genetic structure emerging from STRUCTURE results. Therefore, considering that SAPP 206 shares the same mitotype with NAPP and that SAPP and EAST belong to the same STRUCTURE 207 cluster, the scenario was designed hypothesizing that, after an initial split between NAPP and EAST, 208 SAPP was generated by admixture of EAST and an unsampled ghost population, which merged with 209 NAPP. CAPP merged relatively recently with NAPP considering the likely post-glacial connection 210 between these two groups of populations (Magri et al., 2015). The admixture rates ra and 1-ra are 211 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.

219

220 **RESULTS**

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222 Genetic diversity and population structure

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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.

CpSSRs displayed 12 (Pt71936), 17 (Pt30141), and 6 (Pt30249) size variants, which combined into 164 haplotypes and a mean haplotypic diversity over populations of h=0.935. The haplotype richness per population (Hr_{37}) ranged from 10 (COR) to 28 (ROM) (Fig. 3b, Table S1.1). Genetic 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.

252

253 Approximate Bayesian computation to infer demographic history

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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.

262 Under scenario B, SAPP originated at t_2 by an admixture event between EAST and a ghost population. 263 The ghost population merged with NAPP at t_3 and gave the largest contribution (ra=0.780) to the 264 formation of SAPP. The median values of the effective population sizes were 41800, 15500, 97900, 265 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 266 267 population was estimated to be 14.5 and 32.5 times lower than those of NAPP and EAST, 268 respectively, suggesting an expansion event at t_4 . The results also indicated that the demographic 269 expansion continued in the following events, which led to the formation of SAPP at t₂. More recently, 270 a bottleneck is likely to have given rise to the formation of CAPP at t_1 .

271 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.

- 279
- 280 **DISCUSSION**
- 281
- 282 Spatial distribution of genetic diversity
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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).

287 The first area is located in the southern Apennines (Fig. 1), from the southernmost population (GAM) 288 to the latitude of CIL and LAU, with the highest haplotype and allelic richness recorded for the TDP 289 population. The only Apennine silver fir populations regularly included in genetic surveys at the 290 biogeographical scale were those from Calabria, which often showed higher genetic variation than 291 northern ones (Bergmann et al., 1990; Vicario et al., 1995; Liepelt et al., 2002; Longauer et al., 2003; 292 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 293 294 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 295 296 southern Apennines and indicate that the upper latitudinal limit of this refugial area was at ~40.5° N, 297 i.e. the latitude of CIL and LAU. These two populations have retained high genetic diversity during 298 the postglacial period despite erosion to their current small, highly-isolated state. In particular, CIL is

composed of few hundred individuals and located ~40 km from the nearest stand (Rovelli, 1995; Di
Pietro & Fascetti, 2005). Nevertheless, CIL still maintains levels of genetic diversity comparable to
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 fits well with palaeobotanical data (Vescovi et al., 2010; Magri et al., 2015). Besides its conservation 312 313 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).

A third area might also have acted as a refugium in the Italian Peninsula. Bayesian clustering results,
 genetic differentiation from surrounding populations, and DIYABC estimate that CAPP diverged from
 NAPP ~66,000 yrs BP, all point towards the local persistence of silver fir during the last glacial period
 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 palaeobotanical records showing a marked demographic reduction of silver fir since 70,000 yrs BP 329 in central Italy (Follieri et al., 1998). Although northern and central Apennine populations are 330 331 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.

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337 Genetic structure and demographic history

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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 Park (VDC, CEP, COR and TOS) and ABS are separated by only 90 km, an area comprising the Gran 344 345 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 detected (G'_{ST} = 0.22, 0.21, 0.25 and 0.15, respectively, Table S1.3). According to DIYABC estimates, 347 348 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

351 different recolonization routes (Larsen & Mekic, 1991; Scaltsoyiannes et al., 1999; Linares, 2011; 352 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 353 354 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 355 356 represents a steep discontinuity at different time scales. For example, it is considered as the tectonic 357 separation between the northern Apennines Arc and the southern Apennines-Calabrian Arc (Satolli & 358 Calamita, 2008), and an ecotone between different precipitation patterns that have had a large, long-359 lasting influence on vegetation dynamics in the Italian Peninsula (Comborieu-Nebout et al., 2015).

360 Populations along the rear edge of silver fir distribution are indeed separated by neat genetic 361 boundaries, as previously hypothesized, but the present study demonstrates their location is not where 362 they were previously thought. In particular, we found a genetic similarity between populations from 363 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 364 365 Liepelt et al. (2002) and Longauer et al. (2003). The former hypothesized a possibly extensive trans-366 Adriatic gene flow via pollen homogenizing chloroplast haplotype frequencies over long distance. 367 Our data support this hypothesis as the most likely scenario, considering that SAPP was generated by 368 the admixture of EAST and a population originating from NAPP. These ancient gene pools would 369 have diverged long before the last glacial period, corroborating the hypothesis of a separation lasting 370 for several Quaternary glacial cycles (Liepelt et al., 2009). The median admixture rate estimated (78% 371 from NAPP vs. 22% from EAST) is compatible with an introgression via-pollen from the Balkan into 372 the northern Apennine gene pool having shaped, together with the isolation between populations at 373 the two extremes of the Apennine chain, the genetic layout of southern Apennine populations between 374 340,000 and 90,000 yrs BP. Geological studies demonstrated that the Apulian platform connected 375 several times to the Balkans by a trans-Adriatic land bridge (Patacca et al., 2008), and the presence 376 of shared haplotypes in the Balkan and Italian Peninsulas for other tree species (e.g. Bagnoli et al., 377 2016) supports the idea of effective gene flow being not rare across what is nowadays considered as378 a geographic barrier.

379 The scenario depicted by our intensive genetic survey of Apennine populations provides new insights 380 into the Quaternary history of silver fir. Among them, two seem particularly relevant for studying 381 adaptive responses of rear edge silver fir populations. First, a solid knowledge about past 382 demographic patterns can improve the study of local adaptation. So far, it has been based on 383 considering a main separation between Alpine and Apennine populations in silver fir (Mosca et al., 384 2014), which seems quite unlikely from our results. Incorrectly considering demographic scenarios 385 can hinder the study of local adaptation by introducing biases in selection tests (Nielsen, 2005). In 386 addition, silver fir tree-ring growth series in Italy show three differentiated groups of populations: 1) 387 the western Alps, 2) the northern Apennines and the eastern Alps, and 3) the central and southern 388 Apennines (Carrer et al., 2010). This subdivision partially corresponds to the genetic structure found 389 in our survey, with some exceptions. In fact, central Apennine populations are genetically similar to 390 northern populations but show growth responses comparable to the ones from southern populations, 391 raising several questions about the relative strength of past migration dynamics vs. adaptation to 392 climate in highly heterogeneous regions.

393 Second, the existence of a sharp genetic boundary in the central Apennines poses the basis for 394 studying possible south-to-north adaptive gene flow in this area. With ongoing climate warming, 395 genotypes able to perform well in warmer and drier conditions are expected to be selectively 396 advantaged both in local persistence and during latitudinal and altitudinal migrations, in particular at 397 Mediterranean latitudes (Fady et al., 2016). In this context, the genetic boundary found in central 398 Italy, corresponding to an area where ecological conditions markedly change (Brunetti et al., 2004; 399 Comborieu-Nebout et al., 2015), might represent an ideal location for studying the role of gene flow 400 in promoting adaptive responses to climate change (Kremer et al., 2012). In such a study, it should 401 be noted that, although Mediterranean silver fir provenances are often considered less sensitive to 402 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).

Our study has revealed that unexpected processes have shaped the spatial distribution of silver fir 405 406 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 changing environmental conditions (Gazol et al., 2015) and their adaptive potential might be 413 414 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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435 **REFERENCES**

- 436 Aussenac, G. (2002) Ecology and ecophysiology of circum-Mediterranean firs in the context of
 437 climate change. *Annals of Forest Science*, **59**, 823-832.
- 438 Bagnoli, F., Tsuda, Y., Fineschi, S., Bruschi, P., Magri, D., Zhelev, P., Paule, L., Simeone, M.C.,
- 439 González-Martínez, S.C. & Vendramin, G.G. (2016) Combining molecular and fossil data to
- 440 infer demographic history of *Quercus cerris*: insights on European eastern glacial refugia.

441 *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.
- 446 Brousseau, L., Postolache, D., Lascoux, M., Drouzas, A.D., Källman, T., Leonarduzzi, C., Liepelt,
- 447 S., Piotti, A., Popescu, F., Roschanski, A.M., Zhelev, P., Fady, B. & Vendramin, G.G. (2016)
- 448 Local adaptation in European firs assessed through extensive sampling across altitudinal
 449 gradients in Southern Europe. *PLoS ONE*, **11**, e0158216.

- Brunetti, M., Maugeri, M., Monti, F. & Nanni, T. (2004) Changes in daily precipitation frequency
 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,
- K., Maiorano, L. & Laborde, H. (2014) Revisiting tree-migration rates: *Abies alba* (Mill.), a case
 study. *Vegetation History and Archaeobotany*, 23, 113-122.
- Combourieu-Nebout, N., Bertini, A., Russo-Ermolli, E., Peyron, O., Klotz, S., Montade, V.,
 Fauquette, S., Allen, J., Fusco, F., Goring, S., Huntley, B., Joannin, S., Lebreton, V., Magri, D.,
 Martinetto, E., Orain, R. & Sadori, L. (2015) Climate changes in the central Mediterranean and
 Italian vegetation dynamics since the Pliocene. *Review of Palaeobotany and Palynology*, 218,
 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.

470	Dering, M., Sękiewicz, K., Boratyńska, K., Litkowiec, M., Iszkuło, G., Romo, A. & Boratyński, A.
471	(2014) Genetic diversity and inter-specific relations of western Mediterranean relic Abies taxa
472	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.
- 483 Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using
 484 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.

- Follieri, M., Giardini, M., Magri, D. & Sadori, L. (1998) Palynostratigraphy of the last glacial period
 in the volcanic region of central Italy. *Quaternary International*, 47/48, 3-20.
- Gazol, A., Camarero, J.J., Gutiérrez, E., Popa, I., Andreu-Hayles, L., Motta, R., Nola, P., Ribas, M.,
 Sangüesa-Barreda, G., Urbinati, C. & Carrer, M. (2015) Distinct effects of climate warming on
 populations of silver fir (*Abies alba*) across Europe. *Journal of Biogeography*, 42, 1150-1162.
- Hampe, A. & Petit, R.J. (2005) Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters*, 8, 461-467.

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

- Hansen, O.K., Vendramin, G.G., Sebastiani, F. & Edwards, K.J. (2005) Development of
 microsatellite markers in *Abies nordmanniana* (Stev.) Spach and cross-species amplification in
 the *Abies* genus. *Molecular Ecology Notes*, 5, 784-787.
- Hu, F.S., Hampe, A. & Petit, R.J. (2009) Paleoecology meets genetics: deciphering past vegetational
 dynamics. *Frontiers in Ecology and the Environment*, 7, 371-379.
- Jakobsson, M. & Rosenberg, N.A. (2007) CLUMPP: a cluster matching and permutation program for
 dealing with label switching and multimodality in analysis of population structure.
 Bioinformatics, 23, 1801-1806.
- Kalinowski, S.T. (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures
 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.

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

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

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

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

- Liepelt, S., Bialozyt, R. & Ziegenhagen B. (2002) Wind-dispersed pollen mediates postglacial gene
 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

- expansion and its genetic imprints in *Abies alba* (Mill.) A synthesis from palaeobotanic and
 genetic data. *Review of Palaeobotany and Palynology*, **153**, 139-149.
- Linares, J.C. (2011) Biogeography and evolution of *Abies* (Pinaceae) in the Mediterranean Basin: the
 roles of long-term climatic change and glacial refugia. *Journal of Biogeography*, **38**, 619-630.
- Longauer, R., Paule, L. & Andonoski, A. (2003) Genetic diversity of southern populations of *Abies alba* Mill. *Forest Genetics*, **10**, 1-9.
- Magri, D., Agrillo, E., Di Rita, F., Furlanetto, G., Pini, R., Ravazzi, C. & Spada, F. (2015) Holocene
 dynamics of tree taxa populations in Italy. *Review of Palaeobotany and Palynology*, 218, 267284.
- Mosca, E., González-Martínez, S.C. & Neale, D.B. (2014) Environmental versus geographical
 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**, 197544 218.
- Nieto Feliner, G. (2014) Patterns and processes in plant phylogeography in the Mediterranean Basin.
 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.

- Petit, R.J., El Mousadik, A. & Pons, O. (1998) Identifying populations for conservation on the basis
 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.
- Piovani, P., Leonardi, S., Piotti, A. & Menozzi, P. (2010) Conservation genetics of small relic
 populations of Silver fir (*Abies alba* Mill.) in northern Apennines. *Plant Biosystems*, 144, 683691.
- Postolache, D., Leonarduzzi, C., Piotti, A., Spanu, I., Roig, A., Fady, B., Roschanski, A., Liepelt, S.
 & Vendramin, G.G. (2014) Transcriptome versus genomic microsatellite markers: Highly
 informative multiplexes for genotyping *Abies alba* Mill. and congeneric species. *Plant Molecular Biology Reporter*, **32**, 750-760.
- Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using
 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.
- Ruosch, M., Spahni, R., Joos, F., Henne, P.D., van der Knaap, W.O. & Tinner, W. (2016) Past and
 future evolution of *Abies alba* forests in Europe comparison of a dynamic vegetation model
 with palaeo data and observations. *Global Change Biology*, 22, 727-740.

- Satolli, S. & Calamita, F. (2008) Differences and similarities between the central and the southern
 Apennines (Italy): Examining the Gran Sasso versus the Matese-Frosolone salients using
 paleomagnetic, geological, and structural data. *Journal of Geophysical Research*, **113**, B10101.
- Scaltsoyiannes, A., Tsaktsira, M. & Drouzas, A.D. (1999) Allozyme differentiation in the
 Mediterranean firs (*Abies*, Pinaceae). A first comparative study with phylogenetic implications. *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.,
- Allen, J.R.M., Carraro, G., Conedera, M., Joos, F., Lotter, A.F., Luterbacher, J., Samartin, S. &
 Valsecchi, V. (2013) The past ecology of *Abies alba* provides new perspectives on future
 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.
- Vendramin, G.G., Lelli, L., Rossi, P. & Morgante, M. (1996) A set of primers for the amplification
 of 20 chloroplast microsatellites in *Pinaceae*. *Molecular Ecology*, 5, 595-598.
- Vescovi, E., Ammann, B., Ravazzi, C. & Tinner, W. (2010) A new Late-glacial and Holocene record
 of vegetation and fire history from Lago del Greppo, northern Apennines, Italy. *Vegetation History and Archaeobotany*, 19, 219-233.
- Vicario, F., Vendramin, G.G., Rossi, P., Liò, P. & Giannini, R. (1995) Allozyme, chloroplast DNA
 and RAPD markers for determining genetic relationships between *Abies alba* and the relic
 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	consequ	uences in	Eur	opean lar	ch.	PL	os on	VE, 1 () , e0127	516.			

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598 Supporting Information

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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

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

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- 621 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
- 622 contributions from all authors, who revised and approved the manuscript.
- 623

Table 1 Posterior probability of each tested demographic scenario and its 95% confidence interval based on the logistic estimate according to DIYABC. In Step 1 analysis, scenarios 1, 2, and 6 are based on an ancient separation between populations from the Apennines and Eastern Europe, scenario 3 strictly represents STRUCTURE results (Fig. 3c,d), and scenarios 4 and 5 consider an admixed origin of southern Apennine populations (see details in the Appendix S2). Scenarios from Step 2 analysis 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								
А	0.2680	0.2252-0.3109						
В	0 7320	0 6891-0 7748						

633

634

636 Table 2 Parameter estimates for the best demographic scenario based on approximate Bayesian637 computation.

6	3	8

				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
NCAPP	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
NSAPP	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}
NEAST	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
t3	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

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

650 FIGURE LEGENDS

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

654

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

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



666 Figure 1



671 Figure 2



675 Figure 3