

# Environmental effects on fine-scale spatial genetic structure in four Alpine keystone forest tree species

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2	Alpine keystone forest tree species
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23	Running title: Environmental effects on FSGS in Alpine trees

#### 24 Abstract

25 Genetic responses to environmental changes take place at different spatial scales. While 26 the effect of environment on the distribution of species' genetic diversity at large 27 geographical scales has been the focus of several recent studies, its potential effects on 28 genetic structure at local scales are understudied. Environmental effects on fine-scale 29 spatial genetic structure (FSGS) were investigated in four Alpine conifer species (five to 30 eight populations per species) from the eastern Italian Alps. Significant FSGS was 31 found for 11 out of 25 populations. Interestingly, we found no significant differences in 32 FSGS across species but great variation among populations within species, highlighting 33 the importance of local environmental factors. Inter-annual variability in spring 34 temperature had a small but significant effect on FSGS of Larix decidua, probably 35 related to species-specific life-history traits. For Abies alba, Picea abies and Pinus 36 *cembra*, linear models identified spring precipitation as a potentially relevant climate 37 factor associated with differences in FSGS across populations; however, models had 38 low explanatory power and were strongly influenced by a *P. cembra* outlier population 39 from a very dry site. Overall, the direction of the identified effects is according to 40 expectations, with drier and more variable environments increasing FSGS. Underlying 41 mechanisms may include climate-related changes in the variance of reproductive 42 success and/or environmental selection of specific families. This study provides new 43 insights on potential changes in local genetic structure of four Alpine conifers in the 44 face of environmental changes, suggesting that new climates, through altering FSGS, 45 may also have relevant impacts on plant microevolution.

- 47 Keywords: environmental change, climate, FSGS, Single Nucleotide Polymorphisms,
- 48 Alpine plants

#### 50 Introduction

51 Environmental changes, including climate change, are rapidly modifying plant 52 community composition (Franks et al. 2013). For example, during the last decades, the 53 Alps experienced an increase in minimum temperature of about 2°C, three times the 54 global average (IPCC 2014), with changes being more acute at higher altitudes 55 (Haeberli & Beniston 1998; Auer et al. 2007; Brunetti et al. 2009; Philipona 2013). 56 Global warming has also increased the frequency and intensity of extreme events (e.g., 57 floods; Wilhelm et al. 2013). As a consequence, range shifts and vegetation community 58 changes are strongly affecting Alpine ecosystems (Theurillat & Guisan 2001; Thuiller 59 et al. 2005; Pauli et al. 2007). Environmental effects on genetic variation of Alpine 60 keystone plants, such as forest trees, are still understudied. So far, research has focused 61 on the macro-geographic scale of population genetic structure while environmental 62 effects at local scale, i.e. within natural populations, have often been neglected (Manel 63 & Holderegger 2013; Richardson et al. 2014). Several recent studies point to genetic 64 variation within natural populations as a potential source of evolutionary change that 65 could mitigate new environmental impacts (see references in Scotti et al. 2016; 66 Valladares et al. 2014).

Abiotic factors, such as climate or soils, shape the genetic makeup of individuals, populations and species at different spatial scales (Turner et al. 2010; Fischer et al. 2013). In Alpine plants, temperature and precipitation together with soils (calcareous vs. siliceous) play a central role in shaping plant genetic variation at macrogeographical spatial scales (Alvarez et al. 2009; Manel et al. 2012; Mosca et al. 2012a, 2014). Temperature and plant physiology are strongly correlated, influencing plant growth and survival. Several studies suggested that forest tree species may require

74 exposure to chilling  $(0-10^{\circ}C)$  or freezing  $(<0^{\circ}C)$  temperatures to acquire maximum 75 cold tolerance (Bigras et al. 2001; Beck et al. 2004; Søgaard et al. 2009; reviewed in 76 Strimbeck & Kjellsen 2010), and many species also need a chilling period to prevent 77 budburst during midwinter warm periods (Harrington 2010). Moreover, the increase of 78 temperature associated with climate change causes shifts in the timing of bud burst (i.e., 79 earlier flushing; Bissolli 2006), extending the length of the growing season, with shifts 80 in the flowering phenology affecting plant fecundity, an important fitness component 81 (Vitasse et al. 2011). Recent studies suggest that, in the absence of sufficient plasticity, 82 environmental changes will affect the distribution of genetic diversity at the macro-83 geographical scale (e.g., Jaramillo-Correa et al. 2015), due to species migration and/or 84 genetic adaptation in situ (reviewed in Aitken et al. 2008). We hypothesize that 85 phenological and physiological changes caused by new environments will also have 86 important consequences for genetic structure at the micro-geographical scale.

87 Fine-scale spatial genetic structure (FSGS), i.e., the non-random distribution of 88 genotypes within populations, is determined by the combined effects of dispersal 89 (Hardy & Vekemans 1999), genetic drift and natural selection (Epperson 1990; Rousset 90 2004). High FSGS often just reflects higher relatedness between neighboring 91 individuals, compared to more distant ones, due to restricted dispersal (i.e., isolation by 92 distance, IBD; Wright 1943), while low FSGS is associated with a random distribution 93 of genotypes. Typically, life-history traits related to pollen and seed dispersal determine 94 the strength of FSGS (Hardy et al. 2006), with wind-pollinated and/or outcrossing 95 species showing lower FSGS than animal-pollinated and/or selfing species (Vekemans 96 & Hardy 2004; Dick et al. 2008). In addition, FSGS is higher in fragmented/peripheral 97 populations than in continuous/core ones (Gapare & Aitken 2005; De-Lucas et al. 2009;

98 Yao et al. 2011; Leonardi et al. 2012; Pandey & Rajora 2012). Importantly, Audigeos et 99 al. (2013) showed that divergent selection caused by heterogeneous microenvironments 100 (i.e., seasonally flooded bottom lands versus seasonally dry soils) can also shape the 101 genetic structure within populations of forest trees. In such cases, isolation by 102 environment (IBE) is expected (Nosil et al. 2008; Andrew et al. 2012). IBD and IBE are 103 not mutually exclusive and can both contribute to FSGS (Van Heerwarden et al. 2010). 104 The strength of FSGS is relevant for plant populations at evolutionary time scales as 105 strong FSGS can lead to bi-parental inbreeding and thereby decrease genetic diversity 106 (reviewed in Heywood et al. 1991).

107 Despite several recent FSGS studies (e.g., Duminil et al. 2016; Fajardo et al. 108 2015; Sork et al. 2015; Torroba-Balmori et al. 2017), the effects of environmental 109 factors on spatial genetic structure within populations are yet to be investigated. 110 Different non-exclusive processes could contribute to environmental effects on FSGS, 111 being climate effects of particular relevance in Alpine species, where spring phenology 112 is triggered by climate factors and not by photoperiod (Zohner et al. 2016). First, high 113 spring temperatures can result in early growth resumption after winter, which in turn 114 leads to longer growing seasons (Cleland et al. 2007; Hänninen & Tanino 2011; 115 Linkosalo et al. 2006). Early spring warming can increase flowering synchrony (Wang 116 et al. 2016), which could in theory blur FSGS but would also make trees more prone to 117 late frost events (increasing the variance of reproductive success and thus FSGS, see 118 next point). Second, because climate is strongly associated with fecundity, it can affect 119 the variance of reproductive success, a key mating parameter that strongly influences 120 FSGS (see, for example, De-Lucas et al. 2009). Sites with more favorable years for 121 growth and reproduction (e.g., cool years with high spring precipitation; see, for 122 example, Oberhuber 2004 for *Pinus cembra*), with few or no late frosts, may reduce the 123 variance of reproductive success, and in turn FSGS, by allowing most trees to 124 contribute to reproduction, while in sites experiencing hotter, drier years, many trees 125 might be effectively sterile, increasing FSGS. Third, masting events (i.e., the inter-126 individual synchronization of seed crops in particular years) are driven by climate 127 factors, in particular spring/summer temperatures and rainfall (Bisi et al. 2016; Kelly et 128 al. 2013). Climate could then affect the variance of reproductive success, and thus 129 FSGS, in forest trees with marked masting, such as Larix decidua, Picea abies or Abies 130 alba. Fourth, local environment can also affect genetic structure patterns due to uneven 131 post-dispersal mortality. For example, low spring precipitation could favor high 132 mortality due to intraspecific competition, selecting particular families with higher 133 competition ability (e.g., Vizcaíno-Palomar et al. 2014), and thus also increasing FSGS. 134 Hence, environmental factors responsible for phenological and physiological traits 135 could, at least in theory, influence both IBD and IBE processes, thus affecting the 136 strength of FSGS.

137 The knowledge on which environmental factors may contribute to FSGS would 138 improve our understanding on how changing environmental conditions affect genetic 139 variation and fitness within natural plant populations. Twenty-five natural populations 140 of A. alba, L. decidua, P. abies and P. cembra were sampled across the eastern part of 141 the Italian Alps. For each population, we examined FSGS using Single Nucleotide 142 Polymorphism (SNP) markers and determined differences across species and across 143 populations within species due to non-climatic factors known to be relevant in Alpine 144 environments (i.e., biogeographic regions, elevation and soils). Then, we estimated the 145 effects of different climatic variables related to temperature and precipitation, which are

146 closely associated to plant physiology and growth, on FSGS while considering other147 relevant factors in Alpine ecosystems.

148

#### 149 Material and methods

# 150 <u>Study species and sampling</u>

151 Alpine forests are characterized by the presence of several conifer species, with natural 152 populations growing across a wide altitudinal range (c. 800–2,250 m a.s.l.). The eastern 153 part of the Italian Alps is covered by pure and mixed stands of A. alba and P. abies, 154 substituted by pure stands of L. decidua and P. cembra, and mixed pine forests of P. 155 cembra and Pinus mugo, at higher elevation. Alpine conifer species have different 156 preferences for light, temperature and water availability. A. alba tolerates a wide range 157 of soils but it is sensitive to moisture availability and temperature (Mauri et al. 2016). P. 158 abies is a shade-tolerant species and has its optimum on deep, nutritious and humid 159 soils (Skrøppa 2003). L. decidua is a typical pioneer that grows on disturbed soils but it 160 suffers from the competition with other species (Matras & Pâques 2008). P. cembra is 161 well adapted to the severe upper subalpine climate (Ulber et al. 2004). Moreover, 162 Alpine conifer species show different strategies to cope with environmental stress. For 163 example, L. decidua is a deciduous species that sheds its needles, reducing winter 164 transpiration to 2.3% of its annual photosynthetic carbon gain (Havranek & Tranquillini 165 1995), whereas P. cembra has strong roots that penetrate a large volume of soil in their 166 search for water. Bisi et al. (2016) found marked inter-annual variation in cone 167 production, with years resembling masting events in P. abies, A. alba and L. decidua, 168 whereas *P. cembra* had less inter-annual variation in cone-crop size. All species are 169 wind-pollinated and seeds are exclusively dispersed by wind in A. alba, L. decidua and

170 P. abies, whereas in P. cembra seed dispersal is tightly associated with a bird, the 171 European nutcracker (Nucifraga caryocatactes), and establishment is strongly affected 172 by biotic interactions (Neuschulz et al. 2017). In this study, five to eight natural 173 populations per species (total of 25 populations) of A. alba, P. abies, L. decidua and P. 174 cembra, four Alpine conifer species with distinct life-history traits (Table S1), were 175 sampled in the eastern Italian Alps (Figure 1), covering their environmental variation in 176 altitude and soils (see Table S2). For each population, coordinates of each tree (latitude, 177 longitude, and elevation, as provided by GPS; Trimble Technologies, Sunnyvale, CA, 178 USA), geographic position of the sampling site (East or West) relative to the Adige 179 River, which constitutes an important biogeographic barrier (Thiel-Egenter et al. 2011), 180 and soil types (calcareous or siliceous), were recorded in the field. Fresh needles were 181 sampled from 65 adult trees in each population for molecular analyses (see Mosca et al. 182 2012a for sampling details).

183

## 184 <u>Climatic variables and ecological indexes</u>

185 For each sampling site, monthly and annual cumulative precipitation for the period 186 1981-2010 were obtained from the European Climate Assessment & Dataset time series 187 (spatial resolution of 0.25 degrees; Haylock et al. 2008), and temperature data from 188 daily reconstructed MODIS LST time series data for the period 2002-2012 (resolution -189 pixel size- of 250 m; Neteler 2010, 2005). In conifers, high temperatures from late 190 winter to early spring cause an earlier initiation of cambial activity and consequently a 191 longer annual growth period (Rossi et al. 2007). Cambial activity is also regulated by 192 rainfall and photoperiod (Begum et al. 2013). Thus, we focused the analysis of climate 193 effects on FSGS on the minimum April temperature (*tmin*) and the spring (April 1<sup>st</sup> to

194 June  $30^{\text{th}}$ ) precipitation (*precQ2*). These two variables were uncorrelated (Pearson's r of 195 0.10) and thus appropriate to be combined in linear models (see below). We also 196 included inter-annual variability in spring temperature, defined as the mean of the 197 standard deviation of March, April and May average temperatures between 2000 and 198 2010 (Zohner et al. 2016). Sites with high inter-annual variability in spring temperature 199 have also a higher probability to experience late frost events. Moreover, to account for 200 the effect of winter temperature on plant growth resumption, two ecological indexes 201 were calculated using daily mean temperature: chilling degree days (CDD) and freezing 202 degree days (FDD). Since in Alpine conifers cambial activity occurs only above a mean 203 daily temperature of 5.8-8.5°C (Rossi et al. 2007), we calculated CDD as the number of days with mean temperature between 0°C and 5°C in the period from January 1<sup>st</sup> to 204 205 March 31<sup>st</sup>. Moreover, as Alpine trees only suffer damage from freezing below -10°C 206 (Neuner 2014), we computed FDD as the number of days with mean temperature below 207 -10°C in the period from November 1<sup>st</sup> to the end of February (Greuell et al. 2015). 208 CDD and FDD were calculated for each year and averaged for the period 2000-2010. 209 Both averages for the period 2000-2010 and outlier years/periods (i.e., years/periods 210 with low correlation with the average), which in our case corresponded to year 2005 for 211 CDD and period 2006-2007 for FDD, were used in the models (see below).

212

### 213 <u>SNP genotyping</u>

For each species, a SNP-genotyping assay was designed based on SNPs obtained from Sanger re-sequencing with PCR primer pairs from *P. taeda* (see details in Mosca et al. 2012b; Scalfi et al. 2014; Di Pierro et al. 2016). SNP genotyping was carried out at the Genome Center of the University of California, Davis, using the Golden Gate platform

218	(Illumina, San Diego, CA, USA). After standard filtering and visual inspection with
219	GenomeStudio software (Illumina, San Diego, CA, USA), a total of 231, 233, 455 and
220	214 high-quality SNPs were retained for FSGS analyses in A. alba, L. decidua, P.
221	cembra and P. abies, respectively.

### 223 <u>Genetic diversity and differentiation</u>

Expected heterozygosity,  $H_{\rm E}$ , was computed for each population following Nei (1978) and averaged for each species. Global *F*-statistics ( $F_{\rm IS}$  and  $F_{\rm ST}$ ) and pairwise genetic differentiation ( $F_{\rm ST}$ ) among populations within species were calculated following Weir & Cockerham (1984). Significant levels for  $F_{\rm IS}$  and pairwise  $F_{\rm ST}$  were obtained by 10,000 permutations. All genetic diversity and differentiation statistics were computed using SPAGeDi v1.4 (Hardy & Vekemans 2002) and Arlequin v3.5 (Excoffier et al. 2010).

231

# 232 <u>Fine-scale spatial genetic structure (FSGS)</u>

233 Fine-scale spatial genetic structure (FSGS) was analyzed by linear regression of 234 pairwise kinship coefficients on the logarithm of inter-tree distances using SPAGeDi. 235 The average kinship coefficients were calculated for six distance classes with similar 236 tree-pair numbers (Table S3), following Nason's method (reported in Loiselle et al. 237 1995). The significance of the regression slope, b-log, was assessed by 10,000 238 permutations. For each population, we computed the Sp statistic, defined as the negative 239 ratio between the regression slope, *b*-log, and  $(1-F_1)$ , where  $F_1$  is the mean kinship 240 coefficient in the first distance class (Vekemans & Hardy 2004). Previous FSGS studies 241 in tree species have revealed Sp-values varying from 0.03934, indicating strong FSGS

242 in Vouacapoua Americana, over Sp=0.00031, indicating weak FSGS in Virola michelii, 243 to non-significant, undetectable FSGS (reviewed by Vekemans & Hardy 2004). To test 244 for FSGS differences among species and among populations within species, a standard 245 ANOVA was performed in R environment (R v3.3.1, R Core Team 2016). In addition, 246 Student's t-tests and Pearson's r, also performed in R environment, were used to 247 investigate correlation of FSGS with soil types and biogeographic regions, and 248 elevation, respectively (i.e., main non-climatic factors that may also affect FSGS in the 249 Alpine ecosystems).

250

#### 251 Effects of climate on FSGS

252 Linear regression models were used to investigate a potential association of 253 temperature/precipitation variables (April minimum temperature, *tmin*; inter-annual 254 variability in spring temperature, SD springTemp; and cumulative spring precipitation, 255 precQ2) and ecological indexes (CDD and FDD, see above) with FSGS, as evaluated 256 by Sp, while considering other relevant factors (i.e., species, soil types and 257 biogeographic regions; see above). Species, soil types and biogeographic regions were 258 introduced in the models as covariates, while climatic variables and ecological indexes 259 as linear predictors of Sp. The step function, a standard stepwise selection method based 260 on the minimum Akaike's Information Criterion (AIC) was used to select which 261 variables/factors would remain in the final model that best fitted the data. Previous to 262 analyses, continuous variables were normalized using the *scale* function. Final models 263 were fitted using the *lm* function. All analyses were performed in R computing 264 environment.

266 **Results** 

267

#### 268 <u>Genetic diversity and differentiation</u>

269 Genetic diversity ( $H_E$ ) was similar across tree species, except for significantly lower 270 values in L. decidua (mean  $H_E=0.170$  in L. decidua vs. mean  $H_E=0.237-0.264$  in the 271 other tree species; Student's t-test=-14.7086, P<0.00001). Genetic diversity was also 272 similar for conspecific populations sampled at sites with contrasting elevation, soil type 273 and geographic position with respect to the Adige River, an important biogeographic 274 barrier (Table 1). Inbreeding coefficients ( $F_{IS}$ ) were close to zero in all species, ranging 275 from -0.132 in L. decidua to 0.027 in P. abies (Table 1). Genetic differentiation among 276 populations was lower in A. alba and P. abies (F<sub>ST</sub> values of 0.0092 and 0.0068, 277 respectively) than in *P. cembra* and *L. decidua* (*F*<sub>ST</sub> of 0.0191 and 0.0255, respectively). 278 Most pairwise  $F_{ST}$  values were highly significant, with the highest values often 279 involving pairs of populations from different biogeographic regions (Table 1).

280

#### 281 <u>Fine-scale spatial genetic structure (FSGS)</u>

282 Significant fine-scale spatial genetic structure (FSGS) was found for 11 out of 25 283 populations tested (44%), involving one (P. cembra) to four (A. alba) populations per 284 species (Table 2 and Figure 2). Average Sp ranged from 0.0018 in P. cembra to 0.0035 285 in L. decidua (Table 2 and Figure 3), but differences among species were not significant 286 (as shown by ANOVA; F-value=0.377, P=0.771). Interestingly, however, Sp varied 287 greatly among populations within species, for example from 0.0005 (P-value for b-log 288 of 0.3819, i.e., no significant FSGS detected) to 0.0113 (P-value for b-log of 0.0003, supporting a relatively strong FSGS) in L. decidua (Table 2), highlighting the 289

290	importance of local site environmental factors. No overall significant correlation of Sp
291	with soil types, biogeographic regions or elevation was found (Figure S1).

# 293 Effects of climate on FSGS

294 The best linear model associated FSGS, as evaluated by Sp, with inter-annual variability 295 in spring temperature (SD springTemp), mean chilling degree days (CDD) and CDD of 296 the extreme year 2005 (CDD<sub>2005</sub>), while retaining species and soil types as significant 297 fixed factors (adjusted  $R^2=0.413$ , P=0.018; Table 3, Model A). This model showed a 298 significant positive effect of SD\_springTemp (P=0.008) and mean CDD (P=0.083) on 299 FSGS while CDD<sub>2005</sub> had a negative effect (P=0.011). Among the fixed factors, a 300 significant species effect for L. decidua (P=0.006) and P. cembra (P=0.033) as well as 301 for soil type (P=0.042) was found. Removing SD springTemp variable rendered the 302 model non-significant (Table 3, Model B). Further exploratory analyses for L. decidua, 303 the species with the strongest fixed effect, revealed that SD\_springTemp was 304 significantly correlated with FSGS in this species (Pearson's correlation of 0.807; 305 P=0.015; Figure 4), while for other climate variables and ecological indexes 306 correlations were not significant. For the other species, the best linear model removing 307 L. decidua (Table 3, Model C) included only a significant negative effect of spring cumulative precipitation (*precO2*) on FSGS (adjusted  $R^2$ =0.2163, P=0.034), with no 308 309 significant fixed factors. However, this relationship was not significant when P16, a P. 310 *cembra* outlier population with very low *precQ2* and very high Sp, was removed from 311 the analyses (Table 3, Model D).

313 **Discussion** 

314

315 By studying within-population genetic structure in four keystone Alpine tree species 316 with distinct life-history traits, we aimed at improving our understanding of the 317 complex interactions between geography, environment and genetics at local scales. 318 Importantly, most of the genetic variation found in forest trees resides within 319 populations (Scotti et al. 2016) and this variation may prove to be of great value for 320 evolutionary responses to climate change. Although identified correlations of FSGS 321 with climate factors were weak and sensitive to the choice of populations and variables, 322 our study suggests that, depending on the species, changes in inter-annual variability in 323 spring temperatures and spring rainfall regimes may have consequences for population 324 microevolution in Alpine forests.

325

# 326 <u>Fine-scale spatial genetic structure (FSGS) across species</u>

327 The strength of FSGS did not vary significantly among the four studied Alpine conifer 328 species although it was slightly lower in *P. cembra* and the strongest was detected in *L*. 329 decidua. Usually, differences in FSGS among species have been related to differences 330 in life-history traits that influence pollen and seed dispersal (see, e.g., Dick et al. 2008, 331 Vekemans & Hardy 2004). In our study, differences in seed dispersal (by wind in A. 332 alba, L. decidua and P. abies vs. by birds in P. cembra) did not affect FSGS. Both 333 wind- and bird- mediated seed dispersal can render long dispersal distances. In the 334 specific case of *P. cembra*, which produces cones with large seeds that are an attractive 335 food source for the European nutcracker (Tomback 2005), seed dispersal up to 15 km 336 from the mother tree has been observed (Mattes 1982). Seed dispersal by wind can also

reach long distances, for example up to about 50% of seeds went over 3 km in *Fraxinus excelsior* (Bacles et al. 2006). Other factors such as soil types, elevation or biogeographic regions did also not affect patterns of FSGS. These results contrast with a study by Nardin et al. (2015) in *L. decidua*, where the intensity of FSGS was found to vary with elevation. However, the authors interpreted their findings rather as the effect of human activities and recent re-colonization than as an effect of changing environmental conditions along the altitudinal gradient.

344

#### 345 Environmental effects on FSGS

346 Interestingly, we found more variation in FSGS among populations of the same species 347 than among species, highlighting the importance of local site environmental factors. 348 Overall, our results suggested that, depending on the species considered, high inter-349 annual variability in spring temperature (L. decidua) or lower spring precipitation (A. 350 alba, P. cembra and P. abies) may result in increased FSGS. Although the latter 351 correlation was weak and sensitive to the choice of populations, the result was in 352 agreement with our expectations. It also pointed to a potential effect of climatic 353 extremes since the significant correlation was driven by an outlier (P. cembra) 354 population living in exceptionally dry conditions. Our study is exceptional in that it 355 includes a large number of sites (total of 25 populations) for which FSGS could be 356 assessed, but still this sample size is insufficient to reveal weak effects associated with 357 extreme environmental conditions. Thus, the associations between FSGS and climate 358 discussed below, although pointing to relevant environmental factors to be considered 359 in further studies, must also be taken with caution.

360 High variability in inter-annual spring temperatures is associated to a higher risk361 of late frost events that, in turn, could increase the variance of reproductive success and

362 thus FSGS in L. decidua. Interestingly, L. decidua is the only deciduous species in our 363 study and the one that flushes and flowers the earliest (Zohner & Renner 2014; Table 364 S1). In this species, cones are produced every 3-6 years (Poncet et al. 2009 and personal 365 observations), and specific responses to climate in masting patterns may have also 366 enhanced FSGS. In contrast to L. decidua, spring cumulative precipitation, especially in 367 very dry sites, seemed to play a more relevant role than temperature in shaping FSGS in 368 the other species. Lower precipitation in spring can both reduce the number of 369 individuals that contribute to reproduction and increase post-dispersal mortality, 370 resulting in unequal representation of genotypes in the next generation and, as 371 suggested in this study, greater FSGS. Mathiasen & Premoli (2013) obtained similar 372 results for *Nothofagus pumilio*, where high elevation populations growing under harsher 373 environmental conditions showed stronger FSGS than low elevation populations. 374 However, N. pumilio is a partly clonal species and other factors such as disturbance 375 regimes played also a role in their study, impeding direct comparisons.

376 Other environment-related population features could also underlie the observed 377 FSGS patterns. From those, the potentially most important is population density. Lower 378 density populations are expected to have higher FSGS (Vekemans & Hardy 2004). 379 Thus, environment could interact indirectly with FSGS by modifying population 380 density, for example if low-density populations typically grow in environments with 381 lower spring precipitation and/or less adequate soils. However, correlations between the 382 climate factors selected in the linear models in this study and a proxy for population 383 density in regular stands (basal area in m<sup>2</sup>/ha) for the four Alpine tree species studied 384 were not significant (Table S2), allowing us to discard this possibility.

#### 386 Expected trends under climate change

387 In the Alpine region, climate change is expected to increase temperature (about +2.7°C 388 in spring at the end of the 21<sup>st</sup> century) but not to change overall annual precipitation, 389 according to current models (Gobiet et al. 2014). However, a different distribution of 390 the precipitation along the year, with less precipitation in summer (-20.4%) and more 391 precipitation in winter (+10.4%) is also predicted (Gobiet et al. 2014). Reduced water 392 availability during spring and summer could be exacerbated by the expected reduction 393 of snow amount and duration (i.e., an upward snowline shift of over 300-600 m by the 394 end of the 21<sup>st</sup> century; Gobiet et al. 2014). Our results suggests that these changes (i.e., 395 reduced water availability during reproduction and seedling establishment) may result 396 in increased FSGS in Alpine tree species, affecting tree population microevolution, as 397 higher FSGS may, in the long-term, increase population inbreeding (Frankham et al. 398 2002). Climate is also expected to become more unpredictable in the Alps, with sudden 399 decreases in temperature after cambial reactivation becoming more frequent (late spring 400 frosts). This may enhance FSGS in temperature-sensitive Alpine species, as our study 401 suggests for L. decidua. Bisi et al. (2016) showed that spring and summer temperatures 402 and precipitations of one and two years prior to seed maturation influence cone-crop 403 size in Alpine conifer species. Based on their model and taking into account climate 404 change scenarios, they do not expect marked changes in masting patterns in Alpine 405 forests. However, inter-annual spring temperature variability was not taken into account 406 in their study.

407

408 Climate and other environmental changes will have profound impacts on Alpine409 ecosystems. We suggest that new environments will not only modify how genetic

diversity is distributed among populations at large geographical scales but will also
affect, in complex ways, how fine-scale spatial genetic structure builds within
populations, being relevant for plant microevolution.

413

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#### 683 Data Accessibility

- 684 SNP genotypes and sampling locations for A. alba, P. cembra and L. decidua, Dryad
- 685 Digital Repository: doi:10.5061/dryad.tm33d
- 686 SNP genotypes and sampling locations for *P. abies*, Dryad Digital Repository:
  687 doi:10.5061/dryad.n818s
- 688 Environmental data used in linear models associating climate with FSGS, Dryad Digital
- 689 Repository: doi:10.5061/dryad.6d831
- 690

# 691 Author Contributions

EM and EADP collected the needle samples. EM and SCGM realized statistical
analyses with the contributions from EADP and KBB. EM, EADP and KBB were
responsible for figure preparation. EM and SCGM wrote the manuscript with

695 contributions from DBN, EADP and KBB. All authors read, edited and approved the

696 final manuscript.

698 Figure legends

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Figure 1. Sampling sites for four Alpine conifer forest species: *Pinus cembra*, *Larix decidua*, *Abies alba* and *Picea abies*. Population codes and site description are given in
Table S2. The blue line indicates the Adige River, a natural biogeographic barrier.

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Figure 2. Average kinship coefficient ( $F_{ij}$ ) plotted against mean geographical distance between individuals in each distance class, for each of 25 populations sampled in four Alpine conifer forest species. Significant autocorrelograms are indicated with an asterisk.

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Figure 3. Box plots showing *Sp* statistics for each population across species. Greater
variability in FSGS among populations within species than among species is observed.

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Figure 4. (A) Regression of inter-annual variability in spring temperature (*SD\_springTemp*, defined as mean standard deviation of March, April and May temperatures from 2000-2010) and FSGS, as evaluated by *Sp*, in *Larix decidua*. (B) A scatterplot showing no significant relationship in the other species; the discontinuous line indicates *Sp*=0.

**Table 1**. Genetic diversity ( $H_E$ ), pairwise genetic differentiation ( $F_{ST}$ ), and population inbreeding ( $F_{IS}$ ), for each of 25 populations in four

719 Alpine forest tree species. Numbers in italics are significant at 0.001 level, after 10,000 permutations. The biogeographic region (Geo) is

720 indicated as E (East side) or W (West side) with respect to the Adige river; soil types are calcareous (C) and siliceous (S).

Species	ID	ID	ID	<b>N</b> 7	Caa	Elevation	Soil	E	$H_{\rm E}$		Doimuia	o Fam						
Species	ID	11	Geo	[m]	type	<b>F</b> IS	mean	SD	- Pairwis	e r st								
A. alba									P1	P2	P3	P4	P5	P6				
	P1	65	W	1,168	С	-0.031	0.241	0.162		-	-	-	-	-				
	P2	65	W	1,187	С	-0.049	0.259	0.156	0.0099		-	-	-	-				
	P3	65	W	1,581	С	-0.052	0.250	0.154	0.0099	0.0038		-	-	-				
	P4	65	E	1,288	S	-0.005	0.248	0.152	0.0101	0.0079	0.0084		-	-				
	P5	65	W	940	S	-0.066	0.243	0.158	0.0118	0.0084	0.0042	0.0121		-				
	P6	63	E	1,340	С	-0.026	0.252	0.151	0.0133	0.0126	0.0081	0.0085	0.0094					
L. decidua									P7	P8	P9	P10	P11	P12	P13	P14		
	P7	64	E	1,707	С	-0.117	0.172	0.165		-	-	-	-	-	-	-		
	P8	65	W	1,883	S	-0.051	0.173	0.156	0.0181		-	-	-	-	-	-		
	P9	63	E	1,906	S	-0.132	0.182	0.159	0.0360	0.0288		-	-	-	-	-		
	P10	65	W	1,881	С	-0.062	0.152	0.159	0.0319	0.0082	0.0377		-	-	-	-		
	P11	65	W	1,480	S	-0.123	0.176	0.161	0.0451	0.0251	0.0236	0.0302		-	-	-		
	P12	65	W	2,144	S	-0.125	0.181	0.159	0.0481	0.0274	0.0292	0.0371	0.0014		-	-		
	P13	64	W	1,631	S	-0.112	0.158	0.159	0.0315	0.0074	0.0411	0.0113	0.0281	0.0332		-		
	P14	65	W	2,217	S	-0.066	0.164	0.153	0.0290	0.0055	0.0347	0.0083	0.0209	0.0240	0.0046			
P. cembra									P15	P17	P18	P19	P16					
	P15	63	W	2,053	S	-0.007	0.247	0.153		-	-	-	-					
	P17	65	E	2,032	С	-0.018	0.242	0.157	0.0155		-	-	-					
	P18	65	W	2,149	S	-0.035	0.238	0.159	0.0150	0.0057		-	-					
	P19	65	E	1,885	S	0.027	0.214	0.167	0.0104	0.0214	0.0193		-					
	P16	61	E	2,227	С	-0.001	0.246	0.155	0.0273	0.0252	0.0183	0.0345						
P. abies									P20	P21	P22	P23	P24	P25				

P20	64	Е	1,242	S	-0.007	0.266	0.157		-	-	-	-	-
P21	62	E	1,701	S	-0.005	0.261	0.161	0.0024		-	-	-	-
P22	65	W	1,683	S	-0.006	0.262	0.158	0.0102	0.0107		-	-	-
P23	65	W	1,757	С	-0.005	0.267	0.156	0.008	0.0083	0.0046		-	-
P24	64	Е	1,879	S	0.022	0.266	0.158	0.0043	0.0051	0.0089	0.0071		-
P25	64	Е	1,805	С	0.0004	0.264	0.158	0.0057	0.0043	0.0112	0.006	0.0049	

**Table 2.** Fine-scale spatial genetic structure (FSGS) in four Alpine conifer species; *N*:
sample size; *b*-log: slope of the regression of kinship with the logarithm of the distance; *F*<sub>1</sub>: mean kinship of the first distance class (0-10 m, except for *L. decidua* that is 0-20
m); *P*-value was calculated using 10,000 permutations and significant values at 0.05
level are given in italics.

Species	ID	N	<i>b</i> -log	<i>P</i> -value	<b>F</b> <sub>1</sub>	Sp
A. alba	P1	65	-0.0064	0.0009	0.0123	0.0064
	P2	65	-0.0029	0.0488	0.0088	0.0029
	P3	65	-0.0001	0.4556	0.0019	0.0001
	P4	65	-0.0025	0.0404	0.0105	0.0026
	P5	65	-0.0032	0.0315	0.0114	0.0033
	P6	63	0.0010	0.6673	0.0112	-0.0010
Overall					0.0094	0.0024
L. decidua	P7	64	-0.0107	0.0003	0.0523	0.0113
	P8	65	-0.0005	0.3819	0.0097	0.0005
	P9	63	-0.0043	0.0415	0.0374	0.0044
	P10	65	-0.0045	0.0220	0.0182	0.0046
	P11	65	-0.0017	0.2045	0.0232	0.0018
	P12	65	-0.0022	0.2610	0.0313	0.0022
	P13	64	-0.0019	0.1468	0.0177	0.0019
	P14	65	-0.0014	0.2156	0.0123	0.0015
Overall					0.0253	0.0035
P. cembra	P15	63	-0.0006	0.3790	0.0139	0.0006
	P16	61	-0.0085	0.0002	0.0385	0.0088
	P17	65	-0.00002	0.4846	0.0104	0.0000
	P18	65	0.00177	0.8634	0.0041	-0.0018
	P19	65	-0.00142	0.2888	0.0165	0.0014
Overall					0.0167	0.0018
P. abies	P20	64	-0.0010	0.2675	0.0057	0.0010
	P21	62	-0.0034	0.0119	0.0082	0.0034
	P22	65	-0.0042	0.0073	0.0119	0.0042
	P23	65	-0.0034	0.0192	0.0091	0.0034
	P24	64	-0.0011	0.1935	0.0045	0.0011
	P25	64	-0.0016	0.1792	0.0067	0.0016
Overall					0.0077	0.0025

**Table 3**. Best linear model correlating climatic factors and FSGS, as identified by AIC, after step-wise selection. A: complete model based
on all variables; B: model with the same variables as A except for *SD\_springTemp*; C: model without *Larix decidua*; D: model without *L. decidua* and with outlier population P16 removed. *SD\_springTemp*: inter-annual variability in spring temperature; *precQ2*: spring
cumulative precipitation; *CDD*: mean chilling degree days; *CDD*<sub>2005</sub>: chilling degree days in 2005.

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Variable estima	tes			Fixed facto	ors				
	02		<i>CDD</i> <sub>2005</sub>	Species			Soils	Adj. $R^2$	<i>P</i> -value
SD_spring1emp	precQ2	CDD		L. decidua	P. abies	P. cembra	Siliceous		
0.7084	-	1.3325	-0.7468	3.0173	1.6046	2.5575	-0.8550	0.4134	0.0182
-	-	1.0482	-0.4932	2.5459	2.1263	1.9972	-1.2503	0.1588	0.1655
-	-0.5150	-	-	-	-	-	-	0.2163	0.0344
-	-0.2494	-	-	-	-	-	-	0.0622	0.3517
	Variable estima SD_springTemp 0.7084 - - -	Variable estimates           SD_springTemp         precQ2           0.7084         -           -         -           -         -           -         -           -         -0.5150           -         -0.2494	Variable estimates           SD_springTemp         precQ2         CDD           0.7084         -         1.3325           -         -         1.0482           -         -0.5150         -           -         -0.2494         -	Variable estimates           SD_springTemp         precQ2         CDD         CDD2005           0.7084         -         1.3325         -0.7468           -         -         1.0482         -0.4932           -         -0.5150         -         -           -         -0.2494         -         -	Variable estimates       Fixed factor $SD\_springTemp$ $precQ2$ $CDD$ $CDD_{2005}$ Species         0.7084       -       1.3325       -0.7468       3.0173         -       -       1.0482       -0.4932       2.5459         -       -0.5150       -       -       -         -       -0.2494       -       -       -	Variable estimates         Fixed factors $SD\_springTemp$ $precQ2$ $CDD$ $CDD_{2005}$ Species           0.7084         -         1.3325         -0.7468         3.0173         1.6046           -         -         1.0482         -0.4932         2.5459         2.1263           -         -0.5150         -         -         -         -           -         -0.2494         -         -         -         -	Variable estimates         Fixed factors $SD\_springTemp$ $precQ2$ $CDD$ $CDD_{2005}$ Species           0.7084         -         1.3325         -0.7468         3.0173         1.6046         2.5575           -         -         1.0482         -0.4932         2.5459         2.1263         1.9972           -         -0.5150         -         -         -         -         -         -           -         -0.2494         -         -         -         -         -         -	Variable estimates         Fixed factors $SD\_springTemp$ $precQ2$ $CDD$ $CDD_{2005}$ Species $Soils$ 0.7084         -         1.3325         -0.7468         3.0173         1.6046         2.5575         -0.8550           -         -         1.0482         -0.4932         2.5459         2.1263         1.9972         -1.2503           -         -0.5150         -         -         -         -         -         -           -         -0.2494         -         -         -         -         -         -	Variable estimatesFixed factors $SD\_springTemp$ $precQ2$ $CDD$ $CDD_{2005}$ SpeciesSoilsSoils $Adj. R^2$ 0.7084-1.3325-0.74683.01731.60462.5575-0.85500.41341.0482-0.49322.54592.12631.9972-1.25030.15880.21630.21630.0622

# Supporting Information

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**Table S1.** Overview of life-history traits for the four Alpine tree species (*Abies alba*, *Larix decidua*, *Picea abies*, *Pinus cembra*) included in the study.

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**Table S2.** Sampling site description, including geographic location, elevation (m a.s.l.),
a proxy for population density in regular stands (basal area in m<sup>2</sup>/ha), biogeographic
regions (Geo) with respect to Adige River (E: East, W: West), soil type (C: calcareous;
S: siliceous), inter-annual variability in spring temperature (*SD\_springTemp*) and spring
precipitation (*precQ2*).

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**Table S3.** Distance classes (in meters) used for fine-scale spatial genetic structure
(FSGS) analyses; average number of tree pairs per population per species included in
each distance class is given between parentheses.

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Figure S1. Box plots and a scatter-plot showing non-significant differences in FSGS, as
evaluated by *Sp*, across (A) biogeographic regions (West or East of the Adige River),
(B) soil type (Calcareous or Siliceous) and (C) elevation; the discontinuous line in the
scatter-plot indicates *Sp*=0.









Picea abies









