Environmental effects on fine-scale spatial genetic structure in four 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.

46

Introduction

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

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74   exposure to chilling (0–10°C) or freezing (<0°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 other

147   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 1st to

194   June 30th) 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

204   days with mean temperature between 0°C and 5°C in the period from January 1st to

205   March 31st. 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 1st 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>

214   For each species, a SNP-genotyping assay was designed based on SNPs obtained from

215   Sanger re-sequencing with PCR primer pairs from *P. taeda* (see details in Mosca et al.

216   2012b; Scalfi et al. 2014; Di Pierro et al. 2016). SNP genotyping was carried out at the

217   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.

222

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

224   Expected heterozygosity, H_E, was computed for each population following Nei (1978)

225   and averaged for each species. Global F-statistics (F_{IS} and F_{ST}) and pairwise genetic

226   differentiation (F_{ST}) among populations within species were calculated following Weir

227   & Cockerham (1984). Significant levels for F_{IS} and pairwise F_{ST} were obtained by

228   10,000 permutations. All genetic diversity and differentiation statistics were computed

229   using SPAGeDi v1.4 (Hardy & Vekemans 2002) and Arlequin v3.5 (Excoffier et al.

230   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.

265

Results

Genetic diversity and differentiation

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

Fine-scale spatial genetic structure (FSGS)

Significant fine-scale spatial genetic structure (FSGS) was found for 11 out of 25 populations tested (44%), involving one (*P. cembra*) to four (*A. alba*) populations per species (Table 2 and Figure 2). Average *Sp* ranged from 0.0018 in *P. cembra* to 0.0035 in *L. decidua* (Table 2 and Figure 3), but differences among species were not significant (as shown by ANOVA; *F*-value=0.377, P=0.771). Interestingly, however, *Sp* varied greatly among populations within species, for example from 0.0005 (*P*-value for *b*-log 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

13

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).

292

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_{2005}), 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_{2005} 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

308   cumulative precipitation (*precQ2*) on FSGS (adjusted R^2=0.2163, *P*=0.034), with no

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).

312

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

337   reach long distances, for example up to about 50% of seeds went over 3 km in *Fraxinus*

338   *excelsior* (Bacles et al. 2006). Other factors such as soil types, elevation or

339   biogeographic regions did also not affect patterns of FSGS. These results contrast with a

340   study by Nardin et al. (2015) in *L. decidua*, where the intensity of FSGS was found to

341   vary with elevation. However, the authors interpreted their findings rather as the effect

342   of human activities and recent re-colonization than as an effect of changing

343   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 risk

361   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^2/ha) for the four Alpine tree species studied

384   were not significant (Table S2), allowing us to discard this possibility.

385

Expected trends under climate change

387 In the Alpine region, climate change is expected to increase temperature (about +2.7ºC

388 in spring at the end of the 21st 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 21st 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 Alpine

409 ecosystems. We suggest that new environments will not only modify how genetic

410 diversity is distributed among populations at large geographical scales but will also

411 affect, in complex ways, how fine-scale spatial genetic structure builds within

412 populations, being relevant for plant microevolution.

413

428

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682

683   **Data Accessibility**

690

691   **Author Contributions**

692   EM and EADP collected the needle samples. EM and SCGM realized statistical

693   analyses with the contributions from EADP and KBB. EM, EADP and KBB were

694   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.

697

Figure legends

699

700 **Figure 1.** Sampling sites for four Alpine conifer forest species: *Pinus cembra*, *Larix*

701 *decidua*, *Abies alba* and *Picea abies*. Population codes and site description are given in

702 Table S2. The blue line indicates the Adige River, a natural biogeographic barrier.

703

704 **Figure 2.** Average kinship coefficient (F_{ij}) plotted against mean geographical distance

705 between individuals in each distance class, for each of 25 populations sampled in four

706 Alpine conifer forest species. Significant autocorrelograms are indicated with an

707 asterisk.

708

709 **Figure 3**. Box plots showing *Sp* statistics for each population across species. Greater

710 variability in FSGS among populations within species than among species is observed.

711

712 **Figure 4.** (A) Regression of inter-annual variability in spring temperature

713 (*SD_springTemp*, defined as mean standard deviation of March, April and May

714 temperatures from 2000-2010) and FSGS, as evaluated by *Sp*, in *Larix decidua*. (B) A

715 scatterplot showing no significant relationship in the other species; the discontinuous

716 line indicates *Sp*=0.

717

718   **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).

721

Species	ID	N	Geo	Elevation [m]	Soil type	F_{IS}	H_E mean	SD	Pairwise F_{ST}							
A. alba									P1	P2	P3	P4	P5	P6		
	P1	65	W	1,168	C	-0.031	0.241	0.162		-	-	-	-	-		
	P2	65	W	1,187	C	*-0.049*	0.259	0.156	*0.0099*		-	-	-	-		
	P3	65	W	1,581	C	*-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	C	-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	C	*-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	C	*-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	C	-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	C	-0.001	0.246	0.155	*0.0273*	*0.0252*	*0.0183*	*0.0345*				
P. abies									P20	P21	P22	P23	P24	P25		

P20	64	E	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	C	-0.005	0.267	0.156	*0.008*	*0.0083*	*0.0046*		-	-
P24	64	E	1,879	S	0.022	0.266	0.158	0.0043	*0.0051*	*0.0089*	*0.0071*		-
P25	64	E	1,805	C	0.0004	0.264	0.158	*0.0057*	0.0043	*0.0112*	*0.006*	*0.0049*	

722

723 **Table 2.** Fine-scale spatial genetic structure (FSGS) in four Alpine conifer species; N:

724 sample size; b-log: slope of the regression of kinship with the logarithm of the distance;

725 F_1: mean kinship of the first distance class (0-10 m, except for *L. decidua* that is 0-20

726 m); P-value was calculated using 10,000 permutations and significant values at 0.05

727 level are given in italics.

728
729

Species	ID	N	b-log	P-value	F_1	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

730 **Table 3**. Best linear model correlating climatic factors and FSGS, as identified by AIC, after step-wise selection. A: complete model based

731 on all variables; B: model with the same variables as A except for *SD_springTemp*; C: model without *Larix decidua*; D: model without *L.*

732 *decidua* and with outlier population P16 removed. *SD_springTemp*: inter-annual variability in spring temperature; *precQ2*: spring

733 cumulative precipitation; *CDD*: mean chilling degree days; CDD_{2005}: chilling degree days in 2005.

734

Model	Variable estimates				Fixed factors				Adj. R^2	*P*-value
					Species			Soils		
	SD_springTemp	*precQ2*	*CDD*	CDD_{2005}	*L. decidua*	*P. abies*	*P. cembra*	Siliceous		
A	0.7084	-	1.3325	-0.7468	3.0173	1.6046	2.5575	-0.8550	0.4134	0.0182
B	-	-	1.0482	-0.4932	2.5459	2.1263	1.9972	-1.2503	0.1588	0.1655
C	-	-0.5150	-	-	-	-	-	-	0.2163	0.0344
D	-	-0.2494	-	-	-	-	-	-	0.0622	0.3517

735

736   **Supporting Information**

737

738   **Table S1.** Overview of life-history traits for the four Alpine tree species (*Abies alba*,

739   *Larix decidua*, *Picea abies*, *Pinus cembra*) included in the study.

740

741   **Table S2.** Sampling site description, including geographic location, elevation (m a.s.l.),

742   a proxy for population density in regular stands (basal area in m^2/ha), biogeographic

743   regions (Geo) with respect to Adige River (E: East, W: West), soil type (C: calcareous;

744   S: siliceous), inter-annual variability in spring temperature (*SD_springTemp*) and spring

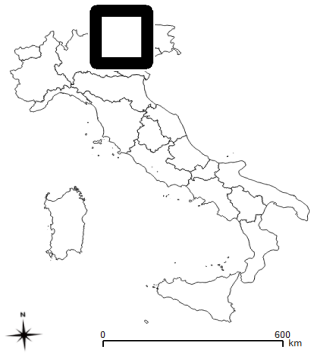745   precipitation (*precQ2*).

746

747   **Table S3.** Distance classes (in meters) used for fine-scale spatial genetic structure

748   (FSGS) analyses; average number of tree pairs per population per species included in

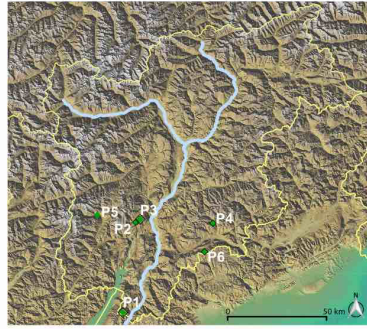749   each distance class is given between parentheses.

750

751   **Figure S1.** Box plots and a scatter-plot showing non-significant differences in FSGS, as

752   evaluated by *Sp*, across (**A**) biogeographic regions (West or East of the Adige River),

753   (**B**) soil type (Calcareous or Siliceous) and (**C**) elevation; the discontinuous line in the
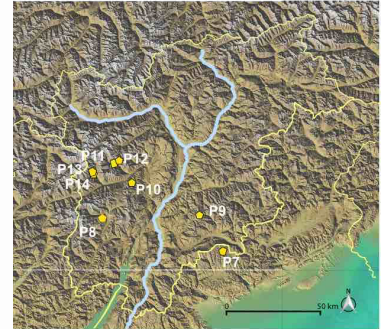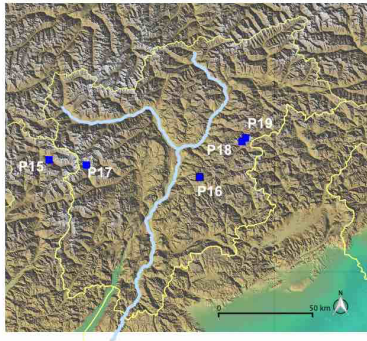
754   scatter-plot indicates *Sp*=0.

755

Abies alba

Larix decidua

Pinus cembra

Picea abies

A

Sp

Larix decidua

3.70 3.80 3.90 4.00 4.10 4.20 4.30 4.40

B

Sp

△ *Abies alba*
◆ *Picea abies*
● *Pinus cembra*

3.70 3.80 3.90 4.00 4.10 4.20 4.30 4.40

Average SD of spring temperatures (°C)