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

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Running title: Environmental effects on FSGS in Alpine trees

Abstract

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

47 Keywords: environmental change, climate, FSGS, Single Nucleotide Polymorphisms,
48 Alpine plants
49

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

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

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

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

Despite several recent FSGS studies (e.g., Duminil et al. 2016; Fajardo et al. 2015; Sork et al. 2015; Torroba-Balmori et al. 2017), the effects of environmental factors on spatial genetic structure within populations are yet to be investigated. Different non-exclusive processes could contribute to environmental effects on FSGS, being climate effects of particular relevance in Alpine species, where spring phenology is triggered by climate factors and not by photoperiod (Zohner et al. 2016). First, high spring temperatures can result in early growth resumption after winter, which in turn leads to longer growing seasons (Cleland et al. 2007; Hänninen & Tanino 2011; Linkosalo et al. 2006). Early spring warming can increase flowering synchrony (Wang et al. 2016), which could in theory blur FSGS but would also make trees more prone to late frost events (increasing the variance of reproductive success and thus FSGS, see next point). Second, because climate is strongly associated with fecundity, it can affect the variance of reproductive success, a key mating parameter that strongly influences FSGS (see, for example, De-Lucas et al. 2009). Sites with more favorable years for growth and reproduction (e.g., cool years with high spring precipitation; see, for

example, Oberhuber 2004 for *Pinus cembra*), with few or no late frosts, may reduce the variance of reproductive success, and in turn FSGS, by allowing most trees to contribute to reproduction, while in sites experiencing hotter, drier years, many trees might be effectively sterile, increasing FSGS. Third, masting events (i.e., the inter-individual synchronization of seed crops in particular years) are driven by climate factors, in particular spring/summer temperatures and rainfall (Bisi et al. 2016; Kelly et al. 2013). Climate could then affect the variance of reproductive success, and thus FSGS, in forest trees with marked masting, such as *Larix decidua*, *Picea abies* or *Abies alba*. Fourth, local environment can also affect genetic structure patterns due to uneven post-dispersal mortality. For example, low spring precipitation could favor high mortality due to intraspecific competition, selecting particular families with higher competition ability (e.g., Vizcaíno-Palomar et al. 2014), and thus also increasing FSGS. Hence, environmental factors responsible for phenological and physiological traits could, at least in theory, influence both IBD and IBE processes, thus affecting the strength of FSGS.

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

149 **Material and methods**

150 Study species and sampling

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

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

Climatic variables and ecological indexes

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

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

SNP genotyping

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

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

Genetic diversity and differentiation

Expected heterozygosity, H_E , was computed for each population following Nei (1978) and averaged for each species. Global F -statistics (F_{IS} and F_{ST}) and pairwise genetic differentiation (F_{ST}) among populations within species were calculated following Weir & Cockerham (1984). Significant levels for F_{IS} and pairwise F_{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).

Fine-scale spatial genetic structure (FSGS)

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

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

Effects of climate on FSGS

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

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

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

Effects of climate on FSGS

The best linear model associated FSGS, as evaluated by *Sp*, with inter-annual variability in spring temperature (*SD_springTemp*), mean chilling degree days (CDD) and CDD of the extreme year 2005 (CDD₂₀₀₅), while retaining species and soil types as significant fixed factors (adjusted $R^2=0.413$, $P=0.018$; Table 3, Model A). This model showed a significant positive effect of *SD_springTemp* ($P=0.008$) and mean CDD ($P=0.083$) on FSGS while CDD₂₀₀₅ had a negative effect ($P=0.011$). Among the fixed factors, a significant species effect for *L. decidua* ($P=0.006$) and *P. cembra* ($P=0.033$) as well as for soil type ($P=0.042$) was found. Removing *SD_springTemp* variable rendered the model non-significant (Table 3, Model B). Further exploratory analyses for *L. decidua*, the species with the strongest fixed effect, revealed that *SD_springTemp* was significantly correlated with FSGS in this species (Pearson's correlation of 0.807; $P=0.015$; Figure 4), while for other climate variables and ecological indexes correlations were not significant. For the other species, the best linear model removing *L. decidua* (Table 3, Model C) included only a significant negative effect of spring cumulative precipitation (*precQ2*) on FSGS (adjusted $R^2=0.2163$, $P=0.034$), with no significant fixed factors. However, this relationship was not significant when P16, a *P. cembra* outlier population with very low *precQ2* and very high *Sp*, was removed from the analyses (Table 3, Model D).

Discussion

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

Fine-scale spatial genetic structure (FSGS) across species

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

Environmental effects on FSGS

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

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

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

Other environment-related population features could also underlie the observed FSGS patterns. From those, the potentially most important is population density. Lower density populations are expected to have higher FSGS (Vekemans & Hardy 2004). Thus, environment could interact indirectly with FSGS by modifying population density, for example if low-density populations typically grow in environments with lower spring precipitation and/or less adequate soils. However, correlations between the climate factors selected in the linear models in this study and a proxy for population density in regular stands (basal area in m²/ha) for the four Alpine tree species studied were not significant ([Table S2](#)), allowing us to discard this possibility.

Expected trends under climate change

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

Climate and other environmental changes will have profound impacts on Alpine 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.

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682

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**

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

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.

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.

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.

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 Alpine forest tree species. Numbers in italics are significant at 0.001 level, after 10,000 permutations. The biogeographic region (Geo) is 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	N	Geo	Elevation [m]	Soil type	F_{IS}	H_E		Pairwise F_{ST}							
							mean	SD								
<i>A. alba</i>	P1	65	W	1,168	C	-0.031	0.241	0.162	P1	P2	P3	P4	P5	P6		
	P2	65	W	1,187	C	<i>-0.049</i>	0.259	0.156	<i>0.0099</i>	-	-	-	-	-		
	P3	65	W	1,581	C	<i>-0.052</i>	0.250	0.154	<i>0.0099</i>	<i>0.0038</i>	-	-	-	-		
	P4	65	E	1,288	S	-0.005	0.248	0.152	<i>0.0101</i>	<i>0.0079</i>	<i>0.0084</i>	-	-	-		
	P5	65	W	940	S	<i>-0.066</i>	0.243	0.158	<i>0.0118</i>	<i>0.0084</i>	<i>0.0042</i>	<i>0.0121</i>	-	-		
	P6	63	E	1,340	C	-0.026	0.252	0.151	<i>0.0133</i>	<i>0.0126</i>	<i>0.0081</i>	<i>0.0085</i>	<i>0.0094</i>	-		
<i>L. decidua</i>	P7	64	E	1,707	C	<i>-0.117</i>	0.172	0.165	P7	P8	P9	P10	P11	P12	P13	P14
	P8	65	W	1,883	S	<i>-0.051</i>	0.173	0.156	<i>0.0181</i>	-	-	-	-	-	-	-
	P9	63	E	1,906	S	<i>-0.132</i>	0.182	0.159	<i>0.0360</i>	<i>0.0288</i>	-	-	-	-	-	-
	P10	65	W	1,881	C	<i>-0.062</i>	0.152	0.159	<i>0.0319</i>	<i>0.0082</i>	<i>0.0377</i>	-	-	-	-	-
	P11	65	W	1,480	S	<i>-0.123</i>	0.176	0.161	<i>0.0451</i>	<i>0.0251</i>	<i>0.0236</i>	<i>0.0302</i>	-	-	-	-
	P12	65	W	2,144	S	<i>-0.125</i>	0.181	0.159	<i>0.0481</i>	<i>0.0274</i>	<i>0.0292</i>	<i>0.0371</i>	0.0014	-	-	-
	P13	64	W	1,631	S	<i>-0.112</i>	0.158	0.159	<i>0.0315</i>	<i>0.0074</i>	<i>0.0411</i>	<i>0.0113</i>	<i>0.0281</i>	<i>0.0332</i>	-	-
	P14	65	W	2,217	S	<i>-0.066</i>	0.164	0.153	<i>0.0290</i>	0.0055	<i>0.0347</i>	<i>0.0083</i>	<i>0.0209</i>	<i>0.0240</i>	0.0046	
<i>P. cembra</i>	P15	63	W	2,053	S	-0.007	0.247	0.153	P15	P17	P18	P19	P16			
	P17	65	E	2,032	C	-0.018	0.242	0.157	<i>0.0155</i>	-	-	-	-			
	P18	65	W	2,149	S	<i>-0.035</i>	0.238	0.159	<i>0.0150</i>	<i>0.0057</i>	-	-	-			
	P19	65	E	1,885	S	0.027	0.214	0.167	<i>0.0104</i>	<i>0.0214</i>	<i>0.0193</i>	-	-			
	P16	61	E	2,227	C	-0.001	0.246	0.155	<i>0.0273</i>	<i>0.0252</i>	<i>0.0183</i>	<i>0.0345</i>	-			
<i>P. abies</i>									P20	P21	P22	P23	P24	P25		

722

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	<i>0.0102</i>	<i>0.0107</i>		-	-	-
P23	65	W	1,757	C	-0.005	0.267	0.156	<i>0.008</i>	<i>0.0083</i>	<i>0.0046</i>		-	-
P24	64	E	1,879	S	0.022	0.266	0.158	0.0043	<i>0.0051</i>	<i>0.0089</i>	<i>0.0071</i>		-
P25	64	E	1,805	C	0.0004	0.264	0.158	<i>0.0057</i>	0.0043	<i>0.0112</i>	<i>0.006</i>	<i>0.0049</i>	

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*₁: 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	<i>N</i>	<i>b</i> -log	<i>P</i> -value	<i>F</i> ₁	<i>Sp</i>
<i>A. alba</i>	P1	65	-0.0064	<i>0.0009</i>	0.0123	0.0064
	P2	65	-0.0029	<i>0.0488</i>	0.0088	0.0029
	P3	65	-0.0001	0.4556	0.0019	0.0001
	P4	65	-0.0025	<i>0.0404</i>	0.0105	0.0026
	P5	65	-0.0032	<i>0.0315</i>	0.0114	0.0033
	P6	63	0.0010	0.6673	0.0112	-0.0010
Overall					0.0094	0.0024
<i>L. decidua</i>	P7	64	-0.0107	<i>0.0003</i>	0.0523	0.0113
	P8	65	-0.0005	0.3819	0.0097	0.0005
	P9	63	-0.0043	<i>0.0415</i>	0.0374	0.0044
	P10	65	-0.0045	<i>0.0220</i>	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
<i>P. cembra</i>	P15	63	-0.0006	0.3790	0.0139	0.0006
	P16	61	-0.0085	<i>0.0002</i>	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
<i>P. abies</i>	P20	64	-0.0010	0.2675	0.0057	0.0010
	P21	62	-0.0034	<i>0.0119</i>	0.0082	0.0034
	P22	65	-0.0042	<i>0.0073</i>	0.0119	0.0042
	P23	65	-0.0034	<i>0.0192</i>	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; *CDD₂₀₀₅*: chilling degree days in 2005.
734

Model	Variable estimates				Fixed factors				Adj. R^2	P-value
	<i>SD_springTemp</i>	<i>precQ2</i>	<i>CDD</i>	<i>CDD₂₀₀₅</i>	Species			Soils		
					<i>L. decidua</i>	<i>P. abies</i>	<i>P. cembra</i>	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

Supporting Information

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.

Table S2. Sampling site description, including geographic location, elevation (m a.s.l.), a proxy for population density in regular stands (basal area in m²/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*).

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.

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.

Abies alba

Larix decidua

Pinus cembra

Picea abies