Genomic vulnerability to rapid climate warming in a tree species with a long generation time

Running title: Stone pine vulnerability to climate change

List of authors: Benjamin Dauphin[1*], Christian Rellstab[1], Max Schmid[2], Stefan Zoller[3], Dirk N. Karger[1], Sabine Brodbeck[1], Frédéric Guillaume[2], and Felix Gugerli[1*]

ORCID: B.D. 0000-0003-0982-4252, C.R. 0000-0002-0221-5975, D.N.K. 0000-0001-7770-6229, FE.G. 0000-0003-3878-1845, FR.G. 0000-0003-0874-0081, M.S. 0000-0001-7197-4775, S.B. 0000-0002-4481-7384.

Institutional affiliations:

[1]Swiss Federal Research Institute WSL, Birmensdorf, Switzerland

[2]Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

[3]Genetic Diversity Centre, ETH Zurich, Zurich, Switzerland

[*]Corresponding Authors: +41 44 739 26 56, benjamin.dauphin@wsl.ch

+41 44 739 25 90, felix.gugerli@wsl.ch

Abstract

The ongoing increase in global temperature affects biodiversity, especially in mountain regions where climate change is exacerbated. As sessile, long-lived organisms, trees are especially challenged in terms of adapting to rapid climate change. Here, we show that low rates of allele frequency shifts in Swiss stone pine (*Pinus cembra*) occurring near the treeline result in high genomic vulnerability to future climate warming, presumably due to the species' long generation time. Using exome sequencing data from adult and juvenile cohorts in the Swiss Alps, we found an average rate of allele frequency shift of 1.23×10^{-2}/generation (i.e. 40 years) at presumably neutral loci, with similar rates for putatively adaptive loci associated with temperature (0.96×10^{-2}/generation) and precipitation (0.91×10^{-2}/generation). These recent shifts were corroborated by forward-in-time simulations at neutral and adaptive loci. Additionally, in juvenile trees at the colonisation front we detected alleles putatively beneficial under a future warmer and drier climate. Notably, the observed past rate of allele frequency shift in temperature-associated loci was decidedly lower than the estimated average rate of 6.29×10^{-2}/generation needed to match a moderate future climate scenario (RCP4.5). Our findings suggest that species with long generation times may have difficulty keeping up with the rapid climate change occurring in high mountain areas and thus are prone to local extinction in their current main elevation range.

Keywords: Allele frequency shift, Alps, climate change, conifer, ecological genomics, genomic offset, local adaptation, risk of non-adaptedness

Introduction

Climate change has manifold effects on biodiversity (Scheffers et al., 2016). Increasing temperature and changes in precipitation affect the sustainability of alpine ecosystems (Ernakovich et al., 2014) and in some cases pose a considerable challenge to the competitive ability and physiological limits of organisms (Alexander, Diez, & Levine, 2015). As a result, plant populations have to migrate beyond their current range or adapt to changing environmental conditions to avoid local extinction (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; Cotto et al., 2017; Steinbauer et al., 2018). These evolutionary trajectories are partly governed by the adaptive potential of species, especially the amount of standing genetic variation that confers putatively beneficial variants for climate-related traits (Barrett & Schluter, 2008). Moreover, the ratio between generation time and the pace of climate change is critical because it can impose an adaptational lag (Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008). Evaluating associations of allele frequencies in fitness-relevant genes with the local environment (Rellstab, Gugerli, Eckert, Hancock, & Holderegger, 2015) can provide the basis for determining the degree of genomic vulnerability (i.e. genomic offset) of species and help to predict the required response to future climate conditions (Fitzpatrick & Keller, 2015; Gárate-Escamilla, Hampe, Vizcaíno-Palomar, Robson, & Benito Garzón, 2019; Pina-Martins, Baptista, Pappas, & Paulo, 2018). Although it has been shown that the alpine flora responds to climate warming through both accelerated elevational changes and increased growth of plant communities (Pauli et al., 2012; Steinbauer et al., 2018), little is known about how populations of species with long generation times, such as trees, cope with rapid climate change.

Swiss stone pine (*Pinus cembra* L.) is an emblematic keystone species of the treeline ecotone (Körner, 2012) and occurs in the subalpine vegetation zone throughout the Central European Alps and the Carpathian Mountains (Fig. 1a). Due to its low competitive ability, the species is largely restricted to high elevations along the upward colonisation front of trees (Fig. 1b; Lingua, Cherubini, Motta, & Nola, 2008), where extreme climate events, such as severe frost, strong wind desiccation or recurrent heat waves, can occur (Gruber, Zimmermann, Wieser, & Oberhuber, 2009; Wieser, Gruber, & Oberhuber, 2014). The species is common across the central parts of its distribution, although it has experienced substantial range contractions over the last two centuries due to the decline of its main dispersal vector, the Eurasian Nutcracker (*Nucifraga caryocatactes* (Linnaeus, 1758); Neuschulz, Merges, Bollmann, Gugerli, & Böhning-Gaese, 2018), in combination with high grazing and harvesting

pressures from Alpine pasture farming (Vittoz, Rulence, Largey, & Freléchoux, 2008). As a result, its range is fragmented, particularly in peripheral parts, but populations still show high levels of gene flow facilitated by wind pollination (Salzer & Gugerli, 2012). Swiss stone pines reach maturity after 40–60 years in natural stands and viable cones are produced irregularly over the years (Zong et al., 2010). Selection is expected, as in most trees (Petit & Hampe, 2006), to be strongest in the earliest life stages, both in established stands (i.e. rejuvenation) and above the treeline (i.e. colonisation; Savolainen, Kärkkäinen, & Kuittinen, 1992). Swiss stone pines are among the oldest trees of the Alpine Arc (i.e. frequently older than 500 years; Zhao et al., 2018) and they grow in heterogeneous habitats, making the species an ideal study system for addressing the impact of climate change on the adaptive response of alpine species with long generation times. For this purpose, we reconstructed past climate conditions of Swiss stone pine populations (Fig. 1c–e) and investigated the genomic basis of climate adaptation using environmental association analysis (EAA; Rellstab et al., 2015) through space and time. We determined rates of historical allele frequency shifts (i.e. absolute difference in allele frequency between age cohorts) over two centuries and compared them with the estimated shifts needed to cope with projected future warmer and drier conditions (Fig. 1e–g). With this approach, we could assess the potential adaptational lag of Swiss stone pine under different future climate scenarios and evaluate the species' vulnerability to local extinction.

Materials and methods

Sampling design

In summer and fall 2014, we sampled seven populations of *P. cembra* growing under a broad range of environmental conditions and covering the two main phylogeographic lineages of the species in Switzerland (Fig. 1c). At each locality, we haphazardly sampled 20 georeferenced trees, minimum 30 metres apart, from each of three cohorts: an adult (LA) and a juvenile (LJ) cohort at low elevation (main elevation range), and a juvenile cohort at high elevation (HJ) at the colonisation front around 350 m above the low-elevation cohorts (Fig. 1d, Table S1). With this sampling design, we were able to address whether genetic variation is associated with contrasting environmental conditions and to quantify differences in allele frequencies (AFs) between age cohorts (LA–LJ, LA–HJ) and between juvenile cohorts at different elevations (LJ–HJ; Fig. 1d). The ages of adult trees were estimated by taking increment cores and counting the annual rings of each tree using a binocular microscope. For juvenile trees, ages were

estimated in the field by counting annual shoot increments, and saplings with an age of 10–20 years were selected. In total, we collected needles of 420 trees for molecular analyses.

Pooled exome capture sequencing and filtering of putatively paralogous genes

We performed DNA extraction, library preparation and exome capture as described by Rellstab, Dauphin, Zoller, Brodbeck, & Gugerli (2019). This study originally targeted ~25,000 mostly annotated contigs based on a 69.4 Mbp transcriptome, obtained sequence information of around ~15,000 contigs after basic filtering, and finally identified a well-supported set of 4,950 single-copy contigs containing almost 15,000 single-nucleotide polymorphisms (SNPs).

In short, DNA was extracted from 15–20 mg of desiccated needle samples, and 55 ng of high-quality DNA from every sample was used to produce equimolar DNA pools (n = 20 individuals for each of the 21 cohorts, 1100 ng in total per cohort pool). We generated barcoded libraries (average size 550 bp) using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) and subsequently performed probe hybridisation using the MYcroarray myBaits Custom Capture Kit (Arbor Biosciences, Ann Arbor, MI, USA), including a PCR amplification step of 14 cycles. The 21 hybridised libraries were submitted for sequencing on three lanes of an Illumina HiSeq 4000 (paired-end reads of 150 bp) at the Functional Genomics Center Zurich (FGCZ, Zurich, Switzerland) and Fasteris (Geneva, Switzerland; Table S2). We trimmed and filtered raw reads with TRIMMOMATIC 0.35 (Bolger, Lohse, & Usadel, 2014), mapped the remaining reads back to the transcripts of the reference transcriptome that contained probe bases using Bowtie 2.3.0 (Langmead, Trapnell, Pop, & Salzberg, 2009), and performed the SNP calling using GATK 3.8 (McKenna et al., 2010) with ploidy set to 40, a depth $\geq 40\times$, and a mapping quality/depth ratio ≥ 0.25. AF estimates of pooled sequencing data were validated using two individually sequenced HJ cohorts (Rellstab et al., 2019). To conduct subsequent analyses, we created four SNP datasets (all cohorts, HJ + LJ, HJ + LA, and LJ + LA; Table S3). To remove weakly supported SNPs and SNPs in putatively paralogous genes, we applied three additional filters to assemble the final SNP datasets (Table S3). We removed SNPs: (i) from putatively paralogous contigs (Rellstab et al., 2019) using the HDplot approach (McKinney, Waples, Seeb, & Seeb, 2017) based on excess heterozygosity and deviation from usual allele balance, (ii) with missing data > 3 (pools, i.e. 14%), and (iii) with a minor allele frequency (MAF) $\leq 2.5\%$ in at least one population (i.e. at least one chromosome in one of the pools). In downstream analyses, we used these four SNP datasets in either AF or read count data format.

Genetic structure and diversity

To characterise neutral genetic variation among populations, we performed a principal component (PC) analysis from AFs of all cohorts using the *prcomp* function from the stats package in R (R Development Core Team, 2020). Next, we carried out a hierarchical clustering analysis from the dissimilarity matrix Omega (dij = 1−ρij) generated with BAYPASS 2.1 (Gautier, 2015; see below) using the *hclust* function from the stats R package. We assessed genetic diversity within each cohort by calculating the proportion of polymorphic loci (PPL) and expected heterozygosity H_e (Fischer et al., 2017), and we tested for significant differences among cohorts using a paired *t* test from the stats R package (Table S4). Pairwise genetic differentiation (F_{ST}; Table S5) among cohorts was estimated from read count data with the poolfstat R package (Hivert, Leblois, Petit, Gautier, & Vitalis, 2018), and pairwise geographic distances were calculated from latitude and longitude using the geosphere R package (Hijmans, Williams, Vennes, & Hijmans, 2017). Using these two distance matrices, we then tested for patterns of isolation by distance for each cohort (IBD, $F_{ST}/[1–F_{ST}] \sim \ln[\text{distance}]$, Fig. S2; Rousset, 1997) with 500 permutations in a Mantel test using the vegan R package (Oksanen, Blanchet, Kindt, Legendre, & O'Hara, 2011).

Environmental change over time

For each sampling location, we compiled four datasets to characterise the environmental conditions that cohorts encountered during the time of their establishment (see Table S1 for age estimates) and will encounter in the future. For data on the current climate, we used CHELSA V.1.2 (reference period 1979–2013; http://chelsa-climate.org/future/; Karger et al., 2017) with a spatial resolution of about 650 m × 650 m over the European Alps (i.e. horizontal grid spacing of 0.00833° or 30 arc-seconds). From the centroid of each cohort, we derived yearly climate variables, as well as monthly values for the period corresponding to the growing season (May–October) of the species: mean/maximum/minimum temperature and precipitation sum. We also considered all bioclimatological variables for habitat characterisation that are supplied by CHELSA V.1.2 (Karger et al., 2017). For past environmental data corresponding to the period when adult cohorts established, we used long-term series of HISTALP (period 1780–2014; Chimani, Böhm, Matulla, & Ganekind, 2011; Chimani, Matulla, Böhm, & Hofstätter, 2013) for spatial and temporal downscaling of CHELSA V.1.2 temperature and precipitation data using a combination of climatologically aided interpolation (Willmott & Robeson, 1995) and the change factor method (Anandhi et al., 2011). We defined the past reference period of each adult cohort as the average age of adult trees (sample year [2014] –

mean cohort age ± 17 years, corresponding to the duration of the reference period 1979–2013; Table S1), or as the oldest period available when their age exceeded the start of temperature and precipitation time series. We first calculated the delta (anomaly) of the monthly mean temperature and precipitation sum of the HISTALP time series for every low- and high-elevation site as: $\Delta T_H = mean(T_H) - T_H$ (1) and $\Delta P_H = mean(P_H) - P_H$ (2), where T_H is the monthly mean temperature and P_H the monthly precipitation sum derived from the HISTALP data. Then, we performed the downscaling of the CHELSA V.1.2 data as follows: $T_{downscaled} = mean(T_C) - \Delta T_H$ (3) and $P_{downscaled} = mean(P_C) - \Delta P_H$ (4), where T_C is the monthly mean temperature and P_C the monthly precipitation sum from the CHELSA V.1.2 data.

From these downscaled CHELSA V.1.2 time series, we derived four climate variables to retrace past conditions during the establishment of today's adult trees: yearly mean temperature and precipitation sum, and mean temperature and summed precipitation over the growing season (May–October). For future data, we used the $CHELSA_{cmip5}$ts (available for RCP4.5 and RCP8.5; Karger, Schmatz, Dettling, & Zimmermann, 2020) based on the five most informative models (Sanderson, Knutti, & Caldwell, 2015) in the Alps for the reference period 2061–2080. Projected future climate variables were taken from five global circulation models (GCMs), which were downscaled to 1 km × 1 km resolution using an additive (for temperature), or multiplicative (for precipitation) change factor method using CHELSA V.1.2 as a baseline. The five selected models originate from the CMIP5 collection of model runs used in the IPCC's 5th Assessment Report (Stocker et al., 2013). GCMs are, however, often based on similar code which consequently results in similar outputs. We therefore chose models with only a small amount of interdependence to include a realistic representation of uncertainty in climate projections. Model selection was based on model interdependence in ensembles (Sanderson et al., 2015). The five models from which data were taken are: CESM1-BGC, run by the National Center for Atmospheric Research (NCAR), Boulder, CO, USA; CMCC-CM, run by the Euro-Mediterranean Center on Climate Change (CMCC), Lecce, Italy; MIROC5, run by the University of Tokyo, Japan; ESM-MR25, run by the Max Planck Institute for Meteorology (MPI-M), Hamburg, Germany; and ACCESS1-0, run by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and Bureau of Meteorology (BOM), Australia. The representative concentration pathway (RCP) trajectory accounts for anthropogenic activities, i.e. RCP4.5 (radiative forcing of 4.5 W/m^2 in 2100) represents a scenario with the peak of greenhouse gas (GHG) emissions in 2040 followed by a decline, while RCP8.5 (radiative

forcing of 8.5 W/m^2 in 2100) models GHG emissions that continue to rise up to 2100 without mitigation measures.

To characterise topography, we derived ten variables (Leempoel et al., 2015) at the individual tree level from the SwissALTi3D using SAGA 6.2 (Conrad et al., 2015): elevation, aspect, slope, profile curvature, morphometric protection index, vector ruggedness measure, visible sky, diffuse and direct solar radiation, and topographic wetness index. Because we assessed genomic data at the cohort level (i.e. AFs from pooled exome capture; see above), we averaged topographic variables from the 20 individuals of each cohort to account for the habitat variance and spatial heterogeneity. In total, we examined 35 climate variables and 10 topographic variables. To avoid redundant information and minimise multicollinearity in EAA (see below), we performed pairwise Pearson's correlations between the 45 environmental variables and applied several rules for retaining independent variables: (i) maximum Pearson's correlation coefficient r was set to |0.7| (Dormann et al., 2013), (ii) yearly variables that were highly correlated ($r \geq |0.7|$) with monthly (growing season, May–October) average variables were removed, (iii) when two monthly average variables were highly correlated ($r \geq |0.7|$), we selected the one we considered biologically more relevant to test our hypotheses (e.g. monthly mean temperature instead of monthly minimum temperature), and (iv) secondary variables (e.g. morphometric protection index) that were highly correlated ($r \geq |0.7|$) with primary variables (e.g. slope) were removed (Fig. S3, Tables S6, S7). For EAA, we kept only the two variables monthly mean temperature and monthly precipitation sum over the growing season (May–October), which showed a consistent trend (positive or negative) across cohorts when comparing LA with LJ and LJ with HJ, using the *wilcox.test* function from the stats R package.

Detecting putatively adaptive loci

Adaptive genetic variation was investigated with EAA using both continuous and categorical explanatory variables (Table S8). For the first type of analyses (continuous variables), we tested for a linear correlation between either of the two environmental descriptors selected above (monthly mean temperature and monthly precipitation sum over the growing season) and pooled AFs with LFMM 2.0 (Caye, Jumentier, Lepeule, & François, 2019) or pooled read counts with BAYPASS 2.1 (Gautier, 2015). LFMM integrates neutral genetic structure as K latent (random) factors and is combined with rigorous statistics that take into account false-positive associations (François, Martins, Caye, & Schoville, 2016). We imputed missing data with the function *imputePCA* (Josse & Husson, 2016) to generate a complete matrix for assessing the singular value deposition. We ran *lfmm_ridge* with the analytical algorithm from

$K = 1$ to $K = 6$ for each of the two environmental variables and assessed the genomic inflation factor (λ) for each K value (Table S8). Then, based on true AFs without imputed missing data, the z scores were calculated with latent factors using the function *lm*, and *P* values were adjusted based on λ and the χ^2 distribution (François et al., 2016). To take into account false discoveries, we applied the Benjamini-Hochberg algorithm with a false discovery rate (FDR) of 0.05 (Benjamini & Hochberg, 1995). λ differed only slightly among *K*s using unadjusted *P* values. Therefore, based on the number of genetic clusters visualised in the first three principle components (Fig. S1) and the hierarchical clustering tree (Fig. S4), we chose $K = 4$ as the optimal number of latent factors. BAYPASS can also handle data generated from pooled sequencing and evaluates the strength of associations with the log-transformed Bayes Factor (BF). This method has been shown to be robust because it accounts for size and read depth in pools, and because it takes population genetic structure into account using the scaled covariance matrix Ω (Fig. S4; Gautier, 2015). We therefore analysed the read count datasets under the core model that identifies overly differentiated SNPs based on the X^TX genetic differentiation statistics (Günther & Coop, 2013), and subsequently, the auxiliary model that tests for associations between corrected AFs (Ω) and each environmental variable. To identify "significant" associations, we followed Jeffreys' rule (Jeffreys, 1961), where $BF > 10$ is considered significant ("strong evidence"). We performed 10 independent runs (with different initial seeds) under the auxiliary model for each covariable and computed the median of BF through 10 convergent analyses. We visually inspected the congruence between independent runs and the median and calculated the pairwise Pearson correlation coefficient *r* for comparison.

For the second type of analyses (categorical variable, i.e. age cohort or elevation), we applied three different EAA methods. First, we tested the significance of AF differences between LA and LJ and between LJ and HJ cohorts using LFMM and BAYPASS with the auxiliary covariate model, as described above. We coded age and elevation as binary variables, with values –1 (young; low, referring to warm or dry) or +1 (adult; high, referring to cold or wet) for each sample. Further, we investigated adaptive genetic variation with a sign test to track consistent AF differences between LA and LJ cohorts and between LJ and HJ cohorts. For the sign test, we used transformed AFs (corrected for population structure) from the scaled covariance matrix Ω generated with BAYPASS. Only loci with a consistent median AF difference (positive or negative) between the respective cohorts were kept. We then illustrated overlap among the five EAAs and temporal/elevational analyses (Fig. S5) using the

VennDiagram R package (Chen & Boutros, 2011), but used the combination (i.e. union) of putatively adaptive SNPs for further analyses. Conversely, we generated a putatively neutral dataset comprising those SNPs that were not detected as putatively adaptive in the analyses described above. We annotated top candidate genes associated with temperature and precipitation based on the *P. cembra* transcriptome (Rellstab et al., 2019) and inspected their gene ontology (GO) terms in view of local adaptation to abiotic variables (Tables S9–S10).

Observed allele frequency shift over time

We visualised the absolute difference in AFs in putatively neutral and adaptive loci between old and young cohorts in each site and their relationship with time (age of LA minus age of LJ or age of HJ). The LA–LJ comparison reflects a pure temporal effect within a given location (Figs. 1d, 2), while LA–HJ combines a temporal and spatial (elevational) effect on the AF shift (Figs. 1d, S6, S7). We expressed the AF shift as the variation per year (ΔAF / year; Tables S11–S13) or generation (ΔAF / generation, where generation time corresponds to 40 years) and inferred linear regressions (ΔAF ~ Δage) for the putatively neutral loci and for the temperature- and precipitation-associated loci (LA + LJ dataset; Table S3).

Simulated allele frequency shift in response to climate change

We used forward-in-time, stochastic, individual-based simulations with Nemo-age (Cotto, Schmid, & Guillaume, 2020) to model the neutral and adaptive evolutionary dynamics of *P. cembra* for three selected populations with a computationally implementable population size (EN, EC, and WN) since early postglacial colonisation (i.e. from ~12,000 years ago to present day; Vescovi et al., 2007). Using this theoretical framework, we intended to demonstrate conceptually that the observed AF responses are realistic and reproducible. We accounted for the specific life history of *P. cembra* and density-dependent processes using key demographic properties similar to natural populations, in particular generation time and number of adult trees (Fig. S8). Populations were modelled at neutral and adaptive loci. Seedlings were subjected to selection by two abiotic environmental factors, temperature (T) and precipitation (P), which changed over time based on empirical data (see above for climate reconstruction). Environmental change over time triggered quantitative trait evolution, and ultimately fed back on population density (i.e. hard selection). At the end of the simulations, genotypes of the entire populations were stored and the AF shift was analysed similar to the empirical (observed) approach. In total, we simulated all 18 combinations of 3 selection intensities, 2 quantitative trait architectures (levels of redundancy), and 3 population-specific climate scenarios for

12,223 years (see below), with 10 replicates per combination. Each year, the following life-cycle events took place in the order listed: density regulation (removal of seedlings and juvenile trees depending on adult tree number), stage transitions (according to the matrix population model), mating (sexual reproduction of hermaphroditic adult trees), and viability selection (on seedlings).

The life history was simulated as comprising three stages (Fig. S8), with seedlings (n_1, one year old), non-reproducing juveniles (n_2, 2–41 years old), and sexually reproducing adults (n_3, older than 41 years; Tomback, Holtmeier, Mattes, Carsey, & Powell, 1993; Zong et al., 2010). Stage transitions were based solely on the individuals' ages. Yearly survival rates were estimated from other *Pinus* life histories provided on COMPADRE (Salguero-Gómez et al., 2015), and adjusted for computational efficiency. To do so, we used increased seedling survival ($\sigma_1 = 1$) and reduced fecundity ($\varphi = 50$) to reach a reasonable number of surviving individuals within the range of values provided by COMPADRE (e.g. 3–1700 for fecundity). We also chose values for the survival rates (in the absence of competition and selection) for juveniles ($\sigma_2 = 0.9$) and for adult trees ($\sigma_3 = 0.99$) within the range of COMPADRE values, while keeping $\sigma_2 < \sigma_3$, as in natural tree populations. This adjusted life cycle led us to consider only those seeds which survived predation and diseases, eventually germinated, and had a realistic chance of reaching adulthood, while avoiding to create seeds that died immediately when reaching the seedling stage. With this life history conceptualisation, we were able to recover key demographic properties similar to those estimated from tree ring data, i.e. most adult trees were between 100 and 200 years old and only a few trees were older than ~300 years (Table S1). Each population reached a carrying capacity through intra-specific density regulation when seedling and juvenile survival declined with increasing adult number (n_3), following the Ricker function (Ricker, 1954): $c_t = a \times e^{-bn_{3(t)}}$ (5), where a is a constant equal to 1 and b is the competition coefficient. By adjusting the strength of intra-specific competition (b), we could align the total number of adult trees at equilibrium to meet empirical conditions (Table S14). To do so, we used the known density and population size of the Rautialp site (EN; Salzer, 2011), with its uneven cohort structure, and extrapolated the number of non-reproducing juvenile and reproductive adult trees for the two other populations based on aerial photos delineating their occurrence areas (Table S14). We utilised a geographic information system (ArcMap, ESRI, CA, USA) to conduct the spatial analysis of populations.

We simulated each population from post-glacial colonisation to the present day and exposed it to yearly climate conditions (i.e. temperature and precipitation). We started with

burn-in simulations over 12,000 years, with constant average climate conditions, but between-year climatic fluctuations around the mean. Each year, a random value was picked from a normal distribution as follows: $\Delta\theta = N(0, \varepsilon_2)$ (6), which was added to the average climate condition of the respective trait and population. As a result, climatic fluctuations were independently and identically distributed for simplicity. After 12,000 years, we used the yearly temperature and precipitation data (Fig. 1e) and simulated an additional 233 years (1780–2013). Climate data were rescaled for uniform inter-annual variance to ensure meaningful comparisons between traits and populations. While temperature data covered the period 1780–2013, precipitation data were only available for 1801–2013. We therefore generated precipitation data for 1780–1800 using the population-specific mean and variance.

We simulated the evolution of single quantitative traits in response to climate change (z_1 for temperature, and z_2 for precipitation) by applying viability selection on seedlings (n_1) using a Gaussian fitness function as follows: $w_1(z) = e^{\frac{-(z_1-\theta)}{2\times\omega^2}}$ (7). Individual seedling survival ($w_1(z)$) was maximised when a seedlings' trait value (z) was identical to the phenotypic optimum (θ), and declined with increasing distance between z and θ depending on the variance of the fitness function ω^2 (which is inversely related to the strength of selection). We used rescaled temperature and precipitation data as our phenotypic trait optima (θ) such that environmental change over time translated into changes in the phenotypic optima and eventually caused trait evolution. We simulated three scenarios with increasing selection intensity: $\omega^2 = 1$, $\omega^2 = 0.1$, and $\omega^2 = 0.01$ (Table S15). Each quantitative trait (z_1 and z_2) was controlled by 50 unlinked, additive, bi-allelic loci, and individual trait values were obtained by adding up allelic effects across all 50 loci and both homologue copies, without random environmental effects for simplicity. We modelled quantitative trait loci with a mutation rate of $\mu_q = 10^{-7}$ and a house-of-card mutation model such that the same two allelic effect sizes were present at all 50 loci: α_{min} and α_{max} (8). The choice of the allelic effect sizes controlled the maximum range of potential phenotypes, the redundancy of the quantitative trait, and thus AF responses to selection. The difference between the two effect sizes ($\alpha_{max} - \alpha_{min}$) determined the range of possible phenotypes, $z_{div} = z_{max} - z_{min}$ (9), that could be reached when either all small-effect alleles were fixed, $z_{min} = 50 \times 2 \times \alpha_{min}$ (10), or all large-effect alleles, $z_{max} = 50 \times 2 \times \alpha_{max}$ (11). This maximum range of phenotypes (z_{div}) was further indicative of the genetic redundancy (polygenicity) of our quantitative traits, as the number of loci necessary to realise a certain trait change Δz, which fed back on the selection strength per locus (Yeaman, 2015). When redundancy was small, i.e. small allelic effects α_{max}, more pronounced

AF shifts were necessary at each locus to reach a new trait optimum θ because it would then lie closer to z_{max} and less genotypes exist to reach it (see also Láruson, Yeaman, & Lotterhos, 2020). We simulated two scenarios of quantitative trait architectures, a high- and a low-redundancy scenario with z_{div} = 10 and z_{div} = 5, respectively. We initialised each population with trait values very close to the local phenotypic optima, such that populations were locally adapted and did not experience directional selection before anthropogenic climate change took place. To do so, we initialised the frequency of large-effect alleles with random samples from empirical AFs (LA cohort) of the respective population and trait. We also had to adjust allelic effect sizes for each trait, each population, and each genetic architecture separately to reach $z_{ini} = \theta_{ini}$, while maintaining a constant z_{div}.

In addition to adaptive loci, we simulated 100 unlinked, bi-allelic neutral loci with a mutation rate of $\mu_n = 10^{-7}$ to mimic neutral SNPs. Initial AFs were randomly sampled from the empirical, population-specific AFs of LA at presumably neutral loci. As AFs of neutral and adaptive loci in our simulations were nearly stable over time given the large population sizes, we could recover neutral and adaptive AFs at the end of our simulations for comparison with empirical data.

Tendency in observed allele frequencies

From the results of the five EAA approaches (only temporal analysis, LA–LJ), we assembled a unified list of putatively adaptive SNPs and used untransformed AFs to quantify the degree of similarity between HJ and LA or LJ for each temperature- and precipitation-associated locus. We defined T_{HJ}, which is the tendency of a locus in HJ to be similar to LJ (compared to LA) as $T_{HJ} = \frac{2\times\left(AF_{HJ}-\frac{AF_{LA}+AF_{LJ}}{2}\right)}{AF_{LA}-AF_{LJ}}$ (12), where AF is the allele frequency averaged over the cohort. In equation 12 (illustrated in Fig. S12), T_{HJ} is positive when AF_{HJ} is closer to AF_{LJ} than to AF_{LA}, T_{HJ} is negative when AF_{HJ} is closer to AF_{LA} than to AF_{LJ}, and T_{HJ} is zero when AF_{HJ} is exactly between AF_{LA} and AF_{LJ}. Knowing that LA individuals experienced colder and wetter conditions during their establishment period than did LJ, we assumed that the most frequent alleles of the LA cohorts are putatively beneficial under a colder and wetter climate. Conversely, frequent alleles of the LJ cohorts are considered beneficial under warmer and drier conditions. T_{HJ} therefore depicts whether a locus in HJ is rather beneficial under a warmer and drier climate (as in LJ) or beneficial under a colder and wetter climate (as in LA, and similarly expected in HJ under current conditions). A large number of loci with a positive T_{HJ} therefore indicates that these loci were already subject to selection by a warmer and drier climate, similar

to what LJ cohorts currently experience. We present these tendency values across loci on a decimal logarithmic scale to better compare the patterns between temperature- and precipitation-associated loci. Furthermore, we indicate the sum of loci above and below the 95% confident interval from a normal distribution using the *qnorm* R function.

Genomic vulnerability under future climate conditions

To evaluate the genomic vulnerability (i.e. genomic offset) to future climate conditions of juvenile cohorts growing within the main elevation range (low elevation, LJ), we calculated the risk of non-adaptedness (RONA; Rellstab et al., 2016). This value quantifies the theoretical AF shift needed to cope with climate change, using past, present, and future data of monthly mean temperature and precipitation sum during the growing season. To calculate RONA values, a linear relationship between AFs at significantly associated loci and environmental variables from EAA is first established using linear regressions. In a second step, the AFs theoretically needed to cope with future climate conditions are calculated and the difference between present and theoretically needed AFs is determined. We implemented this method in R (customised R script). Unlike in the original publication on RONA (Rellstab et al., 2016), we used all loci that were significant in the EAA to compare with the past AF shifts at temperature- and precipitation-associated loci with the same sample sizes (i.e. number of loci). We used data on all 14 cohorts (LA and LJ) in the linear regressions with the respective climate values during their establishment, thereby accounting for the temporal trends in climate conditions. For each locus, population and climate variable, we calculated RONA for the two greenhouse gas emissions scenarios (RCP4.5 and RCP8.5) and calculated weighted averages (from the R^2 of linear regressions; Pina-Martins et al., 2018) for each population, climate scenario and environmental variable (Fig. S13). Next, we checked for homoscedasticity of the resulting RONA values with a Bartlett's test and transformed the data to account for normality of residuals using the natural logarithm. We performed a two-way analysis of variance (ANOVA) with cohorts and climate scenarios, and their interaction, as independent variables (Table S16) using the stats R package. Climate models were nested within climate scenarios. To put RONA values into perspective with the past realised AF shifts, we time-corrected the historical AF shift (ΔAF_{corr_time}) for each population as:

$$\Delta AF_{corr\_time} = \frac{mean(\Delta AF)}{mean\ (\Delta Age)} \times \left(Ref_{future} - mean\left(Establishment\ time_{juveniles}\right)\right) \quad (13),$$

where ΔAF is the average absolute difference of AFs between LA and LJ, ΔAge is the average difference between the age of juvenile (LJ) and adult trees (LA), Ref_{future} is the median of the

reference period of future scenarios (the year 2070), and *Establishment time*$_{\text{juveniles}}$ is the estimated year of birth of juvenile trees (LJ).

Results

Genetic structure and diversity

From the pooled exome capture sequencing, we obtained 167,438 SNPs (in 14,937 contigs) in 420 individuals from 21 cohorts (i.e. 20 individuals per cohort) of seven populations. Of these, we retained, depending on the dataset, 7,382–10,036 SNPs in 3,069–3,612 contigs (20–24%) after reducing to the single-copy contigs identified by Rellstab et al. (2019) based on heterozygote excess and deviation from allele balance (McKinney et al., 2017), and filtering for missing data (NAs ≤ 3) and minor allele frequency (≥ 2.5%; Tables S2, S3). Average sequencing depth per cohort was 182.2×, with a range of 40 to 4865×. Population genetic structure was consistent with the biogeographic origin of cohorts (Höhn et al., 2009); the two major lineages (east and west) separated along the first axis in the principal component (PC) analysis (PC1 = 14.2%; Fig. S1). Genetic diversity, measured as the proportion of polymorphic loci (PPL, 0.536–0.724) and expected heterozygosity (H_e, 0.079–0.098), was similar across populations and cohorts, and exhibited no reduction for juvenile cohorts (HJ) at high elevation (i.e. colonisation front; Table S4). Pairwise genetic differentiation (F_{ST}) between locations was low (< 0.001–0.099; Table S5) and significantly correlated with geographic distances, indicating isolation by distance (Mantel r = 0.402–0.573, P < 0.05; Fig. S2a–c).

Environmental change over time

We characterised past, present, and future climate conditions at the location of each cohort by applying climatologically aided interpolation downscaling (Anandhi et al., 2011; Willmott & Robeson, 1995). Multivariate analyses showed that habitat characteristics were independent of biogeographic origin (Fig. S3). Next, environmental variables with inconsistent signs across paired cohorts (positive or negative difference in comparisons LA–LJ and HJ–LJ) were excluded, leaving monthly mean temperature and precipitation sum during the growing season (May–October) for subsequent environmental association analysis (EAA). As expected, LJ cohorts experienced warmer and drier conditions than HJ cohorts during the period when they both established, 12–16 years ago (ya), and also compared with LA cohorts during the time when these adult trees established 110–240 ya (Fig. 1f,g; Table S1).

Detecting putatively adaptive loci

In the EAAs using continuous environmental variables, we found 118 and 105 SNPs significantly associated with temperature, and 506 and 362 SNPs with precipitation for the temporal (LA–LJ) and elevational (HJ–LJ) comparisons, respectively (Fig. S5a–d). Additionally, in the EAAs using categorical variables, we found 40 and 38 SNPs significantly associated with age and elevation for the temporal (LA–LJ) and elevational (HJ–LJ) comparisons, respectively (Fig. S5a–d). Finally, we detected 367 SNPs with consistent AF shifts for LA–LJ, and 410 for HJ–LJ, representing additional loci putatively under selection. Using all associated SNPs from these five EAA approaches, we obtained a modest overlap of SNPs associated with the environment between temporal and elevational comparisons (Fig. S5e–f), suggesting distinct selective pressures between temporal (LA–LJ) and spatial (HJ–LJ) environmental comparisons. The 204 SNPs shared between the two comparisons were located in 129 genes, some of them with a link to abiotic factors such as water deprivation, heat stress or light intensity (Tables S9, S10).

Observed allele frequency shift over time

To quantify AF shifts at the local scale, we investigated AF differences between co-occurring adult (LA) and juvenile (LJ) cohorts, which reflects a pure temporal effect, and between LA and HJ cohorts, which captures a temporal and elevational effect in the AF shifts. Combining the two comparisons (LA–LJ and LA–HJ), we determined an average rate of AF shift between age cohorts (AF shift hereafter) of 1.26×10^{-2} per generation (i.e. 40 years, SD = 0.38×10^{-2}) at presumably neutral SNPs (i.e. loci not significantly associated in EAAs; Table S11). For the purely temporal analysis (LA–LJ), we found an average AF shift of about 1.23×10^{-2}/generation (SD = 0.36×10^{-2}) for the 8,687 presumably neutral SNPs (Table S11). The 504 temperature-associated SNPs had a slightly higher average rate of AF shift (0.96×10^{-2}/generation for LA–LJ, SD = 0.44×10^{-2}) than the 884 precipitation-associated loci (0.91×10^{-2}/generation for LA–LJ, SD = 0.35×10^{-2}; Tables S12, S13), but both groups of loci behaved similarly to neutral loci as seen from the similar L-shaped histograms (Figs. 2a–c, S6, S7). Note that the empirical neutral SNP set may still comprise loci under selection, e.g. related to selective—including biotic—drivers unaccounted for in our study. It is such potentially adaptive loci still occurring in the putatively neutral SNP set that may underlie the extended tail in the histogram of the neutral AF shifts, leading to a slightly higher average AF shift in putatively neutral than in associated SNPs. Overall, this pattern conforms to the assumption of polygenic adaptation

(Csilléry, Rodríguez-Verdugo, Rellstab, & Guillaume, 2018) to warmer and drier conditions, with many loci of minor effect involved in the adaptive response.

Simulated allele frequency shift in response to climate change

To demonstrate that our empirical results are realistic and reproducible, we carried out forward-in-time individual-based simulations with Nemo-age to model the neutral and adaptive evolutionary dynamics in three selected populations (EN, EC, and WN) of Swiss stone pine since early postglacial colonisation into our study area (i.e. from ~12,000 years ago to present day; Vescovi et al., 2007). Accounting for the species' life history and estimated current population census sizes (Fig. S8, Table S14), we found AF shifts between paired cohorts (i.e. LA–LJ) that were similar to empirical observations (Fig. 2d–f), especially with a medium selection intensity ($\omega^2 = 0.1$) and a high-redundancy genetic architecture (i.e. a polygenic response; Figs. S9–S11, Table S15). As expected, at temperature- and precipitation-associated loci, simulated AF shifts among paired cohorts slightly increased with stronger selection intensity ($\omega^2 = 0.01$), but its overall effect in combination with genetic architecture was modest (Figs. S10, S11). Specifically, the WN (Kandersteg) population showed the highest rate of AF shift ($\omega^2 = 0.1$, high redundancy) at temperature-associated loci, and the EC (Davos) population exhibited the highest shift ($\omega^2 = 0.01$, high redundancy) for precipitation-associated loci (Figs. S10, S11). The AF shift at neutral loci was uniform across populations (Fig. S9).

Tendency in observed allele frequencies

To observe whether HJ cohorts at the upper colonisation front already experienced selection for alleles beneficial under warmer and drier conditions (i.e. those more frequent in LJ), or/and whether they harbour parental alleles formerly selected for colder and wetter conditions (i.e. those more frequent in LA), we investigated the degree of similarity in AF among age cohorts for each climate-associated locus based on the temporal EAA (LA–LJ; N = 504 and 884 for temperature and precipitation, respectively). We calculated a tendency index T_{HJ} (see Methods and conceptual diagram in Fig. S12), which indicates whether the AF at a given locus in HJ is closer to LJ ($T_{HJ} > 0$; warmer and drier) or to LA ($T_{HJ} < 0$; colder and wetter), and to what extent adaptive signals vary over space and time among cohorts. We found symmetric distributions of T_{HJ} values in HJ, with a small excess of cold-related (Fig. 3a) and dry-related alleles (Fig. 3b) for temperature- and precipitation-associated loci, respectively. In other words, we found alleles in HJ that are beneficial under the current colder and wetter climate

524 characteristic of high elevations, as well as alleles that are beneficial under the warmer and
525 drier climate expected under future climate change.

526 **Genomic vulnerability under future climate conditions**

527 To evaluate genomic vulnerability of populations to climate change, we quantified the
528 theoretical AF shift needed to cope with projected future scenarios, based on the observed shift
529 in the past. To do so, we assessed the risk of non-adaptedness (RONA; Rellstab et al., 2016) of
530 each LJ cohort for temperature and precipitation under two future greenhouse gas emissions
531 scenarios (2061–2080, RCP4.5 and RCP8.5; Karger et al., 2017). We found significant
532 differences in RONA estimates among populations (cohort comparison: ANOVA $P < 0.01$;
533 Fig. S13, Table S16). In the case of temperature (Figs. 4a, S14), our results indicate substantial
534 genomic vulnerability even for the emissions scenario RCP4.5, which includes modelled
535 mitigation measures to limit temperature increase. Under this scenario, we determined a
536 required AF shift that is at least three-fold higher (mean = 6.29×10^{-2}/generation) than the rate
537 at which populations have shifted their AFs for neutral (2.17×10^{-2}/generation) or putatively
538 temperature-associated loci (1.69×10^{-2}/generation) in the past. As expected, this trend was
539 exacerbated under a scenario without mitigation measures (RCP8.5; mean required AF shift =
540 9.91×10^{-2}/generation). These two scenarios equally affected LJ cohorts throughout our study
541 range (Fig. S14, Table S16). Although precipitation anomalies have increased in the last
542 decades (Scherrer, Begert, Croci-Maspoli, & Appenzeller, 2016), our results only partially
543 captured these events (Fig. 1e–f). In fact, predictions about future precipitation based on the
544 two RCP scenarios were very similar, which is the likely reason why we found only small
545 differences in the respective RONA values. Moreover, RONA values for precipitation-
546 associated loci were substantially lower than those projected from temperature-induced
547 responses (Figs. 4b, S14), being at most twice as high as the realised AF shift of the past (mean
548 required AF shift = 3.36×10^{-2}/generation for RCP4.5 and 3.58×10^{-2}/generation for RCP8.5).

549 ## Discussion

550 **Allele frequency shifts over time**

551 Adaptation to environmental change can occur rapidly within natural populations (Shaw &
552 Etterson, 2012), but this has to be put into perspective with species' generation times (Aitken
553 et al., 2008; Jump, Hunt, Martínez-Izquierdo, & Peñuelas, 2006). Long-lived organisms with
554 long generation times are often subject to an adaptational lag to current (Browne, Wright, Fitz-
555 Gibbon, Gugger, & Sork, 2019) or future climate conditions (Wilczek, Cooper, Korves, &

Schmitt, 2014). By combining dendrochronological measurements, climate modelling and extensive exome sequencing, our study is, to our knowledge, the first to empirically quantify shifts of allele frequency (AF) at neutral and adaptive loci in a long-lived species. We report these generational shifts in Swiss stone pine, a keystone species of the treeline ecotone, and show that AF shifts at putatively adaptive loci are small, i.e. in the same range as at presumably neutral loci, which are not significantly associated in environmental association analysis (EAA; Fig. 2a–c, Tables S11–S13). Consistent with studies of other tree species, climate adaptation appears to be a genome-wide process, largely involving subtle shifts in AFs of many genes (Hornoy, Pavy, Gérardi, Beaulieu, & Bousquet, 2015; Lind et al., 2017). Importantly, our findings are corroborated by forward-in-time simulations that conform to the presumably polygenic nature of climate adaptation (Csilléry et al., 2018), with small effects in AFs at a relatively large number of loci (Fig. 2d–f). This also suggests possible redundancy in the adaptive response to climate change, whereby several genes or pathways may contribute to eco-physiological responses (Yeaman, 2015). Our annotation of candidate genes significantly associated with temperature and precipitation reveals a large variety of biological functions involved in responses to biotic and abiotic stressors, as well as in mechanisms for regulating gene expression (e.g. resistance to disease and heat stress; Tables S9, S10). This finding highlights the importance of climate-induced stresses for juvenile trees at low and high elevations and corroborates the physiological responses (e.g. in growth and establishment) previously observed under climate change in natural stands (Vittoz et al., 2008). Looking at population-specific patterns of simulated data, the strength of selection at medium intensity ($\omega^2 = 0.1$) led to the highest AF shifts between age cohorts at precipitation-associated loci, while differences in temperature-associated loci were mostly influenced by the genetic architecture (i.e. EN and WN populations; Figs. S7, S9–S11).

Genomic vulnerability under future climate change

Understanding the genetic basis of adaptation to climate change remains a major task (Franks & Hoffmann, 2012), and evaluating genomic vulnerability can help determine the possible fate of natural populations under scenarios of future climate change (Fitzpatrick & Keller, 2015). Our estimates of the risk of non-adaptedness (RONA) for temperature and precipitation show that the rates of AF shift theoretically required to cope with predicted future conditions are substantially higher than those that have been realised in the past. Even under a moderate future climate scenario including mitigation measures (RCP4.5), the estimated mean AF shift required for the temperature-associated loci is at least three-fold higher than the rate at which

589 populations have shifted their AFs in the past (Fig. 4a). Although the AF shift was not
590 specifically related to time, and while climate zones are difficult to compare, previous studies
591 looking at a predictive time-span of 95–140 years estimated similar RONA values for a
592 projected composite climate measure (0.05–0.48 for *Betula nana*; Borrell, Zohren, Nichols, &
593 Buggs, 2020) or for a projected mean temperature (0.10–0.30 for *Eucalyptus microcarpa*
594 [RCP8.5; Jordan, Hoffmann, Dillon, & Prober, 2017], 0.09–0.30 for *Quercus* spp. [scenario
595 A1B; Rellstab et al., 2016], and 0.07–0.38 for *Quercus suber* [RCP8.5; Pina-Martins et al.,
596 2018]). However, the age at which these species start to reproduce varies substantially, and the
597 mismatch between generation time and pace of climate change has strong consequences on the
598 coincidence of a species' realised versus fundamental ecological niche (Rumpf et al., 2019).
599 Note that the polygenic nature of adaption is only partly captured in this study, and beneficial
600 alleles with small effect sizes may temper RONA estimates, as they are largely unaccounted
601 for in these calculations.

602 According to future climate scenarios (Stocker et al., 2013), models predict a
603 temperature increase of about 1.5–3.5°C in the European Alps by the end of the century. Given
604 an average temperature lapse rate of 0.6°C/100 m (i.e. moist adiabatic) of elevational increase,
605 and assuming that Swiss stone pine cannot adapt through AF shifts alone, this situation requires
606 a considerable acceleration of upward movement for the species, in the range of what has been
607 reported in other shorter-lived alpine species (Pauli et al., 2012; Steinbauer et al., 2018).
608 Overall, our findings suggest that species with long generation times may have difficulty
609 keeping up with the rapidly changing climate, due to their low rates of AF shift, even though
610 they exhibit high levels of standing genetic variation (Savolainen, Lascoux, & Merilä, 2013).
611 Combined with limited migration potential, as in the bird-dispersed Swiss stone pine
612 (Neuschulz et al., 2018), such genomic vulnerability implies a high risk of local extinction in
613 its current main elevation range.

614 **Adaptive alleles at the species' colonisation front**

615 A way for species to cope with environmental change is to migrate to new areas with more
616 suitable habitats under the changed environmental conditions. As recently reported, terrestrial
617 species showing migration along elevational gradients are too slow to follow the pace of
618 isotherm changes, especially in regions with warm climates (Lenoir et al., 2020). In addition,
619 uphill migration in mountain ecosystems is particularly limited by heterogeneous topography,
620 shallow soil for seed germination and root development, and the negative effect of competition
621 in a reduced area (Lingua et al., 2008). Phenotypic plasticity plays a prominent role in the

evolutionary response, especially in long-lived organisms, but can also promote the persistence of old individuals that are maladapted to current environmental conditions (Maherali, Caruso, Sherrard, & Latta, 2010; Oostra, Saastamoinen, Zwaan, & Wheat, 2018). Many long-lived organisms are sessile and require a dispersal vector (e.g. birds, insects, water, and wind) for migration. Although the Eurasian nutcracker as the natural vector of Swiss stone pine mostly disperses seeds locally (Neuschulz et al., 2018), juvenile trees—with low density at the colonisation front—show similar levels of overall (i.e. neutral) genetic diversity (Dauphin et al., 2020) as the adult and juvenile cohorts in the main elevation range (Table S4). This was to be expected, as juvenile trees at high elevation most likely reflect the first generation of adult progenitors at lower elevations, and thus have not yet undergone major long-term demographic processes (e.g. genetic drift) that would reduce overall genetic diversity (Elleouet & Aitken, 2019). However, as a result of selection imposed by the changing climate, alleles beneficial under warmer and drier conditions compared with those the progenitors of high-elevation juveniles experienced during their establishment are already found uphill at the colonisation front (Fig. 3). This illustrates the spatio-temporal interplay between migration and adaptation at the local scale and shows that both evolutionary forces are relevant for species with long generation times in the context of climate change. In this study, we put into perspective the evolutionary potential of newly selected trees at the colonisation front to overcome, at the species level, the ongoing rapid warming. We highlight the substantial genomic vulnerability of juvenile cohorts occurring in the current main elevation range, implying a high risk of local extinction under projected future conditions.

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Author contributions

FE.G. acquired funding. B.D., C.R., and FE.G. designed the conceptual approach. C.R., FE.G., M.S. and S.B. carried out the field work, and S.B. performed the dendrochronological and molecular laboratory work. S.Z. performed the bioinformatic analyses. B.D. and D.N.K. generated and analysed topographic and climate data. B.D. and C.R. analysed the genomic data. FR.G and M.S. carried out simulations. B.D. wrote the manuscript, with major contributions from C.R., FE.G and M.S. All authors read and approved the final version of the manuscript.

Data sharing and data accessibility

Raw sequence data used in this study will be made available in the European Nucleotide Archive (ENA) upon acceptance [XXX]. Allele frequency and environmental datasets used will be uploaded to the Dryad Digital Repository upon acceptance [XXX], together with the R codes applied for their analyses. Climate data used in this study are available in the CHELSA data archive (chelsa-climate.org) and the EnviDat Repository (envidat.ch).

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Figure legends

Figure 1 Geographic and climatic characterisation of locations of cohorts sampled to investigate adaptive genetic variation in the alpine tree species *Pinus cembra*. a, Distribution range of *P. cembra* in the European Alps and Carpathian Mountains (grey area; Caudullo, Welk, & San-Miguel-Ayanz, 2017) and study sites (squares) within its Swiss range (rectangle). b, Typical habitat of *P. cembra* along the Aletsch glacier (population WZ, western Switzerland). Photo credit: Felix Gugerli. c, Locations of sampling sites (squares) of *P. cembra* within its native Swiss range (solid black lines denote northern and southern range limits). The digital elevation model originates from SwissALTi3D (www.geoadmin.ch). The dashed line represents the contact zone of the two main genetic lineages of *P. cembra* found in the Swiss Alps (Fig. S1; Höhn et al., 2009). d, Sampling design within locations, considering elevational and temporal contrasts and their combination; HJ = high-elevation juvenile cohort, LJ = low-elevation juvenile cohort, and LA = low-elevation adult cohort. Ages refer to estimates averaged over sampled trees (Table S1). e, Time series of temperature and precipitation data at locations of low-elevation cohorts, with the interpolated tendency over two centuries, including predictions for the future (greenhouse gas emissions scenarios with [RCP4.5] and without [RCP8.5] anthropogenic mitigation; Karger et al., 2017). f–g, Comparison of monthly mean temperature and monthly precipitation sum during the growing season (May–October; log-transformed values for precipitation); f, between low-elevation adult (LA) and juvenile (LJ) cohorts, and g, between juvenile cohorts from low (LJ) and high elevations (HJ), depicted with different colours and symbols. Temperature and precipitation data in f and g represent conditions during the respective establishment periods of adult and juvenile trees.

Figure 2 Observed and simulated allele frequency shifts over time for neutral and putatively adaptive loci in *Pinus cembra*. Histograms of absolute values and averages (inset) of observed allele frequency shifts in all paired cohorts at low elevation (LA–LJ) for presumably a, neutral loci (N = 8,687), b, temperature-associated loci (504), and c, precipitation-associated loci (884). *P* values represent the significance for linear regressions between mean cohort age and mean allele frequency shift per year (i.e. corrected for age). Absolute values of allele frequency shifts at low elevation (LA–LJ) are simulated for three selected populations, Davos (EC), Rautialp (EN), and Kandersteg (WN), based on medium selection intensity ($\omega^2 = 0.1$) and a high redundancy of genetic architecture. Histograms of the three merged populations represent d, neutral loci (N = 6,000), e, temperature-associated loci (1,500), and f, precipitation-

928 associated loci (1,500). Allele frequency shifts refer to the absolute difference in allele
929 frequency between age cohorts.

930 **Figure 3** Allele frequency tendencies for putatively adaptive loci in high-elevation juvenile
931 cohorts. Tendency of allele frequencies in high-elevation juvenile cohorts (HJ), a, at
932 temperature-associated loci (N = 504) and b, at precipitation-associated loci (884) compared
933 with low-elevation adult cohorts (LA; negative values) and low-elevation juvenile cohorts (LJ;
934 positive values). Associated loci are based on the temporal EAA only (Fig. S5a,c). Details on
935 the calculation of the tendency index (T_{HJ}) are given in Fig. S12. Numbers indicate the sum of
936 loci above and below the 95% confidence interval (dashed lines), and light and dark colours
937 refer to the values within and outside the confidence interval, respectively.

938 **Figure 4** Risk of non-adaptedness (RONA) of *Pinus cembra* in low-elevation juvenile cohorts.
939 RONA represents the theoretically required allele frequency shift to match future climate
940 conditions. Shown are average RONA values across putatively adaptive loci for the low-
941 elevation juvenile (LJ) cohorts under two different climate scenarios (RCP4.5 and RCP8.5;
942 during the growing season May–October). RONA of each cohort to a, future mean temperature
943 based on 504 associated loci and b, future precipitation sum based on 884 associated loci. Error
944 bars represent the standard error of the mean (SE). Horizontal lines represent the past realised
945 allele frequency shifts (time-corrected) for neutral (dashed grey) and adaptive loci (dashed-
946 dotted black).