Dendrochronologia 38 (2016) 26–37

Contents lists available at ScienceDirect

Dendrochronologia

journal homepage: www.elsevier.com/locate/dendro

Original article

The effect of individual genetic heterozygosity on general homeostasis, heterosis and resilience in Siberian larch (*Larix sibirica* Ledeb.) using dendrochronology and microsatellite loci genotyping

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ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form 19 January 2016
Accepted 25 February 2016
Available online 3 March 2016

Keywords:
Dendrochronology
Tree ring width
Radial growth
Individual heterozygosity
Microsatellite markers
Heterosis
Homeostasis
Climate change
Environmental stress

ABSTRACT

The genetic mechanisms underlying the relationship of individual heterozygosity (IndHet) with heterosis and homeostasis are not fully understood. Such an understanding, however, would have enormous value as it could be used to identify trees better adapted to environmental stress. Dendrochronology data, in particular the individual average radial increment growth of wood measured as the average tree ring width (AvTRW) and the variance of tree ring width (VarTRW) were used as proxies for heterosis (growth rate measured as AvTRW) and homeostasis (stability of the radial growth of individual trees measured as VarTRW), respectively. These traits were then used to test the hypothesis that IndHet can be used to predict heterosis and homeostasis of individual trees. Wood core and needle samples were collected from 100 trees of Siberian larch (*Larix sibirica* Ledeb.) across two populations located in Eastern Siberia. DNA samples were obtained from the needles of each individual tree and genotyped for eight highly polymorphic microsatellite loci. Then mean IndHet calculated based on the genotypes of eight loci for each tree was correlated with the statistical characteristics of the measured radial growth (AvTRW and VarTRW) and the individual standardized chronologies. The analysis did not reveal significant relationships between the studied parameters. In order to account for the strong dependence of the radial growth on tree age the age curves were examined. An original approach was employed to sort trees into groups based on the distance between these age curves. No relationship was found between these groups and the groups formed based on heterozygosity. However, further work with more genetic markers and increased sample sizes is needed to test this novel approach for estimating heterosis and homeostasis.

1. Introduction

The concept of individual homeostasis in a heterogeneous environment as indicated by the low impact of environmental factors (temperature, precipitation, etc.) on individual development was first introduced by Walter Cannon (1929). It was further developed into the concept of developmental homeostasis (Dobzhansky and Wallace, 1953), genetic homeostasis (Lerner, 1954), developmental stability (Mather, 1953; Thoday, 1955) and phenotypic stability (Lewis, 1954). The concept was based on the observation that individuals with higher individual heterozygosity (IndHet) were characterized by a more stable growth pattern and less impacted by environmental factors, such as, for instance, temperature and precipitation (see Livshits and Kobyliansky, 1985 for early review). The concept was revisited and reevaluated multiple times, but still needs additional studies and experimental data to improve our understanding of the molecular basis and genetic mechanisms underlying individual homeostasis and heterosis (see for more recent review Woolf and Markow, 2003; Hochholdinger and

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http://dx.doi.org/10.1016/j.dendro.2016.02.005
1125-7865/

Hoecker, 2007; Fridman, 2015; Lippman and Zamir, 2007; Nicoglou, 2015; Peirson, 2015).

Stable growth pattern and the problem of individual response to environmental stress should receive special attention in light of global climate change. Long-term changes in climates as well as short-term fluctuations in weather are of special concern for long-lived, sessile plant species such as forest trees, because unlike freely moving organisms, such as most animals and some plants they cannot purposefully search for a favorable habitat and move to it, and have to withstand environmental stresses during their lifetime as long as, for instance, 300–400 years on average and up to maximum 750 years for Siberian larch (*Larix sibirica* Ledeb.) (Vaganov et al., 2006). Conifers, such as pine, larch and spruce, are the keystone species of the boreal forest ecosystems that could be both significantly affected by global climate change and at the same time play a very important role in the mitigation of climate change effects due to their ability to store large amounts of carbon (Kasischke and Stocks, 2000; Soja et al., 2007; Nelson et al., 2008; Chen and Luo, 2015; Gauthier et al., 2015). Conifers have a substantial adaptive capacity at the individual tree level due to the high phenotypic plasticity and at the population level due to the high genetic variation (Hamrick, 2004; Santos-del-Blanco et al., 2013). However, genetic mechanisms of this high adaptability at both individual and population levels are still not fully understood. Siberian larch was selected for study here as it is one of the major boreal tree species in Eurasia (Kobak et al., 1996; Abaimov, 2010; Shuman et al., 2011).

We consider two main hypotheses for the genetic mechanisms that may explain why individuals with higher IndHet could be less impacted by environmental factors and demonstrate higher heterosis: (1) overdominance (see review by Hansson and Westerberg (2002)), and (2) dominance, because highly heterozygous individuals by definition have lower levels of inbreeding and less inbreeding depression (see, e.g., David, 1999; Reed et al., 2012; Gonzalez-Varo et al., 2012; Abrahamsson et al., 2013). Both these genetic mechanisms could be responsible for the stable growth of individual trees with higher IndHet and their resistance to fluctuations in the environment, i.e., homeostasis can be associated with heterosis due to either the higher fitness of heterozygotes because of dominance (when the detrimental or less favorable recessive alleles that weaken the individual adaptability in homozygotes are masked and do not affect the individual fitness in heterozygotes) and/or overdominance (when heterozygotes have higher fitness than any of homozygotes). Either case would lead to the natural selection of trees with higher IndHet, and one can expect that trees that are more resistant to (and more independent from) the environmental stress would have both a more stable development and a higher IndHet. Maladaptive seedlings and trees would occur in the population, however, as a genetic segregation load that could be a heavy price that a population would need to pay to maintain a high level of heterozygosity (Altukhov, 1991). Therefore, we expect also that there is an optimal level of IndHet. Exceeding this optimal level may lead to an increase of the segregation load and thus IndHet can be regulated by selection making extremely heterozygous trees less adaptive and less stable.

In addition, several variants of certain multimeric enzymes can be formed in heterozygotes, which acting together may be more efficient than the single form of the enzyme found in homozygotes (Berger, 1976). In this case, heterosis and homeostasis can be due to overdominance of heterozygotes. More heterozygous individuals are better adapted according to the theory of balancing selection in favor of heterozygotes. The mechanisms of heterosis and homeostasis are poorly understood, however, and available data are very contradictory.

Both heterosis and homeostasis have been studied in different organisms, including tree species and using different traits and genetic markers, such as allozymes (e.g., Ledig et al., 1983; Mitton and Grant, 1984; Strauss, 1986; Bush et al., 1987; Strauss and Libby, 1987; Zouros et al., 1988; Jelinski, 1993; Gonzalez-Varo et al., 2012), microsatellites or so-called simple sequence repeats—SSRs (e.g., Abrahamsson et al., 2013; Zgaga et al., 2013), as well as single nucleotide polymorphisms – SNPs (e.g., Govindaraju et al., 2009; Chelo and Teotonio, 2013). Correlation of IndHet with various physiological, morphological and biochemical traits of heterosis and homeostasis (stable development) was estimated in these studies. Traits used included bilateral asymmetry (see Livshits and Kobyliansky, 1991; Parsons, 1992; Leung et al., 2000 for early reviews and more recent Kurbalija et al., 2011; Weisensee, 2013), growth rate (Ledig et al., 1983; Mitton and Grant, 1984; Strauss, 1986; Bush et al., 1987; Strauss and Libby, 1987; Zouros et al., 1988; Jelinski, 1993), and skeletal meristic traits (Zink et al., 1985).

The main objective of our study was to examine relationships between the level of heterosis and homeostasis measured using dendrochronology traits, such as the average tree ring width (AvTRW) and the variance of tree ring width (VarTRW), and IndHet measured with genome wide genetic markers, such as microsatellite loci (SSRs). In this initial study we used random (and, therefore, likely intergenic) genomic SSRs that are supposedly selectively neutral genetic markers. Microsatellite loci were chosen because they are highly informative and relatively inexpensive for measuring genome-wide individual heterozygosity (but see Väli et al., 2008). They have high mutation rate, high levels of polymorphism, relatively uniform distribution across the genome, broad representation, and are relatively simple to detect and to genotype (e.g., Schlötterer, 2000).

In our study we used a novel approach to address homeostasis from perspectives of two disciplines —dendrochronology and population genomics (Gonzalez-Martinez et al., 2006; Krutovsky and Neale, 2005; Krutovsky, 2006). This approach allows us to more effectively study the adaptability of natural populations to global climate change (King et al., 2013), and how genetic variation may be affected (Pauls et al., 2013). For the first time here we propose to use tree ring data to estimate stability and homeostasis. The AvTRW and VarTRW parameters are particularly useful because they likely correlate with very important environmental and climatic factors such as precipitation, temperature, and length of growth period (Vaganov et al., 1996, 1999, 2006).

The main task in our study was to test the hypothesis that IndHet is associated with AvTRW and VarTRW. In the early genetic studies some evidence was obtained suggesting that IndHet is positively associated with heterosis—a higher viability and stronger adaptive traits were observed in hybrids obtained from crossing parents that were genetically different and distant from each other. It was expressed as higher resistance to environment change or stress, increased growth rate and biomass growth, etc. (Schnable and Swanson-Wagner, 2009; Schnable and Springer, 2013; Feng et al., 2015).

If more heterozygous trees are characterized by a more stable homeostasis, then their development should be less dependent on the environment. Therefore our expectation was to find a negative correlation between IndHet and VarTRW. If AvTRW can be considered as an adaptive trait, then one can expect a positive correlation between IndHet and AvTRW due to heterosis.

There may, however, be an optimal level of IndHet resulting in nonlinear relationships between IndHet with AvTRW and VarTRW. High IndHet can lead to an increased the segregation load in the population and cause an imbalance in the individual development. On the other hand, low IndHet may result from inbreeding, in which frequency of homozygotes for unfavorable recessive alleles increase. This in turn could adversely affect AvTRW, causing a negative correlation of the level of homozygosity with AvTRW and also disrupt homeostasis. The latter would be manifested as a positive correlation between the level of homozygosity and VarTRW.

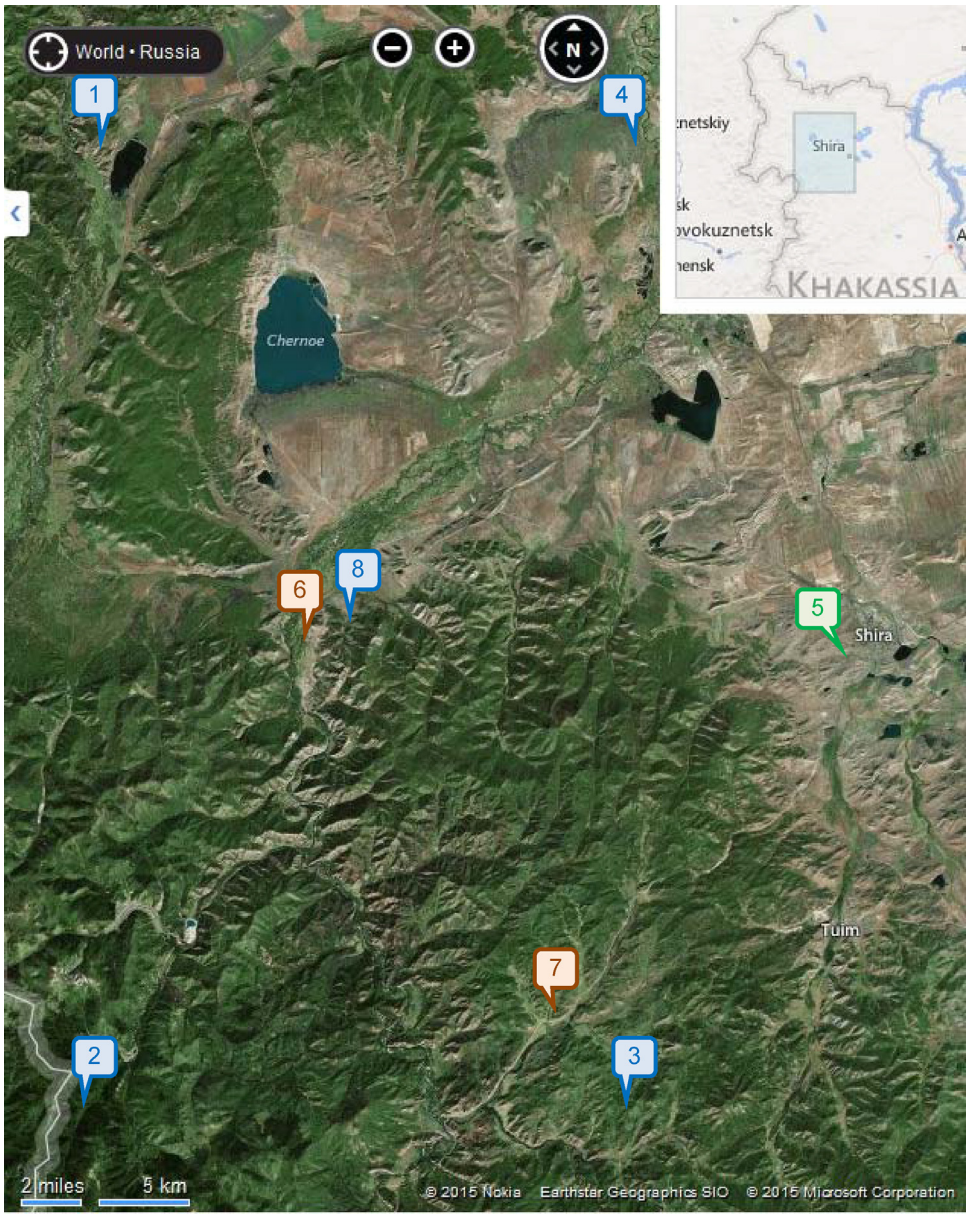

Fig. 1. Map of the study area. Numbers1–4 and 8 indicate grid points for climatic data CRU, 5—the middle of the square grid for meteorological station "Shira", 6 and 7—dendrochronological polygons for populations Efremkino (EFR) and Berenzhak (BER), respectively.

Table 1
Correlations between various climatic data series for the time interval 1966–2012.

Sites	Temperature					Precipitation					PDSI				
	Fall	Winter	Spring	Summer	Year	Fall	Winter	Spring	Summer	Year	Fall	Winter	Spring	Summer	Year
1–2	0.996	0.998	0.998	0.991	0.998	0.967	0.968	0.950	0.928	0.954	0.853	0.823	0.891	0.924	0.908
1–3	0.993	0.996	0.998	0.990	0.996	0.886	0.843	0.843	0.834	0.896	0.781	0.688	0.783	0.880	0.815
1–4	0.999	0.999	0.999	0.998	0.999	0.961	0.930	0.948	0.945	0.965	0.888	0.824	0.923	0.937	0.915
2–3	0.999	0.999	0.999	0.998	0.999	0.954	0.932	0.949	0.954	0.966	0.818	0.776	0.877	0.921	0.896
2–4	0.997	0.999	0.997	0.992	0.998	0.963	0.958	0.924	0.938	0.949	0.815	0.758	0.916	0.917	0.898
3–4	0.996	0.998	0.998	0.993	0.998	0.947	0.968	0.893	0.942	0.953	0.918	0.865	0.914	0.938	0.920
1-Mean	0.998	0.999	0.999	0.997	0.999	0.973	0.960	0.965	0.951	0.972	0.936	0.908	0.943	0.964	0.948
2-Mean	0.999	1.000	0.999	0.998	0.999	0.992	0.990	0.988	0.984	0.987	0.925	0.913	0.963	0.970	0.965
3-Mean	0.998	0.999	0.999	0.998	0.999	0.967	0.960	0.952	0.963	0.975	0.936	0.908	0.934	0.965	0.947
4-Mean	0.999	1.000	0.999	0.998	1.000	0.987	0.989	0.969	0.984	0.986	0.964	0.941	0.982	0.978	0.974
1-Shira	0.965	0.974	0.964	0.901	0.977	0.328	0.287	0.276	0.372	0.362					
2-Shira	0.967	0.970	0.965	0.914	0.975	0.359	0.403	0.339	0.514	0.420					
3-Shira	0.967	0.971	0.968	0.920	0.977	0.372	0.610	0.441	0.611	0.479					
4-Shira	0.967	0.975	0.967	0.911	0.979	0.417	0.507	0.433	0.516	0.452					
Mean-Shira	0.968	0.973	0.967	0.914	0.977	0.376	0.460	0.381	0.523	0.439					

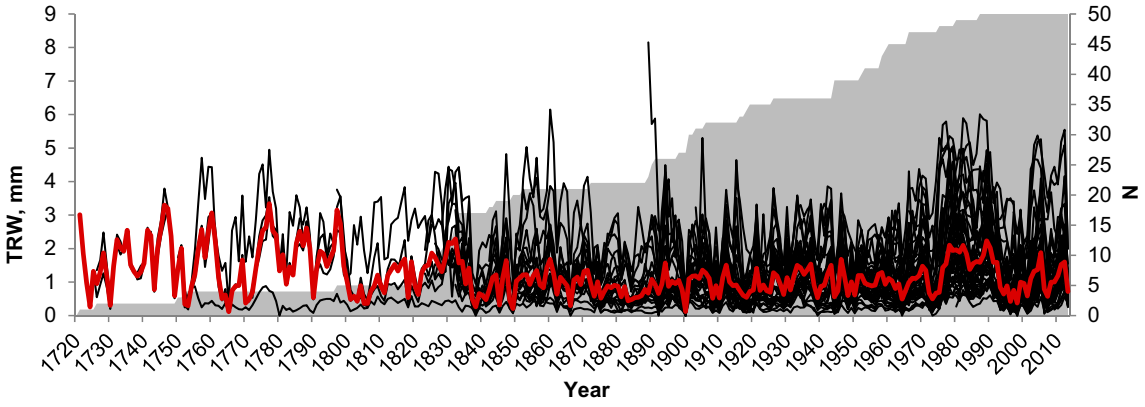

Fig. 2. Tree ring width (TRW) of the individual trees and the local measured chronology (red line) in the BER population along the years measured. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

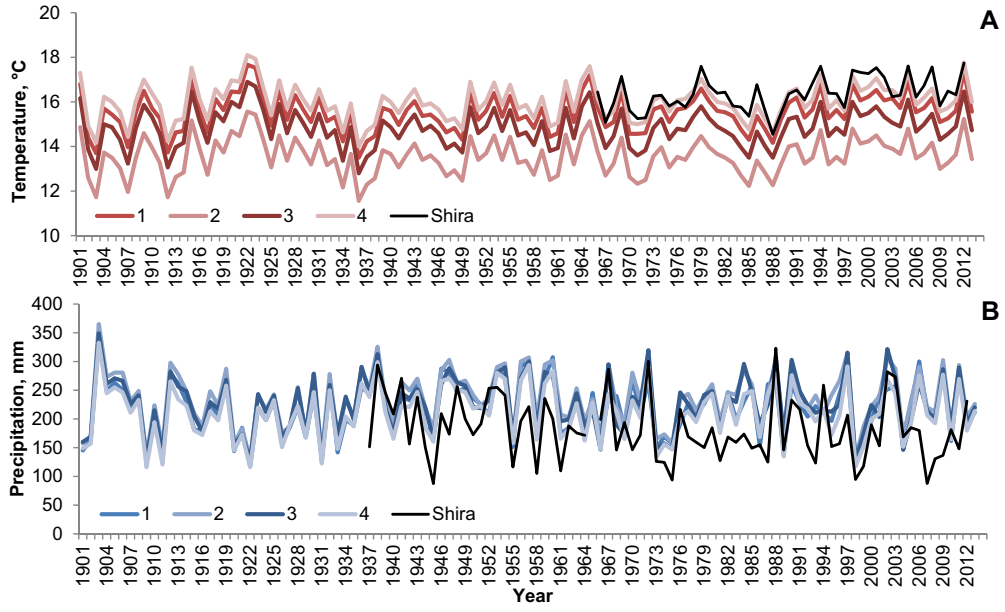

Fig. 3. Summer temperature and precipitation in the study area based on data from different sources (see Section 2).

Table 2
Microsatellite loci genotyped in Siberian larch in this study.

Locus	Motif	Annealing T (°C)	Number of alleles[a]	Fragment size, bp	References
bcLK056	$(AG)_{20}$	Touchdown	12/10	140–200	Isoda
bcLK066	$(TG)_{12}$	63–53 °C	5/4	140–172	and
bcLK224	$(AG)_{17}$		9/4	130–168	Watanabe
bcLK260	$(TG)_{14}(AG)_9$		5/5	80–126	(2006)
bcLK232	$(AG)_{19}$		10/4	135–178	
bcLK235	$(TC)_9(AC)_2AG(AC)_{14}$	58 °C	9/15	168–220	
UBCLXtet-1-22	$(TATC)_9(TA)_{12}$		8/3	175–250	Chen et al. (2009)
UAKLly6	$(GT)_{17}$		13/9	212–264	Khasa et al. (2000, 2006)

[a] First number is a number of microsatellite alleles published earlier; second one is a number of alleles discovered in this study.

Dendrochronological and genetic data were collected for the same individual trees to assess AvTRW, VarTRW, and IndHet and to test these hypotheses.

2. Materials and methods

2.1. Plant material

Wood cores of Siberian larch were collected in July 2014, from the following two populations in the Shira region of Khakasia: (1) the predominantly larch forest mixed with pine and some birch trees on a gentle southeastern slope (2–5°, 600–700 m a.s.l.) near the Shira-Berenzhak highway (this population is denoted as "BER"; Fig. 1); and (2) the larch light forest on a steep western slope (up to 30°, 600–800 m a.s.l.) from the top to the base of the hill in the vicinity of the Efremkino village (this population is denoted as "EFR"; Fig. 1). The distance between the BER and EFR populations is approximately 25 km. Fifty trees of approximately similar age were randomly sampled in each population according to the dendrochronological principles (standing apart mature trees with

minimal nonclimatic impacts) (Cook and Kairiukstis, 1990), taking also into account availability of live branches to collect needles for DNA isolation. Two wood cores were taken from each tree to measure tree rings. Needles were also collected from the same trees for DNA isolation and genotyping.

2.2. Tree-ring width data processing

Initial extraction of wood cores and measurement of the tree-ring width (TRW) were performed using standard procedures (Cook and Kairiukstis, 1990). A semi-automated device LINTAB-5 and a specialized program TSAP Win were employed (Rinntech, 2011). Cross-dating of the original series was performed using the COFECHA program (Holmes, 1999). About five cores from each population were partially broken because the larch wood in the study area was particularly brittle. Consequently, the time series obtained from these cores were missing from two to three rings. For further work the estimates for these cores were adjusted using the ARSTAN program (Cook and Krusic, 2005). This was accomplished by constructing a 20-year spline, on which the TRW fluctuations observed on the duplicate core from the same tree were superimposed. The mean time series for each tree were obtained by averaging measured TRW values for duplicate cores (Fig. 2).

Most cores did not pass through the pith due to the frequently observed offset of the pith from the geometric center of the tree cross-section and the sampling imperfection. The pith was also damaged in 2–3 trees per population. We estimated the number of missing innermost rings (pith offset, PO) using the radius of curvature and the width of the innermost available rings, while taking into account the cross-dating results for duplicate cores from the same tree (Duncan, 1989; Esper et al., 2008). Using the ARSTAN program we plotted the age trend curves for each tree using the following two approaches: (1) spline having the length equal to 67% of the length of the series and (2) an exponential function or in the case of this resulting in negative values on the exponential curve, a linear function.

The calculation of the distance between the age curves $A(t)$ was carried out for the age interval 6–127 years (using the median *Me* of the parameter *PO* and the cambial age *T* of the trees measured in the year 2014). The distances Δ_{ij} were calculated for each pair of *i* and *j* tree using the formula:

$$\Delta_{ij} = \frac{1}{t_2 - t_1 + 1} \sum_{t=t_1}^{t_2} |A_i(t) - A_j(t)| \quad , (1)$$

where $t_1 = \max\left(PO_i, PO_j, Me(PO)\right)$ and $t_2 = \min\left(T_i, T_j, Me(T)\right)$ are the common borders for the considered trees in the certain age interval, taking into account the above restrictions. The resulting Table of the distances was employed to perform hierarchical cluster analysis of the local set of trees. The clustering at each step was performed using the method of complete linkage.

Standardization of the raw tree-ring width data was processed in two steps with ARSTAN. At the first step, age trends described above were removed, thus standard (*std*) individual series and generalized (averaged) chronologies were obtained. At the second step, we removed autocorrelation of the first order (*ac1*) and obtained residual (*res*) individual series and chronologies.

Statistical characteristics of individual series and chronologies used included mean value (*mean*, that is AvTRW for the raw data), standard deviation (*stdev*, that is VarTRW for the raw data), mean coefficient of sensitivity (*sens*), autocorrelation of the first order (*ac1*), expressed population signal (*eps*), interseries average correlation coefficient (*rbar*), and correlation of individual series with their master chronology (*R*). Significance of differences between different groups of trees was tested using Student's *t*-distribution.

Table 3
Genetic variation of eight microsatellite loci in two Siberian larch populations.

Population[a]	Parameter	bcLK056	bcLK224	bcLK066	bcLK260	bcLK235	UBC-1-22	UAKLly6	bcLK232	Mean ± SE
BER	N_a	10	4	4	5	15	3	9	4	6.8 ± 1.5
	N_e	6.2	2.8	1.4	2.1	8.8	1.2	5.6	1.7	3.7 ± 1.0
	H_o	0.340	0.180	0.260	0.340	0.560	0.040	0.380	0.420	0.315 ± 0.056
	H_e	0.839	0.637	0.270	0.517	0.886	0.185	0.821	0.407	0.570 ± 0.095
	F	0.595	0.717	0.037	0.343	0.368	0.784	0.537	−0.032	0.419 ± 0.106
EFR	N_a	9	3	4	5	9	3	7	3	5.4 ± 0.9
	N_e	5.4	1.8	1.2	1.4	4.3	1.4	4.3	1.2	2.6 ± 0.6
	H_o	0.420	0.200	0.180	0.120	0.440	0.260	0.320	0.140	0.260 ± 0.043
	H_e	0.816	0.455	0.168	0.287	0.768	0.295	0.767	0.165	0.465 ± 0.099
	F	0.486	0.561	−0.073	0.582	0.427	0.120	0.583	0.154	0.355 ± 0.089
Mean ± standard error (SE) over both populations BER & EFR	N_a	9.5 ± 0.5	3.5 ± 0.5	4.0 ± 0.0	5.0 ± 0.0	12.0 ± 3.0	3.0 ± 0.0	8.0 ± 1.0	3.5 ± 0.5	6.1 ± 0.9
	N_e	5.8 ± 0.4	2.3 ± 0.5	1.3 ± 0.1	1.7 ± 0.3	6.5 ± 2.2	1.3 ± 0.1	4.9 ± 0.7	1.4 ± 0.2	3.2 ± 0.6
	H_o	0.380 ± 0.040	0.190 ± 0.010	0.220 ± 0.040	0.230 ± 0.110	0.500 ± 0.060	0.150 ± 0.110	0.350 ± 0.030	0.280 ± 0.140	0.288 ± 0.035
	H_e	0.828 ± 0.011	0.546 ± 0.091	0.219 ± 0.051	0.402 ± 0.115	0.827 ± 0.059	0.240 ± 0.055	0.794 ± 0.027	0.286 ± 0.121	0.518 ± 0.068
	F	0.540 ± 0.055	0.639 ± 0.078	−0.018 ± 0.055	0.463 ± 0.120	0.398 ± 0.030	0.452 ± 0.332	0.560 ± 0.023	0.061 ± 0.093	0.387 ± 0.067

[a] 50 trees were genotyped in each population. N_a – number of different alleles; N_e – number of effective alleles = $1/\sum_{i=1}^{n} p_i^2 1$; H_o – observed heterozygosity = number of heterozygotes/*N*; H_e – expected heterozygosity = $1 - \sum_{i=1}^{n} p_i^2$; *F* – fixation index = $(H_e - H_o)/H_e = 1 - (H_o/H_e)$; where *N* is number of trees genotyped, and p_i is the frequency of the *i*th allele in the population.

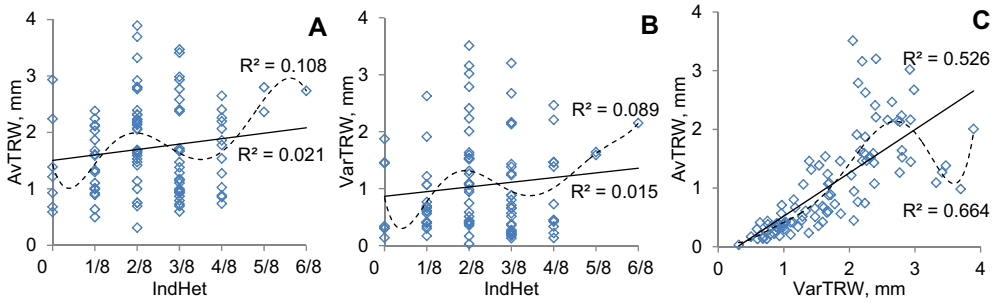

Fig. 4. Correlation of the average tree ring width (AvTRW) and the variance of tree ring width (VarTRW) with individual heterozygosity (IndHet) of trees, and AvTRW vs. VarTRW measured in two populations (50 trees each) combined.

2.3. Climatic data

Monthly climatic data for dendroclimatological analysis were obtained from the Climatic Research Unit (CRU) database (http://climexp.knmi.nl/selectfield_obs2.cgi) for a grid with a step of 0.5° for the four points that are closest to the dendrochronological polygons (Fig. 1) for the period 1901–2014. The following data were used: the average temperature, total precipitation, and the Palmer Drought Severity Index (PDSI). Climate variables were compared at different points, as well as with the instrumental data from the weather station "Shira" for temperature (1966–2012) and precipitation (1937–2012). Correlation coefficients were calculated for the following periods: September-November, December-February, March-May, June-August and for the full-year period from September to August (Table 1).

The interannual changes of temperature and precipitation for the most important summer period are illustrated in Fig. 3. While the CRU data are well-correlated among each other, the correlation with the data from the weather station "Shira" is much lower, especially for precipitation. This discrepancy may be because (1) the CRU data were obtained by interpolation from other sources, possibly reflecting regional climate rather than weather at a specific point or (2) the possibility of inaccurate instrumentation or human error at the weather station during a certain period. We decided to use the CRU climatic data for further analysis because these data have longer duration and are expected to have higher reliability over the full period.

The BER sampling population is located 7 km from grid point 3, whereas the sampling population EFR is 3 km from the center of the area (point 8) bounded by the neighboring grid points. For the climatic response analysis we used data for grid point 3 for the BER chronologies and the averaged data for points 1–4 for the EFR chronologies.

2.4. Genotyping with nuclear microsatellite loci

To estimate genetic polymorphism of the two populations of Siberian larch and individual tree heterozygosity, we used the eight best performing and the most polymorphic nuclear microsatellite loci (SSRs) that were previously developed for Japanese larch (*L. kaempferi* Sarg.)—loci *bcLK*, and for alpine larch (*L. lyallii* Parl.) and western larch (*L. occidentalis* Nutt.)—loci *UAKly* and *UBCLX* (Table 2), and then adapted for the Siberian larch (Oreshkova et al., 2013). The characteristics of these markers and the PCR conditions of their amplification are presented in Table 2.

Individual samples of total DNA were extracted from 100 to 200 mg of needles per tree. Extractions were performed according to the standard protocol for plant tissues using cetyltrimethylammonium bromide, CTAB (Devey et al., 1996).

The fragment analysis and sizing of the amplified individual alleles of the microsatellite loci and their genotyping were done using 6% polyacrylamide gel electrophoresis (PAGE) in Tris-EDTA-borate electrode buffer. Gels were stained in ethidium bromide solution and visualized using the system of gel documentation. The frag-

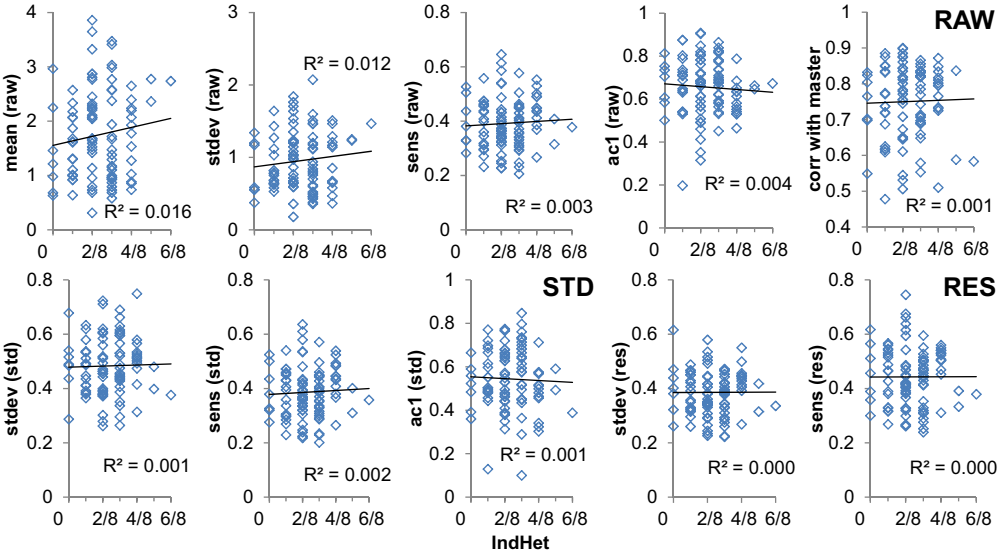

Fig. 5. Scattering diagrams of the studied statistical characteristics for the measured and standardized individual chronologies of radial increment growth with the individual heterozygosity (IndHet) of trees parameter.

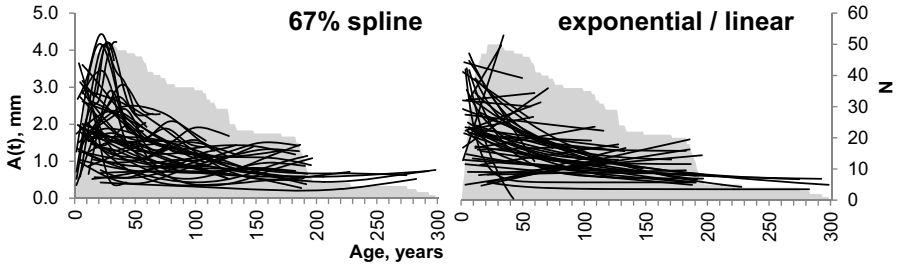

Fig. 6. Age curves for the population BER, calculated using different methods. A(t), mm—age curve (function of age trend) of radial growth in millimeters, N—number of trees for each age.

ment lengths were determined by comparison with the standard DNA ladder (plasmid p*BR*322 DNA digested by the HpaII restriction enzyme) using the Photo-Capt software. To more precisely determine the lengths of the PCR fragments (microsatellite alleles) multiple comparisons of variants of each locus were performed by running them on the same gel. Genetic diversity parameters including individual heterozygosity were estimated using the GenAlEx 6.41 software (Peakall and Smouse, 2006).

2.5. Correlation analysis

All relationships between variables were analyzed using Pearson's correlation coefficients. Significance of correlation was tested using Student's *t*-distribution. We also applied multifactorial analysis of variance using the Variance Components ANOVA/ANCOVA module in the STATISTICA software (StatSoft Inc., Tulsa, OK, USA) to estimate relationship of IndHet with AvTRW and VarTRW (using IndHet as a dependent variable, population as fixed effect, and AvTRW and VarTRW as random effects), but it gave results similar to the correlation analysis, therefore, these data are not presented here.

3. Results

Genetic variation was high in both populations across all loci, varying from 3 to 15 alleles per locus (Table 3). Observed heterozygosity (H_o) varied from 0.040 to 0.560 per locus and was 0.315 and 0.260 on average for all loci in BER and EFR populations, respectively.

Table 4
Correlations between average tree ring width (AvTRW), variance of tree ring width (VarTRW) and individual heterozygosity of trees (IndHet).

Population[a]	Parameter	AvTRW/VarTRW	IndHet/AvTRW	IndHet/VarTRW
BER	*R*	0.805	0.215	0.265
	P	0.000[a]	0.134	0.063
EFR	*R*	0.660	0.203	0.203
	P	0.000[a]	0.156	0.158
Combined (BER + EFR)	*R*	0.726	0.146	0.122
	P	0.000[a]	0.147	0.225

[a] 50 trees were genotyped in each population. R—correlation coefficient, P—significance level ($^*P < 0.001$).

Both parameters AvTRW and VarTRW had positive, but weak and statistically nonsignificant correlations with IndHet (Table 4, Fig. 4). At the same time, AvTRW and VarTRW were positively correlated at a highly significant level. Relationships of IndHet were estimated using absolute values for measured parameters (*raw*) of the individual series of radial growth, as well as with two types of standardized (*std* and *res*) parameters (Table 5, Fig. 5). All correlation coefficients were close to zero and nonsignificant.

Since the radial growth largely depends on the tree age, a phenomenon referred to as the age trend, we also compared the groups of trees characterized by different levels of IndHet with the groups (clusters) of trees characterized by different age curves, determined by hierarchical classification using two methods of age curves estimation (spline/exponential function). The obtained age curves and

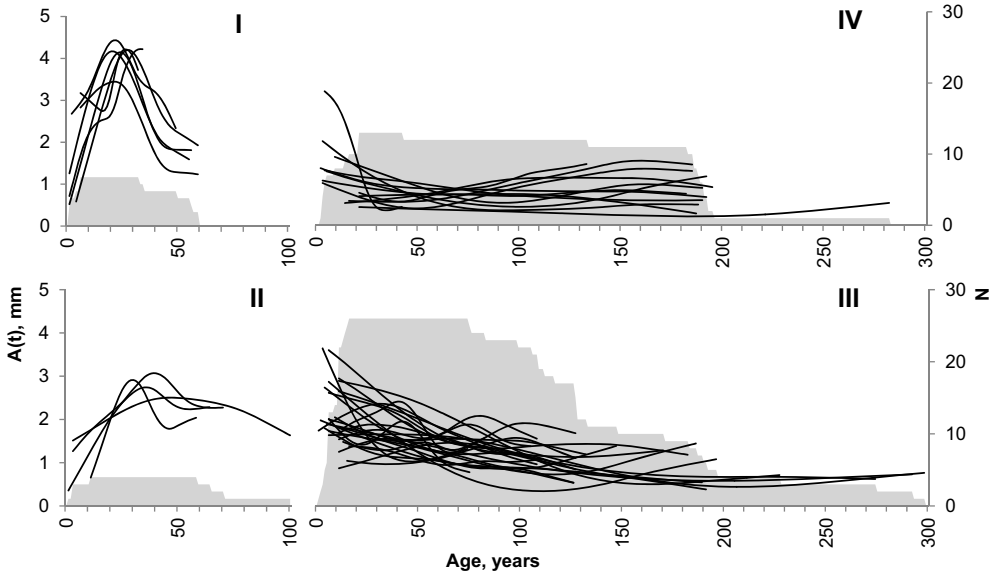

Fig. 7. Clusters of age curves calculated as splines.

Table 5
Correlations of individual heterozygosity (IndHet) of trees with their radial increment growth statistics in two populations (BER and EFR).

Population	Parameter	raw					*std*			*res*	
		mean (AvTRW)	*stdev* (VarTRW)	*sens*	*ac1*	*R*	*stdev*	*sens*	*ac1*	*stdev*	*sens*
BER	*r*	0.215	0.222	0.109	−0.142	−0.173	0.045	0.088	−0.117	0.047	0.050
	p	0.134	0.122	0.449	0.325	0.231	0.757	0.542	0.420	0.744	0.732
EFR	*r*	0.172	0.202	0.119	−0.035	0.190	0.017	0.115	−0.068	0.059	0.006
	p	0.234	0.159	0.412	0.809	0.186	0.907	0.426	0.637	0.684	0.969
Combined	*r*	0.126	0.111	0.054	−0.062	0.024	0.023	0.048	−0.038	0.005	0.002
(BER + EFR)	*p*	0.213	0.272	0.597	0.540	0.814	0.822	0.635	0.710	0.964	0.985

r—correlation coefficient with IndHet, *p*—significance level (other parameters and abbrviations are explained in Materials and methods).

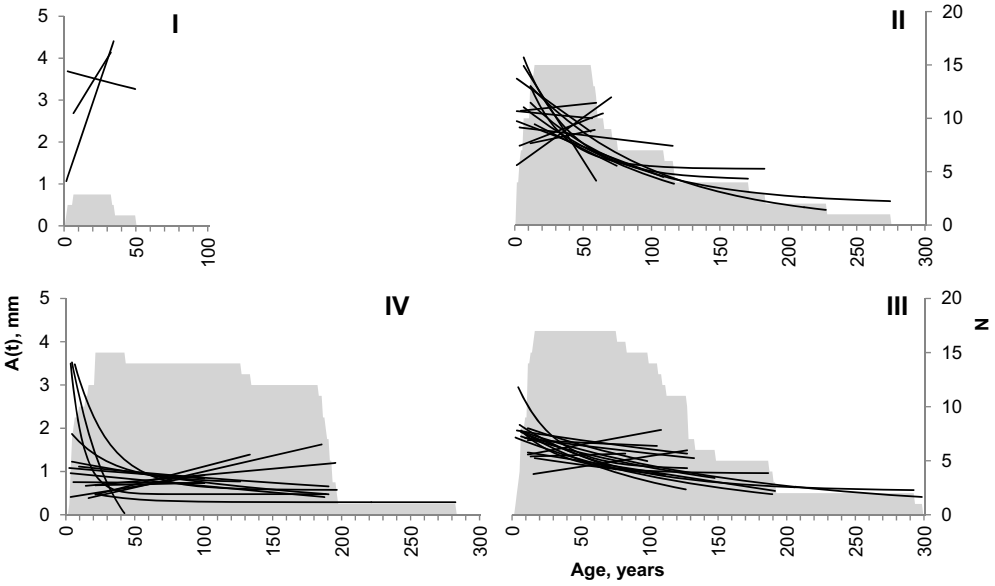

Fig. 8. Clusters of age curves calculated as exponential and linear functions.

the depth of the dataset aligned by the cambial age, i.e., the number of trees for each age, are shown in Fig. 6, the cluster subsets are shown in Figs. 7 and 8, and the dendrograms of classification are shown in Fig. 9. Different methods of calculating the age curves yielded significantly different results of classification, although certain common patterns may be found. Nevertheless, no common patterns in the distribution of trees with different IndHet were found in either case.

Each population (BER and EFR) was then partitioned into two subsets after removing trees younger than 50 years from the analysis. The first subset "low IndHet"—with the index of individual heterozygosity in the range of 0–0.25, and the second subset "high IndHet"—with IndHet in the range of 0.375–0.75. For each subset standard dendrochronological procedures were then performed, and the generalized standard (*std*) and residual (*res*) chronologies were obtained. The statistical characteristics of the chronologies obtained using the ARSTAN software are shown in Table 6. For each subset, standard dendroclimatological analysis was carried out. Correlation coefficients of the chronologies with the monthly total precipitation, average temperature and the PDSI were found to be significant for some months (Fig. 10).

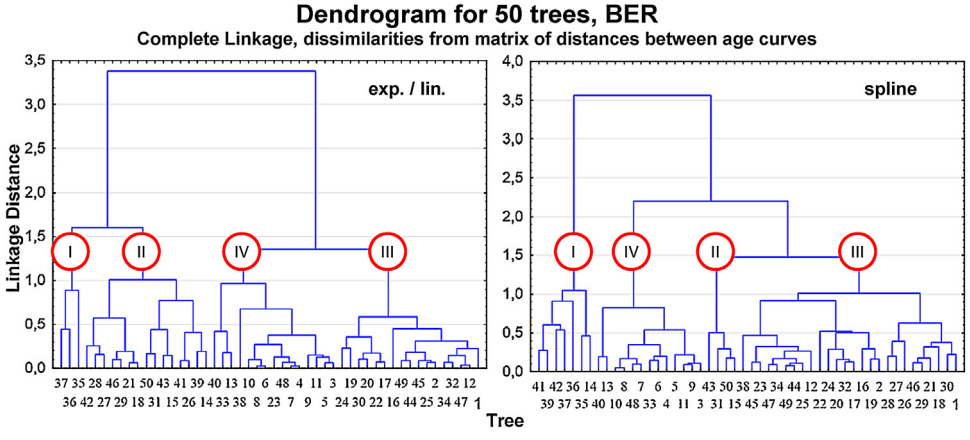

Fig. 9. Hierarchical dendrograms for the BER population dataset (clusterization is based on the age curves).

Table 6
The mean values and standard deviations (mean ± standard deviation) of statistics for original (*raw*) and standardized (std and *res*) radial growth chronologies of two populations (BER and EFR) partitioned for groups with low and high individual heterozygosity (IndHet) of trees.

Type of chronology	Statistics	Chronology			
		BER		EFR	
		low IndHet	high IndHet	low IndHet	high IndHet
raw	mean (AvTRW)	1.42 ± 0.63	1.37 ± 0.68	0.80 ± 0.72***	1.85 ± 0.77**
	stdev (VarTRW)	0.74 ± 0.26	0.75 ± 0.34	1.13 ± 0.45	1.11 ± 0.45
	sens	0.36 ± 0.07**	0.39 ± 0.07**	0.44 ± 0.11	0.47 ± 0.10
	ac1	0.66 ± 0.14	0.65 ± 0.15	0.67 ± 0.14**	0.59 ± 0.12**
	rbar	0.57 ± 0.12*	0.62 ± 0.15*	0.56 ± 0.10	0.59 ± 0.08
	eps	0.94 ± 0.05***	0.96 ± 0.02***	0.94 ± 0.04***	0.97 ± 0.01***
	R	0.74 ± 0.10	0.75 ± 0.10	0.69 ± 0.14***	0.76 ± 0.08***
std	*stdev*	0.48 ± 0.09*	0.51 ± 0.12*	0.53 ± 0.11	0.51 ± 0.10
	sens	0.36 ± 0.07**	0.39 ± 0.07**	0.43 ± 0.11	0.46 ± 0.10
	ac1	0.57 ± 0.14	0.56 ± 0.17	0.54 ± 0.11***	0.43 ± 0.14***
	rbar	0.56 ± 0.13**	0.62 ± 0.15**	0.57 ± 0.09**	0.62 ± 0.09**
	eps	0.93 ± 0.05***	0.96 ± 0.02***	0.94 ± 0.03***	0.97 ± 0.01***
res	*stdev*	0.37 ± 0.06*	0.39 ± 0.07*	0.43 ± 0.09	0.45 ± 0.09
	sens	0.43 ± 0.08*	0.46 ± 0.08*	0.48 ± 0.12	0.49 ± 0.10
	rbar	0.62 ± 0.09	0.65 ± 0.10	0.60 ± 0.07	0.62 ± 0.09
	eps	0.94 ± 0.06*	0.96 ± 0.03*	0.95 ± 0.03**	0.97 ± 0.01**
Number of cores		41	54	55	27

Significance level of differences between groups with low and high individual heterozygosity.
* $p < 0.10$.
** $p < 0.05$.
*** $p < 0.01$.

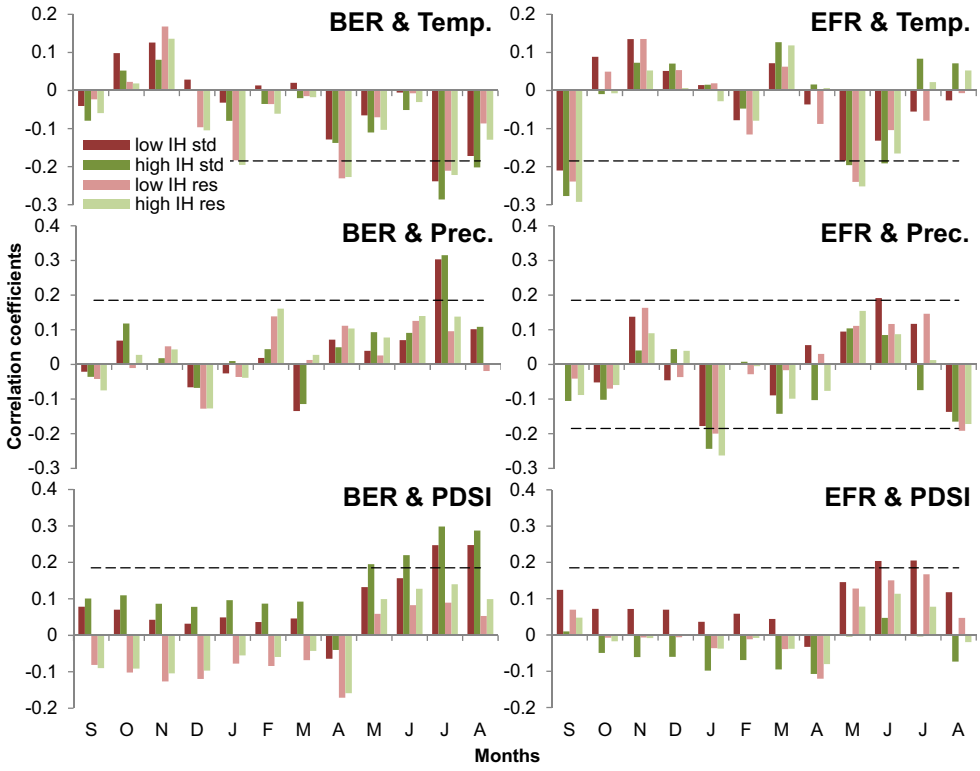

Fig. 10. The climatic response in the chronologies of the two local population datasets (BER and EFR) with lower and higher heterozygosity. Dotted line indicates the significance threshold for $P < 0.05$.

4. Discussion

The highly significant and positive correlation between AvTRW and VarTRW was interesting. This phenomenon can be explained if under unfavorable conditions most (if not all) trees grow slower regardless of their genotype, but under favorable conditions some trees may respond better via increased radial growth.

It is difficult to draw a conclusion about the relationships of AvTRW and VarTRW parameters with IndHet based on the data presented here. Although the correlations were nonsignificant, they were nonlinear rather than linear (Fig. 4). Therefore, the effect of individual heterozygosity could be very complex, and there may be an optimal intermediate level, when low individual heterozygosity could be as detrimental as a very high value (Altukhov et al.,

1986; Altukhov, 1996, 1998, 1999; Altukhov and Sheremet'eva, 2000; Altukhov and Moskaleichik, 2006; Olano-Marin et al., 2011; Thoß et al., 2011).

ABTempts to reveal the relationships between IndHet and individual series statistical characteristics and age curve groups did not give significant results. Use of generalized chronologies of subsets with low and high IndHet was more successful. The most significant and stable differences were found for expressed population signal (*eps*), which was higher for more heterozygous chronologies at all stages of standardization (Table 6). The same but less significant regularity was observed for the interseries correlation coefficients (*R*) and sensitivity (*sens*) coefficients. These patterns suggest a trend towards more pronounced common external signals in trees with higher heterozygosity because both *R* and *eps* are measures of common variation of individual growth series in the chronology, especially since *eps* can be interpreted as a measure of closeness between individual series and theoretical chronology of entire population (Wigley et al., 1984). As common environmental factors become more extreme, the populations exhibit a higher synchrony in growth patterns of individual trees and thus the common signal (Cook, 1985; Briffa and Jones, 1990). In the same environment, a common signal also depends on tolerance of plants to local conditions (Mérian and Lebourgeois, 2011). Autocorrelation (*ac1*) in the heterozygous chronologies, on the contrary, was lower (although this difference was significant in only one population): that is, the radial growth in the current year was less dependent on growth in the previous year. Therefore, on the basis of identified trends, we can assume that for trees with higher heterozygosity there was a more pronounced effect of factors common for the entire population (climate, general characteristics of the landscape and the soil), especially climatic variables with their high-frequency variation. For less heterozygous trees, the impact of individual stress factors, such as microenvironment and competitive relationship, was more important, which can be cautiously interpreted as their individual development is less stable.

Climatic response varied depending on heterozygosity. There was a stronger negative response to the warm season temperatures for the data subsets with high IndHet in both populations and a stronger positive response to the PDSI and the spring-summer precipitation, as a factor decreasing water deficit stress in plants, in the BER population. On the contrary, in the more humid and thus less extreme environmental conditions of the EFR population, the positive effect of increased precipitation and less severe drought (PDSI) was more pronounced for the data subset with low IndHet. The dendroclimatic analysis, however, generally confirmed an expected pattern of positive relationship between heterozygosity and common signal strength in moderately extreme conditions of water availability.

The lack of correlation between IndHet and characteristics of radial growth can be explained by the ascertainment bias caused by typically selecting only the most polymorphic microsatellite markers in the genome, which may lead to reduced sensitivity for judging genome-wide levels of genetic diversity. Väli et al. (2008) tested this potential limitation of microsatellite-based approaches by correlating nucleotide diversity in noncoding regions of eight different carnivore populations assessed by sequencing 10 introns (5.4–5.7 Kb) in 20 individuals of each population with mean multilocus heterozygosities based on microsatellite genotyping (10–27 markers) of the same animals. Although there was a positive correlation between microsatellite marker heterozygosity and nucleotide diversity at the population level, no significant correlation was found at the individual level. These results imply that the variability of microsatellite marker sets typically used in population studies may not accurately reflect the underlying genomic diversity. This suggests that researchers should consider using resequencing-based approaches for assessing genetic diversity when accurate inference is critical, as it may be in our case.

Another problem could be associated with a relatively high frequency of null-alleles that can mask heterozygotes. The high *F*-values observed in several loci in both populations (Table 3) can be a signature of null-allele presence. Inbreeding can also inflate *F*-values, and self-pollination seems higher in larch compared to other conifers (Knowles et al., 1987; Oreshkova et al., 2013), but it cannot explain uneven distribution of *F*-values across loci.

SSR markers alone did not allow us to discriminate two main hypotheses: overdominance vs. dominance, but only to test the association of IndHet with the average tree-ring width (AvTRW) and with the variance of the tree-ring width (VarTRW) used as proxy traits for heterosis and homeostasis, respectively. In the following studies we plan to use also supposedly adaptive genetic markers, i.e., microsatellites closely linked with functional and adaptive genes, and sequence data—that are SNPs in the coding (preferably nonsynonymous SNPs) regions, as well as supposedly selectively neutral SNPs in noncoding regions for comparison. A description of the different types of genomic markers proposed in our study and also recommended for the study of the impact of global climate change on the genetic variability of populations and species is provided in Angeloni et al. (2012).

5. Conclusions

Dependence of some radial growth characteristics of Siberian larch trees on their individual heterozygosity was investigated. Application of different approaches demonstrated that partitioning the populations into two groups (subsets) with low and high individual heterozygosity, respectively, and the subsequent comparison of their chronologies provided additional valuable information. It can be assumed that radial growth of trees with high IndHet responded more strongly to the climatic changes because of their faster recovery after extreme stress. On the contrary, radial growth of trees with low IndHet is more autoregressive and is more affected by continuously acting stress factors. In our further work we plan to increase the number of loci to make them more genome wide for more accurate estimation of individual heterozygosity and for better detection of environmental signals.

Acknowledgments

We thank Dr. Alexei A. Ibe (Forest Protection Centre of Krasnoyarsk Territory, Krasnoyarsk, Russia) for help with sampling and Dr. Judy Brooks (Blinn College, College Station, Texas, USA) and Dr. Tom D. Byram (Texas A&M Forest Service, College Station, Texas, USA) for proofreading and significantly improving the original manuscript. This study was supported by research grants No. 14-04-01462 from the Russian Foundation for Basic Research and No. 14.Y26.31.0004 from the Government of the Russian Federation and by the President Grant No. НШ-3297.2014.4 for Government Support of Young Russian Scientists and the Leading Scientific Schools of the Russian Federation.

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