

Supplementary material

Table S1. Brief characteristics of climate and soils of the 6 forest natural regions in Lithuania based on Galvonaite et al. [94].

| FRN | Name | Briefly | Alt., m a. s.l. | Mean annual T °C | Mean warmest month T, °C | Mean coldest month T °C | Annual precipit- tation, mm | Snow cover duration, days | Soils |
|---|------------------------------|-----------------------------------|--------------------|------------------------|-----------------------------------|----------------------------------|-----------------------------------|------------------------------------|--------------------------------------|
| 1 | Western highland | Wettest, cooler, rich soils | 150-200 (234) | 6.3-6.7 | 17.0-17.5 | -3.2 to - 2.9 | 810-820 | 75-90 | Medium rich, albeluvisols |
| 2 | Midland lowland, north | Drier, rich soils | 50-70 (85) | 6.5-7.0 | 17.4-18.1 | -3.6 to - 3.1 | 560-700 | 75-90 | Rich, cambisols |
| 3 | North-east highland | Coldest, dry, poor | 150-200 (289) | 6.1-6.7 | 17.7-18.0 | -4.8 to - 3.8 | 610-690 | 90-105 | Poor, albeluvisols |
| 4 | Sea-side lowland | Mildest, wet, poor | 10-50 (78) | 7.4- 8.0 | 17.8-18.4 | -1.9 to - 1.4 | 770-800 | 60 | Very poor, arenosols, podsoles |
| 5 | Midland lowland, south | Mild, dry, rich | 50-80 (104) | 7.1-7.4 | 18.0-18.1 | -3.6 to - 3.1 | 600-640 | 65-80 | Rich, cambisols, podsoles |
| 6 | South-east highland | Cold, dry, poor | 150-220 (259) | 6.8 | 17.9 | -3.7 | 700 | 90 | Very poor, arenosols |
| FRN- Forest natural region, Alt. – altitude common range (maximum). Soils – dominant soil type. Data from 1981 to 2010. T is temperature. | | | | | | | | | |

Table S2. Molecular characteristics of the nuclear and chloroplast microsatellite loci used for the Scots pine DNA analysis. The repeat motive size is given in bp after the slash. For allele size the range and the most frequent alleles are given.

| Locus | Repeat motif, bp | Allele size (most frequent), bp**** | Forward primer (5'-3') Reverse Primer (3'-5') | References |
|---|---------------------|-------------------------------------|---|-------------------------|
| Nuclear microsatellites (P_{syl}= EST-SSR, rest = genomic SSR) | | | | |
| P _{syl} 16 | (AT)7 / 2 bp | 198-220 (202) | GCTCTGCCCATGCTATCACT TGATGCTACCCAATGAGGTG | Sebastiani et al. [75]* |
| P _{syl} 18 | (GCA)7 / 3 bp | 291-309 (300) | ACTACCTGGCATTTCGTCCTG GGATCTGGTCCATTTCGTGT | Sebastiani et al. [75] |
| P _{syl} 2 | (GC)5 / 2 bp | 198-224 (208) | TTGCTTTTGCAGAACATTTCG GTCCTGCAGGCAATCAAAAT | Sebastiani et al. [75] |
| P _{syl} 25 | (GCA)5 / 3 bp | 218-221 (221) | CAGCACGCGTTCTTTGTATC ACCGTTGCTCGTTGTCTTCT | Sebastiani et al. [75] |
| P _{syl} 42 | (TC)9 / 2bp | 167-177 (173) | CAACTTCAGCCTTGCAACAA CGACTTCATTGGAACACCA | Sebastiani et al. [75] |
| P _{syl} 44 | (CGG)5 / 3 bp | 160-177 (171) | TCCAAGTTCGGTTCCTTGTC GACACGATGGATTCCCTGAT | Sebastiani et al. [75] |
| P _{syl} 57 | (ACC)7 / 3 bp | 191-209 (200) | CCCCACATCTCTACAGTCCAA TGCTCTTGGATTGTGTGCTG | Sebastiani et al. [75] |
| PtTX 4001 | (GT)15 / 2 bp | 201-237 (217) | CTATTTGAGTTAAGAAGGGAGTC CTGTGGGTAGCATCATC | Auckland et al. [77] |
| PtTX 4011 | (CA)20 / 2 bp | 195-283 (261) | GGTAACATTGGGAAAACACTCA TTAACCATCTATGCCAATCACTT | Auckland et al. [77]*** |
| SPAC 11.4 | (AT)5(GT)19 | 135-165 (139) | TCACAAAACACGTGATTACACA GAAAATAGCCCTGTGTGAGACA | Soranzo et al. [76]** |
| SPAC 12.5 | (GT)20(GA)10 / 2 bp | 121-193 (155) | CTTCTTCACTAGTTTCCTTTGG TTGGTTATAGGCATAGATTGC | Soranzo et al. [76] |
| SPAC 7.14 | (TG)17 (AG)21 | 181-253 (213) | TTCGTAGGACTAAAAATGTGTG CAAAGTGGATTTTGACCG | Soranzo et al. [76] |
| Chloroplast microsatellites**** | | | | |
| Pt71963 | (T)16 (T)14 / | 138-146 (143, | TTCATTGGAAATACACTAGCCC | Vendramin et al. [42] |
| Pt15169 | (C)8(T)8A(T) | 120-128 (123,124) | CTTGGATGGAATAGCAGCC | Vendramin et al. [42] |
| Pt30204 | (A)12(G)10 | 138-144 (141) | TCATAGGGGAAGATCCTCTTT | Vendramin et al. [42] |

*- EST SSR loci developed for *Pinus sylvestris*. **- nuclear SSR loci developed for *Pinus sylvestris*. ***- nuclear SSR loci developed for *Pinus taeda*. ****- cpSSRs for *Pinaceae*, all with 1 bp SSR motive. *****- allele range and most frequent allele from our study.

Table S3. Proportion of mitochondrial DNA mitotypes A and B in natural Scots pine populations within Lithuania.

| Pop.No. | Pop. name | Alt. (m) | 295 bp (B) | 300 bp (A) |
|--|--------------|----------|-------------|-------------|
| 1 | Plunge | 122 | 3 | 17 |
| 2 | Tryskiai | 118 | 7 | 12 |
| 3 | Kurtuvenai | 108 | 10 | 10 |
| 4 | Varniai | 169 | 6 | 15 |
| 5 | Mikieriai | 80 | 5 | 15 |
| 6 | Geguzinė | 60 | 9 | 11 |
| 7 | Rokiskis | 127 | 10 | 10 |
| 8 | Salakas | 164 | 17 | 3 |
| 9 | Grazute | 183 | 11 | 9 |
| 10 | Labanoras | 174 | 9 | 12 |
| 11 | Azvintis | 177 | 12 | 8 |
| 12 | Juodkrante | 27 | 3 | 17 |
| 13 | Darbenai | 40 | 4 | 12 |
| 14 | Sveksna | 38 | 3 | 17 |
| 15 | Pagegiai | 25 | 4 | 16 |
| 16 | Vaisvydava | 87 | 15 | 5 |
| 17 | Braziukai | 82 | 7 | 13 |
| 18 | Punia | 81 | 9 | 11 |
| 19 | Ancia | 122 | 6 | 13 |
| 20 | Veisieiai | 138 | 8 | 12 |
| 21 | Trakai | 122 | 7 | 13 |
| 22 | Druskininkai | 153 | 11 | 9 |
| 23 | Raseiniai | 70 | 0 | 20 |
| Proportion of A and B mitotypes % | | | 38.6 | 61.4 |

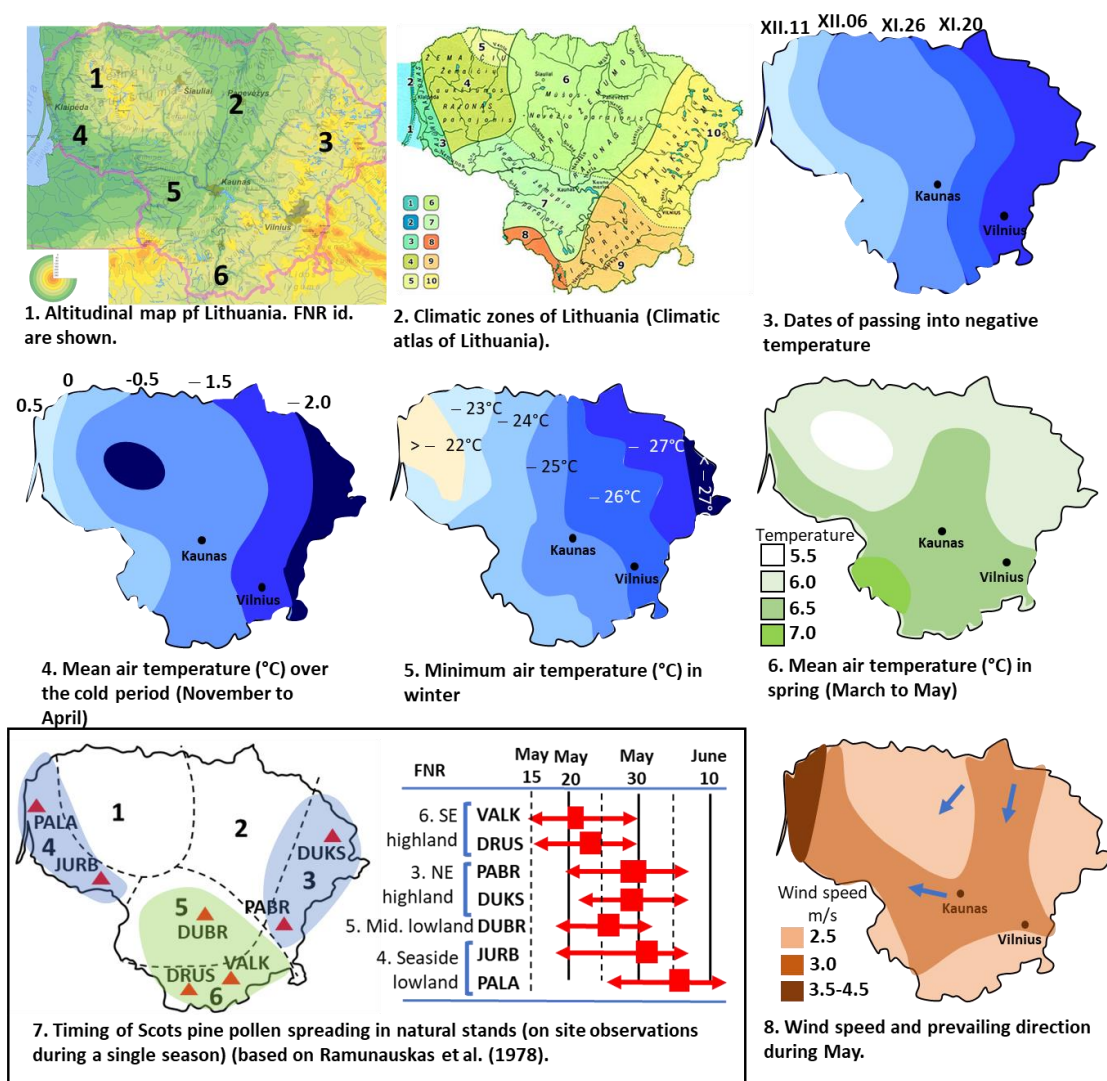


Figure S1. The main climatic characteristics regarding frost hardness and timing of spring budburst for Scots pine in Lithuania. Maps 1 and 2 taken from Climate atlas of Lithuania [94]. FRN are forest natural regions, i.e., ecoclimatic zones used in forestry and basically reflecting altitudinal variation and continentally gradients similarly as in general climatic zoning in map 2. Maps 3 to 6 and 8 are modified from Galvonaite et al. [94], where the climatic data averages the period over 1980–2011. The map 7 is modified from an empirical study by Ramanauskas et al. [73], where during a single spring onsite observation on budburst timing were carried out simultaneously in different parts of Lithuania (arrows for start and end of pollen spread, the red boxes for mass pollen shed). The map 8 gives prevalent direction and strength of winds during pollination time, which may indicate possible zones of more frequent mating (we outlined these putative zones with different colors in the map 7). Lines on map 7 show forest natural regions.

Pop. 5 MIKE (northern midland lowland, FNR 2)



Pop. 12 JUOD (Nerija spit, FNR 4, sandy dunes)



Pop. 3 KURT, FNR 1 western highland.



Pop. 19, ANCI FNR 6 south-eastern highland



Figure S2. Actual view of the sampled stands in various eco-climatic zones of Lithuania (forest natural regions, FNR). Pop. 5 MIKE typical Scots pine forests in north-eastern highland with pine on the overstory and spruce in the understory. Pop. 12 JUOD is in the forest foots between sandy dunes with accumulations of richer upper soil layers (myrtilossum ground vegetation, some oaks in the understudy). Pop. 3. KURT – typical mixed Scots pine

with Norway spruce stands on richer soils in the western highland (717 is a plus tree, FNR 1). Pop. 19 ANCI – Pure Scots pine stands on poorest sandy soils with Juniper in the understory common on the south-eastern highland.

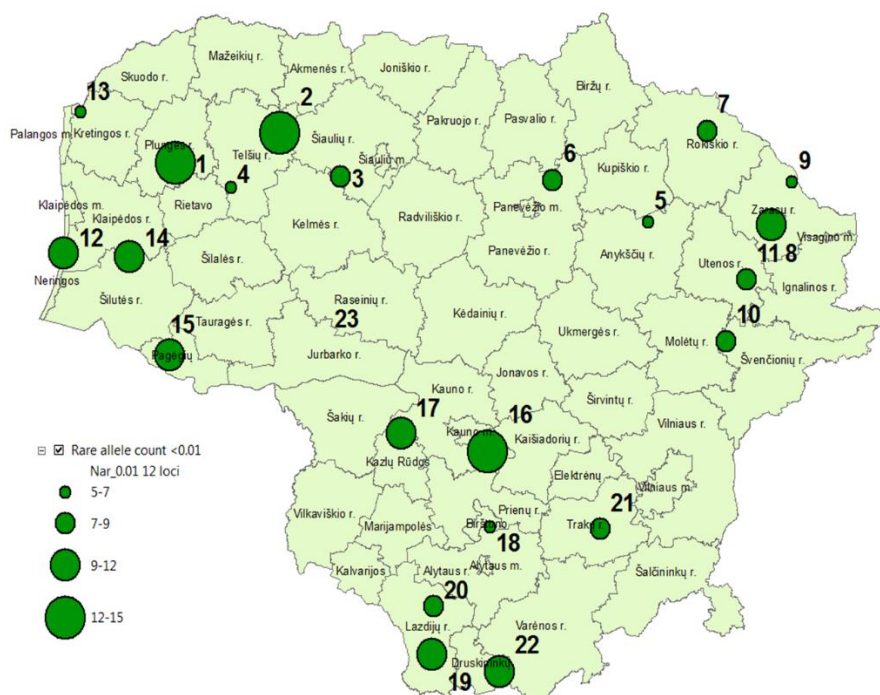


Figure S3. Population frequency of rare alleles (alleles with frequency <0.01 in whole material).

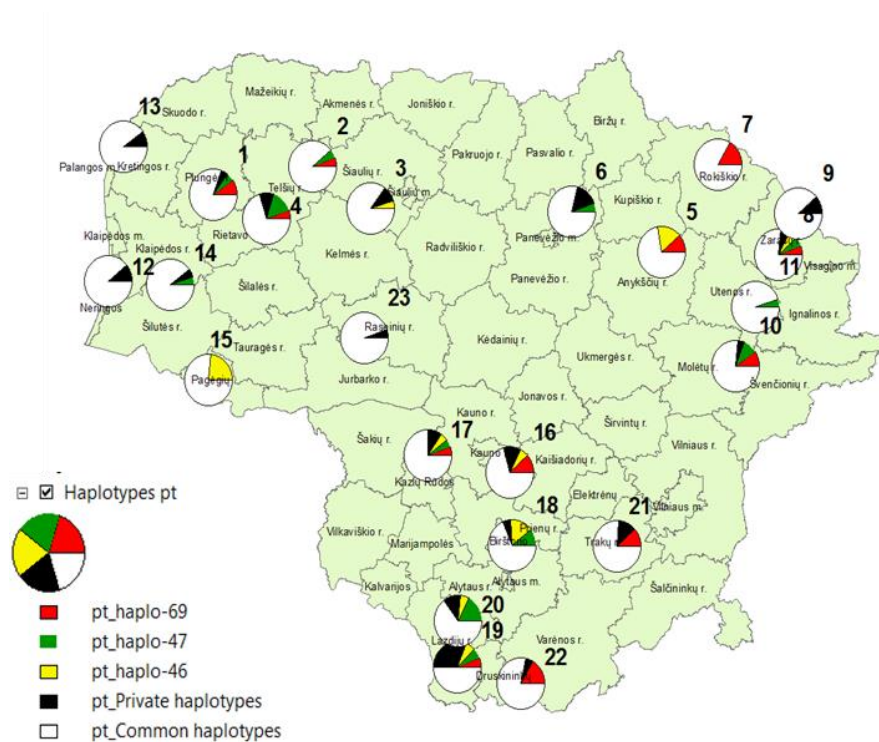


Figure S4. Geographical distribution of four most frequent clusters making up to 61 % of all haplotypes, private and shared haplotypes based on three cpSSR loci of pt series. Paternally inherited haplotypes are highly shared among populations studied (compare common haplotypes in white).