

Communication

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Communication

Genomic Data Suggests Pathways of Modern White Poplar (*Populus alba* L.) Range Formation in the Postglacial Era

Natalya S. Gladyshevskaya¹, Mikhail I. Popchenko^{1,2}, Maxim A. Kovalev^{1,*}, Vsevolod V. Volodin^{1,3}, George S. Krasnov^{1,3}, Alina S. Bogdanova^{1,4}, Dmitry S. Karpov^{1,3}, Nadezhda L. Bolsheva¹ and Anna V. Kudryavtseva^{1,3}

¹ Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32 Vavilova, Moscow 119991, Russia

² Institute of Geography, Russian Academy of Sciences, Staromonetny Pereulok, 29/4, 119017 Moscow, Russia

³ Center for Precision Genome Editing and Genetic Technologies for Biomedicine, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov Str., 32, 119991 Moscow, Russia

⁴ National Research University Higher School of Economics, Myasnitskaya Str., 20, 101000, Moscow, Russia

* Correspondence: kovalev_maksim_2002@mail.ru

Abstract: The white poplar (*Populus alba* L.) is an economically significant tree species with a natural distribution spanning extensive region of Eurasia. Nevertheless, there is currently no hypothesis regarding the historical shaping of this range. In this study, we collected and sequenced 36 individuals of white poplar from disparate regions of Russia and Kazakhstan. Additionally, we employed available genomic data of white poplars from Italy, Hungary, and China. A genomic approach was employed to collate data on the location of glaciers in different periods, along with information on the natural and artificial distribution of white poplar. This enabled the formulation of the first hypothesis regarding the formation of the modern range of this plant. It is hypothesized that during the period of maximum glaciation, three refugia existed: the South European, Transcaucasian, and Altai-Middle Asian refugia. Post-glacial migration from these refugia led to the formation of modern populations of *P. alba* in Eastern Europe (including the European part of Russia), the Caucasus, and Siberia, respectively.

Keywords: *Populus alba*; natural range; historical formation; Russia; Europe; genomics; Quaternary glaciation; glaciers

1. Introduction

Poplars are a widespread genus of woody plants with pronounced economic value. They have long been used as a source of pulp and wood, and the International Poplar Commission (IPC) was established in 1947 to promote the sustainable management of fast-growing trees by its 38 member countries [1]. At the same time, due to their fast growth, poplars can be used to combat climate change as they store atmospheric carbon in their wood [2,3]. Poplars have traditionally been used for landscaping rural and urban areas, including in Russia. This is partly due to their ability to withstand heavy soil pollution, which allows poplar to be used for phytoremediation, cleaning both urban and industrial soils [4,5]. Finally, poplars are used by scientists as model objects among trees - for example, *Populus trichocarpa* became the first tree the genome of which was sequenced in 2006 [6]. Due to all these reasons, the geographical distribution of selected valuable poplar species has become wider, through introduction in selected regions.

White poplar (*Populus alba*) is a well-known member of the genus with a native range from Central and Southern Europe to Xinjiang and the Western Himalayas (<https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:776573-1>, accessed 13 October, 2024), and is currently present on five continents due to its biological characteristics that allow it to be grown as a source of cellulose and lignin or for landscaping in urban areas. Poplars are dioecious plants, much less common than monoecious plants. The ARABIDOPSIS RESPONSE REGULATOR 17 (*ARR17*) gene, located in the sex-determining region (SDR), is the main regulator of sex, which ensures female phenotype development [7], while male development is regulated by suppression of this gene expression [8]. White poplar is an exception in the sense that males do not have genetic mechanisms to suppress *ARR17* expression, but instead have a deletion of this gene [9], making it possible to use genome editing tools to change the sex of a tree by inserting or deleting the gene, which could allow the cultivation of trees of the preferred sex to, for example, reduce economic losses by reducing the number of trees of the preferred sex.

The complex history of white poplar range formation, including the reduction of its habitat area during the glacial period and the subsequent development of new territories, has led to the formation of a large intraspecific genetic diversity, which arose both because of range disjunction and the need to adapt to the conditions of very diverse regions in terms of climate and biota. Populations from different regions are characterized by very contrasting indicators of growth rate, winter hardiness, frost resistance, drought tolerance, salt tolerance, as well as resistance to pests and pathogens, primarily those that cause stem rot. In addition, populations of different origins differ in their ability to vegetative reproduction and the set of symbiotic microorganisms, which is very important in connection with the increasing use of biotechnology methods in the cultivation of these tree species. The current intraspecific diversity has both great practical applications in breeding work and can be used to reconstruct the history of white poplar dispersal after the retreat of glaciers, to identify primary and secondary ranges of the species, the history of origin and dispersal of individual forms, and to solve problems of intraspecific systematics.

In this study, we present the first insights into the formation of the modern *P. alba* region during the postglacial era, based on the genomes of 36 white poplar individuals collected in Russia and Kazakhstan, and using available genomic data of white poplars from Italy, Hungary, and China. Our findings shed light on the evolutionary history of this species in the East European Plain, the Caucasus, and Western Siberia. The data may also be employed to model the range dynamics of other species of the genus in Eurasia and North America, as poplars exhibit analogous strategies in the development of their populations.

2. Results

To understand the process of modern white poplar range formation, we used a genomic approach. To do so, we collected genome data from plants originating from a range of countries and geographic locations and then evaluated clusters of genetically similar samples based on SNP profiling. First, we mapped the reads to the reference genome of white poplar and then performed SNP calling as described in the Materials and Methods section. For the most samples (73 of 87) more than 85% reads were successfully mapped to the reference sequence. About 23 million SNPs (at least in one sample) were identified. Based on the profiles of per-sample SNP occurrence, we performed PCA analysis (Figure 1). The phylogenetic tree corresponding to this graph is shown in Supplementary Figure S1.

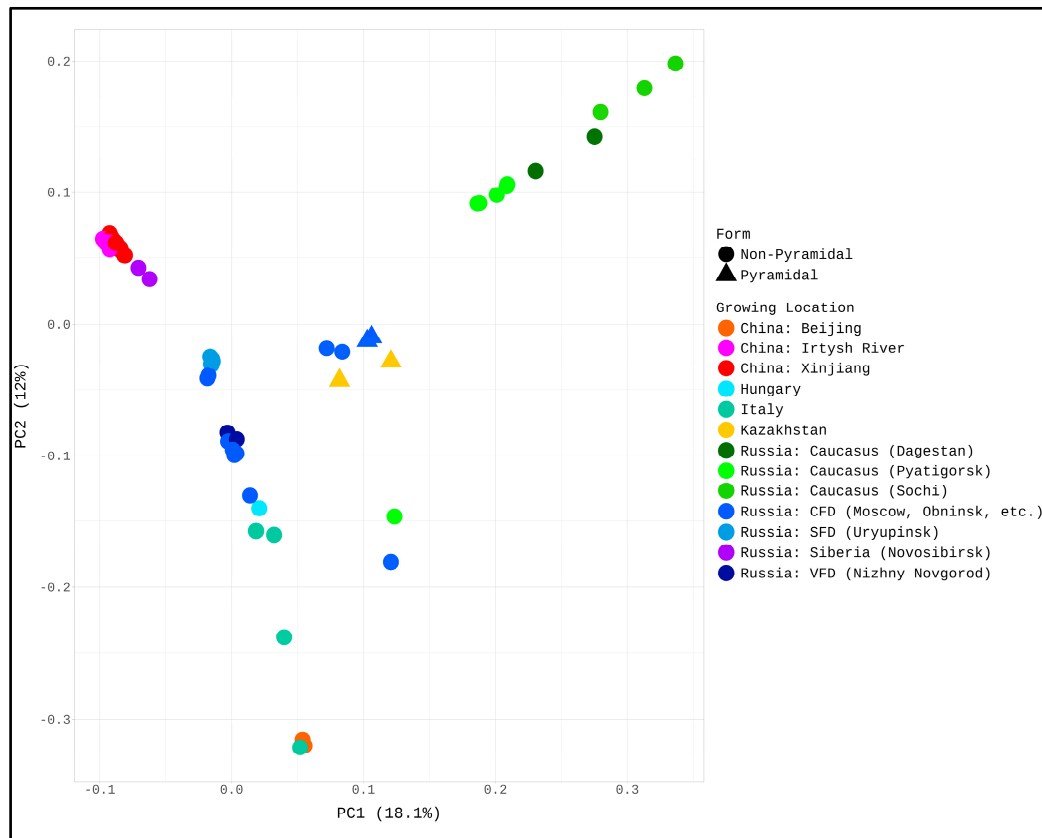


Figure 1. PCA plots are based on SNP profiling. Three clusters were identified, one of which corresponds to samples from China (Xinjiang Province and Irtysh River Valley) and Western Siberia (Novosibirsk), the second to plants from the Caucasus (Sochi, Pyatigorsk, and Dagestan), and the third to all other samples.

Based on this PCA graph, we identified three clusters. First, samples collected in the Caucasus (in the vicinity of Sochi, Pyatigorsk, and in Dagestan) were most strongly separated from all other points and formed a separate cluster. Chinese (growing in Xinjiang and the Irtysh River valley) and Siberian trees were also grouped separately. All other plants formed a large third cluster. We will now note that the Italian specimens are furthest away in it, as well as plants from Beijing (which were imported). And 6 poplars, 4 of which were pyramidal (these are all pyramidal poplars in our study), formed a separate subcluster inside this third cluster.

Having studied the PCA plot, we hypothesized that there were probably 3 ancestral populations according to the 3 identified clusters. Based on these genotypes, the entire modern diversity of white poplars could have been formed. To test this hypothesis, we used the NGSAdmix program as written in the Materials and Methods section, the results of which are presented in Figure 2.

We also used NGSAdmix with the number of genotypes $k = 2, 4, 5$, and 6 , and the results are shown in Supplementary Figures 1, 2, 3, and 4, respectively. However, while at $k = 3$ the split corresponds to geography (Europe, Caucasus, and Chinese regions), at $k = 4$ Chinese poplars split into two genotypes, and at $k = 5$ a cluster appears in the Southern Federal District, where it is unlikely that a refugium existed during glaciation due to the harsh climatic conditions. Thus, we hypothesize that there were three major refugia in terms of the global population of *P. alba*.

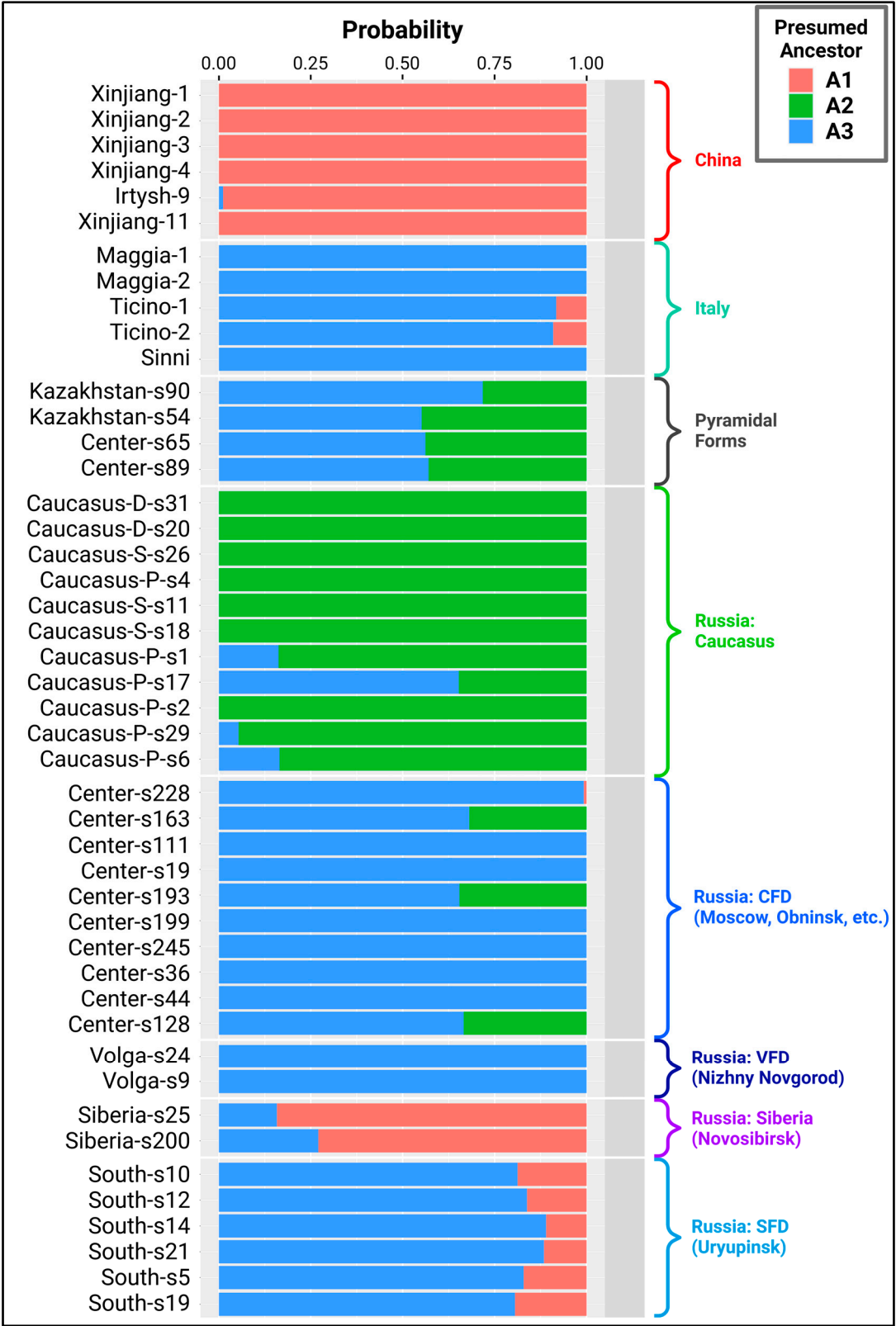


Figure 2. Results of population mixing analysis using the NGSAdmix program if all plants belong to three clusters and, accordingly, have three main ancestors (A1, A2, A3). A1 is the most likely ancestor of Chinese poplars, A2 of Caucasian poplars, and from A3 came Italian plants as well as poplars living in central Russia. Siberia-s13 is absent from this scheme because it is a *P. alba* x *P. tremula* hybrid.

We interpreted the results of this analysis as follows. A1 is the most probable ancestor of poplars in China, largely in Western Siberia, and has also contributed to the gene pool of plants in the vicinity of Uryupinsk. A2 is a key ancestor of trees in the Transcaucasia and Caucasus, and its participation

in the artificial settlement of CFD territories and the creation of pyramidal forms is also notable. A3 is an ancestor of poplars from Italy, a key member of the gene pool of the central and lower Volga region, secondary area of central part of Russia, as well as contributing to populations in south of Western Siberia and the Caucasus and to the creation of pyramidal forms, known as the variation *pyramidata sovietica*. We reveal our theory of white poplar distribution in more detail in the discussion section.

3. Discussion

The family Salicaceae separated about 128 million years ago. It includes 54 genera and about 1,400 species, with most genera represented by a small number of species and distributed in Southeast Asia, the likely center of origin of the family [10]. The most evolutionary successful genera are *Salix* and *Populus*. Their common ancestor about 60-65 million years ago underwent Salicoid whole genome duplication, which affected approximately 92% of the genome and resulted in more than 8000 pairs of paralogous genes [6]. This is probably why the genera *Salix* and *Populus* were able to spread almost all over the Northern Hemisphere, especially in the boreal regions, and now number around 450 and 50 species, respectively [11,12].

The genus *Populus* is divided into four sections: Abaso, Turanga, *Populus* and ATL, the latter including representatives of the traditional sections Aigeiros, Tacamahaca and Leucoides. Abaso is the most primitive section, including *P. mexicana*, common in Mexico. Turanga includes *P. euphratica*, known to grow in deserts in the Middle East and named after the Euphrates River. *Populus* and ATL are the most evolutionary advanced groups. *Populus* includes the aspens: *P. tremula*, *P. tremuloides*, as well as *P. alba*, *P. tomentosa*, *P. qionghdaoensis*, and some other poplars. The ATL group includes *P. trichocarpa*, *P. balsamifera*, *P. deltoides*, *P. nigra*, *P. lasiocarpa*, and others [13]. Interestingly, poplars, like willows, are dicotyledonous plants, and some representatives of the *Populus* section, including *P. alba*, have a ZW system of sex determination, while most poplars are characterized by an XY system [14,15]. Moreover, only 2 species are tropical in the genus *Populus*, namely *P. qionghdaoensis* and *P. ilicifolia*, distributed in Hainan and Africa (Kenya and Tanzania), respectively [15]. *P. trichocarpa* and *P. balsamifera* are distributed in temperate forests of North America. *P. trichocarpa* is spread along the west coast of the United States and Canada, while *P. balsamifera* occurs somewhat northward, from Alaska to Labrador; however, there is a significant degree of hybridization between these closely related species in the habitat overlap zone [16–19]. *P. tomentosa* is a Chinese endemic [20]. The range of *P. alba* extends from Western Europe to Altai and Xinjiang, bounded in the north by mixed forests and taiga, where the climate is too cold for it, and in the south by steppes, semi-deserts and deserts, where it is too dry, as can be seen in Figure 3. *P. alba* did not spread across the Altai, probably due to the harsh sharply continental climate of Eastern Siberia and competition from other species [21–24].

We did not find articles describing a possible scenario for the formation of the modern range and population structure of *P. alba*, there was only a research devoted to Central and Southern Europe. It was shown that the two main refugia in this region were in Italy and Romania. At the same time, *P. tremula* survived in more northerly and harsh conditions, near the ice shield of the glaciated Alps. When the glaciers retreated, the ranges of both species expanded, and in the contact zone they began to hybridize actively, forming the natural hybrid *P. x canescens* [25]. White poplars in China, growing in the Irtysh River basin, have also been studied. Interestingly, the heterozygosity of populations there is lower than in Italy and Hungary [26]. In addition, in this region, as in Europe, *P. alba* also hybridized significantly with *P. tremula*. This process, in turn, was significantly limited by plastid-nuclear incompatibility [27].

We will also consider analogous research for two other species, namely *P. cathayana* in China and *P. balsamifera* in Canada and USA, just for example.

A recent study of *P. cathayana* revealed 4 genetically distinct populations, named by the authors for growing in the Southwest (NW), Northwest (SW), and North China (NC), and the Taihang Mountains (TH). Splitting of the ancestral population began 1649 thousand years ago (kya), with one branch dividing 1430 kya into TH and NW and the other branch splitting 987 kya into NC and SW. The distribution and adaptation of *P. cathayana* has been linked to climate changes [28].

Another interesting evolutionary story concerns the postglacial distribution of *P. balsamifera* in North America after the last glacial maximum, which occurred about 18,000 years ago. The authors identified three demes, namely the Central, Northern, and Eastern ones. The Central deme is the most genetically diverse, occupies the largest area, and is probably directly related to the ancestral population in the refugium. The Northern deme occupies Alaska, and the Eastern deme inhabits Quebec and Labrador [18]. In another paper, it was shown that the boundary between the Eastern and Central demes is clearer than between the Central and Northern demes (there referred as Western), which can probably be explained by the earlier separation of the Eastern deme from the general evolutionary branch [19].

Quaternary glaciation appears to have been a significant force in shaping the present-day range of *P. alba*. It began 2.58 million years ago and has continued to modern times, with periods of glaciation alternating with much warmer periods of interglaciation [29,30]. For example, the last glaciation ended 11,700 years ago, followed by the Holocene, which is an interglacial period [31,32], and the next glaciation is projected to begin in 50,000 years [33]. During the maximum glaciation glaciers were in the northern part of Europe (most of Great Britain, the territory of modern Germany, Poland, Belarus, northern Ukraine and part of Russia), also the centers of glaciation were mountain systems: the Alps and the Caucasus. But in Eastern Siberia glaciation also occurred but covered quite a little percentage of all surface - probably, the dry continental climate did not allow the formation of a glacier [34–36].

Based on our findings and all the above information, as well as on the data on the current distribution of white poplar we formulated a hypothesis of how the formation of its modern range occurred, which is shown in Figure 3, and the numbers in this section of the text coincide with the numbers in the figure for ease of perception.

In the late Pliocene, about 2.5 million years ago, a single population of white poplar probably existed across vast territories of Eurasia. However, after a series of glaciations in the Pleistocene, this tree became extinct in a significant part of its historical range, surviving, among others, in three regions on the territory of Russia and neighboring countries. These regions, those we consider as refugia, were never covered by glaciers and correspond to putative ancestors A1, A2, and A3. The first (5) is the Altai Region and mountains of Central Asia, located in present-day Russia, Kazakhstan, China (Xinjiang), Kyrgyzstan, Uzbekistan, Tajikistan and Afghanistan, which matches A1. The second (4) is Transcaucasia (since the Caucasus Mountains themselves were covered by glaciers at that time and the southern coast of the Caspian Sea on the territory of modern Azerbaijan, Armenia, eastern Turkey and northern Iran, it conforms to ancestor A2. The third region (3) was in the north of Africa and in the south of Europe, on the Iberian, Apennine and Balkan peninsulas, on some large islands, including Sicily, Sardinia and Crete, and on peninsula Asia Minor, settling on the territory of modern Spain, France, Italy, Croatia, Greece, Turkey and some other countries, and corresponded to ancestor A3. We named these regions Altai-Middle Asian refugium, Transcaucasian refugium, and South European refugium, respectively.

When glaciers began to retreat, white poplars started to spread outside these refugia. It was the third, Southern European refugium that became the most important for the formation of poplars in the European part of Russia. Migrations (6) from it in the northeastern direction led to the settlement of a significant part of the East European Plain, including the vicinities of Nizhny Novgorod and Uryupinsk. At the same time, steppes (10) in the south of modern Ukraine and the Pre-Caucasus, as well as semi-deserts and deserts (11) in the territory of modern Russia, Kazakhstan and other countries became a natural obstacle to the spread of poplar in certain directions. It penetrated the respective regions only along river valleys (12), such as the Don (12a), Volga (12b), and Ural (12c). From the European part of Russia, poplar migrated further eastward to Western Siberia, making a certain contribution to the gene pool of this region, as can be seen from the results of genomic analysis of samples collected near Novosibirsk in Figure 3. In parallel with this migration, plants from the Black Sea coastline of Turkey reached the Caucasus, when the glaciers retreated from here. This event had an impact on the local genofond, as some of the Caucasian samples are also carriers of European genes.

The main contribution to the gene pool of modern populations of white poplar in the Caucasus comes from the Transcaucasian refugium. When the glacier in the Caucasus receded, trees from Transcaucasia penetrated northward (7), but did not spread further due to steppe and semi-desert zones north of the Caucasus Mountains.

The Altai-Middle Asian refugium may have expanded somewhat (8) after the end of glaciation; the poplars living there are the ancestors of modern Chinese plants and, to a large extent, the poplars of Western Siberia. There was also their migration westward, at least to the East European Plain, since all plants collected in the vicinity of Uryupinsk are carriers of Altai genes.

We separately note that although the plants we collected in the Central Federal District (Moscow and Moscow Region, Obninsk, Tula, etc.) are genetically close to other European populations and also have a certain percentage of Caucasian genes, in these regions, poplars appeared as a result of introduction in the nineteenth and twentieth centuries (9), so this part of their habitat cannot be called natural.

All pyramidal poplars, two of which were collected by us in Kazakhstan and two in CFD, are artificially bred based on European and Caucasian genotypes and are genetically relatively close to each other.

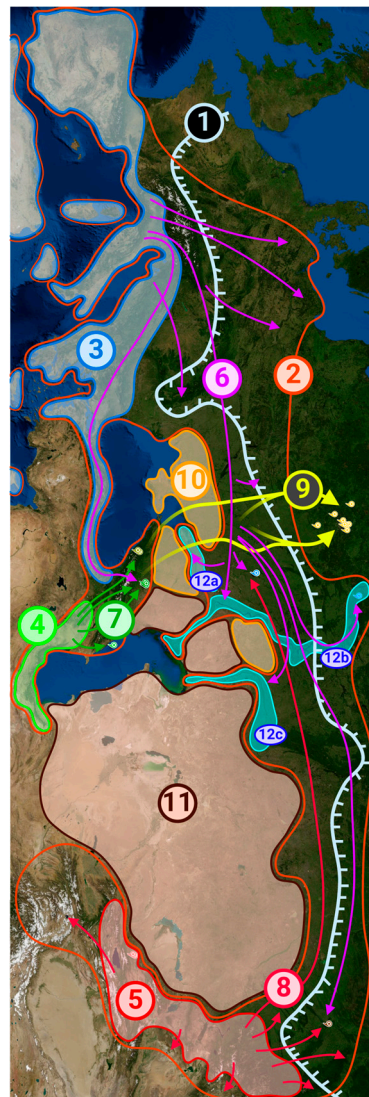


Figure 3. Map of the historical formation of the modern white poplar region in the postglacial era. Lines, areas, arrows and numbers: pale blue (1) - glacier boundary during maximum glaciation; orange (2) - boundaries of the modern natural range of the white poplar; blue (3) - Southern European refugium; bright green (4) - Transcaucasian refugium; red (5) - Altai-Middle Asian refugium; purple arrows (6) - migration routes from the Southern European refugium to the European part of Russia

and then eastward to Siberia, as well as to the Caucasus through northern Turkey; dark green arrows (7) - settlement of the Caucasus Mountains from the Transcaucasian refugium; pink arrows (8) - paths of local range expansion as well as global migration westward from the Altai-Middle Asian refugium; yellow arrows (9) - artificial settlement of the CFD regions (Moscow and its surroundings) by mostly artificially bred poplars on the basis of European and Caucasian genotypes; yellow-orange (10) - steppes (in Pre-Caucasus and southern Ukraine), which are a natural barrier to poplar settlement; brown (11) - semi-deserts and deserts (in Kazakhstan and other Central Asian countries), where white poplars do not live; bright blue (12) - river valleys, where white poplars settle (even if they are surrounded by steppe or desert): 12a - Don, 12b - Volga, 12c - Ural. For ease of location, the map is placed so that the west is facing up. The colors and placement of the labels are the same as in Figure 4.

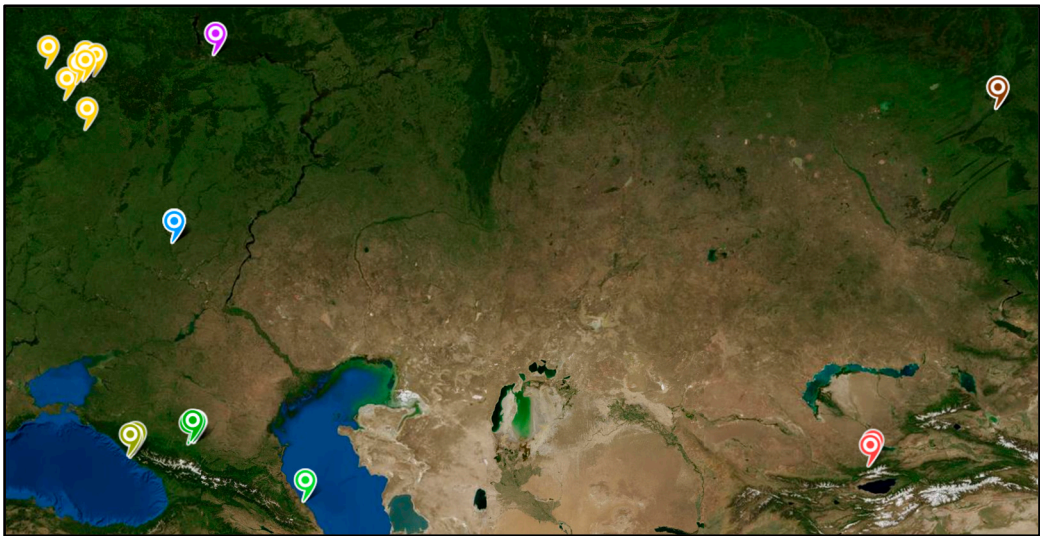


Figure 4. The geographical areas from which samples were obtained for the purposes of this study. Detailed information about the samples, including the growing coordinates of each tree, can be found in Table 1. Color coding of labels: Yellow-Orange - Central Federal District (Moscow and Moscow region, Obninsk, Tula, are collectively named as Center-sX, where X is the unique sample identifier); Blue - Uryupinsk (Southern Federal District, named as South-sX); Purple - Nizhny Novgorod (Volga Federal District, named as Volga-sX); Yellow-Green - Sochi (Southern Federal District, named as Caucasus-S-sX); Green - Pyatigorsk and surroundings (North Caucasian Federal District, named as Caucasus-P-sX); Gray-Green - Republic of Dagestan (North Caucasian Federal District, named as Caucasus-D-sX); Brown - Novosibirsk (Siberian Federal District, named as Siberia-sX); Red - Almaty and surroundings (Kazakhstan, named as Kazakhstan-sX).

Table 1. List of sampled poplar trees, their labeling during the study, coordinates of growth, and assigned sex.

Number	Growing region	Name	Coordinates	Sex of the tree
1	Central Federal District, Moscow	Center-s19	55.793039, 37.556753	female
2	Central Federal District, Moscow	Center-s36	55.830375, 37.547010	male
3	Central Federal District, Moscow	Center-s44	55.665872, 37.589880	male
4	Central Federal District, Moscow	Center-s65	55.668454, 37.584296	male

5	Central Federal District, Ozninsk	Center-s89	55.093246, 36.618053	female
6	Central Federal District, Moscow	Center-s111	55.674906, 37.558021	male
7	Central Federal District, Moscow	Center-s163	55.750744, 38.061924	female
8	Central Federal District, Moscow	Center-s193	55.668355, 37.614051	female
9	Central Federal District, Moscow	Center-s199	55.528115, 37.196700	female
10	Central Federal District, Tula	Center-s245	54.204123, 37.618254	male
11	Central Federal District, Moscow	Center-s128	55.611070, 37.552098	male
12	Central Federal District, Moscow**	Center-s228	55.969725, 35.691320	female
13	Volga Federal District, Nizhny Novgorod	Volga-s9	56.349087, 44.059552	male
14	Volga Federal District, Nizhny Novgorod	Volga-s24	56.346479, 44.061316	male
15	Southern Federal District, Uryupinsk	South-s5	50.790670, 41.977279	male
16	Southern Federal District, Uryupinsk	South-s10	50.813251, 41.992686	female
17	Southern Federal District, Uryupinsk	South-s12	50.811208, 41.988316	female
18	Southern Federal District, Uryupinsk	South-s14	50.810218, 41.979661	female
19	Southern Federal District, Uryupinsk	South-s19	50.785039, 41.985022	*
20	Southern Federal District, Uryupinsk	South-s21	50.787698, 41.985552	female
21	North Caucasus Federal District, Pyatigorsk	Caucasus-P-s1	44.027603, 43.067865	female
22	North Caucasus Federal District, Pyatigorsk	Caucasus-P-s2	44.027049, 43.063490	female
23	North Caucasus Federal District, Pyatigorsk	Caucasus-P-s4	44.027473, 43.063530	*

24	North Caucasus Federal District, Pyatigorsk**	Caucasus-P-s6	44.042940, 42.932274	female
25	North Caucasus Federal District, Pyatigorsk**	Caucasus-P-s17	44.042521, 42.963468	male
26	North Caucasus Federal District, Pyatigorsk**	Caucasus-P-s29	43.972696, 42.789123	female
27	Southern Federal District, Sochi	Caucasus-S-s11	43.616446, 40.052978	male
28	Southern Federal District, Sochi	Caucasus-S-s18	43.563056, 39.793006	male
29	Southern Federal District, Sochi	Caucasus-S-s26	43.491915, 39.896625	female
30	North Caucasus Federal District, Republic of Dagestan	Caucasus-D-s31	41.867246, 48.556359	male
31	North Caucasus Federal District, Republic of Dagestan	Caucasus-D-s20	41.876808, 48.538710	female
32	Republic of Kazakhstan, Almaty	Kazakhstan-s90	43.309556, 76.887625	female
33	Republic of Kazakhstan, Almaty**	Kazakhstan-s54	43.164237, 76.760445	female
34	Siberian Federal District, Novosibirsk**	Siberia-s13	54.820822, 83.120080	male
35	Siberian Federal District, Novosibirsk**	Siberia-s25	54.819091, 83.117505	female
36	Siberian Federal District, Novosibirsk**	Siberia-s200	54.819042, 83.114673	female

* Both female and male generative organs have been found in trees. ** - near this city. Additional info: Siberia-s13 is a triploid hybrid of aspen (*P. tremula*) and white poplar (*P. alba*) obtained by selection from the natural population. Siberia-s25 was accepted by the organization as established on the phenotype of *P. canescens*.

Therefore, we consider Southern Europe, Transcaucasia and the Altai-Middle Asian system as potential refugia where poplar could have survived during glaciations. During glacial retreat, poplar spread to regions suitable for it in terms of climatic conditions. This probably happened repeatedly. Our study is the first hypothesis about the formation of the modern range of white poplar. Further research in this direction involving more data is required.

4. Materials and Methods

4.1. Plant Material

A collection of samples was obtained from 36 trees, representing a range of geographical conditions, for the purposes of the study. The study encompassed a multitude of regions within the European part of the Russian Federation, including the Central Federal District, Volga Federal District, Southern Federal District, and North Caucasus Federal District. Additionally, two specimens were procured from the Republic of Kazakhstan, and three were obtained from the collection of the Central Siberian Botanical Garden of the Siberian Branch of the Russian Academy of Sciences (CSBS SB RAS), situated in the vicinity of the city of Novosibirsk. The geographical distribution of the collected samples is illustrated in Figure 4. To ensure accurate sex determination, samples were collected during the flowering period of poplars.

Branches were cut from the trees and transported for two days before DNA extraction. Leaf material for DNA isolation was collected in laboratory conditions and frozen in 2 ml eppendorf tubes at -70 °C.

4.2. DNA Isolation, Library Preparation, and Whole-Genome Sequencing

For DNA isolation, 0.2 g of leaf material was homogenized using a MagNA Lyser automated homogenizer (Roche, Switzerland) in 1 ml of lysis modified CTAB buffer (100 mM Tris-HCl pH 8.0; 3% CTAB; 3M NaCl; 20 mM EDTA pH 8.0; 1% PVP K30) with 5 µl β-mercaptoethanol and solid beads. The homogenate was incubated at 65°C for 1 h, stirring every 20 min. Two consecutive chloroform purifications were then performed: an equal volume of chloroform was added to the homogenate and centrifuged for 15 min at 10,000 g and 20°C. The aqueous phase was transferred to clean tubes, two volumes of 96% alcohol were added to precipitate DNA and incubated for 60 min at -20°C. Then centrifuged for 20 min at 10,000 g and 4°C. The supernatant was carefully removed without touching the precipitate. The precipitate was washed three times with 70% alcohol and centrifuged for 5 min at 10,000 g and 4°C. DNA was quantified using a Qubit®2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA); quality control was performed on a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA).

The A260/A280 ratio in the DNA samples was 1.8-2.0. Approximately 500 ng of genomic DNA was fragmented into 400 bp double-stranded fragments using the Covaris S220 system (Covaris Inc., Woburn, MA, USA). Double-stranded DNA library was prepared using the VAHTS Universal Plus DNA Library Prep Kit for Illumina V2 (Nanjing Vazyme Biotech Co., Red Maple Hi-tech Industry Park, Nanjing, PRC) according to the manufacturer's recommendations. AMPure XP beads were used to select DNA libraries by size (500-600 bp). Samples were quantified at standard concentration dilution to 4 nM using a Qubit®2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and verified on an Agilent 2100 Bioanalyzer using the High Sensitivity DNA Kit (Agilent Technologies, Santa Clara, CA, USA). Genomic DNA of bacteriophage PhiX was used as an internal control. Sequencing was performed using the NextSeq 500/550 High Output Kit v2.5 (300 cycles) on the Illumina NextSeq 500 platform (Illumina, San Diego, CA, USA) according to the manufacturer's instructions.

4.3. Read Mapping and SNP-Based Analysis

A phylogenetic analysis was conducted based on SNPs profiling, including point SNVs and short insertions/deletions (indels). Reads were mapped to the white poplar reference genome (GCF_005239225) using Bowtie2 [37] with increased sensitivity parameters -D 20 -R 3 -N 0 -L 20 -i S,1,0.50). Secondary (supplementary) alignments were removed with samtools. FreeBayes was used for variant calling [38]. Using per-sample variant allele frequency (VAF) data, principal components analysis (PCA) was performed to identify groups of genetically related samples. Also, the Euclidean pairwise distance matrix between samples was calculated for variant allele frequency (VAF) vectors, and samples were clustered using UPGMA algorithm (with bootstrapping), thus considering the possibility of polyploidy.

The assessment of population mixing was performed using NGSAdmix software, based on the assessment of the probability of genotypes for all SNPs (-K 3) [39]. For the accuracy of the analysis, additional samples of white poplar trees were used, growing in the Xinjiang province of China, as well as in Italy and Hungary. The raw genomic data for these samples was obtained from the NCBI SRA (accession numbers can be found in Supplementary Table 1). These data correspond to several research articles [9,12,40–43].

5. Conclusions

In this study, using a genomic approach and data from different regions of Russia, as well as Italy, Hungary, Kazakhstan, and China, we first hypothesized the formation of the modern range of white poplar. Probably, before glaciation, the range of *P. alba* was uniform and then divided into three refugia: South European, Transcaucasian, and Altai-Middle Asian, meaning that poplar was preserved in places where glaciers did not occur. After the glaciers retreated, populations from these refugia gave rise to modern poplar populations in Europe, the Caucasus, and Siberia, respectively, which eventually spread and restored the integrity of the area. To confirm and refine this hypothesis, further studies aimed at increasing the number of samples and expanding the study areas are needed.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Supplementary Figure S1 depicts the phylogenetic tree corresponding to the PCA plot in Figure 1. Supplementary Figures S2, S3, S4, and S5 show NGSAdmix results with $k = 2, 4, 5$, and 6 , respectively. Supplementary Table S1 contains information about reference *P. alba* genomes used in our study.

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