Genetic Differentiation of *Pinus sylvestris* L. Populations in the Caucasus and Adjacent Regions

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***Abstract**:*

An analysis of the allozymic structure and differentiation (Nei distances, DN_{78}) for 20 Pinus sylvestris L. populations in the Caucasus and adjacent phylogeographic regions has been performed. It has been shown, that groups of pine populations in the Large Caucasus and the Crimea, on the one hand, and in the Little Caucasus, the Pontic Mountains, the Balkans and the Russian Plain, on the other hand, are differentiated at the level of geographic groups of populations (almost geographic races). The differentiation between the populations in the North Caucasus and in the Transcaucasus as well as between the populations in the Large Caucasus and in the Crimea is at the level of populations. The average Nei's distances (0.017–0.024) between the pine population in the Russian Plain, the Caucasus and the Crimea, which are not higher, than a level of geographical population groups, prove, that all fall into the system of the species Pinus sylvestris L. The hypotheses of the East-Asian origin of the Caucasian P. sylvestris and of the existence of the "Large Euxinic circle" their migration ways have been supposed.

Key Terms: *Pinus sylvestris, Caucasus, population, migration, allozyme differentiation, phylogeny.*

Introduction:

One of the key problems in modern evolutionary biology of woody plants is the exploration of chorogenetical structure and differentiation of natural populations in different parts of the range. A progress of systematics, ecology, microevolutionary theory as well as optimization of measures on the protection and the use of gene pool in the forestry depends, in many respects, on the solution of this problem.

Regional peculiarities of the genetic structure of Scotch pine (*Pinus sylvestris* L.) populations in many separate regions of European and Asian parts of the range have been studied earlier by using of allozymic and DNA methods (Yazdani and Rudin, 1982; Gullberg et al. 1985; Petrova et al. 1989, 2005, 2011; Prus-Glowacki et al. 1993; Goncharenko et al. 1993; Sannikov and Petrova 2003, 2012; Sinclair et al. 1999; Soranzo et al. 2000; Cheddadi et al. 2006; Naidenov et al. 2007; Ćelepirović et al. 2009). The most differentiated chorogenetic structure of this species caused by heterogeneity of the factors of landscape paleogeography, phylogeny and microevolution of populations has been revealed in Euxine floristic province, especially in the Carpathians, the Caucasus and in the Crimea (Petrova et al. 2005; Sannikov and Petrova 2012).

Taxonomic status of *Pinus* species of SYLVESTRES subsection inhabiting the Caucasus and the Crimea had not been synonymously determined during almost all the 20th century. Some taxonomists related them to *Pinus sylvestris* L. species on the base morphophenotypic (ecologically modifiable) traits of "standard" herbarium specimens (Shaw 1914; Critchfield and Little 1966), but the others, until very recently, to the Euxine species of *Pinus kochiana* Klotzsch. ex C. Koch. or *Pinus armena* Klotzsch. ex C. Koch. (Bobrov 1978; Orlova 2001). Preliminary allozymic analyses, which we had carried out before (Sannikov and Petrova 2003; Petrova et al. 2005) revealed a negligible average genetic distance between Central Russian and Caucasian populations (DN_{78} = 0.024±0.005) and showed that the last ones undoubtedly belonged to the system of *Pinus sylvestris* L. species.

The aim of this article is to analyse and to generalize tentatively the results of allozymic exploration of structure, geographical differentiation and to determine the taxonomic status of *Pinus sylvestris* L. populations in the Caucasus and adjacent phylogeographical regions.

Materials and Methods:

The investigations have been carried out on 11 *Pinus sylvestris* mountain populations in three phylogeographical regions of the Caucasus (fig. 1): the North Caucasus (Arkhyz, Teberda, North Osetiya, Gunib); the Transcaucasia (Tuapse, Ritsa, South Osetiya, Belokany) and the Little Caucasus (Borjomi, Stepanavan, Tauz). The parameters of allozymic structure of these populations have been compared with the analogous parameters of populations in the adjacent phylogeographical regions of the Russian Plain (Belgorod, Kiev, Moscow, Belaya Kalitva) and of the Asia Minor (Trabzon, Bartin), as well as with the supposed refugium in the Balkan Mountains (Pirin, Rila). On the whole 20 population samples have been analysed (fig. 1).

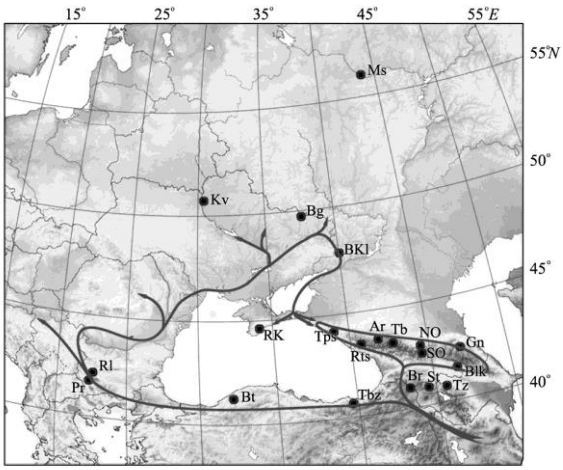

Fig. 1. Distribution of populations samplings of *P. sylvestris* L. in the Caucasus and in the adjacent phylogeographic regions.

Population codes: RK – Roman-Kosh, Arkh – Arkhyz, Tb – Teberda, SO – Northern Osetiya, Gn – Gunib, Tp – Tuapse, Rz – Ritza, Bj – Borjomi, St – Stepanavan, Tz – Tauz, Tbz – Trabzon, Bt – Bartin, Kv – Kiev, Bg – Belgorod, Msc – Moscow.

The allozyme analysis of buds tissues of the lateral shoots of 30–48 trees in each of the populations has been performed for 16 protein loci (including 14 polymorphous ones) of 11 enzyme systems (ADH, GDH, 6-PGD, SKDH, PGM, DIA, GOT, FDH, SOD, SOR, EST-f) according to generally accepted methods (Korochkin et al. 1977). Nei's inbiased genetical distances (Nei 1978, DN_{78}) between local populations were calculated by using the package of BYOSYS programmes (Swofford and Selander 1981). A cluster analysis (UPGMA) has been performed by means of the NTSYS package (Sneath and Sokal 1973). Taxonomic rank of the populations has been determined by using of genosystematic scale which was constructed in accordance with the generelized data of 240 populations samples from the territory of the entire range of *P. sylvestris* (Sannikov and Petrova 2012).

Results and Discussion:

Maximum genetic distances DN_{78} between *Pinus sylvestris* populations in the Caucasus and adjacent regions according to the accepted genosystematic scale do not exceed, in most cases, the level of the geographic race (0,026 – 0,045).

The degree of the mean horogenetic differentiation of populations of the Caucasus and boundary regions is presented by the dendrogram of Nei genetic distances (fig. 2). The group of populations of the Large Caucasus including the North Caucasus (Arkhyz, Teberda, North Osetiya, Gunib), Transcaucasia (Tuapse, Ritsa, South Osetiya, Belokany) and as well as the sample from the Crimea (Roman-Kosh) adjoining them separates distinctly – at the level of strong subdivided geographical group (almost geographical race, DN_{78} = 0.023–0.024) – from the two other groups: the Little Caucasus (Borjomy, Stepanavan, Tauz) and the Russian Plain (Kiev, Moscow, Belgorod, Belaya Kalitva) together with populations closely related to it from the Balkan Mountains (Pirin, Rila) and samples from Asia Minor (Trabzon, Bartin). On the whole the high level of gene pool differentiation of the population groups of the Large Caucasus from the Little Caucasus, on the one hand, and from the Russian Plain,

on the other hand, probably, is due to the long-time influence of different migration barriers: sea as well as mountain and phytocenotic (Sannikov and Petrova 2003).

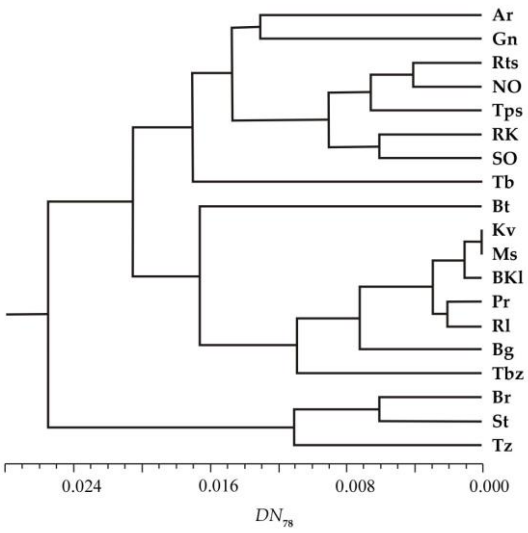

Fig. 2. Dendrogramma of genetic distances of Nei (DN_{78}) between *P. sylvestris* populations in the Caucasus and the adjacent regions. Populations codes see on fig. 1.

Extremely low differentiation of *Pinus sylvestris* populations in the Russian Plain (Kiev, Moscow, Belaya Kalitva) from the Balkan ones (DN_{78} = up to 0,004) as well as their differentiation (that is by an order of magnitude the greatest, DN_{78} – 0,019–0,056) from among the populations of Asia Minor, Apennines, and Iberia (Sannikov and Petrova 2012) allow to suppose that namely the Balkan Mountains is one of their origin centre. The influence of the Balkan refuge which was connected with Anatoliya by the Bosporus neck in glacial phases had an effect on the gene pool of the populations in the Asia Minor (Bartin, Trabzon) subdivided from the Balkan ones at the level of DN_{78} not higher than 0.010–0.016. The local pine populations within the limits of separate phylogeographic region of the Caucasus are differentiated with DN_{78} up to 0.011 with the exception of a small sample from the west part of Asia Minor (Bartin, 0,016).

Thus, the cardinal border of the differentiation of all the populations of *Pinus sylvestris* in the research regions is situated between the Large Caucasus and the Little Caucasus in the zone of valleys of the Kura river and the Riony river. Probably, already since Pleistocene a zone of broad-leaved forests (a “phytocenotic barrier”) which had originated there in the place of the Tertiary sea strait connected Euxine basin with the Caspian sea prevents reliably flows of genes between the pine populations in the southern spurs of the Large Caucasus and in the northern slopes of the Little Caucasus (Sannikov and Petrova 2003).

The mountain island populations of three phylogeographic regions – the Crimea, the North Caucasus and the West Caucasus - within the limits of their regions are differentiated rather poorly: on average, at the level of subpopulation or population (DN_{78} – 0.004–0,014; fig. 2), which is almost half less as between nearly absolute isolates (lacking gene connectivity) in the Ukrainian Carpathians (DN_{78} = 0.021, Sannikov et al. 2011). *Pinus sylvestris* populations within the limits of the Little Caucasus are differentiated (DN_{78} = 0.006–0.011) a little less than within the West Caucasus (DN_{78} = 0.006–0.014) but little more than “microevolutionary young” settlements of *P. sylvestris* in the Russian Plain (DN_{78} up to 0.007).

The hierarchycal analysis of DN_{78} among the groups of six phylogeographic regions showed that mean intergroup differentiation of *P. sylvestris* populations of the Little Caucasus (Borjomi, Stepanavan, Tauz) with the populations of the North Caucasus and the West Transcaucasia reach the level of well-separated geographical races (DN_{78} = 0.031–0.036) and with the populations of the central part of the Russian Plain only a rank of geographical group of populations of (DN_{78} = 0.023–0.024). Thus, this entirely confirms the conclusion which we have made before (Petrova et al. 2005), that there are no population-genetical reasons for distinctinguishing in the Euxine floristic province special “Crimean-Caucasian” species of *Pinus kochiana* Klotzsch. ex C. Koch. or *Pinus armena* Klotzsch. ex C. Koch. (Bobrov 1978; Orlova 2001).

Phylogenetic Wagner tree of *P. sylvestris* populations in the Caucasus and in the adjacent regions constructed by method of

rooting at midpoint of longest path (Nei 1987) can be interpreted as follows (fig. 3).

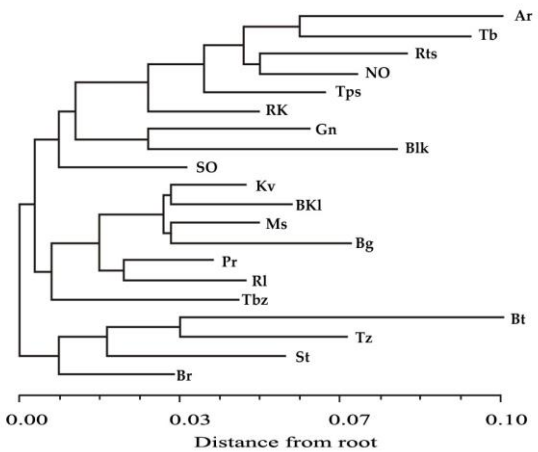

Fig. 3. Phylogenetic tree of *P. sylvestris* populations in the Caucasus and the adjacent regions constructed by the method of rooting at midpoint of the longest path (Nei, 1987). Populations codes see on fig. 1.

Taking into account the data of paleogeography and and flora history (Wulf 1944; Mirov 1967; Goncharova and Cherba 1997; Lomolino et al. 2005) and the greatest phenogenetic similarity of *P. sylvestris* with the species of SYLVESTRES subsection growing near by the south-east of its range *Pinus densiflora* Siebold et Zucc., *P. funebris* Kom. (Sannikov and Petrova 2003, 2012) one can suggest that the Eastern Asia (Japan, North-East China or Northern Mongolia) was the ancestral centre of its origin**.** Populations of *P. sylvestris* may have gradually migrated from the East Asia into the Europe and further into Asia Minor by using two alternative hypothetical ways: 1) along the northern shores of Tethys Sea (Mirov 1967); 2) along the mountain chains of the Central Asia (Altai–Tien Shan–Pamirs–Hindu-Kush) during the Middle Miocene. Greater average genetic distances (DN_{78} = 0.052; Sannikov and Petrova 2012) between the pine populations along the south way rather than along the north ones (DN_{78} = 0.008–0.020) show, that last migration way is more probable. Possibly, *P. sylvestris* could disperse from the Russian Plain onto the Balcans and further into the Asia Minor trough the land necks in the Black Sea and Bosphor Strait which existed during the Middle Miocene.

At the end of the Miocene and in the Pliocene, when the sea strait between Euxine and Caspian basins disappeared, probably two alternative directions of migrations and divergence of *P. sylvestris* populations from the Little Caucasus have been realized (fig.1):

1. The western trend – over the Pontic Mountains (Trabzon, Bartin) onto the Balkan Peninsula (Pirin, Rila) which presented the centre of further fan-shaped dispersal of *P. sylvestris* into the Central Europe, Scandinavia and onto the Russian Plain (Kiev, Belgorod, Moscow, Belaya Kalitva), and served as its refuge in the Pleistocene (Ćelepirović et al. 2009; Sannikov and Petrova 2012).

2. The northern trend – along the watershed of the Kura and the Riony rivers into the Transcaucasian (South Osetiya, Belokany, Ritsa, Tuapse), the North Caucasus (Gunib, North Osetiya, Teberda, Arkhyz) and the Crimea (Roman-Kosh). Migration of *P. sylvestris* to the north was hindered here during the Pliocene and the Pleistocene by the strong disjunction of its islands populations and the vigorous phytocenotic barriers formed by broad-leaved forests between the Little Caucasus and the spurs of the Large Caucasus.

In glacial phases of the Pleistocene *Pinus sylvestris* could settle from the Balkans to the north and the north-east over the eastern foothills of the Carpathians (Grichuk 1982) and further along sand terraces of the Dnieper river and its left tributaries (Psyel, Vorskla) to the north and south of the Russian Plain (s. fig. 1). The Azov Sea, salty soils and swamps of the Manytch depression as well as the Sarmat-Pontic sea earlier undoubtedly hindered the immigration of *P. sylvestris* populations to the Caucasus and into the Crimea from the Russian Plain (Mirov 1967). Nevertheless, one can suppose on the basis of the theory of the conifers hydrochory (Sannikov and Sannikova 2008) that in the glacial phases they could periodically penetrate right up to Kerch Straight and further into the Crimea and onto the Caucasus by means of seeds dispersal with the stream of the Don river or along the drained littorals of the Azov Sea. Thus, on the whole the “Large Euxin circle” of migrations

and gene flows of *P. sylvestris* populations was closed: the Little Caucasus – the Pontic Mountains – the Balkans – the Russian Plain – the Crimea and the Large Caucasus.

Conclusions

1. Horogenetic structure of *P. sylvestris* populations on the Caucasus and in the adjacent phylogeographic regions has been differentiated in the high degree that is conditioned by heterogeneity of the factors of paleogeography and flora history in the Euxine floristic province.

2. Analysis of geographical structure of *P. sylvestris* populations in the Large Caucasus and the Crimea, on the one hand, and of the Little Caucasus, the Russian Plain and the Pontic Mountains, on the other hand, has revealed a high level of their genetic differentiation – on average at the level of well subdivided geographic group of populations (almost geographic race).

3. Genetic differentiation between the population groups of the North Caucasus and of the southern slopes of the Main Caucasus ridge as well as between the populations group of the Large Caucasus and the Crimea is on average at the level of subdivided populations.

4. Degree of the populations differentiation within the limits of the separate phylogeographic regions of the Caucasus is somewhat as less as between these regions

5. The main borderline of the genetic differentiation of *P. sylvestris* populations in the Caucasus is the zone of the former sea strait and modern broad-leaved forests in the valleys of the Kura river and the Riony river between the Large Caucasus and the Little Caucasus.

6. All the populations groups of *Pinus* species of the SYLVESTRES subsection in the Crimea and the Caucasus (with the exception of *P. pityusa* Stev., *P. pallasiana* D. Don., *P. eldarica* Medv.) are differentiated genetically from the populations of *P. sylvestris* in the Russian Plain at a level which is not higher than that of a geographic group of populations (DN_{78} = 0.017–0.024) and fall into the system of this species. There are no basis to distinguish special "Crimean-Caucasian" species of *Pinus kochiana* Klotzsch. ex C. Koch. and *Pinus armena* Klotzsch. ex C. Koch.

7. Analysis of the phylogenetic tree and the possible migrations of *P. sylvestris* populations in the Large Caucasus and in the adjacent phylogeographical regions allows us to suppose the community of the ancestral centre of their origin in the East Asia and the existence of the "Large Euxinic circle" of their migration in the Pleistocene.

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