Comparative analysis of variability of morphological characteristics of European and Japanese larches with their hybrids

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Abstract. Hybridizing European larch with Japanese larch A S Yablokov and M I Dokuchaeva pointed to their better silvicultural and decorative characteristics compared to their parent species. Moreover, E G Bobrov described Larix marschallinii hybrid as a faster-growing species, he also recommended this hybrid larch for afforestation in many countries. In this study, the vegetative and generative organs of European and Japanese larch hybrids are compared with their parent species. Such characteristics as the cones length, the cones width, the number of scales in the cones and the needles length were considered. A comparative analysis of larch trees DNA was also carried out. As a result of the study, we found that by morphological characteristics, the hybrids occupied an intermediate position between the parent species, except for the length of the needles. The spectra samples electrophoregram of larch total DNA indicated some similarity between hybrids and parent species, meanwhile, hybrids have their own distinctive features.

1. Introduction

Many larch species form vigorous hybrids and extended hybrid zones at the junction of their habitats. Most larch species with artificial hybridization produce viable seedlings. Therefore, artificial hybridization of larch, such as European larch (Larix decidua Mill.) and Japanese (Kampfer) larch (L. Kaempferi (Lamb.) Carriere), which habitats are distant from each other, is of great both scientific and practical interest. The European larch covers Central Europe, while Japanese larch has a limited and, moreover, disjunctive habitat in central Japan (Honshu Island) [1].

European larch does not tolerate inundation. Nevertheless, it shows good growth on calcareous, crystalline, phyllite, podzolic, black-earth and rank soils. Japanese larch tolerates cold and dry climate, it is resistant to late spring frosts. Better than any other larch it tolerates shading. In plantations it grows well on podzolic and black-earth soils it also grows better on fresh and thick clay and sandy soils [2].

European larch cones are ovoid-conical or oblong-ovate, 2-4 (up to 6) cm long, 2-2.4 cm in diameter, the cones have the scales located in 6-8 rows. The needles are light green, narrow-linear, soft, 10–40 mm long [2, 3]. Japanese larch cones are 2-3.5 cm long, ovoid, blunt which ripen in October-November. The needles are 15-40 mm long, soft and blunt-pointed [2].
Capper O G [2] indicates that when breeding Japanese larch, one should consider that it needs areas with a coastal climate. In the conditions of the European part of Russia, this larch begins to slow down its growth from 16–20 years of age, while it grows faster being close to the Atlantic Ocean [2].

According to E G Bobrov [4], West European dendrologists have a particular interest in Japanese larch because of its great tolerance in Europe, among other things, Japanese larch hybrids are one of the most common species in parks and arboretums [4].

Among Japanese larch hybrids, some authors including E G Bobrov emphasize Larix marschlinsii Coaz. It is a hybrid of European and Japanese larches and, according to E G Bobrov, it was first described in 1917 in Switzerland. This hybrid attracted so much attention that in 1919 it was described three more times in different countries [4]. This species is considered to be faster growing than a parent species. In the morphological structure it combines the characteristics of both parental species. Larix marschlinsii is recommended for afforestation in many countries.

The hybrids of European larch and Japanese larch were obtained by A. S. Yablokov and M. I. Dokuchaeva in the Ivanteyevsky Dendrological Garden. When describing these hybrids (Yablokov A.S. et al.) [5] it was indicated that “hybrid larch can be widely introduced into forest plantations and forest park zones. The average trunk volume of hybrid larches is 31-41% more than the volume of Siberian larch. In addition to silvicultural properties, larch has highly decorative features such as tender emerald and fragrant needles in spring, which are bright green in summer, in October the trees turn copper-red, the soil in the alley is covered with light yellow fallen soft needles. The alley at this time seems to be transparently-laced and light” [5]. In addition, hybrid larch gives frequent and abundant seed yields with high soil germination [5].

Thus, interspecific hybrids are of great interest in order to create decorative stands; among other things, some authors give hybridization processes important evolutionary significance [6-8]. With a successful combination of parental species, hybrids have wider possibilities of adaptation to various environmental conditions [9-11]. When woody plants are hybridized, the incidence of mutations increases [9, 12], which also has a certain evolutionary significance according to I Yu Koropachinsky and L I Milyutin (2006) [9].

The relevance of the hybrids study is also connected with the fact that “Hybrids of woody plants often differ in greater vitality compared to species” [9].

The aim of this research was to study European and Japanese larches hybrids by their morphological characteristics and to compare hybrids with parent species.

The scientific novelty of this work is the research of larches introduced in the center of the European part of Russia.

2. Methods and Materials
The following objects were studied:

- European larch has been planted in the Moscow at the N V Tsitsin Main Botanical Garden (MBG) since 1953. 38 trees were grown from seeds obtained from Kurnik (Poland). Trees at the age of 15 years old have an average height of 7.6 m, trunk diameter of 8-9 cm [13].

- Dunkeld larch (Larix marschlinsii Coaz; L. kaempferi (Lamb.) Carr. x L. decidua Mill.) is a hybrid of European larch and Kampfer (Japanese) larch and is also introduced in the Moscow Main Botanical Garden.

- Hybrids of European larch and Japanese larch in the Ivanteyevsky dendrological garden are located in block 13, sections “a”, “b” and “c” in the form of an alley. At the age of about 30 years, the height of the trees is 17-18.5 m. the diameter of the trunks is 27-34 cm [5].

- Japanese larch in the Ivanteyevsky Dendrological Garden is located in the southern part of the site. Its homeland is Japan (Honshu Island). It was introduced in the autumn of 1936 by 6-year-old seedlings grown in the Timiryazev Agricultural Academy. The average height of the trees at the time of the study at the age of 40 years is 19 m with a trunk diameter of 24-35 cm. The trees have a wide, regular-shaped crown [5].
Japanese larch has been in the MBG since 1953. 6 samples (49 specimens) were grown from seeds obtained from South Sakhalin. The tree at the age of 36 years old has a height 21.6 m and a trunk diameter 39.0-51.5 cm [13].

The quantitative characteristics of cones and larch needles were investigated, namely the length of the cones, the width of the cones, the number of scales in the cones and the length of the needles. The coefficient of variation (V%) was estimated by the S A Mamaev scale of variability characteristics [14, 15]. Also, when assessing variability, the arithmetic mean value of the attribute (Xav) and the standard error of the arithmetic mean of the attribute (Sx) are presented.

Student’s t criterion was calculated to check the significance of the difference between the obtained average values between European larches and their hybrids.

3. Results and Discussion
Variability of morphological characteristics is presented in the tables 1-4.

Table 1. Comparative analysis of larch variability by the length of needles.

| Species and the place of samples | European larch | Hybrid of European and Japanese larches | Larix marschlinsii | Japanese larch from the Ivanteyevsky dendrological garden | Japanese larch from MBG |
|----------------------------------|----------------|----------------------------------------|-------------------|----------------------------------------------------------|-------------------------|
|                                  | Xav, mm        | Sx, mm                                 | Xav, mm           | Xav, mm                                                  | Xav, mm                 |
| European larch                   | 31.6 ± 0.70    | 22.2                                   | 18.5 ± 0.44       | 19.0 ± 0.25                                              | 18.8 ± 0.67             |
| Hybrid of European and Japanese larches | 30.8 ± 0.40   | 22.5                                   | 15.7 ± 0.25       | 17.5 ± 0.43                                              | 18.8 ± 0.67             |
| Larix marschlinsii               | 22.0 ± 0.40    | 21.0                                   | 25.9 ± 0.37       | 20.2 ± 0.43                                              | 21.3 ± 0.41             |
| Japanese larch from the Ivanteyevsky dendrological garden | 15.7 ± 0.33    | 19.2                                   | 52.1 ± 0.59       | 56.5 ± 0.57                                              | 46.3 ± 1.06             |
| Japanese larch from MBG          | 18.8 ± 0.67    | 43.9                                   | 20.2 ± 0.43       | 21.3 ± 0.41                                              | 21.2 ± 0.45             |

Table 1 shows that on average the European larch needles are longer than that of the hybrids, and that Japanese larch needles are shorter. On this basis, hybrids occupy a middle position but closer to Japanese larch. Meanwhile, the characteristics vary at an average and high level.

The Student’s t-test revealed that between the majority of the studied species, the differences along the length of the needles are significant. However, the differences between the Japanese larch from MBG and its hybrids growing in the Ivanteyevsky dendrological garden are insignificant (t = 0.37). The most significant differences are observed between European larch and Japanese larch, which grow in the Ivanteyevsky dendrological garden (t = 21.4).

Table 2. Comparative analysis of larch variability by morphological features of cones.

| Species and the place of samples | Cones length | Cones width | Number of scales in cones |
|----------------------------------|--------------|-------------|---------------------------|
|                                  | Xav, mm      | Sx, mm      | V%                        |
| European larch                   | 33.1 ± 0.64  | 23.7        | 19.0 ± 0.37               |
| Hybrid of European and Japanese larches | 30.8 ± 0.40  | 15.8        | 20.2 ± 0.43               |
| Larix marschlinsii               | 26.5 ± 0.59  | 22.4        | 35.3 ± 0.78               |
| Japanese Larch, from the Ivanteyevsky dendrological garden | 25.1 ± 0.33  | 13.3        | 42.6 ± 0.34               |
| Japanese larch from MBG          | 22.9 ± 0.54  | 23.5        | 21.1 ± 0.45               |

Tabelle 2 zeigt, dass die durchschnittlich die Nadeln der Europäischen Lärche länger sind als die der Hybriden, und dass die Nadeln der japanischen Lärche kürzer sind. Auf dieser Grundlage, nehmen die Hybriden ein mittleres Position, aber nahezu Japanische Lärche. Zwischen den untersuchten Spezies, die Unterschiede längs der Nadeln sind signifikant. Jedoch, die Unterschiede zwischen der Japanischen Lärche von MBG und seine Hybriden, die wachsen in der Ivanteyevsky dendrologische Garten, sind unwesentlich (t = 0.37). Die meisten signifikanten Unterschiede sind beobachtet zwischen Europäischen Lärche und Japanische Lärche, die wachsen in der Ivanteyevsky dendrologische Garten (t = 21.4).
Thus, as for the length of the cones, the average length of cones in European larch is higher than in hybrids, and in Japanese Larch it is lower. On this basis, hybrids from the Ivanteyevsky dendrological garden are closer to European larch, and *Larix marschlinsii* is closer to Japanese larch.

According to Student’s t-test, the differences along the length of the cones are significant between all the studied larches. The closest along the length of the cones were Japanese larch and *Larix marschlinsii* (*t* = 2.1), and the most significant difference appeared between Japanese larch and European larch (*t* = 11.1).

The cone width among the samples was the most levelled characteristic. Hybrids of European and Japanese larches showed the highest indices on this characteristic, and Japanese larch from the Ivanteyevsky dendrological garden showed the lowest indices.

Assessment of the difference between the average values revealed that the differences between Japanese larch and *Larix marschlinsii* are not significant. This conclusion was confirmed both by the studies carried out in the Ivanteyevsky dendrological garden (*t* = 1.9) and in MBS (*t* = 1.45). Between the rest of the samples, the differences between larches concerning the cone width are significant. Moreover, European larch differs from Japanese larch in the cone’s width less significantly than in other characteristics (*t* = 8.7). Therefore, it is difficult to identify the degree of difference between hybrids and parental species on this basis. The peculiarities of this characteristics are related to the fact that the width of the opened cone depends both on the length of the scales and on the angle of their deviation from the cone’s axis.

The highest average number of scales in cones was observed in the hybrids of European larch and Japanese larch. The lowest indicators for this characteristic are in *Larix marschlinsii*. Moreover, it shows a different degree of variation from low to a high level. Other morphological characteristics of the cones vary at an average and high level.

The Student’s t-test revealed significant differences in the number of scales in the cones for all the studied species. In this case, the most significant differences appeared between hybrid larches (*t* = 21.94), as well as between the European larch and *Larix marschlinsii* (*t* = 17.18).

The revealed significant differences in the studied morphological characters indicate not only differences between European larch and Japanese larch, but also the relative isolation of their hybrid offspring.

We conducted a comparative assessment of European larch and Japanese larch hybrids and their original parental forms based on DNA analysis.

The study was carried out in the genetics laboratory of the Federal budget institution “Russian Center for Forest Protection” of the Federal Forestry Agency of the Ministry of Natural Resources and Ecology in the Russian Federation.

Objects of study were the following:
- specimen No 1 – Japanese larch, tree No 1 (MBG);
- specimen No 2 – Japanese larch, tree No 2 (MBG);
- specimen No 3 – Japanese larch, tree No 3 (MBG);
- specimen No 4 – Japanese larch, tree No 1 (Ivanteyevsky dendrological park);
- specimen No 5 – Japanese larch, tree No 2 (Ivanteyevsky dendrological park);
- specimen No 6 – Japanese larch, tree No 3 (Ivanteyevsky dendrological park);
- specimen No 7 – a hybrid of European and Japanese larches, tree No 1 (Ivanteyevsky dendrological park);
- specimen No 8 – a hybrid of European and Japanese larches, tree No 2 (Ivanteyevsky dendrological park);
- specimen No 9 – a hybrid of European and Japanese larches, tree No 3 (Ivanteyevsky dendrological park);
- specimen No 10 – European larch, tree No 1 (MBG).

The results of these samples DNA analysis are presented on electrophoreograms according to primer 6 (figure 1), primer 85 (figure 2), primer 11 (figure 3) and primer 26 (figure 4).
Figure 1. Electrophoregram of the larch spectra specimen total DNA of primer 6.

According to this electrophoregram, all hybrids are similar to each other and have some common special features different from their parent species and specimens 7 and 8 are almost identical.

Japanese larch shows a common identity between the specimens, while being quite variable on this primer.

Interestingly, in primer 6, Japanese larch from MBG (specimen No 3) is quite similar to European larch (specimen No 10).

Figure 2. Electrophoregram of the larch spectra specimens total DNA of primer 85.
This electrophoregram reveals noticeable differences in the loci of DNA in Japanese larch with hybrids, however, there is a similarity between European larch and its hybrids. A particularly strong similarity to European larch is shown by hybrid tree No 1 (specimen No 7).

![Figure 3. Electrophoregram of the larch spectra specimen total DNA of primer 11.](image)

This electrophoregram again shows the similarity of hybrids with parent species. The greatest similarity of hybrid trees with Japanese larch (specimen No 5 and No 6).

![Figure 4. Electrophoregram of the larch spectra specimen total DNA of primer 26.](image)

The electrophoregram primer 26 shows the similarity of parental species both among themselves and with their hybrids.
Thus, DNA analysis shows the similarity of hybrids with their parent species. Moreover, European larch is quite different from Japanese larch in most primers, which confirms their different taxonomic status.

4. Conclusion
Considering the variability analysis of the European larch and its hybrids, we found that European larch is superior to its hybrids with Japanese larch and Japanese larch in the length of the needles and the length of the cones. At the same time, hybrids occupied an intermediate position according to these characteristics, although the hybrids were closer to Japanese larch in the length of the needles.

In terms of the number of scales the situation is quite different, hybrids of Japanese and European larches have a greater number of scales than both parents; consequently, a heterotic effect can be assumed. On the contrary, in Larix marschlinisii, which is the offspring of hybrid generations, the number of scales is the smallest, that is, we can talk about depression on this basis. Such effects, as a rule, are observed during hybridization of fairly distant species, which is normal considering the spatial distance and isolation of species of European and Japanese larches. All differences on this basis between European and Japanese larches and their hybrids were significant.

The following general conclusion can be drawn from the genetic analysis of Japanese, European larches and their hybrids:

- a fairly high individual polymorphism in Japanese larch should be noted, which was studied in six samples;
- there is a relatively high similarity between the three samples of the studied hybrids, while it is possible to assume that the samples of hybrids 7 and 8 are the offspring of one parent;
- in hybrid plants, according to different primers, there is a fairly high similarity with both parent species, both with European and Japanese larches. At the same time, by the nature of electrophoregrams, hybrids are isolated from parental species and have common characteristics among themselves.

In general, electrophoregrams show the genetic similarity of European larch, Japanese larch and their hybrids within a high individual polymorphism. This confirms the relative historical young age of the genus Larix Mill., that is, the paleontologically relatively recent beginning of the differentiation of the original ancient stem (germinative) species and the formation of the genus. On the example of Larix, we can see a certain rather early stage of the genus formation. Natural area growth has led to differentiation, which is an excellent illustration of classical adaptive radiation. Thanks to it, taxa which are morphologically and genetically still very close to each other began to isolate. Therefore, in hybrids a number of morphological characters occupy an intermediate position between parental forms. At the same time, hybrids occupy extreme positions in terms of the number of scales, which indicates that European and Japanese larches have reached a high genetic isolation and claim the status of independent taxa in the species rank.

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