Study of natural hybridization between *Alnus incana* (L.) Moench. and *Alnus glutinosa* (L.) Gaertn.

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ABSTRACT: Variation of metric and qualitative characteristics of *A. incana* (L.) Moench. and *A. glutinosa* (L.) Gaertn. has been studied in 10 natural populations in West Siberia, Russia and the Czech Republic in connection with the problem of natural hybridization. Morphological peculiarities of the species and their spontaneous hybrids are shown. Twelve leaf characteristics were used, in addition, qualitative characteristics were assessed, such as: type of bark, degree of pubescence of leaves and stems, and presence of a "tuft" in the angles of leaf veins. The reasons for hybridization of these species are discussed.

Keywords: *Alnus* Mill.; natural hybridization; variation; population

Natural hybridization of woody plants has been the objective of research of the specialists all over the world. This phenomenon is typical of a range of genera and study of it allows to solve a great number of theoretical and practical problems.

There are enough data on existence of interspecific hybrids in the genus *Alnus* Mill. s.l. Hybrids between different species of alder are indicated for North America, Europe, the Russian Far East and Japan (Hylander 1957; Mizushima 1957; Steele 1961; Furlow 1979a,b; Bobrov 1980; Bousguet et al. 1989, and others).

Natural hybrids between *A. incana* and *A. glutinosa* are known in Belarus, Latvia, Poland, the Czech Republic, Sweden, Ireland and some other countries. They possess many economically valuable properties. They are characterized by greater drought resistance compared to parent species, less demand for fertility (Kobendza 1956; Kundzinsh 1957), heterosis, high physical-technical properties of wood (Pirags 1962) and heightened resistance to pythium rot (Fér, Šedivý 1963).

In this paper, the morphological characteristics of the species and their hybrids is analyzed, based on actual measurements of intraspecific variation in *A. incana* and *A. glutinosa*, combined with the literature analysis. Possible reasons for hybridization are discussed.

MATERIAL AND METHODS

Alnus glutinosa (L.) Gaertn. 1791, Fruct. et Sem., 2:54

Tree, to 30 m tall and 40 cm diameter; bark dark-brown, cracked.

Young leaves sticky; mature leaves as a rule glabrous, with tufts of hairs in the angles of veins, obovate, round, emarginate or rounded at tip, with broad-cuneate base, (4)5–8(9) cm long and (3)5–7(8) cm wide, with 6–8 pairs of secondary veins, leaf stalks 20–35 mm long. Staminate inflorescences, 3 to 5 in a cluster, hanging, 4–7 cm long; mature infructescences, 3–5, on stalks (Fig. 1).

Sites: lake shores, floodplains, grassy bogs, near springs, roadsides.

Area: broad natural range that includes most of Europe and extends into North Africa, Asia Minor, and western Siberia

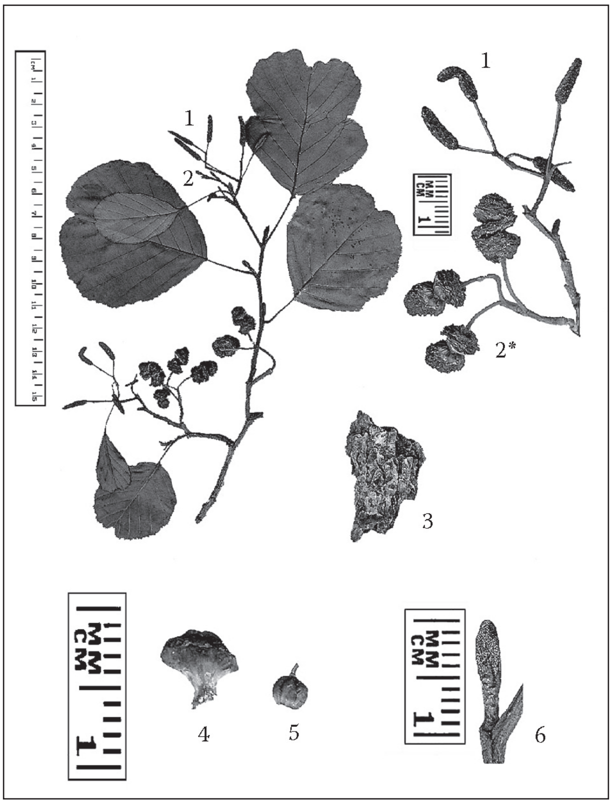

Fig. 1. *Alnus glutinosa*: 1 – staminate inflorescences, 2 – pistillate inflorescences, 2* – mature infructescences, 3 – bark, 4 – cover scale, 5 – fruit, 6 – winter bud. Locality Konyashinskaya – Russia, Tyumen Oblast, motorway Tyumen-Turinskaya Sloboda, 5 km from v. Konyashina, terrace of the Tura River

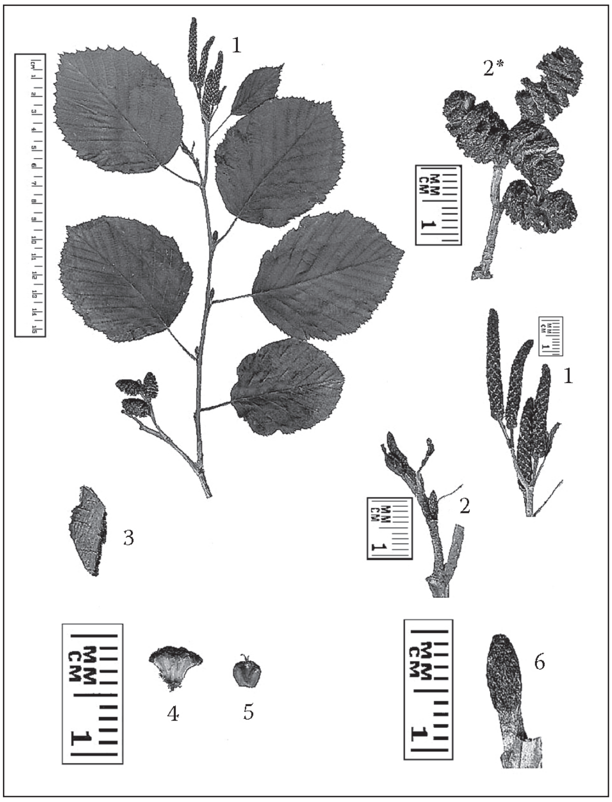

Fig. 2. *Alnus incana*: 1 – staminate inflorescences, 2 – pistillate inflorescences, 2* – mature infructescences, 3 – bark, 4 – cover scale, 5 – fruit, 6 – winter bud. Locality Konyashinskaya – Russia, Tyumen Oblast, motorway Tyumen-Turinskaya Sloboda, 5 km from v. Konyashina, terrace of the Tura River

Chromosome number: 2n = 28 (Chromosome numbers … 1969).

Alnus incana (L.) Moench. 1794, Meth.: 124

Coarse shrub or tree, to 20 m tall and 50 cm diameter, bark smooth, gray or brown. Mature leaves egg-shaped to elliptic, commonly with sharp-pointed tip and broad-cuneate, rounded or slightly heart-shaped base, densely haired to glabrescent below, (4)5–9(11) cm long and (3)4–7(8.5) cm wide, with (9)11–13(15) pairs of secondary veins; stalks 10–30 cm long; mature infructescences commonly sessile, 3–5 (10 and more) in a lateral cluster (Fig. 2).

Sites: streambanks, temporary watercourses along the roads, moist sites at the foot of upland, grassy bogs, felled and burnt areas, abandoned meadows and plough-lands.

Area: North, Middle and Southeast Europe, boundary of West Asia: West Siberia, Caucasus, Lebanon.

Chromosome number: 2n = 28 (Chromosome numbers … 1969).

Material for the study has been collected in 1996, 1998, and 2003 from six populations of *A. incana* and four populations of *A. glutinosa* on the eastern border of the species areas in West Siberia, Russia, and in the Czech Republic (Table 1). Leaves were collected from 15–20 individuals at each population. 5 shoots were taken at lower, middle, and the top part of crown of each tree. 10 leaves were measured from each part. Hybrids were disclosed in populations 4, 6, and 10. The methods of material collection and the measurement procedure followed the work by Banaev and Shemberg (2000).

A leaf is one of the main organs involved in plant functioning. In this regard its characteristics are subjected to a considerable pressure of selection. At the same time, leaf habit plays a crucial role in the systematic of the genus *Alnus* Mill. s.l. This is reflected in rather high resistance of its characteristics to modifying influence of the environment.

In addition, qualitative characteristics were assessed, such as: type of bark, degree of pubescence of leaves and stems, and presence of a "tuft" in the angles of leaf veins. The degree of pubescence was determined on a 5-point scale: glabrous (full absence of hairs) – 0; singly haired (separately standing hairs) – 1; sparsely haired (hairs are scattered on the surface) – 2; densely haired (hairs cover 50% of the surface) – 3; and eriophyllous (hairs cover 100% of the surface) – 4.

Table 1. Sites of material collection

No.	Species	Population	Locality
1	*Alnus incana* (L.)	Kyshtyrlinskaya	Tyumen Oblast, Yalutorovsky Region, vicinities of v. Vinzili, verge of the road
2		Dolmatovskaya	Kurgan Oblast, intersection of the motorway Shadrinsk-Dolmatovo with the Suvarysh River
3		Golovinskaya	Tyumen Oblast, Yalutorovsky Region, vicinities of v. Golovino, terrace of the Pyshma River
4		Konyashinskaya	Tyumen Oblast, motorway Tyumen-Turinskaya Sloboda, 5 km from v. Konyashina, terrace of the Tura River
5		Bobrovskaya	Sverdlovsk Oblast, Baikalovsky Region, vicinities of v. Pelevino, floodplain of the Bobrovka River
6		Lipnovská	the Czech Republic, Šumava Mts. (Bohemian Forest), vicinities of Černá v Pošumaví, Lake Lipno
7	*Alnus glutinosa* (L.)	Kyshtyrlinskaya	Tyumen Oblast, Yalutorovsky Region, vicinities of v. Vinzili, floodplain of the Kyshtyrlinka River
8		Raskatikhinskaya	Kurgan Oblast, Glyadyansky Region, vicinities of v. Raskatikha, the Chernaya River
9		Golovinskaya	Tyumen Oblast, Yalutorovsky Region, vicinities of v. Golovino, terrace of the Pyshma River
10		Lipnovská	the Czech Republic, Šumava Mts., vicinities of Černá v Pošumaví, Lake Lipno

Twelve leaf characteristics were used:

1. length of a leaf blade (A, mm)
2. width of a leaf blade (B, mm)
3. length of a leafstalk (I, mm)
4. number of pairs of lateral veins (N)
5. distance from the base of a leaf blade to its widest part (D, mm)
6. leaf width at the tip (E, mm)
7. upper angle of a leaf (W, degree)
8. lower angle of a leaf (H, degree)
9. leaf coefficient (B/A)
10. D/A
11. I/A
12. E/B.

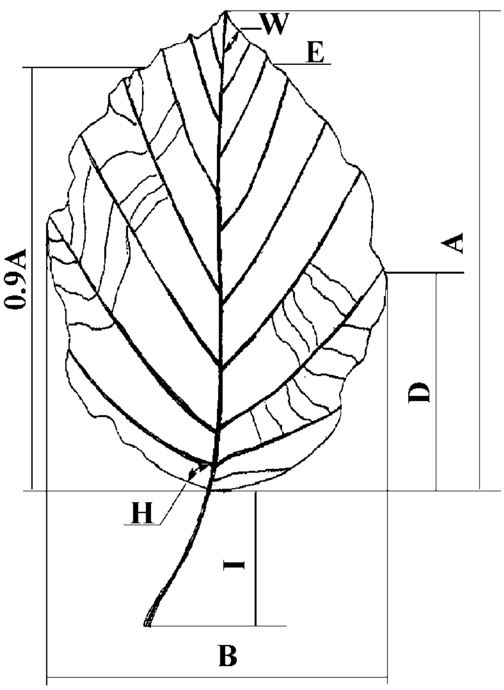

Fig. 3. Leaf characteristic

Endogenous (variation of metamers within an individual) and individual (intrapopulation) forms of variation were analyzed.

The method of major components applied in the study of plan natural hybridization (Adams 1982; Wilson 1992, and others) was used for the identification of spontaneous hybrids and the analysis of their variation.

RESULTS AND DISCUSSION

Endogenous variation

Qualitative characteristics (pubescence of leaves and young stems) of the crown of the species studied are distinguished by a rather high stability. Absence of variation of the degree of leaf pubescence within a young stem is typical for the majority of *A. incana* specimens. Insignificant increase in pubescence of the lower leaf side towards the young stem apex was found in only few individuals (about 5%). However, maximum differences amount to 2 points. For instance, lower leaves are glabrous and upper ones are sparsely haired. The leaves in all specimens studied of *A. glutinosa* had hairs in the angles of the veins – "tufts". This characteristic is stable at the endogenous level.

Metric characteristics change uniformly. For instance, the finest leaves are at the base of young stems. They increase gradually in size along the length of the stem towards the apex. However, the upper leaves are smaller. There is a high correlation between the absolute characteristics at the endogenous level; it is especially common to A, B and D. Length of a leafstalk and a number of pairs of lateral veins are related to a lesser extent to the size of a leaf blade. The correlation between absolute characteristics of an alder leaf testifies to great genetic determination of the leaf shape, lesser dependence on internal conditions of organ formation (e.g., stem size) and greater resistance to modifying influence of the environment (e.g., intensity of natural illumination). In addition, relative characteristics (B/A, D/A) of the crown vary to a lesser extent than absolute A, B and D. Variability of characteristic I/A is comparable to that of the length of a leaf blade, but it is always lower than variability of the length of a leafstalk. The least variation at the endogenous level is typical of the leaf coefficient, number of pairs of lateral veins and D/A.

Intrapopulation variation

Sufficiently high stability of pubescence is typical for *A. glutinosa*. Densely haired or eryophillous forms were found in none of the populations. In most of specimens, leaves and young stems were glabrous or singly haired. Only in Kyshtyrlinskaya population about 20% of specimens were sparsely haired on the upper side of leaves. All plants had "tufts" in the angles of the veins.

Eryophillous forms were not found in *A. incana* either. Densely haired specimens occur very seldom.

Table 2. Variation of morphological characteristics of *A. incana*, *A. glutinosa* and their hybrids

Characteristic	*A. incana*	*A. glutinosa*	Hybrids
	lim / *x*	lim / *x*	lim / *x*
A	42–105 / 70	50–90 / 68	61–80 / 70
B	32–87 / 53	44–85 / 61	50–67 / 58
B/A	0.56–0.95 / 0.77	0.75–1.09 / 0.91	0.82–0.85 / 0.84
I	12–30 / 19	18–35 / 26	16–24 / 14
N	9–15 / 12	6–8 / 6.5	9–10 / 9.7
D	21–62 / 35	30–56 / 40	36–40 / 37
E	10–40 / 19	30–60 / 42	32–35 / 33
I/A	0.18–0.38 / 0.28	0.27–0.49 / 0.37	0.24–0.3 / 0.28
E/B	0.19–0.56 / 0.35	0.55–0.81 / 0.67	0.51–0.7 / 0.59
D/A	0.38–0.61 / 0.50	0.5–0.7 / 0.61	0.5–0.59 / 0.54
W	30–80 / 49	93–160 / 125	70–90 / 80
H	45–105 / 69	45–85 / 61	50–85 / 67

Symbols of the characteristics are in the text, lim – extreme value of a characteristic, *x* – mean value of a characteristic

Table 3. Correlations between characteristics and principal components

Characteristic	1st component	2nd component	Characteristic	1st component	2nd component
A	0.31	–0.94	E	–0.93	–0.15
B	–0.40	–0.80	I/A	–0.77	0.15
B/A	–0.80	0.13	E/B	–0.92	0.15
I	–0.63	–0.46	D/A	–0.86	0.23
N	0.94	–0.16	W	–0.94	0.02
D	–0.49	–0.71	H	0.47	–0.18
Share of component influence				57%	21%

Plants with sparsely haired leaves (up to 90%) and young stems (57–100%) prevail in most of populations. Forms with glabrous and eryophillous stems are sometimes found in the populations. There is often no correlation between pubescence of different surfaces of leaves and stems, which apparently testifies to independence of inheritance of the characteristic. There is not any association of pubescence of vegetative organs with peculiarities of the habitats of *A. incana*.

Most of quantitative characteristics used easily differentiate the species studied. For instance, mean population values of the leaf coefficient differ significantly. In *A. incana* it is 0.75–0.79 and in *A. glutinosa* – 0.91–0.92; I/A – 0.27–0.28 and 0.37–0.39, respectively; E/B – 0.34–0.36 and 0.66–0.68; D/A – 0.49–0.52 and 0.58–0.62. However, these characteristics overlap in separate specimens of the species. Transgression may reach 40% and more. Such characteristics as number of vein pairs and upper leaf angle are species specific (*A. incana* – 9–15 and 30–80 degrees, *A. glutinosa* – 6–8 and 90–160 degrees, respectively) (Table 2).

A factor analysis of characteristics performed in populations with participation of hybrids showed that almost 80% of variation fell on the first and second components (Table 3). There is the closest relationship between the first principal component and N, W, E and E/D characteristics and a rather high correlation of the former with D/A, B/A, I and I/A characteristics. The species studied are separated in the plane of the first two principal components (Fig. 4). By a complex of characteristics hybrids hold an intermediate position or are closer to *A. incana*.

It is rather complicated to describe hybrids as there is a diverse combination of characteristics. For instance, the specimens with the leaf blades typical of *A. incana* may have "tufts" in the angles of veins and cracked bark characteristic of *A. glutinosa*. Moreover, the leaves closer by their shape and some other features to both species occur on the same young stem (Fig. 5). A similar situation was also noted by different researchers (Yurkevich et al. 1963). One of the distinctive and sufficiently stable properties of the hybrids is a number of pairs of leaf veins – 9–10.

According to data of Kundzinsh (1969), the bark of hybrids raised as a result of artificial crossing is greenish- or brownish-gray, remains smooth with age. Separate dark-gray longitudinal cracks up to 1 m long and 3–4 cm wide may appear on the stem at the age of approximately 20. Leaves are somewhat hairy,

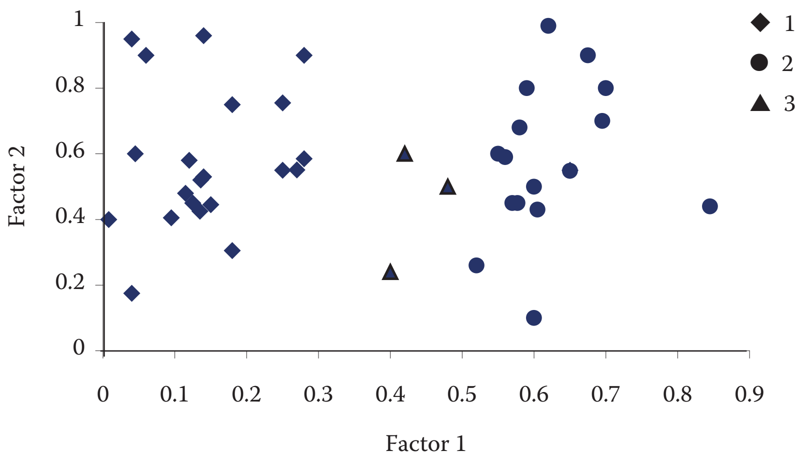

Fig. 4. Distribution of the alder specimens from "hybrid" populations in the plane of the first two principal components (factors): 1 – *A. glutinosa*, 2 – *A. incana*, 3 – hybrids

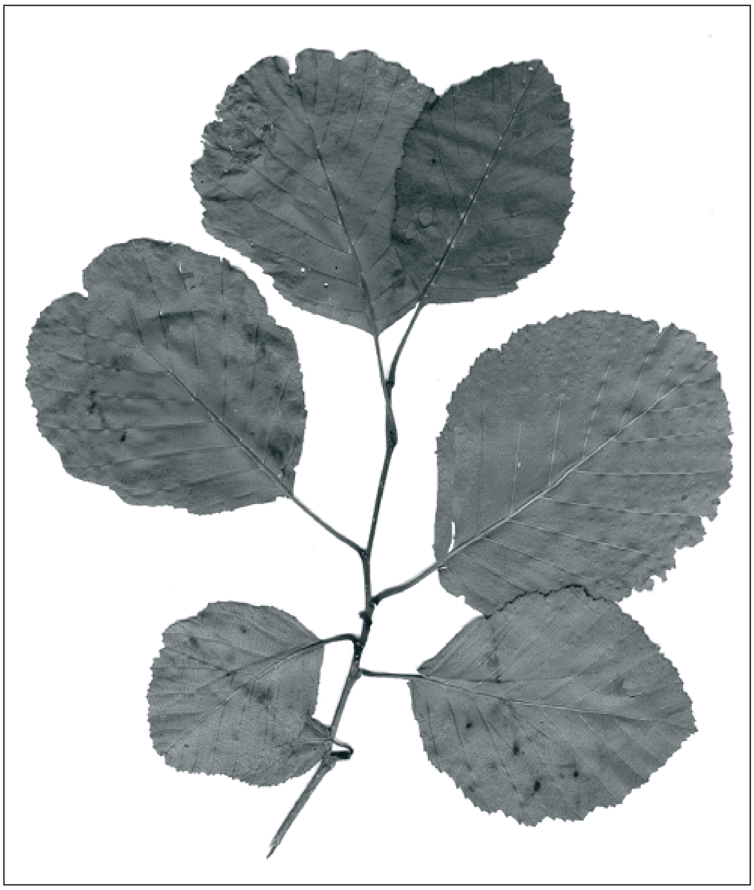

Fig. 5. A specimen of hybrid between *A. incana* and *A. glutinosa*. Locality Lipnovská, the Czech Republic, Šumava Mts. (Bohemian Forest), vicinities of Černá v Pošumaví, Lake Lipno

not shining, yellowish-coloured below. Usually there are 9 pairs of lateral veins. As a rule, hybrids intermediate by size, shape, pubescence of leaves, habit and bark color are in mid-position by time of the onset of certain phenophases, e.g., blooming and leafing.

Parnell (1994) indicated their great affinity with *A. incana* when studying morphological peculiarities of natural hybrids between *A. incana* and *A. glutinosa* in Ireland. Variation of separate characteristics such as length of a leaf blade and angle between the central and lateral veins exceeds variation in both parents. A number of vein pairs in hybrids is distinguished by high stability and on the average amounts to 10.5. A great quantity of glands on the abaxial leaf side is typical for the hybrids compared to both species. In *A. incana*, on the average, 1–3.2 glands occur on 1 mm^2 of the surface chosen by chance; in *A. glutinosa* – about 0.4; and in hybrids – 3–9 glands. Catkins of hybrids are more slender and pointed (Fér, Šedivý 1963).

However, hybrid forms do not often occur, especially in the area of continuous distribution of the species. The main reason for it is considered to be the absence of coincidence of flowering stages (Yurkevich et al. 1963; Parfenov 1980). In Belarus, for instance, *A. incana* begins to blossom, on the average, 6 days earlier than *A. glutinosa*, which provides complete pollination of the plants by proper pollen, although flowering periods of the species overlap. As V.P. Parfenov notes, probability of these species crossing increases in anomalous years, e.g., in those with cold prolonged spring. Moreover, an increase in frequency of hybridization is noticeable on the borders of the areas and in local "island" populations. The author attributes this peculiarity to presence of specific conditions for flowering and pollination phases, as well as to the fact that polymorphic species in adapting are capable to change their structure and pattern of many biological processes.

An analysis of phenological data on *A. incana* and *A. glutinosa* when introducing them beyond their natural ranges allows to conclude that plants keep species specificity for a long time, at least in the first generation. So at the Altai experimental station (Gorno-Altaisk, Russia) *A. incana* began to blossom on the average 7 days earlier than *A. glutinosa*, and flowering periods of the species did not coincide for 1–2 days (Luchnik 1974). By our data, in Central Siberian Botanical Garden, SB RAS (Novosibirsk, Russia) the end of flowering of *A. incana* falls as usual on the beginning of flowering of *A. glutinosa*. Separate samples of *A. incana* due to their biological peculiarities or under influence of microclimatic conditions were in blossom at one time with *A. glutinosa*. Usually *A. incana* begins to blossom a week earlier than *A. glutinosa*.

Apparently besides phenological peculiarities preventing hybridization between *A. incana* and *A. glutinosa*, there are some other mechanisms of their isolation, as these species grow together in a large area in different climatic conditions. The tests on artificial hybridization provide support for it. All researchers engaged in this problem note a difficulty of hybridization between *A. incana* and *A. glutinosa*. For instance, KUNDZINSH (1968) indicated that successful hybridization was only observed in that case when *A. incana* was used as a maternal plant. Hybridization went unrewarded when *A. glutinosa* was a maternal plant. Seeds obtained in the process of hybridization gave viable seedlings, but the quality of seeds was low. Germination amounted to only 12–13%. VÁCLAV (1963) obtained hybrids between these species using 5-year individuals as maternal trees and 25 to 60-year ones as paternal plants. By his data, when crossing young plastic hybrids, the seeds obtained were of high germinating ability and gave late heterotic generation.

Our study has shown that hybrids between *A. incana* and *A. glutinosa* occur very seldom in both European part and West Siberia – on the eastern border of the species areas. Based on the literature, we can say about somewhat greater frequency of hybridization in the northern boundary of their areas (Latvia, Ireland and others).

Hybridization between *A. incana* and *A. glutinosa* should be assigned to the type "B" by MAYR (1974), i.e., more or less fruitful hybrids are sometimes formed between sympatric species, a part of the hybrids cross with one or both parental species.

CONCLUSION

Spontaneous hybridization is observed between *A. incana* and *A. glutinosa*. Hybrids occur sporadically in the area and very rarely in the zone of continuous distribution of the species. Some increase in frequency of hybridization is noted by the northern boundary of the species areas, which is possibly connected to their migration due to the change of climate. Hybrids, as a rule, hold an intermediate position between the species or are closer to the maternal individual (*A. incana*) in complex morphological traits. Great diversity of combinations of characteristics of parental species, even on the young stem of the same tree, is observed in hybrids. One can identify hybrid forms by a number of pairs of leaf veins, leaf coefficient, upper angle of the leaf blade and a range of other absolute and relative characteristics.

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Studie přirozeného křížení olše šedé a olše lepkavé

ABSTRAKT: Na území Ruska (západní Sibiř) a České republiky byla v deseti přirozených populacích olše lepkavé a olše šedé porovnávána proměnlivost vybraných morfologických znaků s ohledem na možnosti přirozené hybridizace obou druhů. Pro porovnávání bylo vybráno 12 znaků na listech a hodnoceny byly další kvalitativní znaky – typ kůry, stupeň ochlupení listu a přítomnost chomáčků v úhlech žilek listu. Dále jsou zde posouzeny možné příčiny hybridizace daných druhů a zhodnoceny morfologické charakteristiky hybridů a jejich proměnlivost.

Klíčová slova: *Alnus* Mill.; přirozená hybridizace; proměnlivost; populace

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