

Natural Hybridisation within Elms (*Ulmus* L.) in Lithuania

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Abstract

Putative natural hybrids (*Ulmus* × *hollandica*) between the Smooth-leaved (*Ulmus minor* ssp. *minor*) and the Wych elm (*Ulmus glabra*) were observed in mixed forests along the rivers and rivulets around the central part of Lithuania. Eleven populations of elms (*Ulmus* L.) were studied to determine 1) the critical groups of phenotypes indicative for their taxonomic identity, 2) the variability of taxa from contact zones. Three characteristics for the trees and twenty one for their leaf, including nineteen applied by WinFOLIA 2004a programme, were used to describe each sample of the fifty eight and to ascertain the degree of affinity between samples. As a result of this approach, four taxa could be distinguished at the contact zones within elms. Three elm species and hybrids, *Ulmus glabra*, *Ulmus minor*, *Ulmus laevis*, and *Ulmus* × *hollandica*, were fairly satisfactorily distinguishable, but there is much overlap between the Wych elm and the Field elm in their characters that can be explained only by the equivalence of their statistically discriminant phenotypic distinctness. On average, the putative natural hybrids between the Smooth-leaved and the Wych elm account for less than two percent of all individuals within group *U. minor* – *U. × hollandica* – *U. glabra*. The possibility of introgressive hybridisation is shown by the outstanding variability of morphological characteristics of the taxa from the contact zones and by the presence of extreme or novel properties of hybrids.

Key words: *Ulmus minor* ssp. *minor*, *Ulmus glabra*, *Ulmus minor*, *Ulmus* × *hollandica*, *Ulmus laevis*, natural hybridisation

Introduction

The most generalised treatment of the complex taxonomical nature of European elms (Richens 1983, Armstrong and Sell 1996), at least in continental Europe, considers the presence of three species in this continent, the European white elm (*Ulmus laevis* Pallas), the Wych elm (*Ulmus glabra* Hudson ≈ *Ulmus montana* Stokes) and the Field elm (*Ulmus minor* Miller *sensu latissimo*), the latter represented by a number of varieties, and some hybrids (Richens 1983, Mitterpergher and La Porta 1991, Mackenthun 2007). Some authors (e.g. Richens 1983) consider it a single variable taxon (the species concept adopted in this study), whereas others have proposed narrower concepts involving three species (Stace 1997), five species (Melville 1975) to over 40 species (Armstrong 1992). The Smooth-leaved elm (*U. minor* ssp. *minor*) is the commonest type of the Field elm in continental Europe today (Richens 1983, Mackenthun 2007). *U. campestris* var. *glabra* Hartig, Planchon, Ascherson and Graebner, *U. campestris* var. *laevis* Spach, Planchon, *U. foliaceae* Gilibert, Sargent (also known as the Mulberry-leaved elm), *U. glabra* (not Hudson) Miller,

Smith, Loudon, Reichenbach, Wilkomm, Schneider, Ley, and *U. nitens* Moench are the synonyms of *U. minor* ssp. *minor*. In Europe, this subspecies has a more southerly distribution than *U. glabra*, and is unknown in Denmark, Sweden, and Norway as a wild tree, though it is said to occur, possibly planted, on the island of Gothland, in the Baltic (Elwes and Henry 1913). Natural hybrids between the Wych elm and the Smooth-leaved elm are common in Europe, as these two species were the most widely planted in the countryside, with the former in northern areas (Scandinavia, northern Britain), and the latter further south. In addition, the Wych elm is sometimes divided into subspecies (Uotila 1997, Myking and Yakovlev 2006), of which the *glabra* (in the south of the species' range) has leaves that are relatively broad, short tapering, with acute lobes present, trees often have a short, forked trunk and a low, broad crown, the *montana* (Stokes) Lindquist (in the north of the species' range – northern Britain, Scandinavia) has leaves that are relatively long, long tapering, without acute lobes, the upper surface of which are strigose (Sherman-Broyles 2007), trees commonly have a long single trunk and a tall, narrow crown. The subspecies are not accepted

by *Flora Europaea* (Tutin et al. 1968), as there is much overlap between populations in these characters and the distinction may be owing to environmental influence, rather than genetic variation.

Hybridization is an important mechanism for producing “evolutionary novelty” and is considered to be a common mode of speciation in plants (Abbott 1992, Futuyma 1998). Morphological data can offer actual information on hybridization related evolutionary and ecological phenomena, because hybrids are not always intermediate between the parental species, but often exhibit extreme and novel characters (Rieseberg and Ellstrand 1993). The percentage of plants with extreme or novel characters increases in later hybrid generations as does the percent of characters that show extreme. Hybrids often show characteristics that are absent from the parents (Rieseberg 1995). At least two independent markers are needed to demonstrate that introgression is occurring, and at least one of them must be able to define the two parental species (Harrison 1990). In the case of *Ulmus laevis*, a major advantage for detailed studies is the absence of interfertile species within the distribution area, removing the complicating factors of hybridisation and introgression. For this reason, *U. laevis* was taken as ‘datum-species’ in this study. Along with the American elm (*U. americana*), *U. laevis* belongs to the *Blepharocarpus* section which seems genetically isolated from the other sections of the genus (Heybroek 1968, 1993, Townsend 1975), whereas the other two European elm species, *U. glabra* and *U. minor*, belong to the subgenus *Ulmus*. *U. laevis* is the oldest elm species in Lithuania (Stockmarr 1970).

It is customary, in sexually-reproducing organisms, to distinguish between species and interspecific hybrids, the latter being usually few in numbers compared with the putative parent species. The genus *Ulmus*, however, is atypical in that, therefore the subdivision of the genus into species must rest entirely on the discovery of discontinuities (Richens 1955). The approach taken here follows the lines being drawn by R. Richens (1983), who followed a fairly simple strategy. He claimed that there are just two species of elms present in the British Isles, *U. glabra* and *U. minor*, and both species hybridise to form the natural hybrid elm, *Ulmus* × *hollandica* Mill. Unfortunately, every individual within the *U. minor* – *U. × hollandica* – *U. glabra* group is able to hybridise again with one of parental species. The process may be continuously repeated from generation to generation and is called introgressive hybridisation. It is suggested that the most probable proximate outcome of such hybridization will be a collapse of hybridizing species and subsequent loss of biodiversity (Seehausen et al.

2008). Decreasing landscape heterogeneity at all spatial scales from microhabitats to biomes most likely elevates the general likelihood of interspecific introgressive hybridization by relaxing ecological divergent selection among species and/or by removing ecological barriers to gene flow between divergently adapted species. By introgression species may acquire new adaptive characters that allow them to colonize new habitats or increase their fitness in their existing niche (Minder et al. 2007). The phenomenon of hybrids having higher fitness than one or both of the parents is known as hybrid vigour or heterosis. The effects of introgressive hybridization among pure and hybrid phenotypes on a morphological variability of the taxa has not yet been studied in native elms. Therefore, the main purpose of the present study was to characterize the phenotypic variability of native elms and to determine 1) the critical groups of phenotypes indicative of their taxonomic identity, 2) the variability of taxa from contact zones. The novelty of the study is presented by the determination of critical group of elm hybrids indicative of its taxonomic identity.

Materials and methods

Morphological Characterization of Elm Taxa

Morphological characters were the main tools used for the identification of elm hybrids in this study. Wych elms generally share a set of characters as follows: stout densely villous petioles, exceeding 2-7 mm in length, the leaf base being strongly oblique with the lowermost lobe strongly overlapping, covering the petiole, leaves equally rough on the upper surface, though rather downy beneath, the leaf margins with few acuminate lobes at a broad apex, the blade having 15 to 18 (20) pairs of lateral nerves, which are often forked up near the edges of the leaf, no corky wings on the twigs, and the absence of sucker shoots. The Wych elm (*U. montana* Stokes) shows little variation in the wild state, but trees growing in dense woods usually bear smaller and thinner leaves than those which stand in the open (Elwes and Henry 1913). The common name Wych comes from Anglo Saxon meaning “with pliant branches” or from Gaelic meaning “drooping”. The name *glabra* means smooth, a probable reference to the fact that the bark of this tree is smoother than that of the Field elm. The common names of the Field elm, the Smooth-leaved elm, or the Narrow-leaved elm (see Mittempergher 1996), contrast the smooth upper surface and narrowness of the leaves with those of the Wych elm, which are rough and broad (Stace 1997, White and More 2003). But it has to be remembered that the juvenile leaves in Field elms are scabrous, and it would probably require only a slight

physiological change for this character to persist to the adult condition (Richens 1955). In this study atypical Wych elms having a long single trunk with smooth bark and the very few leaves with some acute lobes and no more than 16 pairs of lateral nerves were attributed to atypical Wych elms (or non-Dutch elms) or to the subspecies of the Wych elm (*U. glabra* ssp. *montana* (Stokes) Lindquist). The Field elm is supposed to be easily recognizable because of its thick crown of branches that begin low on the long single trunk and the leaves without acute lobes and no more than 8-11 (14) pairs of lateral nerves, having white hair on the back of the leaf blades where the veins meet. The Smooth-leaved elm (*U. minor* ssp. *minor*) was identified by corky ridges on young twigs – so called ‘winged twigs’. This subspecies is variable in the wild state, in the amount of pubescence on the branchlets and leaves, and in the presence or absence of corky ridges on the twigs and branches (Elwes and Henry 1913). Leaves are very unequal at the base and acuminate at the apex. Their margins are biserrate and non-ciliate; lateral nerves have about 12 pairs and are often forked. It was hypothesized by us that Smooth-leaved elms with corky bark should have more pairs of leaf lateral nerves in comparison to those with smooth bark. The European white elm is easily recognized by its leaf that has a single peak and a very unequal basis, with strongly curled leaf-teeth with a double cerrulation, and 12-13 (16) pairs of lateral nerves, 1-2 (0-4) of which may be forked (Hempel and Wilhelm 1889, Elwes and Henry 1913, Ozolinčius 2003, Petrokas and Pliūra 2007). No corky wings on the branchlets of this species (Elwes and Henry 1913). Unshapely leaf of natural hybrids between the Wych elm and the Smooth-leaved elm (i.e. Dutch elms, *Ulmus* × *hollandica* Mill.; Heybroek 1987; Figure 1) is somehow similar to that of European white elms. It is

curly, without acute lobes, the base being very unequal, leaf-teeth with a double-triple cerrulation, but having more than 14 pairs of lateral nerves, which are often forked up near the edges of the leaf. Dutch elms may almost always be recognised by the large corky ridges, which are developed only on the epicormic branches of the trunk (Elwes and Henry 1913). In the Cork-barked elm (*U. minor* ssp. *minor* var. *suberosa* Henry ≈ *U. suberosa* Moench = *U. campestris* var. *suberosa* Wahlenberg), which is the variety of the Smooth-leaved elm, the branchlets in the crown of trees are all corky.

Study Sites and Plant Material

Putative natural hybrids between the Smooth-leaved elm and the Wych elm were observed in mixed forests along the rivers and rivulets around the central part of Lithuania. The research sites of the native elms have been chosen to grasp their coexistence at natural habitats, normally moist or temporarily wet particularly fertile sites, preferably where the taxa are occupying the same or overlapping areas. During the mid-summer of 2011, randomly chosen fully expanded leaves from mature shoots were collected. In total, it was evaluated 399 leaves (5-7 for each individual) from 58 elms from 11 localities (Table 1).

Data on putative natural hybrids between native elms were obtained from transects of three independ-

Table 1. Elms (*Ulmus* spp.) summary. *t* – number of trees, *n* – number of leaves, *l* – % of leaves measured by the Win-FOLIA 2004a programme

Taxon	Forest population	Latitude	Longitude	Tree No.	<i>t</i>	<i>n</i>	<i>l</i>
<i>Ulmus minor</i>	Sušvė	55°12'	23°50'	1, 2, 3, 4, 6			
	Juodkiškiai	55°17'	24°02'	13, 14, 16	10	70	94.3
	Eleonoravas	55°09'	23°03'	44, 45			
<i>U. minor</i> ssp. <i>minor</i>	Juodkiškiai	55°17'	24°02'		15		
	Mikytai	56°07'	21°50'		40	5	33
	Dūkstyna	55°16'	24°50'		18, 19		
	Eleonoravas	55°09'	23°03'		46		
<i>Ulmus glabra</i>	Kunioniai	55°12'	23°47'		8, 10, 12		
	Šilas	55°15'	24°15'		21, 22, 23, 24, 26		
	Turloviškės	54°47'	24°27'		28, 30, 31		
	Juodė	54°54'	25°08'		33, 34	20	137
	Dūkštos	54°50'	24°57'		37, 38		
	Mikytai	56°07'	21°50'		39, 41, 43		
	Eleonoravas	55°09'	23°03'		47		
<i>U. glabra</i> ssp. <i>montana</i>	Juodgiris	55°29'	24°42'		54		
	Turloviškės	54°47'	24°27'		29		
	Juodė	54°54'	25°08'		32	6	40
	Dūkštos	54°50'	24°57'		36		
<i>Ulmus</i> × <i>hollandica</i>	Eleonoravas	55°09'	23°03'		48, 49, 50		
	Sušvė	55°12'	23°50'		5		
	Kunioniai	55°12'	23°47'		7, 9, 11		
	Šilas	55°15'	24°15'		25, 27, 56	13	91
	Dūkštos	54°50'	24°57'		35, 57, 58		
<i>Ulmus laevis</i>	Mikytai	56°07'	21°50'		42		
	Eleonoravas	55°09'	23°03'		51, 52		
	Dūkstyna	55°16'	24°50'		17, 20	4	28
	Juodgiris	55°29'	24°42'		53, 55		

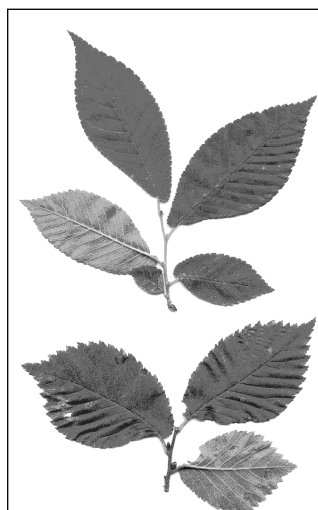


Figure 1. The Dutch elm – ‘Belgica’ (above) and ‘Groeneveld’ (below). Photo by Leo Goudzwaard

ent contact zones at the Šilas, Eleonoravas, and Dūkštos forests (Figure 2, Table 2).

Table 2. The approximate figures of elms at the contact zones of Lithuanian forests

Forest	Site types*	Transect size, m	Elms in total	Number of trees per hectare		
				Field elms	Hybrids	Wych elms
Šilas	Ndp	235 × 5	1991	1804	8	179
Eleonoravas	Lds, Lfs	359 × 5	1855	774	95	986
Dūkštos	Ncl	332 × 5	3747	2169	66	1512

*N – normally moist, L – temporarily wet, c – fertile, d – very fertile, f – particularly fertile, l – light rocks, s – heavy rocks, p – twin rocks (light on heavy)



Figure 2. The transects (in zip lines) for the observation of elm hybrids at the contact zones of Šilas, Eleonoravas, and Dūkštos forests (from top to bottom)

Evaluation and variables

Three descriptors for the trees and twenty one for their leaf, including nineteen applied by the WinFO-LIA 2004a program, were used to evaluate elms in this study.

Stem form and forking was evaluated as the dominance of stem axis whereby the main central stem of the plant is dominant over (i.e. grows more strongly than) other side stems. Ultimate shapes of stems were as follows (Petrokas 2008a): 1 – multi-dominant axes of order one: the union of several stems/branches at the root neck/branch base, 2 – co-dominant axes of order one: at the basal zone of stem/branch – the diameter of any side axis is about half the diameter of the base of union, 3 – decurrent main axis: the axis is bifurcated or trifurcated; lower offshoot of supposed axis is prevailing, 4 – apparent main axis: some offshoot of supposed axis is prevailing at the furcations, 5 – excurrent main axis: there are no furcations of axis – rectilinear axis is prevailing at full height/length.

The privileged repartition of sibling shoots (Troll 1937) on the upper, lateral or basal position of a slanted or horizontal parent shoot is referred to, respectively, as epitony, amphitony or hypotony. In the elms the privileged repartition of sibling axes from order two to the ultimate orders or branching trend (Petrokas 2008b) was defined as the amphitony-epitony, amphitony, or amphitony-hypotony. Amphitony is a frequent feature in rectilinear branches (Caraglio and Barthélémy 1997), e.g., fastigate, upright, and spreading branches, whereas epitony and hypotony are characterized by the predominant development of lateral axes on the convex side of the curved, downwardly or upwardly orientated branches. Tree branching habit (Szalotnay 2006) was defined as 1 – fastigate, 2 – upright, 3 – spreading, 4 – drooping or 5 – weeping branches.

For this study fairly uniform leaf samples which showed consistent characters were collected so that comparisons between elm populations could be made more feasible. Nineteen morphology characteristics of summer leaves were measured or scored by WinFO-LIA 2004a program: lamina area in cm² (A), leaf perimeter in cm (P), vertical leaf length in cm (L), horizontal

leaf width in cm (W), leaf aspect ratio (W/L), form coefficient ($=4\pi A/P^2$), blade length in cm, maximum perpendicular width in cm, position in % of blade length where maximum perpendicular width has been measured, perpendicular width at 50 % of blade height, perpendicular width at 90 % of blade height, lobe angle in degrees at 10 % of blade height, lobe angle (in degrees) at 25 % of blade height, petiole length in cm, petiole area in cm^2 (Figure 3).

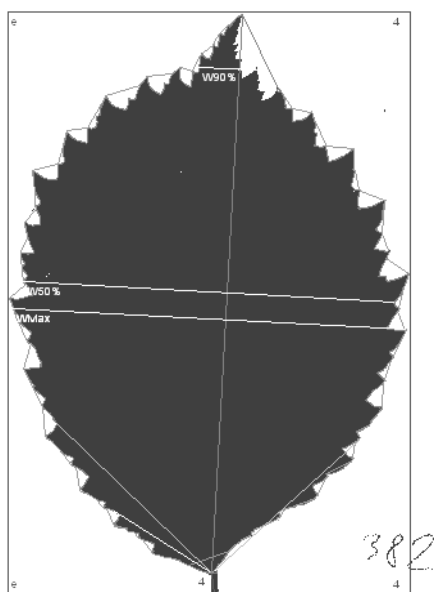


Figure 3. Outline of elm leaf illustrating the characteristics measured (from top to bottom): perpendicular width at 90 % of blade height (W90 %), perpendicular width at 50 % of blade height (W50 %), maximum perpendicular width (WMax), lobe angle at 25 % of blade height, lobe angle at 10 % of blade height, and blade length

Additionally, the leaves were evaluated in relation to the number of lateral nerves and forked lateral nerves at the left and right from the underside of blades. Subsequently, the ratios of all lateral nerves to forked lateral nerves were calculated to obtain characteristics of leaf nervation. Ultimate shape of leaves (Petrokas 2008b) between annual shoots of strongest axes of sequencing orders was evaluated as the number of leaf peaks: 1 – one tip, 2 – two (three apparent tips), 3 – three (3-tipped leaf or five apparent tips), 4 – four (five apparent tips), 5 – five (5-tipped leaf or seven apparent tips), 6 – six (seven apparent tips), 7 – seven or over (7-tipped leaf with seven peaks or over).

Statistical analysis

In order to determine the critical groups of elm phenotypes indicative for their taxonomic identity Stepwise method of SAS Stepdisc procedure was used

for selecting variables (SAS Institute, Inc., SAS/STAT software Release 8). Fourteen variables that best discriminated the four taxa population of Wych elms, Field elms, Dutch elms, and European white elms (presented in Table 3) were then used to perform SAS Factor and G3D procedures and to assess multivariate morphological differentiation between the taxa. Interequivalence of the taxa was predicated on the influence of the strongest factors dominating over the whole of variance explained in principle. Clustering of elm trees based on the three factorial estimates of morphological traits significantly influencing differences between the taxa was realised by applying SAS Cluster (Average method) and SAS Tree procedures. Fourteen variables that were found to differentiate the four taxa population were used to calculate means, standard errors, standard deviations, and the 95 % level confidence intervals for the means (Explore procedure of SPSS 16.0 for Windows®) per taxon and to analyse traits useful for identifying putative hybrid individuals of the Dutch elm.

Table 3. The pattern of factors dominating over the whole of variance explained of four taxa population investigated - Wych elms, Field elms, Dutch elms, and European white elms

Characteristics	Factor1	Factor2	Factor3
	Variance explained, %		
	56	22	13
Stem dominance	-0.33	-0.22	-0.69
Tree branching habit	0.03	-0.18	0.68
Forked lateral nerves of leaf at the left	0.63	-0.71	-0.07
Lateral nerves of leaf at the right	0.53	0.34	-0.23
Forked lateral nerves of leaf at the right	0.63	-0.73	-0.07
Leaf peaks	0.52	-0.28	0.25
Ratio of leaf nervation	-0.17	0.92	0.05
Vertical leaf length	0.96	0.17	0.02
Horizontal leaf width	0.95	0.18	0.01
Leaf aspect ratio	0.94	0.15	-0.12
Position of blade maximum perpendicular width	0.95	0.15	-0.07
Blade perpendicular width at 50 % of blade height	0.92	0.24	0.11
Leaf lobe angle at 25 % of blade height	0.41	0.28	-0.11
Leaf petiole length	-0.12	-0.00	0.58

Results

Ulmus minor and *Ulmus glabra* are two closely related Lithuanian elm species with partially overlapping distributions. Within the area of overlap, there are localities at the forests around the central part of Lithuania where morphological intergradation occurs. Approximate figures of the elm taxa from contact zones are given in Table 2. On average, the putative natural hybrids account for less than two percent of all *Ulmus minor* – *Ulmus* × *hollandica* – *Ulmus glabra* individuals within this group. Every individual within this group is able to hybridise again with its next neighbour. This process is shown not only by the coexistence of those individuals in the same or overlapping populations but also by the outstanding var-

iability of their morphological characteristics and by the presence of extreme or novel characters of hybrids.

The WinFOLIA 2004a programme has analyzed most of the leaves (Table 1). The worst measureability of leaves was characteristic for the Wych elm, because of its multiple leaf-tips, the best – for the Smooth-leaved elm (73.7 vs. 97 %). The results of factor analysis of six taxa population pointed out that the taxa of *U. minor* – *Ulmus* × *hollandica* – *U. glabra* group were largely overlapped under the influence of strongest factors dominating over the whole of variance explained. The group of four European white elms was easily recognizable though. So, it was purposive to reduce six taxa into four – to subsume atypical Wych elms (non-Dutch elms, the subspecies *montana* of the Wych elm) into Wych elms, Smooth-leaved elms into Field elms, European white elms and Dutch elms leaving separately as before. Now the factor analysis procedure was repeated on the fourteen of the variables that best discriminated the four taxa population. Variation in morphology is shown in coordinates of the first, second, and third factors representing, respectively, 56 %, 22 % and 13 % of variance explained (Table 3). European white elms remained together, the putative hybrids, i.e. Dutch elms, passed into separate group, excluding some individuals appearing distant from their group average (Figure 4).

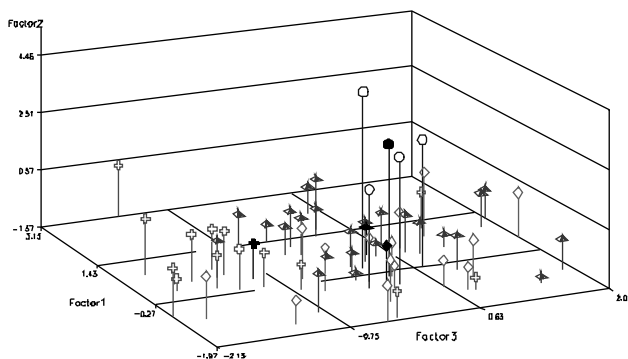


Figure 4. The grouping of elm trees (*Ulmus* spp.) by the strongest factors dominating over the whole of variance explained: *U. glabra* (pyramids), *U. minor* (diamonds), *U. laevis* (balls), hybrids (crosses). The filled symbols show the average of the group specified. Some individuals distant from their group average are indicated with tree No.

Tree No. 57 from Dūkštos forest, the most extraordinary individual from the elms, has been taken as a putative first-generation hybrid between the Wych elm and the Smooth-leaved elm, because of its huge leaves. The biggest leaf (No. 274, Figure 5) having lamina area of 585.4 cm² and leaf perimeter of 167.7 cm was like no one from the rest of elm leaves (see all the leaves in the Figure 5, which are intercomparable).

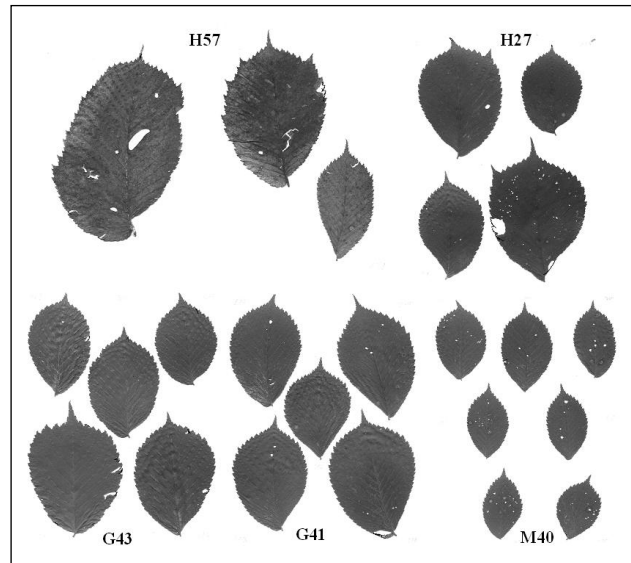


Figure 5. Leaves (in groups indicated by letter and tree No.) of putative hybrids between the Wych elm and the Smooth-leaved elm (H57 – above, left and middle, and H27 – above, right), those of Wych elms (G43 – below, left, and G41 – below, middle), and those of Smooth-leaved elm (M40 – below, right)

Our estimations on first-generation hybrid nature of tree No. 57 were further supported by clustering of the elms based on the three factorial estimates of morphological traits significantly influencing differences between the species (Figure 6).

Putative hybrids No. 40 and 41 from Mikytai forest, developed from stump shoots, were most controversial from the beginning (Figure 5). Consequently, tree No. 40 was subsumed into the Field elm (M), tree No. 41 – into the Wych elm (G). In addition (see the least average distances between clusters in Figure 6), Field elm No. 43 (M) was corrected to Wych elm (G) because of its obovate leaves with long-acuminate to cuspidate apices, and Wych elm No. 27 (G) was corrected to the hybrid (H) because of its self-evident variability of leaf shape (Figure 5). As the last step, the variability of various characters of elm taxa was determined. In general, of the 14 characteristics, only stem dominance, tree branching habit, number of lateral nerves of leaf at the right (from the underside of blades), leaf aspect ratio, and lobe angle at 25 % of leaf blade height (see Table 4) of putative natural hybrids between the Smooth-leaved elm and the Wych elm, i.e. Dutch elms, did not overlapped with those of Field elms and Wych elms. Tree branching habit, number of leaf lateral nerves at the right, and leaf aspect ratio overlapped exclusively with those of European white elms. This is because of partial coincidence of the 95 % confidence intervals for the means.

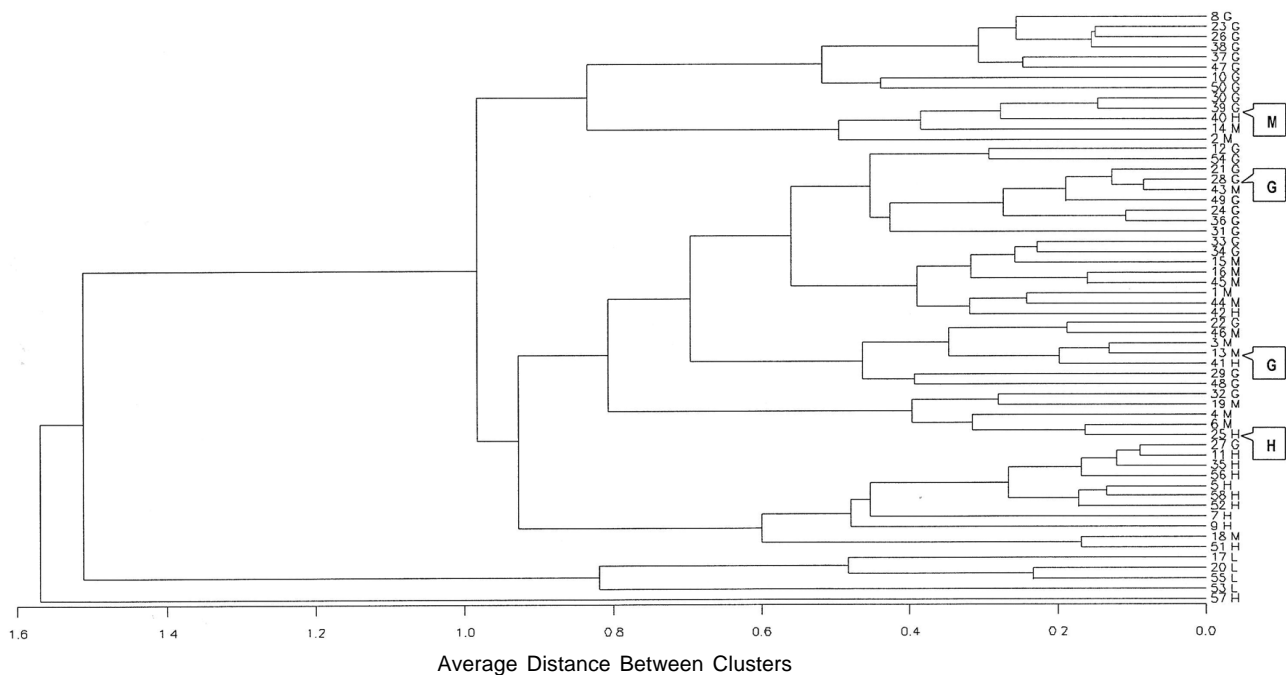


Figure 6. Clustering of elms (*Ulmus* spp.) based on the three factorial estimates of morphological traits significantly influencing differences between the taxa. The letters indicate: L – *U. laevis*, G – *U. glabra*, M – *U. minor*; H – hybrid. All corrected indications are in callouts

The lowest standard deviations of leaf aspect ratio and stem dominance indicated that the data points tended to be very close to the respective means. On average, the best stem dominance was observed for Dutch elms, the worst – for European white elms (excurrent vs. apparent main axes). Leaf aspect ratio (horizontal leaf width / vertical leaf length) of Dutch elms was 0.5 while that of the others – 0.6. Dutch elms were remarkable for their leaf lobe angle at 25 % of blade height – 37.3 ± 0.6 vs. 41.3 ± 0.6 (and 41.2 ± 0.7) degrees for European white elms (and Field elms).

Discussion and conclusions

As a result of this approach, four taxa could be distinguished within the genus *Ulmus* L. at the contact zones of Lithuanian forests. Three elm species and hybrids, *Ulmus glabra*, *Ulmus minor*, *Ulmus laevis*, and *Ulmus* × *hollandica*, were fairly satisfactorily distinguishable, but there is much overlap between the Wych elm and the Field elm in their characters that can be explained only by the equivalence of their statistically discriminant phenotypic distinctness. The latter species differ in some traits and habitat preferences, but hybridize in nature where they grow in close proximity. The two species were found to be porous and moderately differentiated, with the notable exception of some species specific morphological markers that

could point to the existence of genomic regions that are sheltered from introgression.

Putative natural hybrids between the Smooth-leaved elm and the Wych elm account for less than two percent of all individuals within group *U. minor* – *U.* × *hollandica* – *U. glabra*. Such low proportion of hybrid progenies in the contact zones is inconsistent with the high rates of hybridization detected in Europe between native *U. minor* and naturalized *U. pumila* using isozymes (Cogolludo-Agustin et al. 2000) and dominant DNA markers (RAPD and ISSR; Goodall-Copestake et al. 2005). On the other hand, identification of natural hybrids within the native elms is exceedingly difficult and caution is needed when making taxonomic interpretations. Therefore, in contrast to W.P. Goodall-Copestake et al. (2005), we suggest that introgressive hybridisation within the elms should be verified through extremity and novelty of morphological characters of hybrids (sympatric distribution and morphological intermediacy is not enough), because the verification is conducted on unknown phenotypes at natural habitats. Morphological studies carried out in the course of this investigation (Figure 4) show clearly that the Field elm and the Wych elm are distinguished by a number of characters, whose range of variation of each one of them is not entirely separate and distinct. In total, the characters of stem dominance and lobe angle at 25 % of leaf blade height

of hybrid elms, i.e. Dutch elms, did not overlapped with those of Lithuanian elm species, which may show taxonomic dissimilarity to them (Table 4, in bold).

Lithuania. Now we can ask the following questions (according to Minder et al. 2007): (1) what is the genetic structure of the elm hybrid zones? Do they con-

Table 4. Variability characteristics of Lithuanian elms: the means, standard errors, and standard deviations of data sets. N = the number of leaves evaluated. Not overlapping characters of Dutch elm hybrids with those of Lithuanian elm species are in bold

Taxon (N)	Characteristics*													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
The Wych elm (172)	3.8±0.1 0.8	3.2±0.1 0.9	7.2±0.1 1.6	15.3±0.2 2.0	7.3±0.1 1.7	2.0±0.1 1.3	2.2±0 0.4	22.4±0.4 4.3	13.0±0.2 2.4	0.6±0 0.1	11.4±0.3 2.9	12.5±0.2 2.3	40.2±0.4 4.2	1.0±0.1 1.2
The Field elm (115)	4.1±0.1 1.0	3.3±0.1 0.9	6.1±0.1 1.3	15.3±0.2 1.8	6.1±0.1 1.2	1 (constant) 0	2.6±0 0.5	18.1±0.4 4.1	10.5±0.2 2.4	0.6±0 0.1	8.4±0.2 2.3	10.1±0.2 2.3	41.2±0.7 6.8	2.1±0.1 1.6
The Dutch elm (84)	4.9±0 0.3	2.6±0.1 0.6	7.1±0.2 1.8	16.5±0.2 2.1	7.0±0.2 1.8	1.1±0.1 0.7	2.4±0 0.5	22.3±0.5 4.8	11.9±0.3 3.1	0.5±0 0.1	10.7±0.3 2.7	11.5±0.3 2.9	37.3±0.6 5.2	2.0±0.2 1.7
The European white elm (28)	3.8±0.1 0.4	3.0±0.1 0.7	2.3±0.2 0.9	17.0±0.4 2.1	2.2±0.3 1.4	1 (constant) 0	11.6±2.2 10.9	20.2±0.5 2.3	12.3±0.4 2.2	0.6±0 0.1	9.8±0.3 1.6	11.8±0.4 2.0	41.3±0.6 3.1	0.7±0.1 0.6

*1 = stem dominance (1 – multi-dominant axes of order one, 2 – co-dominant axes, 3 – decurrent main axis, 4 – apparent main axis, 5 – excurrent main axis)

2 = tree branching habit (1 – fastigate, 2 – upright, 3 – spreading, 4 – drooping, or 5 – weeping branches)

3 = number of forked lateral nerves of leaf at the left (from the underside of blades)

4 = number of lateral nerves of leaf at the right

5 = number of forked lateral nerves of leaf at the right

6 = number of leaf peaks

7 = ratio of leaf nervation

8 = vertical leaf length in cm (L)

9 = horizontal leaf width in cm (W)

10 = leaf aspect ratio (W/L)

11 = position (in % of blade length) of blade maximum perpendicular width (Figure 3)

12 = blade perpendicular width (in cm) at 50 % of blade height

13 = leaf lobe angle (in degrees) at 25 % of blade height

14 = leaf petiole length (in cm)

The investigated contact zones revealed some extraordinary phenotypes of putative natural hybrids within the elms. Morphological similarities between Dutch elm tree No. 57, and European white elm trees (see *U. laevis* in Figure 6) suggest a hybrid origin of the latter species. Interestingly, Klotzsch, who seems to have been the first botanist to make experiments in crossing forest trees and who was curator of the Berlin Herbarium, crossed the European white elm with the Smooth-leaved elm in 1845, and raised seedlings from both the cross-fertilised seeds and from the seeds of the parent trees, and after eight years' growth under similar conditions the hybrid seedlings were one-third taller than the others (Elwes and Henry 1913). Nevertheless, the contrary seems to be the case of unique crossability barrier, which was found to exist among the European white elm and the European elm species which belong to a different section of the genus (Heybroek 1968, 1993, Townsend 1975, Mittempergher and La Porta 1991, Collin 2003).

We were not able to find the discontinuity of characters that would sufficiently define hybrid phenotypes, however, the individuals mistaken for the hybrids between the Smooth-leaved elm and the Wych elm were identified. Not every finding in this study may be in line with current taxonomic knowledge but the study aims to give an idea of the scope of elms in

sist primarily of intermediate hybrids or of backcross hybrids? (2) Is there evidence for introgression and if yes, is it uni- or bidirectional? (3) What is the extent of introgression? Is it limited to a small proportion of markers? (4) Can morphology be used to predict the structure of hybrid zones? (5) What can we learn about the nature of the species boundary between the two study species based on the analysis of natural hybrid zones? A model for such research has been provided by J.E. Zalapa et al. (2009), who suggested the potential broad applicability of microsatellite markers for hybridization and introgression studies in the genus *Ulmus*. Altogether, biochemical markers, including isozymes (Cogolludo-Agustín et al. 2000), flavonoids (Santamour 1972, Sherman and Giannasi 1988), and DNA markers (Wiegrefe et al. 1994, Kamalay and Carey 1995, Whiteley et al. 2003, Pooler and Townsend 2005), have proven useful to classify *Ulmus* species and to determine genetic identity.

In general, introgression of morphological characters is more restricted than introgression of molecular markers (Rieseberg and Wendel 1993), but molecular markers may provide a good tool to characterize the genetic architecture of hybrid zones. It is meant a baseline for new elm research project in Lithuania is already drawn. For hybridization and introgression studies the data on biochemical and molecular markers should be used to distinguish elm species and their hybrids in contact zones based on phenotype.

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ЕСТЕСТВЕННАЯ ГИБРИДИЗАЦИЯ ИЛЬМОВЫХ (*ULMUS* L.) В ЛИТВЕ

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Резюме

Предполагаемые естественные гибриды – *Ulmus* × *hollandica* – между вязом пробковым (*Ulmus minor* ssp. *minor*) и вязом шершавым (*Ulmus glabra*) наблюдались в смешанных лесах вдоль рек и речушек в центральной части Литвы. Исследовано 11 популяций ильмовых (*Ulmus* L.) с целью определить: 1) критические группы фенотипов, показательные для их таксономического тождества, 2) изменчивость таксонов из контактных зон. Для описания каждого образца (описано 58 образцов) и установления степени близости между ними были использованы 3 характеристики для деревьев и 21 характеристика для их листьев, в том числе 19 характеристик используя программу WinFOLIA 2004a. В результате исследования в зонах контакта выделено 4 таксона ильмовых: 3 вида вяза (*Ulmus glabra*, *Ulmus minor*, *Ulmus laevis*) и гибрид (*Ulmus* × *hollandica*). Таксона довольно удовлетворительно различимы, однако присутствует значительное перекрытие между *Ulmus glabra* и *Ulmus minor*, которое можно объяснить лишь эквивалентностью их статистически дискриминантной фенотипической определенности. В среднем, предполагаемые естественные гибриды между *Ulmus minor* ssp. *minor* и *Ulmus glabra* составляют менее двух процентов всех индивидов внутри группы *Ulmus minor* – *Ulmus* × *hollandica* – *Ulmus glabra*. Возможность интрогрессивной гибридной изоляции показана незаурядной изменчивостью морфологических характеристик таксонов из контактных зон и наличием у гибридов экстремальных или новых качеств.

Ключевые слова: *Ulmus minor* ssp. *minor*, *Ulmus glabra*, *Ulmus minor*, *Ulmus* × *hollandica*, *Ulmus laevis*, естественная гибридная изоляция