Differences in Leaf Morphology between *Quercus petraea* and *Q. robur* Adult and Young Individuals

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The characters of *Quercus robur* and *Q. petraea* leaves are of main taxonomic value and the adult trees of both species can be distinguished on them. However, young individuals, mostly seedlings but also saplings, are told to be undistinguishable or only partly distinguishable on the leaf morphology. The aim of the study was to verify this hypothesis on the basis of biometrical analyses of leaf characteristics of adults trees and saplings in two mixed oak woods, one located close to the north-eastern limit, the other about 400 km inside of the *Q. petraea* range in Poland. The analysis of discriminations and minimum spanning tree on the squares of Mahalanobis distances were analysed to find differences between *Q. robur*, *Q. petraea* and intermediate adults and saplings. The differences between saplings of *Q. robur* and *Q. petraea* were found lower than between adult trees. Nevertheless, the biometrical analysis confirmed determination of saplings in the field.

Keywords morphological diversity, plant variation, biometry, *Quercus*, leaf polymorphism
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1 Introduction

Pedunculate oak – *Quercus robur* L. and sessile oak – *Q. petraea* (Matt.) Liebl. are well distinguishable on the morphological characters of their leaves, acorns and cupulas (Kotschy 1862, Schwarz 1937, Krahl-Urban 1959, Schwarz 1964, Rushton 1983, Amaral Franco 1990, Bacilieri et al. 1995). The leaves, as the most easily accessible parts of the trees and easily measured with utilization of electronic accessories and software have been recently used with high success in extensive biometrical study through Western Europe (Dupouey 1983, Kleinschmit et al. 1995, Kremer et al. 2002). However, the seedlings and even saplings are frequently told to be very similar and quite undistinguishable on the leaf characters (Aas 1993, Rushton 1993, Kremer et al. 2002, Boratynska et al. 2006).

The aim of the present study was the biometrical verification of correctness of in the field determination of adult trees and about 12–18 years old saplings, which appeared spontaneously under canopy of two different, adult mixed stands of *Q. robur* and *Q. petraea* in Poland. The white oaks hybridize frequently (Bacilieri et al. 1996, Dodd and Afzal-Rafii 2004, Boratynska et al. 2006, Giertych 2006). The reviewed biometrical works have been performed predominantly in the Western Europe, partly within range of *Q. pubescens* (Meusel et al. 1965, Jalas and Suominen 1976, Dupont 1990, Boratynski et al. 2006), which could also influence the variation of local populations of *Q. robur* and *Q. petraea*. Central Europe is outside the ranges of oak species other than *Q. robur* and *Q. petraea*, which makes the comparative morphological study much simpler.

2 Material and Methods

2.1 Plant Material and Field Works

The two seed collection stands distinguished for forest gene conservation and seed production (Matras 1996) were selected in: 1) Jamy and 2) Legnica Forest Offices (Table 1). The stands have been thinned as a result of oak decay during 1980–1990 and the cutting down of adult trees and saplings of *Fagus sylvatica* and *Carpinus betulus* (unpublished data of Jamy and Legnica Forest Offices). Natural regeneration appeared in both stands, in Jamy as a result of elimination of competition of other tree species and herbs by soil preparation and in Legnica spontaneously. The saplings in the Jamy stand were formed over 85% of the stand area in 1986. The dead oak trees were removed in subsequent years and the first selective clearings were made in 2000, removing mainly saplings of *Fagus sylvatica*, *Acer pseudoplatanus* and other tree species (personal communications of foresters). The stand in Legnica had not been prepared for self-sowing but, in spite of that, natural regeneration was abundant. The site conditions of both stands are similar (unpublished data of Forest Offices). Both stands are mixed with similar participation of *Q. robur* and *Q. petraea* and only inconspicuous addition of hybrids. The form of mixing is random, stem by stem or rarely 3–4-tree clusters.

The taxonomic status of adult trees was determined over the entire area of both stands. Leaf, acorn and cupula morphology were applied during determination in the field by visual assessment. Every tree was subsequently assigned into one of the three categories: 1) *Q. robur*, 2) *Q. petraea* and 3) hybrid (intermediate morphology). The trees of latter category have been distinguished on the basis of intermediate length of leaf petiole, the shape of leaf blade, the presence/absence of nerves between leaf lobes (Aas 1993, Kremer et al. 2002), and length of cupula peduncle (Schwartz 1937, 1964, Boratynska et al. 2006). The determination was conducted in September, when acorns with cupulas were well visible using binoculars. The trees with not stable characters (see Kremer et al. 2002: 783 and 784, as unclassified) were only scarcely represented in both stands (Table 1). Then plots 120 m long and 40 m wide were established in central parts of each stand. The 59 trees in the Jamy and 95 in the Legnica stands (Table 2) were sampled on the plots for the biometrical verifications. Ten leaves were collected from central parts of long shoots, from south-facing, insolated parts of the crown of every tree, at an altitude of 8–9 m. The leaves from the central part of the first spring longitudinal increment of shoots were used, as the most typically developed (Staszkiewicz 1970,

Table 1. Sampled stands composed of *Quercus robur* and *Q. petraea.*

Locality	Geographic coordinates	Abbreviation	Age	Area [ha]	Participation of taxa [%]		
					Q. robur	*Q. petraea*	Hybrids
Jamy Forest Division, Forestry Jamy, no. 96c	E18°53´ N53°35´	Jamy	125	5.00	57.0	42.2	0.8
Legnica Forest Division, Forestry Karczewiska, no. 315d	E16°10´ N51°19´	Legnica	154	10.83	53.5	44.4	2.1

Table 2. Adult trees and saplings of *Quercus robur*, *Q. petraea* and intermediate individuals on the analysed plots and transects in the stands of Jamy and Legnica.

Taxon	Stand	Number of individuals	
		Trees biometrically analysed on plots	Saplings biometrically analysed on transects
Q. robur	Jamy	23	27
Q. petraea		33	46
Intermediate		3	27
Q. robur	Legnica	35	10
Q. petraea		54	79
Intermediate		6	9

Aas 1993, Rushton 1993, Kremer et al. 2002, Boratynska et al. 2006, Chałupka 2006). A total of 1550 leaves were measured and analysed.

The saplings, individuals higher than 0.3 m (Harmer 2001), growing at a distance of about 1 m from each other were determined. Then, ten leaves from every sapling were collected for biometrical verification in every stand. The 100 saplings were sampled in Jamy and 98 in Legnica stand. A total of 1980 leaves from saplings were biometrically compared (Table 2).

2.2 Leaf Biometry

Leaves were analysed biometrically using 16 measured, 1 evaluated and 7 synthetic characters (Table 3), according to the methods used earlier by Kremer et al. (2002) and Borazan and Babac (2003).

Table 3. Characters of leaves analyzed biometrically.

Number of character	Character
1	Area of leaf blade (A)
2	Circumference of leaf blade (P)
3	Length of leaf blade (LL)
4	Maximum width of leaf blade (LW)
5	Width of leaf blade in mid-length
6	Width of leaf blade in 90% of length
7	Petiole length (PL)
8	Area of petiole
9	Length of apical lobe
10	Width of apical lobe
11	Length of the longest side lobe (a)
12	Length of lobe below the longest side lobe (b)
13	Depth of sinus between lobes a and b
14	Length of the second side nerve
15	Number of lobes (NL)
16	Number of nerves between the lobes (NV)
17	Type of leaf base (BS)
18	$PR = 100 \times PL/(LL + PL)$
19	$PV = 100 \times NV/NL$
20	$LWR = 100 \times LW/LL$
21	$100 \times$ (Width of leaf blade at 90% of length/maximum width)
22	$100 \times$ (Width of leaf blade at mid-length/maximum width)
23	$100 \times$ (Width of leaf blade at 90% of length/width of leaf blade at mid-length)
24	$AP = A/P$

The distributions of character values were verified using Shapiro-Wilks' test and frequency histograms. The average values of particular characters were calculated separately for subpopulations of adult and young individuals of each taxon distinguished in each stand to find their taxonomic importance. The similarity/dissimilarity among adult and young subpopulations of each taxon in each stand was determined using discrimination analysis (Zar 1999, Sokal and Rohlf 2003). The latter was performed on the evaluated (17) and synthetic (18–24) characters only, to avoid a possible influence of environmental differences (Kremer et al. 2002). The square of the shortest Mahalanobis' distances between distinguished subpopulations of adult trees and saplings of *Q. robur*, *Q. petraea* and hybrids has been analysed for each stand separately to find the relationships between them (Sokal and Rohlf 2003).

The leaves were measured with a scanner and WinSeedle software (Regent Inc.). Statistical analyses were performed using STATISTICA 6 software (StatSoft).

3 Results

Most of the leaf characters (Table 3) presented the unimodal frequency distribution. Only length and area of the leaf petiole (characters 7 and 8), the length of the longest side lobe, number of veins between lobes, ratio of petiole length to leaf length and ratio of width of leaf blade at mid-length to maximal width (characters 11, 16, 18 and 22, respectively) have shown a slightly biased frequency distribution.

The character values were found to be predominantly higher for adult trees than for saplings. Additionally, the measured characters had generally higher values in the Legnica than in the Jamy stands (Table 4). In some cases average values of characters for adult trees from Jamy were similar to the sapling characteristics in the Legnica population, as for example in the area of leaf blade, maximal width of leaf blade, width of leaf blade in mid-length, width of leaf blade in 90% of length, length of the longest lobe and lobe below the longest side lobe (characters 1, 4, 5, 6, 11 and 12, respectively) (Table 4). It blurred the differences between species and made difficult the analyses and hybrid identification in the field. Nevertheless, the hybrid adult trees had values of particular characters intermediate between *Q. robur* and *Q. petraea* adult trees, and hybrid saplings intermediate between of *Q. robur* and *Q. petraea* saplings. It concerns mostly such characters as length and area of petiole, length of lobe below the longest side lobe, depth of sinus between lobes a and b, length of second side vein, number of lobes, number of veins between lobes, and type of leaf base (characters 7, 8, 12, 13, 14, 15, 16 and 17, respectively, see Table 4).

The petiole length, number of veins between lobes, type of leaf blade base, ratio of length of petiole to leaf length and number of veins to number of lobes (character 7, 16, 17, 18 and 19, respectively) differed between taxa. The length of petiole (character 7) had significantly higher values for leaves of adult trees of *Q. petraea* and hybrids. The leaves of *Q. robur* had a higher number of veins between lobes (character 16) than of *Q. petraea*, independently of age. The leaves of adult *Q. robur* had a typically auriculate type of blade base and the highest value of this character (17). The ratio of petiole to leaf length (character 18) had the highest values in the group of adult trees of *Q. petraea* and the intermediate individuals. The ratio of number of veins to number of lobes (character 19) had the highest values for adults of *Q. robur* and the lowest for *Q. petraea*.

The average values of some characters differed between populations and between adult trees and saplings within populations (Table 4). The number of characters differing *Q. robur* and *Q. petraea* at a statistically significant level is higher for subpopulations of adults.

Q. robur differed statistically significantly from *Q. petraea* in both, population and age categories in length of petiole, width of apical lobe, depth of sinus between longest and lying below side lobes, number of veins between lobes, proportion of petiole length to leaf length, ratio of number of veins between side lobes to number of side lobes and proportion of width of leaf blade at mid-length to maximal width (characters 7, 10, 13, 16, 18, 19 and 22). More characters of adults differentiated significantly between species in the Legnica than in the Jamy stand. Of particular interest was the fact that the type of leaf blade base, used as one of the most important traits, differed statistically significantly only between adult trees.

The adult trees of *Q. robur* and *Q. petraea* were the most morphologically distant in the discrimi-

Table 4. Average values of characters of *Q. robur*, *Q. petraea* and hybrid subpopulations of mother trees and saplings in Jamy and Legnica stands; 1–24 character numbers as in Table 3; P-JD – *Q. petraea* mother trees in Jamy stand; R-JD – *Q. robur* mother trees in Jamy stand; X-JD – hybrid mother trees in Jamy stand, P-JM – *Q. petraea* saplings in Jamy; R-JM – *Q. robur* saplings in Jamy; X-JM – hybrid saplings in Jamy; P-LD – *Q. petraea* mother trees in Legnica; R-LD – *Q. robur* mother trees in Legnica; X-LD – hybrid mother trees in Legnica; P-LM – *Q. petraea* saplings in Legnica; R-LM – *Q. robur* in Legnica; X-LM – hybrid saplings in Legnica.

Sub-population	Character number																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
P-JD	36.9	37.2	9.8	5.65	4.89	2.48	1.65	0.20	0.64	1.13	3.99	3.34	2.31	1.33	5.93	0.49	3.87	14.28	8.8	58.0	44.0	86.6	52.0	0.97
R-JD	38.3	40.1	10.6	5.86	4.75	2.74	0.86	0.11	0.82	1.38	4.00	2.96	1.73	0.99	5.09	2.49	7.48	7.43	50.8	56.2	46.9	81.3	59.5	0.93
X-JD	36.8	44.5	9.3	6.15	5.33	3.04	1.44	0.18	0.59	1.06	4.45	3.37	2.05	1.07	5.17	1.04	4.35	13.08	21.1	66.1	49.1	86.0	58.4	0.81
P-JM	26.7	31.9	9.3	4.59	3.89	2.18	0.86	0.10	0.45	0.83	3.24	2.62	1.85	1.03	6.15	0.82	2.67	8.43	14.3	49.9	47.3	84.7	57.8	0.82
R-JM	25.7	33.4	9.7	4.51	3.60	2.17	0.66	0.08	0.58	0.93	3.14	2.29	1.39	0.92	5.97	2.27	3.42	6.34	39.3	46.8	47.8	80.1	62.0	0.75
X-JM	29.3	33.4	10.0	4.82	3.95	2.32	0.73	0.09	0.57	1.01	3.36	2.61	1.68	1.03	6.15	1.66	2.68	6.82	28.6	48.5	47.8	81.7	60.2	0.85
P-LD	57.2	41.9	11.6	7.41	6.67	3.33	2.01	0.39	0.52	1.15	4.85	4.09	2.93	1.28	6.84	0.39	3.69	14.87	5.8	63.9	44.9	90.0	50.4	1.34
R-LD	54.3	43.8	12.0	7.34	6.25	3.67	1.04	0.14	0.59	1.29	4.80	3.83	2.52	1.10	5.92	2.68	7.00	8.02	45.8	61.1	49.9	85.0	59.6	1.21
X-LD	62.9	53.2	12.7	8.11	7.16	3.35	2.01	0.27	0.48	1.25	4.48	3.81	2.71	1.14	6.69	1.71	3.34	13.79	27.1	63.7	41.0	88.4	47.5	1.15
P-LM	40.8	35.4	10.2	6.16	5.42	2.66	1.23	0.14	0.50	0.94	4.18	3.45	2.54	1.20	6.58	0.38	2.57	10.73	6.1	60.1	42.9	87.8	49.4	1.11
R-LM	44.3	37.8	10.8	6.36	5.42	2.94	0.94	0.11	0.57	1.08	4.31	3.32	2.31	1.09	6.18	1.33	3.30	7.85	23.7	58.2	46.1	84.9	55.3	1.10
X-LM	37.3	36.4	10.0	6.10	5.40	2.52	0.99	0.11	0.56	1.02	4.15	3.15	2.17	1.08	6.29	0.94	2.55	8.93	15.3	60.9	41.0	88.6	47.1	1.00

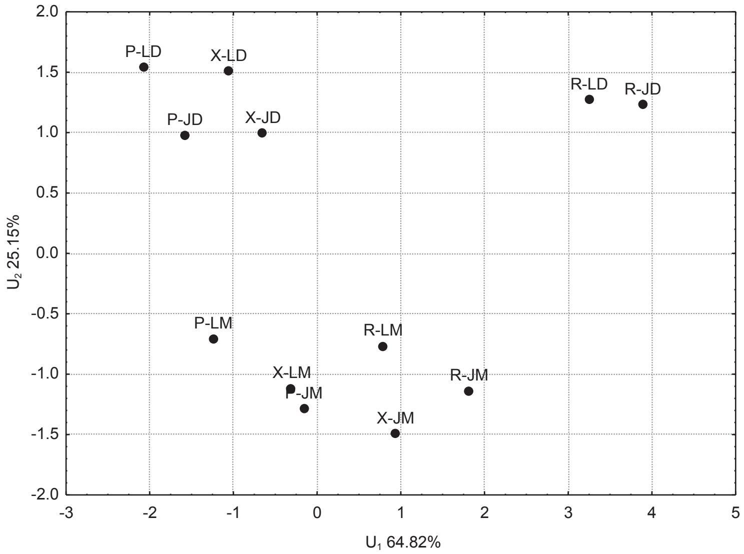

Fig. 1. Result of discrimination analysis based on the calculated characters of leaves among distinguished subpopulations of adult trees and saplings of *Quercus robur*, *Q. petraea* and hybrids in mixed oak populations Jamy and Legnica, plotted along the two first discriminant variables which accounted for 89,97% of the total variation: Jamy: P-JD – *Quercus petraea* adult trees, R-JD – *Q. robur* adult trees, X-JD – hybrid adult trees, P-JM – *Q. petraea* sapling, R-JM – *Q. robur* sapling, X-JM – hybrid sapling; Legnica: P-LD – *Q. petraea* adult trees, R-LD – *Q. robur* adult trees, X-MD – hybrid adult trees, P-LM – *Q. petraea* sapling, R-LM – *Q. robur* sapling, X-LM – hybrid sapling.

nation analysis performed for both populations together (Fig. 1). The adult hybrids were closer to the adults of *Q. petraea*. The saplings of *Q. petraea* and *Q. robur* formed a closed group, placed between groups of adult individuals. The first discriminant variable, which differentiated species, was determined, mostly, by the number of veins between lobes, the proportion of petiole to leaf length, and the ratio of number of veins between side lobes to number of side lobes (characters 16, 18 and 19 respectively). The second discriminant variable differed adult from young individuals (Fig.1), and was determined mostly by petiole length and proportion of petiole to leaf length (characters 7 and 18, respectively).

The discrimination analysis performed separately in each population indicated high differences between adult trees and saplings. In the Jamy stand, the largest differences were between the adults of *Q. robur* and *Q. petraea* (Fig. 2a). The typical saplings of both species differed at a low level, showed a partial overlapping, and were closely related to adult trees. The hybrid saplings were placed between those of typical for species. The two hybrid adult trees had leaves similar to *Q. petraea*, but intermediate acorn and cupula. The first discriminant variable, responsible for 58% of the total variation, was determined mostly by the ratio of petiole to leaf length, and ratio of number of veins to the number of side lobes (characters 8 and 19, respectively). The second discriminant variable, responsible for 37% of the total variation, was determined mostly by the type of leaf blade base and ratio of area to circumference of leaf blade (characters 17 and 24, respectively).

In the Legnica stand, the adult trees of *Q. robur* were the most separated from all other subpopulations (Fig. 2b), mostly by the first discriminating

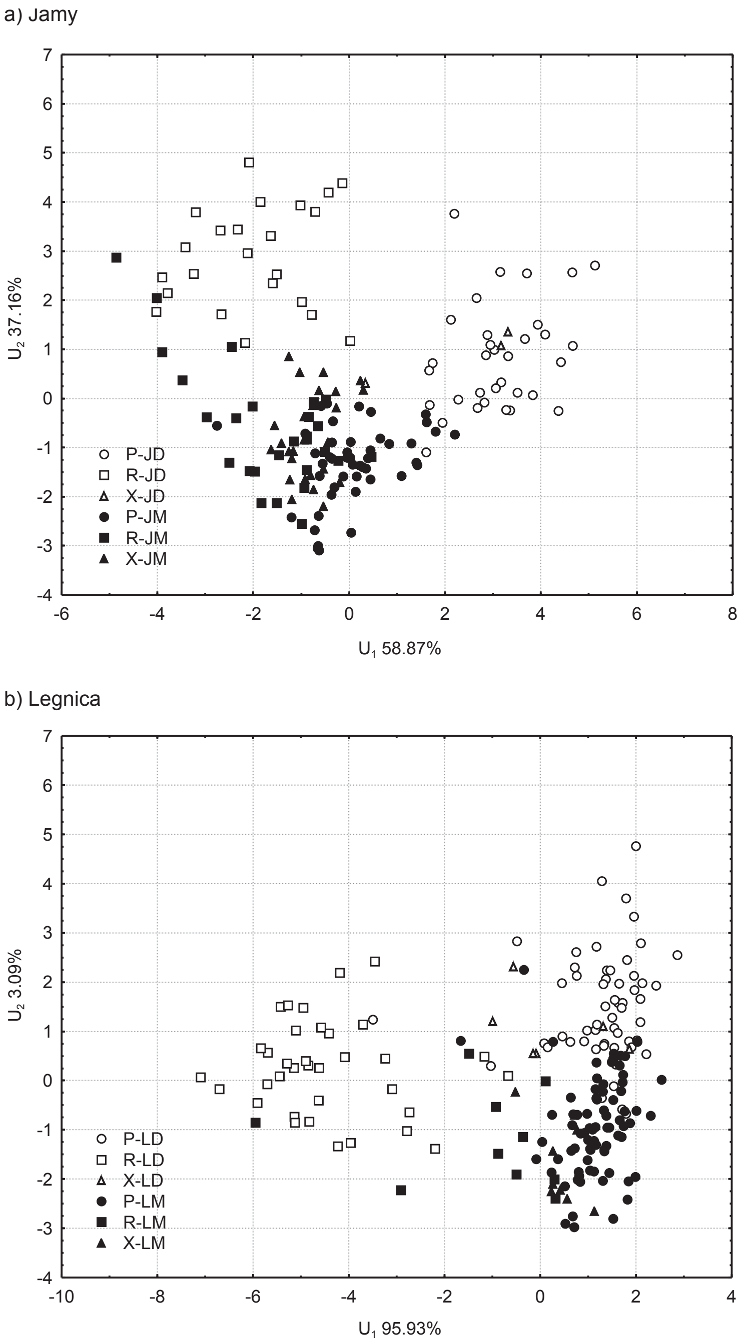

Fig. 2. Result of discrimination analysis based on the calculated characters of leaves for Jamy (a) and Legnica (b) oak populations plotted along the two first discriminant variables; descriptions as in Fig. 1.

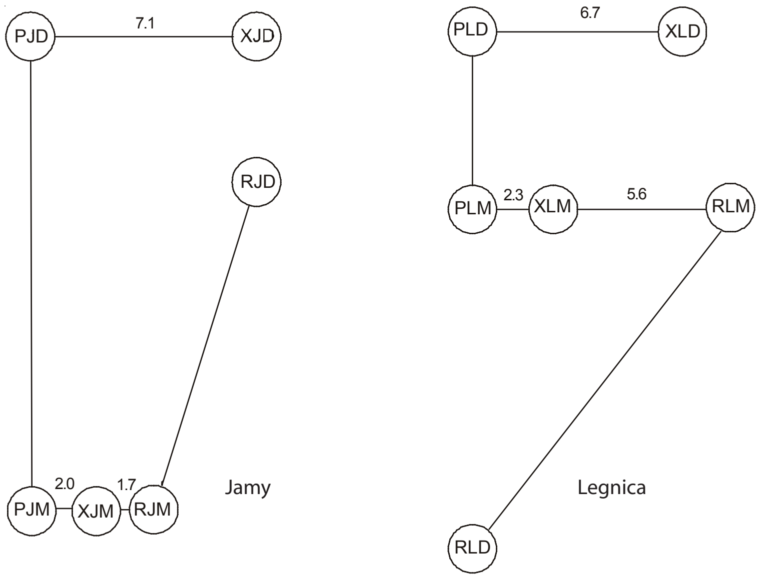

Fig. 3. Minimum spanning tree of distinguished subpopulations of adult trees and saplings of *Quercus robur*, *Q. petraea* and hybrids in oak mixed populations Jamy (a) and Legnica (b) constructed on the basis of the squares of shortest Mahalanobis distances;descriptions as in Fig. 1.

variable, responsible for about 96% of the total variation and determined in the first place by the ratio of number of veins to number of side lobes, and type of leaf blade base (characters 19 and 17, respectively). The adult trees and saplings of *Q. petraea* were the most similar ones, while saplings of *Q. robur* showed a greater difference from adult trees (Fig. 2b). The hybrid adult trees were also closer to *Q. petraea*, while hybrid saplings were dispersed between groups of saplings of *Q. robur* and *Q. petraea* (Fig. 2b).

The analysis of squared Mahalanobis' distances gave similar results. The adult trees were the most distant in both populations. The saplings of typical species joined them and are connected through saplings determined as hybrids, while adult hybrids were more clearly related to typical *Q. petraea* in both populations compared (Figs. 3a and b). The adult trees of *Q. robur* were very distinct in both populations, while *Q. petraea* only in that of Jamy. In the Legnica stand, the adult individuals and saplings of *Q. petraea* were more closely related. In the Jamy stand, the young individuals of *Q. robur* and *Q. petraea* were the closest groups, more distant from adult trees of these species, especially from *Q. petraea* individuals (Figs. 3a and b).

4 Discussion

The biometrical analyses confirmed determination of the species in the field, even in the case of saplings. The latter, however, appeared to be less different than adult trees (see Figs. 1, 2 and 3). *Q. robur* and *Q. petraea* trees turned out to be identified correctly, as in the studies by Staszkiewicz (1970), Kremer et al. (2002) and Kelleher et al. (2005). The determination in the field of the adult hybrid individuals has not been confirmed in the biometrical analyses of their leaves. The leaves of hybrid adults were closer to *Q. petraea*, but during determination in the field also characters of acorns and cupulas were used, and for this reason the field assignments were retained. The position of hybrid adults among individuals of *Q. petraea* (Figs. 2 and 3) seems to indicate, that leaf characters, mainly length of petiole, do not always correlate with characters of acorns and cupulas. This, however, shall be verified in a separate study.

In the case of saplings, the equivocation of species determination in the field based on leaf characters, especially their inclusion into the category of hybrids, was more possible. In spite of that the majority of saplings determined as hybrids in the field, in biometrical verifications were intermediate between *Q. robur* and *Q. petraea*, being nonetheless somewhat closer to *Q. petraea* than to *Q. robur* (Figs. 1, 2 and 3).

The shorter distances between saplings as opposed to adult trees (Figs 1, 2 and 3), confirmed the known rule, that leaves of young individuals of *Q. robur* and *Q. petraea* are more similar. For this reason in this study we did not examine young seedlings, which do not discriminate between species and are comprehended as indistinguishable (Krahl-Urban 1959, Boratynska et al. 2006). The seedlings about 14–15 years old appeared to be distinguishable to the species on the leaf characteristics, but in case of younger one the differences will be lower and the equivocation more possible. The possibility of inclusion of particular individuals into group of morphologically intermediate will be higher in the class of younger seedlings. The higher number of hybrid saplings than adults can be explained by 1) developmental trends of juvenile leaves with the seedling/sapling growth (Aas 1993, Boratynska et al. 2006), and 2) elimination of hybrids in older populations resulting from disruptive selection (Aas 1993, Dupouey and Badeau 1993, Kremer et al. 2002).

When determining the young individuals the complex of characters shall be used. The only single characters, very characteristic and discriminating between adult individuals, cannot be used. The type of leaf base, considered as one of the most important and key characters in distinguishing *Q. robur* and *Q. petraea* leaves (Kotschy 1862, Schwarz 1937, 1964, Bussoti and Grossoni 1997, Kremer et al. 2002, Boratynska et al., 2006), discriminated only between adult trees for example. The leaves of saplings of both species had a similar type of leaf base, so this character cannot be used to identify the young individuals.

The result of our study confirms the possibility of elimination from stand of undesirable species in the class of sapling.

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