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SEED INVOLUCRE VARIATION IN *CARPINUS BETULUS* (CORYLACEAE) IN POLAND

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The paper uses statistical methods to examine whether origination from different Pleistocene centers influences the present-day variation of *Carpinus betulus* in Poland. Twenty-nine populations of the species were sampled in communities of the *Carpinion betuli* alliance in most of the country. Samples of 100 involucres for each population were analyzed for 26 morphological characters. Despite the rather accidental similarities among the sampled populations, their geographic variation confirmed their origin from at least two different refugia, southeast and west.

Key words: *Carpinus betulus*, involucres, morphology, plant geography, plant variation, Holocene migration, migration pathways.

INTRODUCTION

The European hornbeam (*Carpinus betulus* L.) is a transitional species in the Polish flora, reaching its northeastern range limit just beyond the Polish border (Jalas and Suominen, 1976; Boratyńska, 1993). The species is a telocratic taxon which appeared in Central Europe ~5000 years ago and spread throughout Poland ~4000 years ago (Huntley and Birks, 1983; Środoń, 1993; Ralska-Jasiewiczowa et al., 2004). Isopollen maps suggest that the species migrated into Poland from at least two different directions, the southeast and west (Ralska-Jasiewiczowa et al., 2004). Such a migration pattern suggests that *C. betulus* in Poland originated from different Pleistocene refugia, and that this should be reflected in its present-day geographic variation.

The variation of *C. betulus* nuts suggests at least the mid-Tertiary evolution of the species, and morphological differentiation much earlier than Pleistocene (Grossheim, 1940; Jentys-Szaferowa and Bialobrzeska, 1953; Jentys-Szaferowa, 1958, 1960, 1961, 1964). The two different types of nuts visible in its present-day populations have also been found in Pliocene and interglacial deposits (Jentys-Szaferowa, 1958, 1060, 1961, 1964). Isolation of western from southeastern Europe and southwestern Asia by the Paratethis Sea during the Miocene is one hypothetical reason given for the differentiation of nuts (Jentys-Szaferowa, 1964). The shape and dimensions of the nuts, however, are connected with characteristics of the involucres (Białobrzeska, 1966b, 1970).

The present study was intended to verify the hypothesis that populations of *C. betulus* in southeast and central Poland came from different Pleistocene refugia. In that case, the southeastern populations of *C. betulus* in Poland should differ in seed scale morphology from the other populations.

MATERIAL AND METHODS

MATERIALS AND FIELD WORK

Seed scales (involucres), a critical structure for characterization, have been examined biometrically (Jentys-Szaferowa, 1958, 1964; Białobrzeska, 1966a,b, 1970). The morphological characters of involucres have been found not to vary much in material was collected in oak-hornbeam forest associations of the *Carpinion betuli* alliance (Białobrzeska, 1966a,b, 1970). For this reason we collected involucres only in typically developed asso-

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TABLE 1. Locations of the studied populations of *Carpinus betulus*

No	Region	Locality	Forest association	Geographic coordinates	Altitude [m]
1	West Carpathians	Beskid Sądecki, W of Żegiestów, slopes above Poprad	*Tilio-Carpinetum*	N49°20'55" E20°48'13"	410
2	West Carpathians	Beskid Wyspowy, between Młynne and Laskowa	*Tilio-Carpinetum*	N49°45'13" E20°26'11"	360
3	Lowland of Central Poland	Mazowiecka Lowland, Ruszki near Sochaczew	*Tilio-Carpinetum*	N52°15'25" E20°09'00"	80
4	Lowland of Central Poland	Krajna Lake district, Sośno	*Galio-Carpinetum*	N53°22'33" E17°43'33'	130
5	Lowland of Central Poland	Wielkopolska Lowland, N of Jasne Pole	*Galio-Carpinetum*	N51°38'44" E17°34'14"	130
6	West Carpathians	Sandomierz Basin, beach wood reserve in Cyranka	*Tilio Carpinetum*	N50°17'43" E21°34'37"	210
7	Lublin Upland	Roztocze, Wólka Łabuńska, on slopes	*Tilio-Carpinetum*	N23o22'35" E50o37'07"	270
8	Lowland of Central Poland	Wielkopolska Lowland, Nature Reserve Dębina near Wągrowiec	*Galio-Carpinetum*	N52°48'17" E17°08'38"	90
9	Lowland of Central Poland	Wielkopolska Lowland, Owińska, near Potasze	*Galio-Carpinetum*	N52°31'15" E17°02'44"	100
10	Lowland of Central Poland	Wielkopolska Lowland, slopes of Odra River valley near Nowa Sól	*Galio-Carpinetum*	N51°48'22" E15°44'12"	70
11	Lowland of Central Poland	Wielkopolska Lowland, Karczma Borowa	*Galio-Carpinetum*	N51°49'27" E16°37'20"	130
12	Małopolska Upland	Cracow-Częstochowa Upland, Skała Kmity Nature Reserve	*Tilio-Carpinetum*	N50°03'58" E19°42'23"	300
13	West Carpathians	Pogórze Wielickie, Brody near Lanckorona	*Tilio-Carpinetum*	N49°51'20" E19°42'25"	400
14	Lowland of Central Poland	Wielkopolska Lowland, Złoczew, near Nowa Wieś Nature Reserve	*Galio-Carpinetum*	N51°26'24" E18°36'49"	180
15	East Carpathians	Bieszczady, Miklaszki near Komańcza	*Tilio-Carpinetum*	N49°21'23" E22°06'12"	435
16	Małopolska Upland	Cracow-Częstochowa Upland, Zielona Gora Nature Reserve	*Tilio-Carpinetum*	N50°45'40" E19°15'27"	300
17	East Carpathians	Bieszczady, Dwernik, Otryt	*Tilio-Carpinetum*	N49°11'52" E22°37'15"	500
18	East Carpathians	Bieszczady, Bóbrka near Myczkowce, slopes	*Tilio-Carpinetum*	N49°25'02" E22°26'44"	350
19	West Carpathians	Beskid Niski, Szymbark, slopes above Ropa	*Tilio-Carpinetum*	N49°36'48' E21°04'21"	350
20	West Carpathians	Sandomierz Basin, Stale	*Tilio-Carpinetum*	N50°31'33" E21°45'39"	150
21	Lowland of Central Poland	Chełmno-Dobrzyń Lake District, Działdowo, E of Gródki	*Tilio-Carpinetum*	N50°13'49" E20°02'00"	170
22	Bialorussian Lowland	Polesie, Rudka near Chełm Lubelski	*Tilio-Carpinetum*	N51°08'30" E23°25'13"	220
23	Lowland of Central Poland	Podlaska Lowland, Omelno Nature Reserve near Radzyń Podlaski	*Tilio-Carpinetum*	N51°50'07" E22°43'15"	150
24	West Carpathians	Gorce, Tylmanowa-Brzegi, slopes above stream	*Tilio-Carpinetum*	N49°30'45" E20°23'45"	450
25	West Carpathians	Niepołomice Forest, between Chobot and Ispina	*Tilio-Carpinetum*	N50°05'38" E20°22'01"	190
26	Lowland of Central Poland	Krajna Lake District, slopes above Czarny between Okonek and Lędyczek	*Galio-Carpinetum*	N53°32'08" E16°55'08"	120
27	Lowland of Central Poland	Kozienice Forest, near Zagożdżon Nature Reserve	*Tilio-Carpinetum*	N51°32'13" E21°26'10"	140
28	Lublin Uplands	Roztocze, Wólka Abramowska, on slopes	*Tilio-Carpinetum*	N50°43'06" E22°44'01"	280
29	Małopolska Upland	Małopolska Upland, Borki Nature Reserve near Spała	*Tilio-Carpinetum*	N51°31'32" E20°08'58"	160

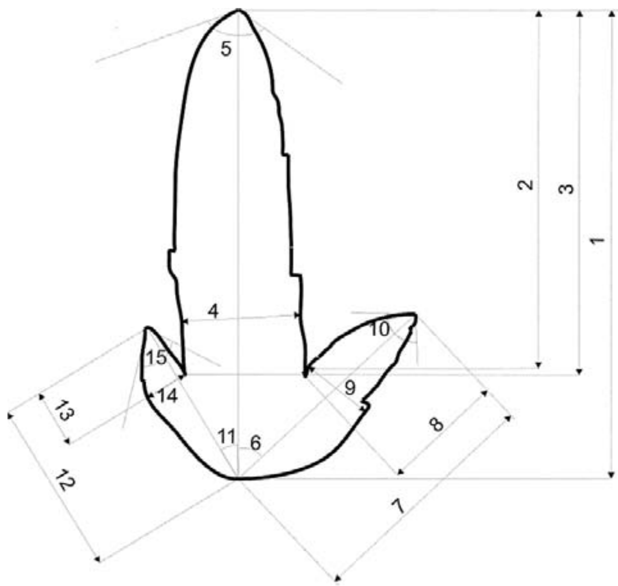

Fig. 1. Measured characters of the involucre of *Carpinus betulus* (for description see Table 2).

ciations of oak-hornbeam forest earlier described by various authors or recognized in the field. The material was gathered in 29 populations (Tab. 1), located mostly on pathways of the main Holocene expansion of the species (Ralska-Jasiewiczowa et al., 2004: Fig. 36). Every population was represented by 100 involucres randomly taken from the forest floor or cut from 25 trees. In the latter case, a single involucre was taken from the midlength of four different infrutescenses of each tree.

BIOMETRICAL VERIFICATION

Involucres of every sample were analyzed for 26 characters (Tab. 2), as other authors have done (Jentys-Szaferowa, 1958, 1964; Białobrzeska, 1966a,b, 1970). The shape of involucres is complicated, involving many detailed measurements as shown in Figure 1.

The statistics for descriptive characteristics of traits (mean, standard deviation, variation coefficient) were calculated for every sample. The variance coefficient of every character was used to eliminate the most variable traits. Cluster analyses and discriminant analyses, including minimum spanning trees, were done to verify the relations between the sampled populations (Marek, 1989; Krzyśko, 1990; Morrison, 1990; Łomnicki, 2000; Zar, 1999; Sokal and Rohlf, 2003).

The values of the measured characters, typically dimensional ones such as lobe length, depth of incisions between lobes, and lobe width (characters 1–4, 7–9, 12–14), were suspected to be influenced by environmental conditions. For that reason these characters were used to calculate the proportions only, and were not used in subsequent analyses (see above); this approach is similar to that of Kremer et al. (2002) on oak leaf variation. Finally, 14 characters were used in the multivariate analyses. The characters used earlier to calculate proportions

TABLE 2. Characters of involucres studied, as depicted Figure 1

No	Character	Precision
1	Length	1 mm
2	Depth of incision between central and outer lobe (distance between top and outer base of central lobe)	1 mm
3	Depth of incision between central and inner lobe (distance between top and inner base of central lobe)	1mm
4	Width of central lobe at base	1 mm
5	Angle of top of central lobe	°
6	Angle between central and outer lobes	°
7	Length of outer lobe	1 mm
8	Distance between top and base of outer lobe	1 mm
9	Width of outer lobe at base	1 mm
10	Angle of top of outer lobe	°
11	Angle between central and inner lobes	°
12	Length of inner lobe	1 mm
13	Distance between top and base of inner lobe	1 mm
14	Width of inner lobe at base	1 mm
15	Angle of top of inner lobe	°
16	Number of teeth of central lobe	
17	Number of teeth of outer lobe	
18	Number of teeth of inner lobe	
19	Inclination of central lobe versus axis	°
20	Shape of central lobe (ratio of length to width at base, characters 1/4)	
21	Shape of outer lobe (ratio of length to width at base, characters 7/9)	
22	Shape of inner lobe (ratio of length to width at base, characters 12/14)	
23	Outer lobe proportion (ratio of lengths of central to outer lobes, characters 1/7)	
24	Inner lobe proportion (ratio of lengths of central to inner lobes, characters 1/12)	
25	Side lobe asymmetry (ratio of lengths of outer to inner lobes, characters 7/12)	
26	Asymmetry of side lobe position (ratio of distances between top of central lobe to bases of outer and inner lobes, characters 2/3)	

TABLE 3. Average values of characters of *Carpinus betulus* involucres (character numbers as in Table 2) in 29 studied populations from Poland

Sample number	Character number																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	34.2	23.6	26.5	9.4	108.0	79.3	14.8	5.5	5.0	66.1	82.5	14.1	6.9	4.9	59.8	4.85	0.64	0.42	2.24
2	32.8	22.7	25.1	9.1	106.8	84.4	14.1	5.8	5.3	71.2	83.3	13.7	6.9	4.7	67.3	5.68	0.88	0.39	1.99
3	33.5	23.4	26.0	8.9	111.0	81.9	15.4	6.5	5.3	69.6	84.0	14.7	8.0	5.0	66.6	4.33	0.66	0.15	2.42
4	37.6	26.3	29.0	9.0	102.5	76.2	17.4	7.3	5.6	65.8	78.3	17.0	8.8	5.0	65.7	4.05	0.80	0.34	2.81
5	35.5	24.5	27.7	8.2	103.9	76.3	15.4	6.1	4.8	61.6	76.8	15.0	7.6	4.4	62.2	5.44	0.80	0.38	2.03
6	39.3	28.0	31.4	9.4	105.0	75.5	17.5	7.9	5.7	65.1	79.7	15.9	8.5	4.9	61.5	6.75	1.35	0.70	2.41
7	34.7	22.9	26.8	9.4	110.0	78.5	16.0	6.3	5.2	64.3	83.5	15.0	8.0	5.2	60.6	5.21	0.93	0.59	2.06
8	34.9	24.8	27.0	9.2	111.2	81.2	15.4	6.7	5.3	71.2	85.2	14.5	7.6	4.9	67.0	3.91	0.99	0.57	1.65
9	32.3	22.0	24.6	8.8	111.5	80.5	14.6	5.5	4.8	69.5	82.9	13.5	6.8	4.6	65.4	4.94	0.61	0.39	2.17
10	35.1	25.0	27.3	9.0	108.8	78.9	15.2	6.7	5.3	73.3	80.2	14.9	8.1	5.0	73.4	3.95	0.91	0.54	1.52
11	32.2	22.0	24.6	9.2	111.5	83.3	14.6	6.1	5.2	68.4	83.8	13.8	7.2	4.7	69.2	3.47	0.75	0.25	1.32
12	32.8	22.4	25.4	8.8	102.4	82.9	13.2	4.9	4.4	66.1	79.9	12.9	6.7	4.6	64.9	3.93	0.68	0.35	1.34
13	35.7	24.3	27.5	8.9	100.7	74.2	15.2	6.2	5.1	58.5	76.5	14.4	7.7	4.8	60.1	6.64	1.13	0.66	1.71
14	31.6	21.2	24.0	7.9	97.3	74.5	14.6	5.9	4.5	57.0	77.6	13.7	7.1	4.2	57.6	3.62	0.63	0.30	1.85
15	30.3	20.1	22.8	9.1	107.4	80.4	13.9	5.6	4.9	66.3	84.0	13.1	6.7	5.0	69.3	5.47	1.03	0.63	1.86
16	34.0	22.7	26.1	8.7	103.0	77.7	15.6	6.3	5.3	69.5	82.4	14.9	7.9	4.9	70.7	5.38	0.64	0.42	2.24
17	29.3	19.9	22.4	8.8	114.5	79.7	13.0	4.9	4.4	67.0	84.1	12.6	6.5	4.6	75.4	3.02	0.56	0.28	1.38
18	32.0	21.6	24.9	9.2	105.5	81.6	14.4	5.8	5.1	64.0	85.0	13.5	7.5	4.9	67.1	4.98	0.84	0.47	1.93
19	36.0	25.2	28.4	9.4	102.7	82.9	14.9	6.1	5.4	66.8	80.3	14.5	7.7	4.9	69.2	4.89	0.94	0.49	1.07
20	35.1	24.4	27.3	9.4	107.6	80.6	15.9	6.9	5.4	64.9	82.4	15.3	8.1	5.1	68.1	5.13	0.67	0.39	1.89
21	32.3	22.4	25.0	9.2	94.9	72.4	14.8	6.1	4.6	59.5	80.6	13.7	7.4	4.6	61.0	6.30	1.39	1.11	1.35
22	34.1	23.6	26.5	9.4	103.8	81.1	15.1	6.1	5.0	62.2	82.5	14.5	7.5	4.9	64.9	4.28	0.72	0.34	1.59
23	33.6	22.6	25.5	8.5	99.3	80.7	14.6	5.7	4.6	61.7	80.8	14.5	7.6	4.6	62.9	2.87	0.54	0.26	2.33
24	32.0	21.8	24.2	9.0	110.0	83.2	13.8	5.5	4.9	69.9	83.4	13.6	6.7	4.8	72.4	4.52	0.62	0.24	2.07
25	31.6	21.7	24.0	8.8	105.8	81.3	13.9	5.5	4.7	61.5	82.1	13.8	7.0	4.6	61.0	4.81	0.73	0.35	1.66
26	32.9	23.2	25.5	9.4	99.3	88.3	14.1	6.1	5.3	71.9	87.1	13.5	7.2	4.8	70.1	3.08	0.88	0.47	1.55
27	32.7	21.3	24.8	9.2	105.1	81.7	14.5	5.0	4.5	68.2	87.1	13.7	7.0	4.8	70.0	4.19	0.92	0.55	1.97
28	33.7	22.0	25.3	9.6	102.6	76.4	15.4	5.6	5.0	66.5	75.5	14.3	7.1	4.8	66.8	4.38	0.72	0.24	1.36
29	32.0	22.6	25.4	7.8	98.7	77.9	14.0	6.3	4.8	57.5	80.2	13.2	7.6	4.3	63.4	4.18	0.70	0.39	1.84
Average	29.28	19.92	22.40	7.80	94.89	72.44	13.02	4.92	4.38	56.97	75.50	12.62	6.49	4.18	57.55	2.87	0.54	0.15	1.07

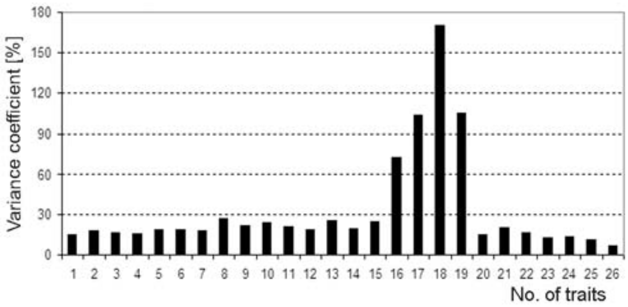

Fig. 2. Average variance coefficient of particular characters (nos. 1–26, as in Tab. 2) of *Carpinus betulus* involucres.

(1–4, 7–9, 12) and the most variable ones, with the highest measurement error (16–19) were omitted from the multivariate analyses. All characters were standardized prior to analyses. The proportions were transformed using the angular function x'= arcus sinus x^{-2}. Statistica 7.1 for Windows software was used for calculations.

RESULTS

CHARACTER EVALUATION

The average values of the measured characters in the sampled populations are shown in Table 3. The number of teeth on lobe sides (characters 16, 17, 18) seemed the most variable character, with very high variance coefficients ranging from 54% and 70% for number of teeth on central lobe, from 77% to as much as 240% in some samples for side lobes, and from 74% to 170% for all the material on average (Fig. 2). Similarly, the angle of the central lobe versus the involucre axis (character 19) showed very high variation. For that reason the characters describing the number of teeth on lobes and the central lobe angle (16–19) were omitted from subsequent analyses. Even though the material was collected exclusively in the forest communities of the *Carpinion betuli* alliance, most of the characters differentiated the samples at a statistically significant level ($p \leq 0.01$). The measured and evaluated characters of the involucres appeared variable, and in subsequent tests only traits with variance coefficients no higher than 30% were accepted. The shape-describing traits, calculated as proportions of measured and/or evaluated characters, were the most stable ones (Fig. 1). Asymmetry of position of side lobes (character 26) had the lowest variance coefficient, 7% on average for all the material and not exceeding 10% in any sample. The inner lobe had a lower position than the outer one except for sample 6 from the Krajna Lake District in northern Poland. Asymmetry of side lobes (character 25) also had a variance coefficient not exceeding 10%. Generally the sample gathered in the Zielona Góra Nature Reserve in the Małopolska Upland (sample 19) showed the lowest variability. Its variance coefficients for shape features (characters 20–26) ranged between 4.6% and 8%.

TABLE 4. Discriminant power tests of the most discriminating characters of involucres of *Carpinus betulus* (character numbers as in Table 2)

No	Character	F	P
5	Angle of top of central lobe	6.29	0.00000
6	Angle between central and outer lobes	3.157	0.00000
8	Distance between top and base of outer lobe	5.179	0.00000
10	Angle of top of outer lobe	5.148	0.00000
11	Angle between central and inner lobes	2.549	0.00001
13	Distance between top and inner lobes	2.714	0.00000
15	Angle of top of inner lobe	5.743	0.00000
20	Shape of central lobe	11.049	0.00000
21	Shape of outer lobe	3.721	0.00000
22	Shape of inner lobe	3.925	0.00000
23	Outer lobe proportion	2.268	0.00016
24	Inner lobe proportion	1.778	0.00719
25	Side lobe asymmetry	1.742	0.00928
26	Asymmetry of side lobe position	5.016	0.00000

GEOGRAPHICAL VARIATION

The discriminating power of all 14 characters used in the statistical analyses was low, and similar for all of them (Tab. 4). Total variability among the 29 samples was described by a dozen discriminating variables. The first two accounted for only 43.5% of total variation. The scatter plot for the two first functions (Fig. 3) grouped two lowland samples – from Krajna Lake district and the Wielkopolska Lowland and from Sandomierz Basin, the latter included with the Carpathians (samples 4, 5, 6, respectively) on one side of the plot, two samples from Bieszczady (15, 17) on the other side, and all the other samples forming one large group between them.

Agglomeration based on the Euclidean distances (Fig. 4) confirmed the separation mentioned above, but also included four others from the lowlands (samples 14, 21, 23, 29) and one from the West Carpathians (13). At the second subdivision, however, the Carpathian populations (2, 12, 17, 19, 24) and one from the Krajna Lake district (sample 26) showed some separation from all the other samples (Fig. 4). The next subgroups of samples covered in turn the lowland and upland regions of central

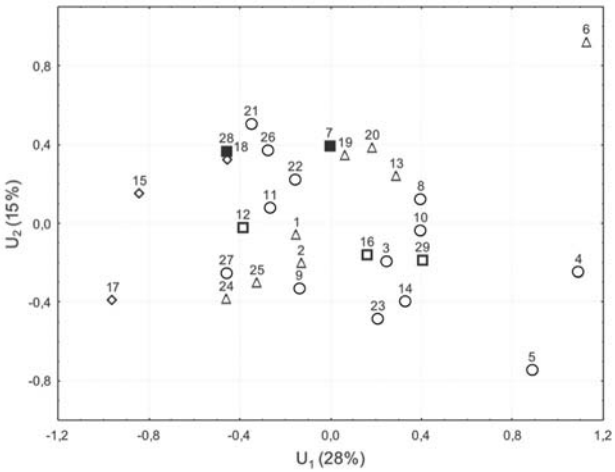

Fig. 3. Result of discriminant analysis based on evaluated and calculated traits of involucres of 29 samples of *Carpinus betulus* (nos. 1–29 as in Tab. 1) plotted against the first two discriminant variables which accounted for 43% of total variation (◇ and △ – mountain population, ○ – lowland population, □ and ■ – upland populations).

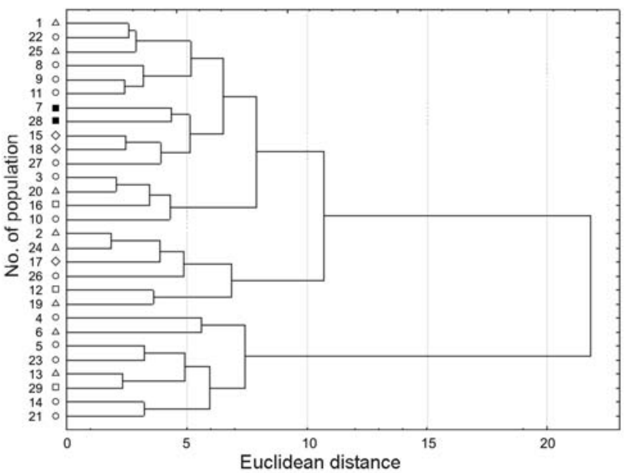

Fig. 4. Dendrogram of *Carpinus betulus* samples (nos. 1–29 as in Tab. 1) constructed on the basis of Euclidean distances (population symbols as in Fig. 3).

Poland (3, 10, 16, 20), the East Carpathians and Lublin Upland (7, 15, 18, 27, 28), and the Wielkopolska and Belarusan Lowlands, Sandomierz Basin and West Carpathians (1, 8, 9, 11, 22, 25).

The minimum spanning tree constructed using the squares of Mahalanobis distances between samples confirmed the geographically unclear pattern of variation (Fig. 5). Most of the distances between samples were statistically significant: ten were significant at less than $p = 0.001$, seven at $p = 0.01$ or $p = 0.05$, and only four were not significant (Fig. 5). Nevertheless, all samples formed one group not divided by exceptionally large distances. Nonsignificant and slightly significant distances linked samples into four slightly differentiated subgroups, some of them similar to those in the agglomeration analysis (Fig. 4).

DISCUSSION

CHARACTERS

The average values of characters found in particular samples were similar to those described by Jentys-Szaferowa (1958, 1964) and by Białobrzeska (1966a, b, 1970). The average values of involucre length (character 1) ranged between samples from 29.3 to 33.6 mm, and corresponded rather to the values for *Circaeo-Alnetum* and *Querco-Betuletum* than to those for *Tilio-Carpinetum*, as given by Białobrzeska (1970).

The average values of central lobe width (character 4) varied between particular samples from 7.80 to 9.61 mm, but the average for the total material was closest to the value characteristic of *Querco-Carpinetum* in the Białowieża Forest (Białobrzeska, 1966b, 1970: 99). The angle of the top of the central lobe (character 10) was higher in our study than the value from the Białowieża Forest. Generally, the top of the central lobes of involucres had an angle greater than 90°, while in data by Białobrzeska (1966b, 1970) its average value in *Querco-Carpinetum* was much lower. The number of teeth on the central lobe (character 16) averaged 4.63 in our study and ranged from 2.87 to 6.75, much higher than reported by Białobrzeska (1970). The differences between our data and those reported by Białobrzeska (1966b, 1970) probably are the result of differences in measurement methods. Surprisingly, the variance coefficients of particular characters from our data and Białobrzeska's data (1996b, 1970) are similar (compare Fig. 2 and Białobrzeska, 1966b).

GEOGRAPHIC VARIATION

The influence of site was minimized by sampling in the same or geographically vicarious communities of the *Carpinion betuli* alliance (Matuszkiewicz, 2001). Nevertheless, the differences between most of the samples were statistically significant (Tab. 4, Fig. 5). This suggests a nonenvironmental influence on the present-day variation of the morphological characters of involucres. The described differences may be the result of geographical isolation and a lack of gene flow during a sufficiently long period, probably before the Holocene.

The genetic diversity of *C. betulus* in Europe appeared lower to the north of the Alps and Carpathians than south of them. The lower genetic

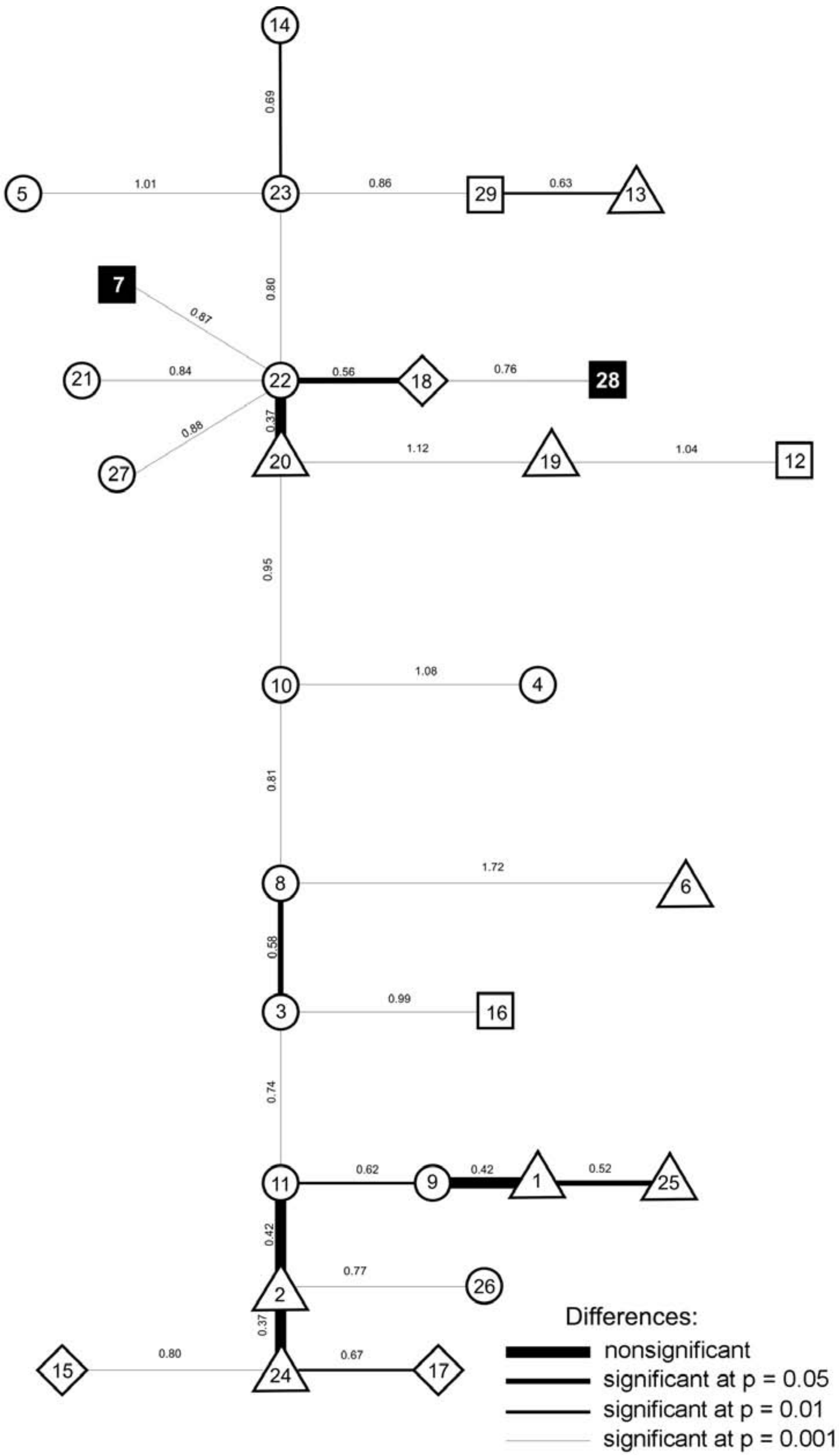

Fig. 5. Minimum spanning tree of *Carpinus betulus* samples (nos. 1–29 as in Tab. 1) constructed on the basis of squares of the Mahalanobis distances (population symbols as in Fig. 3)

variation in Central Europe suggested the bottleneck effect (Coart et al., 2005) and expansion from a single or only a few individuals. The latter probably also affected morphological variability, as has been described for *Juniperus oxycedrus* subsp. *macrocarpa* (Lewandowski et al., 1996; Klimko et al., 2004) and for the chemical composition of plant species of the Asteraceae family (Swenson et al., 1999; Wallaart et al., 2000). The differences between fossil *C. betulus* nuts, described by Jentys-Szaferowa (1964) and earlier mentioned by Grossheim (1940) and Jentys-Szaferowa and Białobrzeska (1953), seems to conform with results on genetic variation (Coart et al., 2005).

The variation of *C. betulus* involucres in Poland is difficult to interpret geographically, like the morphological variation of other tree and shrub species (Staszkiewicz, 1997). The differences and similarities among the compared local populations of *C. betulus* in Poland did not show a simple geographic pattern (Figs. 3–5). The presented results suggest two or three centers of origin of *C. betulus*, as has been described for the variation of samaras of *Acer pseudoplatanus* (Boratyński, 1980) and several species of shrubs (Staszkiewicz, 1997). One well-distinguished group of samples of *C. betulus* covered the East Carpathians, with the most separated samples (15, 18) from Bieszczady (Fig. 3 and 4). *C. betulus* colonized the Bieszczady (and later the Beskid Niski) mountains 4500 years ago. The isopollen map for that period (Ralska-Jasiewiczowa et al., 2004: 76) suggests the arrival of the species from the southeast. Taking into account the ecological character of *C. betulus* (Faliński and Pawlaczyk, 1993; Zarzycki et al., 2002; Didukh et al., 2004), it seems possible that it could migrate along the northeastern forelands of the East Carpathians and then spread to the West Carpathians and the Polish uplands and lowlands. Quite possibly the samples from the Lublin Lowland (7, 28) and from the Kozienice Forest (27) grouped with samples from Bieszczady (Fig. 4) are contemporary traces of that ancient migration.

Five hundred years later, 4000 years ago, *C. betulus* may also have colonized the Beskid Niski Mts. from the southeast (compare Ralska-Jasiewiczowa et al., 2004; see also Gardner, 2002), joining populations already in the Bieszczady. This is suggested by the different character of the West Carpathian populations (samples 2, 19, 24). This group of populations is joined by one from Bieszczady (17), one from the Cracow-Częstochowa Upland (sample 12) and even another from the Krajna Lake District in northern Poland (sample 26). The latter two may be traces of ancient migration, or may be the result of long-distance pollen transport or seed translocation by humans.

The high concentration of pollen grains of *C. betulus* in the Wielkopolska Lowland at 3000–3500 BP (Ralska-Jasiewiczowa et al., 2004) may suggest the arrival of the species from the west. This is supported by the separation of a group of populations from the lowlands of central Poland (4, 5, 14, 21, 23), the northern part of the Malopolska Upland (29) and northern parts of the West Carpathians (6, 13).

Expansion of *C. betulus* from two (or more) centers and subsequent hybridization between populations facilitated by long-distance pollen transport (Ralska-Jasiewiczowa et al., 2004) is probably responsible for the complicated and tangled variation of the species observed at present. It is also quite possible that the migration came in waves from different centers or refugia during particular periods of the Holocene, but this should be confirmed by more detailed studies incorporating additional material from outside Poland.

The rapid expansion of *C. betulus* and colonization of Central Europe started about 4000 BP (Ralska-Jasiewiczowa et al., 2004). Human colonization took place in that period, and influenced the expansion of the European hornbeam by reducing of frequency of other tree species and their role in the forests. Human activity promoted pioneer anemochorous species such as *C. betulus* rather than barochorous trees of the last stages of succession (Ralska-Jasiewiczowa, 1964; Faliński, 1993; Faliński and Pawlaczyk, 1993). The moderate light demands of the hornbeam (Faliński and Pawlaczyk, 1993; Zarzycki et al., 2002) facilitated colonization under canopy of generally higher and more light-demanding tree species such as *Pinus sylvestris* and above all *Quercus robur*, and shifting of the range of *C. betulus* (Ralska-Jasiewiczowa, 1964; Środoń, 1990, 1993; Kuster, 1997). Hornbeam has been well documented as a moderate pioneer species in recent years. It rapidly colonized areas after felling of oak-hornbeam woods in the Białowieża Forest (Bernadzki et al., 1998).

The origin of *Carpinus betulus* in Poland from at least two different centers, visible on isopollen maps, was confirmed in this study of the morphological variation of its involucres.

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