INTRASPECIFIC DIFFERENTIATION OF REED GRASS *CALAMAGROSTIS ARUNDINACEA* (L.) ROTH (POACEAE) POPULATIONS REVEALED BY PEROXIDASE ALLOZYMES

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ABSTRACT

The genetic variation of Reed Grass *Calamagrostis arundinacea* (L.) Roth was investigated in 25 populations in various geographic regions of Poland. A total of 907 individuals were sampled for electrophoretic analysis of peroxidase loci (11 allozymes). Populations were characterised by genetic parameters e.g. heterozygosity level, Wright's fixation index (F) and polymorphism coefficient (Pg). Mean values of interpopulation variability level (GST=0.0310), total genetic diversity (HT=0.4102) and gene flow between populations (Nm=7.805) were also examined. All the populations were polymorphic and they remain in Hardy Weinberg equilibrium.

KEY WORDS: *Calamagrostis arundinacea*, peroxidases, natural populations, Poland.

INTRODUCTION

Species of the genus *Calamagrostis* are highly variable (Mirek et al. 2002; Rutkowski 2002) and considered to be some of the most difficult grass species to identify (Frey and Paszko 1999). Reed Grass, *C. arundinacea* (L.) Roth, is a typical forest species, found in parts of Poland, but less frequently in the south-western part of the country i.e. in Silesia region (Zając and Zając 2001). In mountains it grows in natural habitats of the Sudety Mts., and the Carpathians (Mirek and Piękoś-Mirkowa 2002). It is a typical species for the community of Central-European acidophilic oak forests Calamagrostio arundinaceae – Quercetum petraeae, most closely resembling the continental mixed forests of the class Vaccinio – Piceetea (Brzeg et al. 1989, 2001; Matuszkiewicz 2001).

Because of its attractive seedheads, frequently used in flower arrangements, the species is recommended in North America as easy to be planted (Gilman 1999) as an ornamental grass.

In Poland, *C. arundinacea* used to be regarded as a variable species and differences in respect of panicle morphology enabled distinction of three varieties, including var. *alpina* (Schur) Matuszk., var. *grandiflora* Litv., var. *macrotricha* Litv. (Falkowski 1982). *Calamagrostis* species were also intensively investigated in respect to anatomical features, especially the internal culm structure (Paszko and Krawczyk 2005).

The basic chromosome number in the genus *Calamagrostis* is x=7. Since *C. arundinacea* exhibits the chromosome number of 2n=28 and, it is thought to be a tetraploid species (Frey and Paszko 1999), which manifests a tendency for hybridization with other species, including *C. neglecta*, *C. pseudophragmites*, *C. varia*, *C. canescens* (Falkowski 1982), *C. epigejos*, and *C. villosa* (Krzakowa et al. 2003), forming apomictic complexes with variable chromosome number (Nygren 1962; Tsvelev 1965).

Some of the hybrids have been given a taxonomic rank, e.g. *Calamagrostis* × *hartmaniana* Fries, described originally in Sweden and, in view of intermediate morphological traits, probably representing hybrids of *C. arundinacea* and *C. canescens* (Paszko 2001). Similarly, basing on morphological traits, another species of hybrid origin is known as *Calamagrostis* × *acutiflora* (Schrader) Reichenbach, resulting from *C. arundinacea* × *C. epigejos* crosses (Rebele and Lehman 2001).

Our preliminary study (Krzakowa et al. 2003) permitted to determine genetic differences for species like *C. epigejos, C. arundinacea* and *C. villosa*. This was possible due to examination of electrophoretic variability of peroxidases, regarded as excellent taxonomic markers in other groups of plants (Krzakowa 1993; Kołodziejczak and Krzakowa 2003) as well as a measure of intraspecific variability (Krzakowa 2001), also among grasses (Krzakowa 1996; Krzakowa and Mikulski 1997; Krzakowa and Drapikowska 2000; Krzakowa et al. 2005). In addition, the enzymes are extremely stable (Rassmusen and Kerby 1993). The genetic structure of populations in species of *Calamagrostis* genus remains relatively unknown. Till now, extensive studies on *C. epigejos* have been performed in Germany (Leh-

man 1997) on *C. porteri* ssp. *insperata* (Esselman et al. 1999) in the USA.

Pure populations of *C. arundinacea* in the Karkonosze Mts., (Krzakowa and Dunajski 2007) have exhibited a similar scale of variability, and peroxidases have proven to be also extremely useful in establishing interspecies differences between *C. arundinacea* and *C. villosa*. Each of the species carries its own peroxidase markers (Krzakowa and Dunajski 2007).

Little is known about the genetic subdivision within the species, so the purpose of this study was to estimate the level of genetic variability and differences among *C. arundinacea* populations growing in different habitats and plant associations all over the country.

MATERIAL AND METHODS

Plant material from 25 populations, collected in various geographic regions of Poland (Fig. 1) was tested for peroxidase allozymes. Crude extract from individual plants was subjected to electrophoresis in 11% starch gel, with the lithium-boric buffer system, pH 8.3. Peroxidases (EC 1.11.1.7) were specifically stained with 3-amino-9-ethylcarbazole as in other grass species (Krzakowa 1996; Krzakowa et al. 2006; Krzakowa and Dunajski 2007). In each population, the extent of inbreeding was estimated by means of Wright's fixation coefficient (F), using the formula: F=1-Ho/He, where Ho is observed heterozygosity and He is expected heterozygosity. Polymorphism index was calculated according to Kahler et al. (1980). Gentic similarities and distances were calculated on the basis of alleles (Nei 1977) and genotype frequencies (Hedrick 1974), than illustrated by dendrograms (cluster analysis UPGMA) and dendrite (OUT – the shortest neighbourhood).

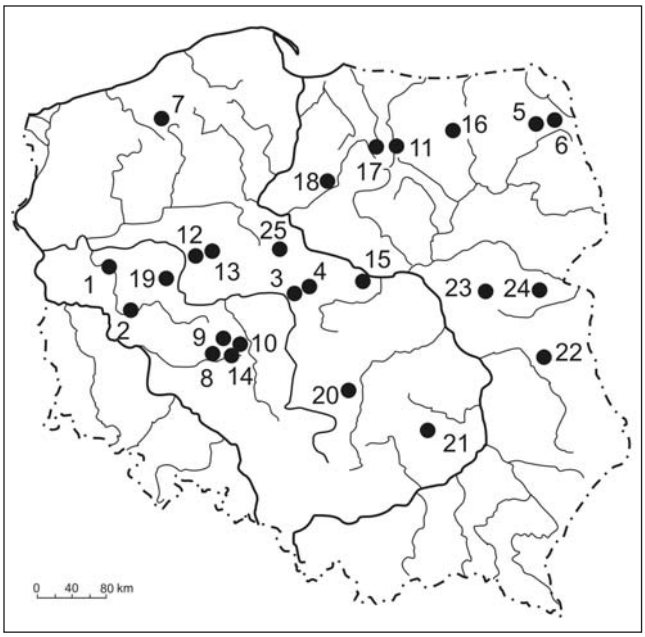

Fig. 1. Geographic distribution of *C. arundinacea* populations examined for genetic variability. Population designation are the same as those given in methods.

Plants were collected from different localities (Table 1).

RESULTS

Electrophoretic separation of peroxidases made it posible to distinguish three polymorphic loci: anodally migrating

TABLE 1. Characteristics of examined populations.

Populations	No of individuals in populations	Locality	Habitat
1	30	Pszczew – forest district Trzciel, N 52°27'10,8'', E 15°45'20,3''	pine forest with very rare *Dianthus gratianopolitanus*
2	30	Wyspa Konwaliowa – forest district Kościan, N 51°59'05,9'', E 16°15'05,8''	acidophilous oak forest
3	30	Kiejsze 1 – forest district Koło, (section 111), N 52°19'22,8'', E 18°45'07,6''	acidophilous oak forest
4	80	Kiejsze 2 – forest district Koło, (section 117) – N 52°19'30,1'', E 18°44'55,6'',	oak forest
5	30	Augustów 1 – forest district Augustów, N 53°47'00,4'', E 22°57'23,3''	pine forest
6	60	Białobrzegi – forest district Augustów, N 53°46'47,1'', E 22°57'49,7''	acidophilous mixed forest (Pino-Quercetum)
7	55	Nizinne near Storków – forest district Szczecinek, N 53°46'55,4'', E 16°35'53,7''	acidophilous beech-forest
8	30	Gliśnica – forest district Krotoszyn, N 51°36'32,3'', E 17°36'43,9''	acidophilous oak forest
9	60	Krotoszyn – forest district Krotoszyn, N 51°45'25,0'', E 17°36'20,2''	acidophilous oak forest
10	30	Roszki 1 – forest district Krotoszyn, N 51°45'43,6'', E 17°36'32,6''	acidophilous oak forest
11	30	Olsztyn – forest district Kudypy, N 53°46'13,8'', E 20°20'57,4''	acidophilous mixed forest
12	30	Dziewicza Góra 1 – forest district Łopuchówko, N 52°29'04,8'', E 17°00'28,9''	acidophilous oak forest
13	25	Dziewicza Góra 2 – forest district Łopuchówko, N 52°29'09,3'', E 17°00'37,2''	acidophilous oak forest
14	60	Roszki 2 – forest district Krotoszyn, N 51°46'42,9'', E 17°36'48,3''	acidophilous oak forest
15	30	Łąck – forest district Łąck, 52°28'44,2'', E 19°38'31,7''	acidophilous mixed forest
16	30	Barczewo – Biskupiec, – forest district Wipsowo, N 53°49'56,9'', E 20°46'23,8''	acidophilous mixed forest
17	30	Olsztyn – forest district Kudypy, N 53°46'16,7'', E 20°23'57,0''	acidophilous mixed forest
18	30	Brodnica – forest district Brodnica, N 53°16'47,1'', E 19°26'53,3''	acidophilous mixed forest
19	30	Jeziory – forest district Mosina, N 52°15'40,1'', E 16°48'11,3''	acidophilous oak forest.
20	30	Piotrków Trybunalski – forest district Piotrków, N 51°25'27,8'', E 19°46'22,6''	acidophilous mixed forest
21	30	Końskie – forest district Barycz, N 51°10'13,3'', E 20°29'13,4''	acidophilous mixed forest
22	31	Leszkowice – forest district Lubartów, N 51°34'05,1'', E 22°37'36,1''	acidophilous mixed forest
23	25	Lipniaki – forest district Radzyń Podlaski, N 51°53'52,4'', E 22°38'17,2''	acidophilous mixed forest
24	31	Zahajki – forest district Międzyrzec Podlaski, N 51°56'38,5'', E 22°51'27,9''	Acidophilous mixed forest
25	30	Miradz – forest district Miradz, N 52°34'56,7'', E 18°10'29,9''	oak forest

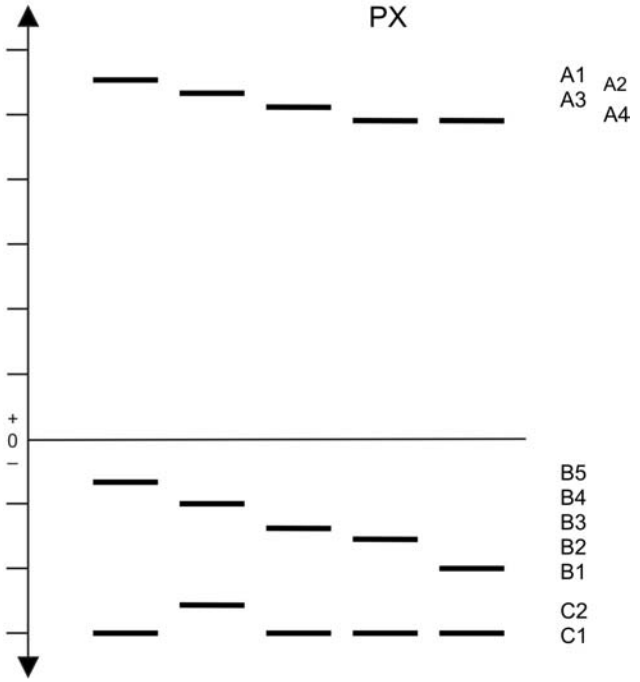

Fig. 2. Zymogram of peroxidase band patterns detected in *C. arundinacea* populations.

locus A with four allozymes, and loci B and C with cathodal migration, comprising, five and two alleles respectively (Fig. 2).

Cathodic peroxidases play a significant role in description of intraspecific variability in various plant groups (Krzakowa 1993, Kołodziejczak and Krzakowa 2003), including grasses (Felder 1976; Krzakowa 1996; Krzakowa and Mikulski 1997; Krzakowa et al. 2006; Krzakowa and Dunajski 2007). The dendrogram constructed on the basis of frequency of alleles of the two cathodic loci show genetically different groups of populations (Fig. 3). The first group consists of two subgroups with 17 populations, the second group is composed of six populations, and the third group includes visibly separated two populations: 21 (Końskie) and 23 (Lipniaki).The shortest connections, i.e. the highest genetic similarity, are between three populations: 1 (Pszczew), 13 (Dziewicza Góra) and 9 (Krotoszyn), each separated by more than 80 km from the others.

Populations compared on the basis of genotype frequencies show radically different connections between populations (Fig. 4). They formed two main groups divided into subgroups. The most similar were four populations: 2 (Wyspa Konwaliowa) and 5 (Augustów), separated by over 400 km, as well as population 14 (Roszki) and 20 (Piotrków Trybunalski), separated by 150 km. Populations

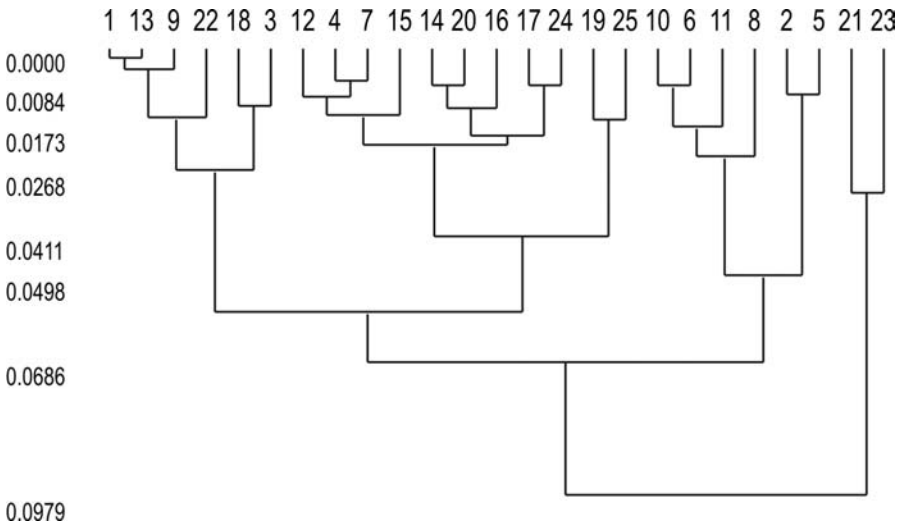

Fig. 3. UPGMA dendrogram illustrates the shortest genetic distances between *C. arundinacea* populations, based on cathodally migrating allozymes frequency (Nei 1977).

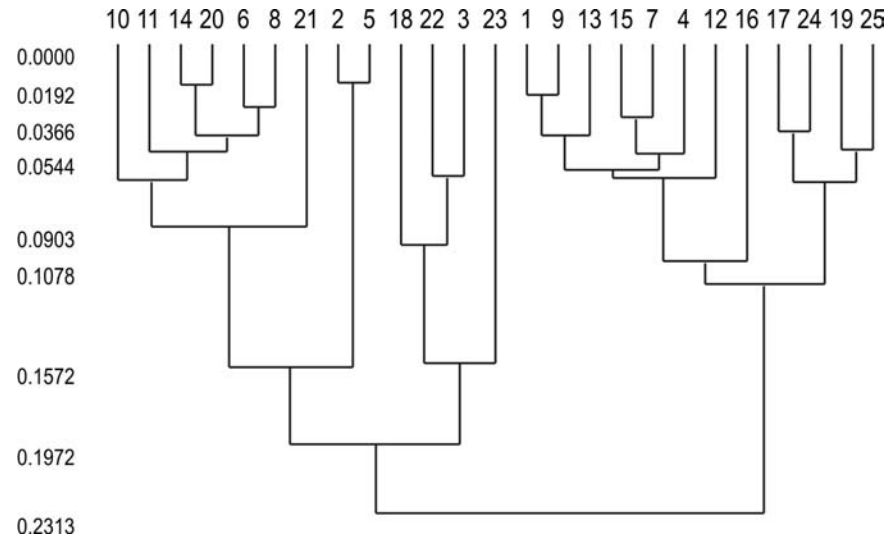

Fig. 4. UPGMA dendrogram based on genotype frequencies (Hedric 1974).

2 and 5 reached the highest level of heterozygosity (H_o=0.733) in locus A and the same values of polymorphism index ($P_{g=}$0.8089 for both populations) in locus B (Table 2).

All examined populations were characterized by low F values, from 0.0249 (for population 19) to 0.5420 (for population 17), which suggests that they remain in Hardy-Weinberg equilibrium. There was no relationship between the value of polymorphism index and observed heterozygosity for particular populations. The most polymorphic populations were 4 (Kiejsze) and 11 (Olsztyn), with P_g=0.8431 and P_g=0.8422 respectively, for both in locus B. The lowest value of P_g=0.5089 characterised population 19 (Jeziory) in locus A.

Populations compared on the basis of genotype frequencies, composed of all anodic and cathodic loci (A+B+C), are illustrated by a dendrite in Figure 5. As was previously seen on dendrograms, the first group, composed of seven populations (10, 11, 14, 20, 6, 8, and 21), is also connected by the shortest distances (Hedrick 1974). Again, the highest genetics similarity is shown by populations 2 (Wyspa Konwaliowa) and 5 (Augustów) as well as 1 (Pszczew) and 9 (Krotoszyn), separated by more than 80 km. Connections between populations 19 (Jeziory) and 25 (Miradz) are also confirmed, although they lie 120 km apart.

Mean values of intra-population variability have proven higher (GST=0.310) than inter-population variability level (DST=0.127), while the total genetic diversity HT amoun-

TABLE 2. Relative measures of genetic differentiation in the studied populations of *C. arundinacea*: Ho – observed heterozygosity; He – expected heterozygosity; F – Wright's fixation index; Pg – proportion of polymorphic loci.

Locus	Populations	He	Ho	F	Pg
PX A	1	0.5422	0.4667	0.1393	0.7222
PX A	2	0.5828	0.7333	-0.2583	0.7222
PX A	3	0.6672	0.4000	0.4005	0.8156
PX A	4	0.5899	0.4125	0.3008	0.7563
PX A	5	0.5789	0.7333	-0.2668	0.7356
PX A	6	0.5357	0.5000	0.0666	0.6939
PX A	7	0.5876	0.5636	0.0408	0.7689
PX A	8	0.5306	0.5333	-0.0052	0.6778
PX A	9	0.5726	0.5333	0.0686	0.7544
PX A	10	0.5461	0.5667	-0.0376	0.6822
PX A	11	0.5872	0.5667	0.0350	0.7533
PX A	12	0.4644	0.3333	0.2823	0.6467
PX A	13	0.5184	0.4400	0.1512	0.6976
PX A	14	0.4661	0.4333	0.0703	0.6106
PX A	15	0.5150	0.5000	0.0291	0.7089
PX A	16	0.4994	0.3667	0.2659	0.6467
PX A	17	0.5094	0.2333	0.5420	0.5933
PX A	18	0.6511	0.5667	0.1297	0.8089
PX A	19	0.2939	0.3333	-0.1342	0.5089
PX A	20	0.5294	0.5333	-0.0073	0.6778
PX A	21	0.5994	0.7333	-0.2234	0.7111
PX A	22	0.6119	0.5484	0.1037	0.7929
PX A	23	0.6536	0.5200	0.2044	0.8192
PX A	24	0.4990	0.3226	0.3535	0.6410
PX A	25	0.5039	0.4000	0.2062	0.6644
PX B	1	0.5800	0.4333	0.2529	0.7489
PX B	2	0.6472	0.4667	0.2790	0.8089
PX B	3	0.5367	0.4000	0.2547	0.7044
PX B	4	0.6784	0.4750	0.2998	0.8431
PX B	5	0.6656	0.4333	0.3489	0.8089
PX B	6	0.6322	0.5000	0.2091	0.7889
PX B	7	0.5929	0.3818	0.3560	0.7617
PX B	8	0.6761	0.4333	0.3591	0.8289
PX B	9	0.5922	0.3667	0.3809	0.7689
PX B	10	0.5889	0.4333	0.2642	0.7689
PX B	11	0.6894	0.5667	0.1781	0.8422
PX B	12	0.5844	0.5333	0.0875	0.7533
PX B	13	0.5184	0.4800	0.0741	0.7040
PX B	14	0.6500	0.4500	0.3077	0.8200
PX B	15	0.6239	0.4333	0.3054	0.7956
PX B	16	0.6528	0.6333	0.0298	0.8111
PX B	17	0.4978	0.4333	0.1295	0.7200
PX B	18	0.5094	0.4000	0.2148	0.7022
PX B	19	0.5128	0.5000	0.0249	0.7044
PX B	20	0.6656	0.5000	0.2487	0.8289
PX B	21	0.6128	0.4667	0.2384	0.8000
PX B	22	0.5146	0.4194	0.1850	0.7201
PX B	23	0.6192	0.4800	0.2248	0.7936
PX B	24	0.5796	0.3548	0.3878	0.7409
PX B	25	0.5394	0.3000	0.4439	0.6889

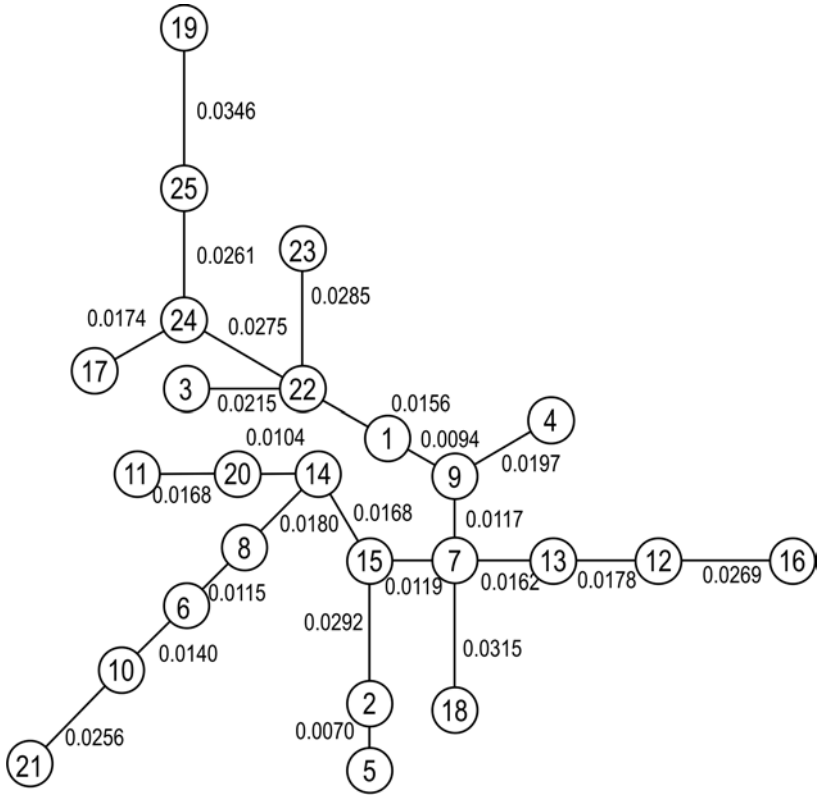

Fig. 5. Dendrite based on all genes frequency in the studied *C. arundinacea* populations.

ted to 0.412. Populations are isolated, therefore gene flow is rather low and achieves a value of Nm=7.80 migrants per population.

DISCUSSION

The genus *Calamagrostis* includes well-defined species. They are recognised on the basis of morphological traits, but show enormous variability and inter-specific hybridization (Paszko 2007). *C. arundinacea* populations are frequently found in natural and semi-natural forest and shrub communities (Celka 2007; Kucharski 2007) in Poland and therefore have not been protected up to now.

The most interesting result of this study is similarity between populations situated a long distance apart from one another, suggesting the same level of gene exchange between individual plants, and panmictic hybridization. It is very possible that limited seed dispersal may generate local genetic structure. Local selection gradients related to topography and soil depth may also play an important role in structuring local genetic variation. A widely distributed species, such as *C. arundinacea,* is assumed to have a relatively low genetic differentiation at a large spatial scale.

All populations were polymorphic, although they were examined in respect of variability of the enzyme system, which is extremely stable (Rassmusen and Kerby 1993). This has inspired us to initiate broader genetic studies on the species in question (Krzakowa and Celka 2007).

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