MORPHOLOGICAL DIFFERENTIATION OF *PRIMULA VERIS* L. POLLEN FROM NATURAL AND CULTIVATED POPULATIONS

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ABSTRACT

Micromorphological characterisation and the comparative statistical analysis of the size of *Primula veris* L. pollen grains collected in three natural and three cultivated populations were done. Observations were carried out with SEM. The obtained measurements were analysed with the use of one-way ANOVA, Kruskal-Wallis Test and the Student-*t* Test. Pollen grains from long-styled ('pin') flower-morphs were mainly 6 colpate and from short-styled ('thrum') flower-morphs 8 colpate. Colpi of some grains from 'thrum' flowers were 'sinuous' and 'circular', and they incised into the apocolpium zone. Ornamentation of 'pin' pollen grains was microreticulate, with lumina up to 0.8 µm wide, and for pollen grains from 'thrum' flowers was reticulate and eureticulate with lumina 1.1-1.7 µm wide. In lumina of mesocolpium area some free columellae were observed. Pollen grains from 'thrum' flower-morphs were more variable in size, both in natural and in cultivated populations, than grains from 'pin' flower-morphs. The differences in mean length (P) and breadth (E) of pollen grains from 'thrum' flowers collected in cultivated populations were statistically important ($F_P = 3.154$ for the critical $F_{0.05} = 3.098$; $K\text{-}W_E = 7.469$ for the critical Test value $_{\alpha=0.05} = 5.991$). Pollen grains from 'thrum' flowers were bigger when coming from plants growing in natural populations ($t_E = 2.784$ for the critical Test value $_{\alpha=0.05} = 2.001$).

KEY WORDS: *Primula veris*, pollen morphology, cultivation, statistical analysis, SEM.

INTRODUCTION

Primula L. is the largest genus of the Primulaceae, comprising more than 400 species distributed on the Northern hemisphere, mainly in China and vicinity. It is also common in most of Europe except the extreme north, and in most of the Mediterranean region, southwards to the Alps (Mabberley 1989; Meusel et al. 1965; Valentine and Kress 1972).

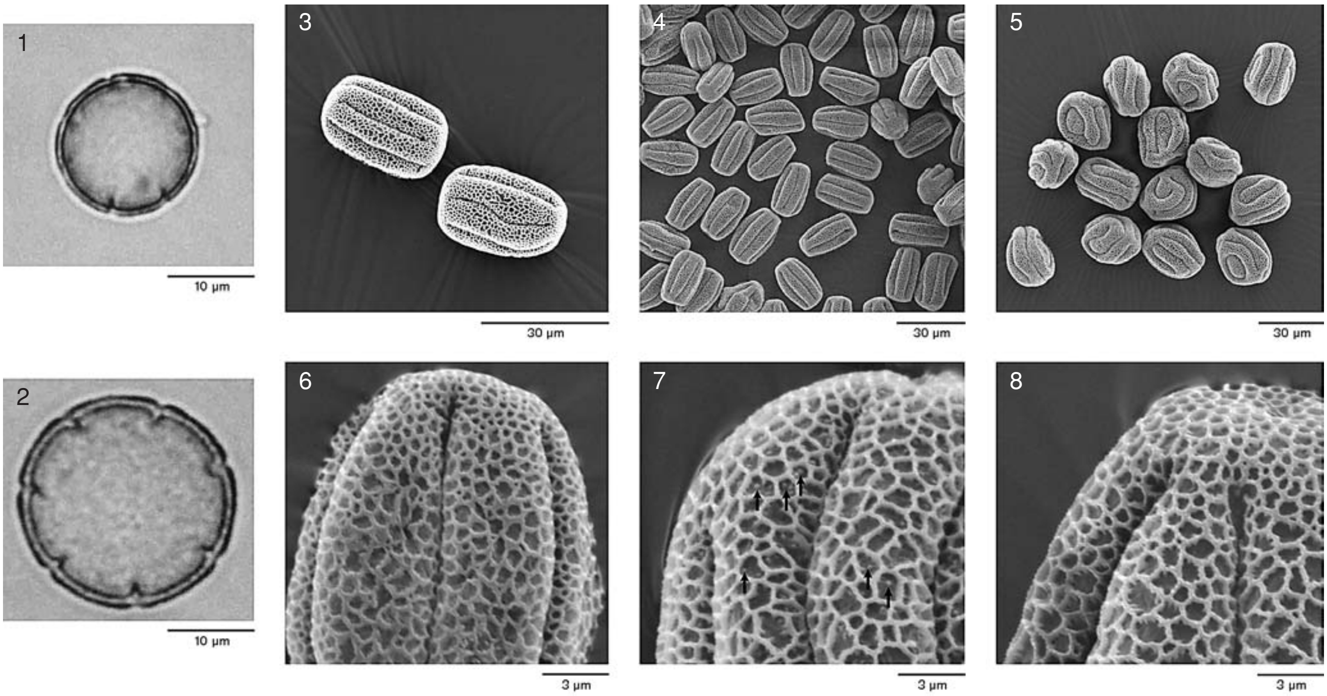
Five species of *Primula* genus grow in natural stands in Poland (Mirek et al. 2002). One of them – *P. farinosa* L. is considered as critically endangered (CR) (Kaźmierczakowa and Zarzycki 2001). *P. veris* L. (cowslip), which is the subject of the presented work, is quite common on almost all Polish lowlands and in lower localities in the mountains (Zając and Zając 2001).

Primula veris, like many others from this genus, is a herostyllic species. The heterostyly promotes cross fertilization over selfing, and is also associated with a certain degree of pollen dimorphism, with different sized pollen grains and variation in sexine sculpture. Most of sections of *Primula* are considered to have pollen of either one of three types, being tricolpate, trisyncolpate, or polycolpate (Fenderson 1986). These three main types differ from each other mainly in the number, shape and distribution of apertures. Tricolpate or trisyncolpate pollen are the prevailing pollen types in *Primula*, found in 31 of the 37 sections, and polycolpate pollen is known to occur only in five sections (Anderberg and Gamal El-Ghazaly 2000).

The aim of the presented study was the micromorphological characterization and the comparative statistical analysis of the size of *Primula veris* pollen grains coming from different natural populations and from cultivated populations derived from natural ones and growing in homogeneous conditions.

MATERIAL AND METHODS

The natural *Primula veris* populations (numbered as 1n, 2n and 3n), from which the pollen grains had been collec-



Figs 1-2. (LM) – *Primula veris* pollen grains in polar view with slightly intruding colpi visible.
Fig. 1. Outline of grain from long-styled flower-morph (×1000).
Fig. 2. Outline of grain from short-styled flower-morph (×1000).
Fig. 3. (SEM) – Elliptic *Primula veris* pollen grains from short-styled flowers with 3 colpi visible (×600).
Fig. 4. (SEM) – Elliptic and rectangular *Primula veris* pollen grains from long-styled flowers with 2-3 colpi visible (×600).
Fig. 5. (SEM) – *Primula veris* pollen grains from short-styled flowers with 'sinous' or 'circular' colpi visible (×600).
Fig. 6. (SEM) – Equatorial view of *Primula veris* pollen grains from 'pin' flowers with long, narrow copli and microreticulate ornamentation of mesocolpium ektexine surface with lumina ca 0.7 µm in diameter (×6200).
Figs 7, 8. (SEM) – Micrographs of *Primula veris* pollen grains from 'thrum' flowers.
Fig. 7. Details of mesocolpium ektexine surface with lumina ca 1.1-1.6 µm in diameter with free columellae in the lumina, and slightly convex mesocolpium (×6200).
Fig. 8. Mesocolpium ektexine surface with lumina ca 1.1-1.7 µm in diameter without free columellae in the lumina and apocolpium area with smaller lumina visible (×6200).

ted, are located in the middle-western part of Poland, in Wielkopolska region: population 1n – in the vicinity of locality Kalina, along the northern shore of Wierzbiczańskie Lake; population 2n – in the vicinity of locality Zagórów, to the East from Skokum, in Nadwarciański Landscape Park, on the edge of Warta valley; and population 3n – in the vicinity of locality Lednogóra, in Lednicki Landscape Park, on the Ostrów Lednicki Island on Lednickie Lake. The cowslip plants grow on them in different environmental conditions. In populations 1n, 2n and 3n, *P. veris* plants are components of the Dactylis glomerata-Calamagrostis epigejos community on humus-mineral soil (pH 7.0), the Trifolio-Agrimonietum (TH. MÜLL. 1961) association on light mineral acid soil (pH 4.7), and the Rhamno-Cornetum sanguinei [KAIS. (1930) PASS. (1957) 1962] = Pruno-Crataegetum (HUECK 1931) association on mineral neutral soil (pH 7.1), respectively (Morozowska 2000). Cultivated *P. veris* populations (numbered as 1c, 2c and 3c) were started from seeds of different origin, collected from the above described three natural localities. All plants grew on the same type of the soil (pH 7.0), with the same water, nutrient elements and light supply.

Voucher specimens are deposited in the herbarium of the Department of Botany at Agricultural University in Poznań.

Primula veris flowers with mature pollen were collected separately from plants with long-styled flower morphs ('pin') and from plants with short-styled flower morphs ('thrum') in natural localisation and from cultivated plants. For each population pollen was sampled from 10 specimen. All examined pollen grains were measured according to their length (P) in longitudinal position and breadth (E) in transverse position. Measurements were done with the use of SEM technique, for 30 replications from each population. For each of the observed features basic position and variability measurements, as well as the variability coefficient were calculated. The influence of pollen origin on the length and breadth for pollen grains coming from natural and cultivated populations was tested by one-way ANOVA. Considering the variance heterogenity, effect of pollen origin on the breadth of pollen grains collected from cultivated plants was tested by Kruskal-Wallis Test. It was also analysed, with the use of the Student-*t* Test, if the dimensions of examined pollen grains from natural and cultivated populations were statistically different (Łomnicki 2000).

Estimation of the P/E ratio allowed to find the category of the shape of the examined cowslip pollen grains. The classification was done according to Erdtman (1952).

Pollen grains were mounted in glycerol jelly for LM studies. Surface sculpture of the examined pollen grains was characterised with use of the SEM technique. The microphotographs were taken with a Philips FEM 515 electron microscope (Laboratory of Electron Microscopy of Adam Mickiewicz University in Poznań).

RESULTS AND DISCUSSION

According to Erdtman (1952) and Moore et al. (1991) pollen grains from *Primula veris* type are usually 6-7-colpate for long-styled forms and 8-colpate for short-styled forms. According to Punt (1976) they are zonocolpate, with number of colpi varying from (5)-6 to 9-(10).

The examined pollen grains from 'pin' flowers were mainly 6-colpate and from 'thrum' flowers 8-colpate, subprolate or prolate-spheroidal, radial symmetrical and isopolar. In polar view they were more or less circular in outline, with slightly intruding colpi (Figs 1 and 2). In equatorial view they were either elliptic or rectangular in outline. Colpi were well defined, long and very narrow, slit-like, quite often slightly sunken, arranged around the equator (Figs 3 and 4). Colpi of some grains from short-styled flowers were 'sinuous' and 'circular' and they often strongly incised into the apocolpium zone, but they did not connect at the poles (Fig. 5). Like other *Primula* species, *P. veris* pollen grains lack margo, which is present in pollen of some other genera of the Primulaceae (Punt et al. 1976; Carrion et al. 1993).

In Primulaceae different surface patterns of pollen grains are due largely to variation in the perforation of the tectum (Nowicke, Skvarla 1977). In genus *Primula* the ornamentation of the sexine is generally reticulate, simply columellate (Punt 1976). Ornamentation of sexine of the examined in our work pollen grains from the long-styled specimens was microreticullate, with muri thin (ca 0.2-0.3 µm) and lumina more or less circular and less often slightly angular in outline, usually up to 0.8 µm wide (Fig. 6). According to Nowicke and Skvarla (1977), the microreticulate surface pattern of pollen grains in *Primula veris* is similar to that of *Hottonia palustris*. In short-styled specimens grains were usually distinctly reticulate, sometimes eureticulate, with muri also thin (ca 0.3 µm) and lumina irregular and angular in outline with perforations 1.1-1.7 µm wide. On some pollen grains from 'thrum' flowers, in lumina of the mesocolpium area free columellae were observed (Fig. 7). Furthermore, the ornamentation of mesocolpium and apocolpium area was similar, with reticulate pattern, but the lumina were smaller in size at the apocolpium area (Fig. 8). In both types of pollen grains the mesocolpus zone was slightly convex.

The biometrical analysis showed that the mean values of P were 16.27 µm (15.84-16.61 µm) for the long-styled and 24.81 µm (24.05-25.34 µm.) for the short-styled flowers. The mean values of E for 'pin' flowers were 13.78 µm (13.60-14.04 µm), and 21.45 µm (20.41-21.97 µm) for 'thrum' flowers (Table 1). According to Punt (1976), the obtained measurements are similar to medium P and E values given for pollen grains from 'pin' flowers and are smaller or similar to the smallest P and E values given for pollen grains from 'thrum' flowers.

The calculated variability coefficient values (V) showed that the examined pollen grains coming from cultivated plants were less variable (V = 2.77-10.69%), than pollen grains coming from plants growing in natural conditions (V = 3.45-18.85%), what might be the result of equalized growing conditions on the experimental plots. Despite the greater variability, the differences observed in size of pollen grains coming from 'pin' and 'thrum' flowers of plants growing in natural populations were not statistically important, in dependence of the examined population. Referring to cultivated populations, the differences in mean P and E values of pollen grains from 'thrum' flowers were statistically important in dependence of the population (Table 2).

The comparison of mean P and E values (Table 1), for both types of pollen grains coming from natural and cultivated populations, showed that the dimensions of pollen grains from 'pin' flowers were bigger when coming from cultivated plants, but these differences were not statistically significant (t_E = 0.823, t_P = 0.910 for the critical test value $_{\alpha=0.05}$ =1.998). Pollen grains from 'thrum' flowers were bigger when coming from plants growing in natural populations and these differences were statistically important referring to E values of pollen grains collected from populations no 2n and 2c (t_E = 2.784 for the critical test value $_{\alpha=0.05}$ = 2.001).

The obtained results showed that *Primula veris* pollen grains from 'thrum' flower-morphs are more variable in size, both in natural and in cultivated populations, than grains from 'pin' flower-morphs of the examined species.

TABLE 1. Mean values for morphological measurements of *Primula veris* L. pollen grains collected from natural and cultivated populations.

No and type of pollen sample	Type of measurement	Mean value (µm)
1-3 n [1] 'pin' flowers	P [3]	15.84-16.12
	E [4]	13.60-13.85
1-3 n [1] 'thrum' flowers	P	24.52-25.34
	E	21.69-21.97
1-3 c [2] 'pin' flowers	P	16.19-16.61
	E	13.60-14.04
1-3 c [2] 'thrum' flowers	P	24.05-25.34
	E	20.41-21.62

(1) – natural population
(2) – cultivated population
(3) – pollen grain length (longitudinal position)
(4) – pollen grain breadth (transverse position)

TABLE 2. Empirical and critical values of F-Snedecor Test and Kruskal-Wallis Test in studies of morphological differentiation of *Primula veris* L. pollen grains from natural and cultivated populations.

No of population and type of pollen sample	Evaluated character	Empirical value of Test	Critical value of Test (α=0.05)
1-3 n [1] 'pin' flowers	P	0.682 [3]	3.100 [3]
	E	0.151 [4]	5.991 [4]
1-3 n [1] 'thrum' flowers	P	0.178	3.094
	E	0.638	5.991
1-3 c [2] 'pin' flowers	P	1.255	3.092
	E	1.697	5.991
1-3 c [2] 'thrum' flowers	P	3.154 *	3.098
	E	7.469 *	5.991

(1) – natural population
(2) – cultivated population
(3) – empirical and critical values of F-Snedecor Test
(4) – empirical and critical values of Kruskal-Wallis Test
* – significance of differences at α = 0.05

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