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Intra- and interindividual variability of selected quantitative features of pollen grain morphology based on the example of *Rosa canina* L. (Rosaceae)

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Abstract: The objective of this study was to investigate the ranges of intra- and interindividual variability on the example of *R. canina*. For this purpose, four flowers were collected randomly (72 flowers in total) from 18 wild shrubs of *R. canina* growing in one population in Poznań (Poland) and then, from each flower, 50 correctly formed pollen grains (200 pollen grains per each individual) were selected. Inter- and intraindividual pollen grain variability was characterised based on 3600 pollen grains. They were analysed for seven quantitative features, i.e. length of polar axis (P), equatorial diameter (E), thickness of the exine along the polar axis (Exp), length of ectocolpi (Le) and P/E, Exp/P, and Le/P ratios. Our study revealed highly significant differences among flowers of the particular *R. canina* individuals with respect to all pollen grain biometrical features. In addition, it showed that the assessment of the full range of variability in pollen grain biometric features within one individual (shrub) was more reliable if we examined several pollen grains from several flowers than for the same number of pollen grains derived from a single flower. We also found statistically significant differences among particular individuals in all pollen grain features. This proves that in order to well characterise a population of a given species from the point of view of palynology, the plant material should derive from a possibly numerous number of individuals (shrubs).

Additional key words: palynology, biometrical analysis, morphological characters, morphological variation

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Introduction

The morphological structure of *R. canina* pollen grains has been described by many palynologists. Pollen grains of this species are radially symmetric, isopolar, 3 (4) – colporate monads (e.g. Reitsma 1966; Eide 1981; Hebda and Chinnappa 1990, 1994; Wrońska-Pilarek and Jagodziński 2009, 2011; Wrońska-Pilarek 2011). The pollen size of the studied species is medium (25–50 μm), very rarely small (10–25 μm). The average length of polar axis (P) amounted to 31.0 (22–42) μm and the mean length of equatorial diameter (E) amounted to 24.9 (16.0–34.0) μm (Wrońska-Pilarek and Jagodziński 2009). Pollen shape (P/E ratio) equals on average 1.26 (1.00–2.13). Pollen grain shapes were mostly subprolate and prolate, or somewhat rarely prolate spheroidal (Wrońska-Pilarek and Jagodziński 2011). Outline in polar view is mostly circular or triangular. In equatorial view pollen grains are elliptic. Exine is two-layered and well marked under the light microscope. The ectexine is thicker than the endexine. Mean exine thickness equals 1.9 μm and ranges from 1.0 to 2.0 μm (Wrońska-Pilarek and Jagodziński 2009). Exine sculpturing pattern is striate and sculpture is well-defined. Striae are high, quite wide (on average: 0.18 μm), cylindrical, and running ± parallel to the colpus (Hebda and Chinnappa 1990; Wrońska-Pilarek 2011). Grooves are quite deep and usually wider than the striae (on average: 0.29 μm). On their bottom numerous (on the average 73 per 25 μm^2) elliptic or circular perforations of varying, relatively substantial diameters are present (Hebda and Chinnappa 1990, 1994; Wrońska-Pilarek 2011). Ueda (1992) classified *R. canina* into the exine sculpturing pattern type II A. Wrońska-Pilarek (2011) assigned this species to two types of exine sculpture – II A and III B. Pollen usually had 3, or very rarely 4 colpori. Colpi are arranged meridionally, regularly, ± evenly spaced. They are usually long, elliptic and ending sharply. Width of colpi are variable, usually greatest in equatorial region. Margins of colpi are irregular, reinforced by costae colpi. Mean length of colpi equals 25.2 μm (18–36 μm), or 81.3% of the length of the polar axis (Wrońska-Pilarek and Jagodziński 2009). Sculpturing of the colpus membranes approaches rugulate. *R. canina* has pollen with or without an operculum (Reitsma 1966; Eide 1981; Wrońska-Pilarek 2011). If the operculum is present, usually is large (about 1/2–1/4 the colpus length) and covers the central part of the colpus. An operculum in *R. canina* is psilate or more rarely rugulate in sculpture (Popek 1996; Wrońska-Pilarek 2011). In some pollen grains of *R. canina* an equatorial bridge is present. Endoapertures are usually located in the middle of the ectocolpus, and with irregular margins.

Papers that examine variability of pollen grain morphological features of the *Rosa* species are very scarce. Such investigations were carried out by the authors of this study (Wrońska-Pilarek and Jagodziński 2009) but they had a different range since they comprised 16 rose species (including *R. canina*) and concerned intra- and interspecific variability, i.e. differences in pollen morphology derived from different shrubs which were growing in several natural sites.

The choice of *R. canina* for the investigations done here was dictated by the fact that it has pollen of well-recognised structure, characteristic for the *Rosa* genus. In the *Rosa* genus taxonomy, it is considered as a "good species" with distinct diagnostic features (Zieliński 1985, 1987; Henker 2000; Kalkman 2004). In addition, plant material for palynological investigations in the form of flowers is easily available because *R. canina* is a very common and widely distributed species occurring frequently both in Poland as well as the entirety of Europe (Klaštersky 1968; Henker 2000; Zając and Zając 2001).

The objective of this study was to investigate, for the first time for any member of the entire Rosaceae family, the range of intraindividual variability of *R. canina* pollen (e.g. the variability of pollen grain morphology inside the flower and among flowers of individual shrubs) and interindividual variability (e.g. the pollen grain morphological variability among individuals within a population of this species), based on the analyses of 7 quantitative features. These investigations were performed on a large sample of pollen grains (3600 in total) which allowed reliable determination of the level of variability with regards to morphological features.

Materials and methods

Flowers were collected from 18 shrubs growing in the outskirts of Poznań, along a distance of about 3 km on Kobylepole and Sowice streets (geographical coordinates – GPS: 52°22'59''N, 17°1'13''E). Shrubs were growing on both sides of the streets at distances of several to several dozen meters. All plant material was collected in the form of shoots with flowers and deposited in the herbarium of the Department of Forestry Natural Foundations (POZNF), Poznań University of Life Sciences (Poland).

Four flowers were collected randomly from each of 18 experimental shrubs (72 flowers in total, flowers were named A, B, C, and D). Only flowers which just bloomed or are about to bloom were used in the study. A sample consisted of 50 correctly formed pollen grains derived from a single flower. In total, 3600 pollen grains were analysed. All samples were acetolysed according to Erdtman's method (1952), slightly modified by Wrońska-Pilarek (1998). The acetolysing mixture was made up of nine parts of acetic acid anhydride and one part of concentrated sulphuric acid and the process of acetolysis lasted 2.5 minutes. The morphological observations were carried out both with light microscope (Biolar 2308, Nikon HFX-DX) and scanning electron microscope (Zeiss 435 VP). Pollen grains were measured using the eyepiece (ocular) with scale and then measurement results were recalculated into micrometers by multiplying them by 2.

Pollen grains were analysed for seven quantitative features, i.e. length of polar axis (P), equatorial diameter (E), thickness of exine along polar axis (Exp), length of ectocolpi (Le) and P/E, Exp/P, and Le/P ratios.

The palynological terminology used in our study follows Punt et al. (2007) and Hesse et al. (2009).

For each pollen grain feature, one-factor analysis of variance (ANOVA) was used to examine differences in the mean values among four flowers harvested from each of the 18 individuals to assess intraindividual variability. Based on all pollen grains harvested for each individual (200 pollen grains) we conducted one-factor analysis of variance to check differences among individuals of *R. canina* (interindividual variablity). When critical differences were noted, multiple comparisons were carried out using Tukey's test for equal sample sizes. Statistical analyses were performed using JMP 8.0 (SAS Institute Inc. Cary, NC. USA; http:// www.sas.com/).

Results

Intraindividual variability of pollen grains

We found statistically significant differences among flowers (4) of the particular individuals (18) for most of the individuals studied for each pollen grain morphological feature. For example length of polar axis (P) was statistically different among flowers for 16 of 18 individuals studied (Table 1). The range between minimal and maximal P value of individual flowers ranged from 4 μm (shrubs No. 6A, 14A) to 16 μm (shrub No. 7D); the P range equals 9.25 μm on average. Differences among minimal and maximal mean values for each flower of the individuals (max to min ratio) ranged from 0.9% (shrub No. 8) to 8.7% (shrub No. 18). Coefficients of variation of P values, estimated as mean value for 4 flowers, ranged from 5.3% (shrub No. 14) to 8.2% (shrub No. 7). Statistically significant differences in length of equatorial diameter (E) among flowers of the particular individuals were found for 13 of 18 shrubs (Table 2). The range between minimal and maximal E value in individual flowers ranged from 6 μm (shrubs No. 2C, 3B, 6A, 9B, 16A, 16B, 16C, 17A, 17C, 17D) to 14 μm (shrubs No. 6D, 9A) and the range equals 9.36 μm on average. Mean differences among minimal and maximal mean values calculated for each flower of the individuals equals 6.2% and ranged from 1.5% (shrub No. 12) to 15.4% (shrub No. 9). Coefficients of variation of E values ranged from 5.8% (shrub No. 16) to 10.9% (shrub No. 10). For 12 of 18 *Rosa* individuals we found statistically significant differences among flowers in P/E (Table 3). Mean differences among minimal and maximal mean values for each flower of the individuals equals 6.1% and ranged from 1.5% (shrub No. 8) to 13.8% (shrub No. 6). It was found that coefficients of variation of P/E ratio, estimated as mean value for 4 flowers, ranged from 7.4% (shrub No. 15) to 10.9% (shrub No. 10). Only for 2 shrubs did we find no statistically significant differences among flowers on the individual shrub in thickness of exine along the polar axis (Exp) (Table 4). The range between minimal and maximal Exp value in individual flowers ranged from 0 μm (shrub No. 8D) to 1.8 μm (shrubs No. 2A, 6D, 8C, 10C, 11C); the mean range equals 0.98 μm. The differences among mean values for particular flowers within individuals were very variable. For example the mean differences among minimal and maximal mean values of Exp for each flower of the individuals ranged from 10.5% (shrub No. 10) to 142.9% (shrub No. 8) and equals on average ca. 54%. The mean coefficients of variation ranged from 28.9% in shrub No. 18 to 44.7% in shrub No. 11. Analysis of variance showed statistically significant differences among flowers with respect to Exp/P ratio for 16 of 18 shrubs (Table 5). The differences between minimal and maximal values obtained for each shrub were similar to these obtained for thickness of exine along polar axis – the differences range from 10% for shrub No. 10 to 142.5% for shrub No. 8, whereas the mean difference for all shrubs studied equals 54.7%. It was found that coefficients of variation of Exp/P ratio, estimated as mean value for 4 flowers, ranged from 29.6% (shrub No. 15) to 44.9% (shrub No. 6). Length of ectocolpi (Le) was significantly different among flowers for 14 of 18 shrubs (Table 6). The range between minimal and maximal Le value in individual flowers ranged from 6 μm (shrubs No. 6A, 15B, 17D) to 14 μm (shrubs No. 3D, 6D, 7D, 11C), while the mean range equals 10.19 μm. Differences among minimal and maximal mean values of Le calculated for each flower of the individuals ranged from 1.1% (shrub No. 12) to 10.9% (shrub No. 16) and equals 6.3% on average. The mean coefficients of variation of Le values ranged from 7.1% (shrub No. 1) to 10.9% (shrub No. 5). Le/P ratio was significantly different among flowers for 5 of 18 individuals studied (Table 7). The mean differences among minimal and maximal mean values of Le/P for each flower of the individuals ranges from 0.6% (shrub No. 3) to 5.6% (shrub No. 8) and equals on average ca. 2.5%. The coefficients of variation ranged from 4.2% in shrub No. 2 to 10.3% in shrub No. 5.

Based on individual flowers, the pollen grain features studied may be ordered from least to most variable as follows: Le/P (mean CV = 5.6%), P (CV = 6.6%), E (CV = 8.3%), Le (CV = 8.4%), P/E (CV = 9%), Exp (CV = 36%) and Exp/P (CV = 36.1%).

Interindividual variability of pollen grains

We found statistically significant differences in all the pollen grains studied among the particular shrubs (Table 8; $p<0.0001$). Based on all the pollen grains studied mean P equals 31.40 (±0.04 SE) μm, E – 26.62 (±0.04) μm, P/E – 1.188 (±0.02), Exp – 0.699 (±0.006) μm, Exp/P – 0.0224 (±0.0002), Le – 28.22 (±0.04) μm and mean Le/P – 0.899 (±0.001).

Table 1. Range (min-max), coefficient of variation (CV) and mean values (±SE) of length of polar axis (P) of *R. canina* pollen grains studied. One way ANOVA's were performed separately for each individual shrub to determine the differences among flowers (A, B, C and D). Same letters indicate a lack of statistically significant differences between analyzed species according to Tukey's a posteriori test (p<0.05)

Shrub No.	Flower A				Flower B				Flower C				Flower D				ANOVA	
	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	F	P
1	28–34	31.60 (0.24)	B	5.27	30–38	32.64 (0.28)	A	6.12	28–38	32.12 (0.25)	AB	5.54	28–36	31.60 (0.27)	B	6.13	3.6212	*0.0141*
2	28–38	33.72 (0.30)	AB	6.34	30–38	34.44 (0.27)	A	5.54	28–40	33.24 (0.34)	B	7.18	30–38	34.28 (0.27)	AB	5.65	3.4055	*0.0187*
3	28–36	32.68 (0.28)	A	6.13	26–36	30.68 (0.28)	B	6.53	28–38	32.88 (0.34)	A	7.29	28–36	32.00 (0.30)	A	6.68	10.7726	*<0.0001*
4	28–36	31.28 (0.27)	B	6.03	28–40	33.32 (0.34)	A	7.24	26–36	31.72 (0.31)	B	6.86	28–36	31.36 (0.30)	B	6.75	9.7696	*<0.0001*
5	24–36	29.72 (0.41)	A	9.80	24–34	28.56 (0.34)	AB	8.49	24–34	28.36 (0.28)	B	7.08	26–34	29.36 (0.26)	AB	6.37	3.8076	*0.0110*
6	28–32	29.88 (0.18)	C	4.36	26–36	31.72 (0.37)	A	8.26	26–36	31.24 (0.34)	AB	7.64	22–34	30.24 (0.37)	BC	8.62	6.9551	*0.0002*
7	24–38	32.24 (0.39)	A	8.47	24–36	30.32 (0.31)	B	7.22	26–38	32.36 (0.41)	A	8.98	22–38	31.96 (0.37)	A	8.24	6.5109	*0.0003*
8	24–34	30.32 (0.28)		6.58	26–34	30.52 (0.27)		6.32	26–36	30.60 (0.31)		7.26	26–36	30.36 (0.28)		6.48	0.2116	*0.8883*
9	24–34	29.52 (0.31)	C	7.44	28–36	31.80 (0.25)	A	5.57	28–38	31.72 (0.32)	A	7.21	26–34	30.60 (0.26)	B	6.09	13.9074	*<0.0001*
10	26–36	31.60 (0.27)	A	6.13	26–36	30.32 (0.26)	BC	6.01	26–34	30.64 (0.30)	AB	7.03	26–32	29.64 (0.23)	C	5.40	9.2952	*<0.0001*
11	28–36	32.32 (0.31)	A	6.77	26–36	30.68 (0.29)	B	6.79	26–36	30.80 (0.32)	B	7.30	26–36	30.08 (0.29)	B	6.84	9.8625	*<0.0001*
12	28–36	31.08 (0.30)		6.78	28–36	31.76 (0.31)		6.81	28–38	31.84 (0.33)		7.38	26–38	31.84 (0.32)		7.05	1.3809	*0.2498*
13	26–34	29.32 (0.25)	B	6.10	26–34	30.24 (0.21)	A	4.93	28–34	30.24 (0.22)	A	5.11	26–38	30.52 (0.28)	A	6.59	4.6192	*0.0038*
14	28–32	30.44 (0.21)	B	4.84	26–34	31.12 (0.22)	B	4.89	26–34	30.36 (0.27)	B	6.34	30–36	32.56 (0.24)	A	5.27	18.6475	*<0.0001*
15	26–34	31.24 (0.25)	B	5.77	28–34	30.68 (0.24)	A	5.53	28–38	32.48 (0.31)	B	6.65	28–34	31.04 (0.22)	B	5.08	9.2093	*<0.0001*
16	28–38	32.68 (0.32)	A	6.95	30–38	32.72 (0.28)	A	6.02	26–38	31.72 (0.45)	AB	9.95	24–38	30.56 (0.37)	B	8.57	8.0089	*<0.0001*
17	28–36	31.36 (0.26)	B	5.97	28–38	32.60 (0.31)	A	6.82	30–38	32.52 (0.25)	A	5.53	28–36	30.72 (0.23)	B	5.22	11.7849	*<0.0001*
18	28–38	32.40 (0.36)	B	7.89	26–36	31.72 (0.26)	B	5.70	28–38	33.76 (0.33)	A	6.84	30–40	34.48 (0.36)	A	7.46	14.5113	*<0.0001*

Table 2. Range (min-max), coefficient of variation (CV) and mean values (±SE) of length of equatorial diameter (E) of *R. canina* pollen grains studied. One way ANOVA's were performed separately for each individual shrub to determine the differences among flowers (A, B, C and D). Same letters indicate a lack of statistically significant differences between analyzed species according to Tukey's a posteriori test (p<0.05)

Shrub No.	Flower A				Flower B				Flower C				Flower D				ANOVA	
	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	F	P
1	20–30	26.24 (0.27)		7.33	22–30	26.56 (0.29)		7.61	20–32	26.20 (0.26)		7.11	22–30	26.00 (0.30)		8.22	0.6791	*0.5658*
2	24–32	29.36 (0.26)	A	6.37	26–34	29.54 (0.23)	A	5.61	24–30	28.04 (0.24)	B	6.03	22–32	28.64 (0.35)	AB	8.63	6.2596	*0.0004*
3	22–32	27.80 (0.30)	AB	7.59	26–32	28.36 (0.24)	A	5.99	24–32	27.28 (0.24)	B	6.24	22–32	27.24 (0.26)	B	6.78	4.0545	*0.0080*
4	22–30	26.80 (0.30)	B	7.98	22–32	27.12 (0.36)	B	9.32	24–32	28.48 (0.25)	A	6.11	22–32	26.60 (0.29)	B	7.78	7.8581	*0.0001*
5	22–32	27.92 (0.36)	AB	9.03	22–34	27.24 (0.39)	AB	10.16	22–30	26.68 (0.29)	B	7.66	22–34	28.20 (0.35)	A	8.86	3.8349	*0.0107*
6	24–30	27.28 (0.23)	A	6.06	18–30	25.64 (0.37)	BC	10.30	20–32	25.96 (0.34)	AB	9.14	16–30	24.56 (0.48)	C	13.87	9.3181	*<0.0001*
7	20–30	26.12 (0.35)	B	9.46	20–30	26.28 (0.36)	B	9.60	18–30	26.08 (0.46)	B	12.39	20–30	27.84 (0.35)	A	8.93	4.9013	*0.0026*
8	22–34	28.40 (0.30)		7.39	22–34	28.64 (0.33)		8.04	24–32	29.08 (0.28)		6.84	24–32	28.64 (0.26)		6.38	0.9462	*0.4193*
9	16–30	23.64 (0.50)	B	14.87	24–30	27.28 (0.23)	A	5.88	20–30	25.84 (0.40)	A	10.82	20–30	26.16 (0.42)	A	11.33	14.7315	*<0.0001*
10	22–32	26.88 (0.42)	A	11.06	20–30	24.08 (0.38)	B	11.13	20–30	24.96 (0.37)	B	10.40	20–30	24.36 (0.38)	B	11.09	10.5880	*<0.0001*
11	20–32	26.20 (0.42)	A	11.46	20–30	24.44 (0.33)	B	9.68	20–30	24.96 (0.35)	AB	9.88	20–28	24.00 (0.30)	B	8.91	7.1631	*0.0001*
12	20–32	25.96 (0.35)		9.66	20–32	25.96 (0.35)		9.66	20–32	26.32 (0.39)		10.45	20–30	25.92 (0.40)		11.02	0.2488	*0.8621*
13	22–30	25.88 (0.32)	A	8.75	20–30	25.72 (0.32)	A	8.89	20–30	24.40 (0.31)	B	9.07	20–30	24.72 (0.37)	AB	10.56	4.8379	*0.0029*
14	20–30	25.64 (0.29)	C	7.99	22–30	26.76 (0.28)	AB	7.38	20–30	26.04 (0.35)	BC	9.37	22–30	27.32 (0.27)	A	7.03	6.3001	*0.0004*
15	20–32	26.96 (0.31)	AB	8.10	22–30	26.84 (0.26)	B	6.75	24–32	27.84 (0.25)	A	6.46	22–30	25.60 (0.23)	C	6.31	12.2179	*<0.0001*
16	24–30	27.40 (0.22)		5.76	26–32	27.44 (0.19)		4.89	24–30	27.20 (0.26)		6.64	20–30	26.92 (0.23)		6.04	1.1081	*0.3470*
17	24–30	26.08 (0.22)	B	5.99	22–30	26.20 (0.27)	AB	7.27	24–30	27.00 (0.22)	A	5.84	24–30	26.04 (0.22)	B	5.91	3.7351	*0.0121*
18	20–30	26.72 (0.28)		7.37	22–30	26.60 (0.21)		5.53	20–30	26.56 (0.27)		7.14	22–30	27.20 (0.28)		7.28	1.2803	*0.2823*

Table 3. Range (min-max), coefficient of variation (CV) and mean values (±SE) of P/E ratio of *R. canina* pollen grains studied. One way ANOVA's were performed separately for each individual shrub to determine the differences among flowers (A, B, C and D). Same letters indicate a lack of statistically significant differences between analyzed species according to Tukey's a posteriori test (p<0.05)

Shrub No.	Flower A				Flower B				Flower C				Flower D				ANOVA	
	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	F	P
1	1.067–1.417	1.209 (0.013)		7.73	1.067–1.455	1.234 (0.013)		7.58	0.938–1.500	1.231 (0.015)		8.37	1.071–1.636	1.223 (0.017)		9.73	0.5737	*0.6329*
2	1.000–1.417	1.153 (0.014)	B	8.74	1.000–1.385	1.168 (0.011)	AB	6.76	1.000–1.429	1.189 (0.015)	AB	8.94	1.063–1.455	1.203 (0.013)	A	7.78	2.7158	*0.0460*
3	1.000–1.500	1.181 (0.015)	A	8.84	0.929–1.308	1.085 (0.012)	B	7.80	1.071–1.462	1.207 (0.011)	A	6.24	1.000–1.417	1.179 (0.014)	A	8.43	17.1154	*<0.0001*
4	1.000–1.455	1.175 (0.017)	B	10.06	1.000–1.545	1.237 (0.017)	A	9.85	0.867–1.308	1.117 (0.013)	C	7.98	0.938–1.364	1.183 (0.013)	AB	7.66	10.7106	*<0.0001*
5	0.867–1.364	1.070 (0.016)		10.83	0.867–1.364	1.056 (0.016)		10.98	0.929–1.273	1.068 (0.013)		8.83	0.875–1.333	1.048 (0.015)		10.33	0.4309	*0.7311*
6	1.000–1.231	1.098 (0.010)	B	6.27	1.000–1.800	1.247 (0.020)	A	11.53	1.000–1.545	1.211 (0.018)	A	10.25	1.000–2.000	1.250 (0.026)	A	14.51	13.7574	*<0.0001*
7	1.000–1.500	1.241 (0.016)	A	9.35	1.000–1.500	1.163 (0.018)	B	10.82	1.000–1.500	1.253 (0.020)	A	11.04	0.786–1.600	1.157 (0.020)	B	12.00	7.5990	*<0.0001*
8	0.923–1.364	1.071 (0.012)		7.96	0.933–1.455	1.072 (0.014)		9.53	0.867–1.286	1.056 (0.013)		8.49	0.929–1.250	1.063 (0.011)		7.13	0.3761	*0.7703*
9	0.929–1.750	1.269 (0.023)	A	12.61	1.000–1.385	1.169 (0.012)	C	7.05	1.000–1.500	1.238 (0.017)	AB	9.67	1.000–1.500	1.180 (0.016)	BC	9.57	7.5949	*<0.0001*
10	1.000–1.500	1.187 (0.018)	B	10.56	0.929–1.636	1.274 (0.022)	A	12.11	1.071–1.500	1.235 (0.014)	AB	8.23	0.929–1.600	1.232 (0.022)	AB	12.62	3.4287	*0.0181*
11	1.000–1.600	1.245 (0.018)		10.12	1.000–1.600	1.265 (0.018)		10.26	1.067–1.455	1.241 (0.014)		7.87	1.071–1.600	1.262 (0.018)		10.20	0.4825	*0.6948*
12	1.000–1.700	1.209 (0.022)		12.69	1.000–1.500	1.232 (0.016)		9.32	1.000–1.500	1.219 (0.017)		9.73	1.000–1.500	1.238 (0.016)		9.27	0.5240	*0.6663*
13	1.000–1.364	1.139 (0.014)	B	8.90	1.000–1.500	1.184 (0.016)	B	9.54	1.000–1.500	1.248 (0.016)	A	8.88	1.000–1.600	1.246 (0.019)	A	10.58	10.3831	*<0.0001*
14	1.000–1.455	1.193 (0.014)		8.18	1.000–1.364	1.167 (0.010)		6.14	1.000–1.500	1.174 (0.016)		9.88	1.000–1.455	1.196 (0.012)		7.36	1.1537	*0.3287*
15	1.000–1.500	1.165 (0.014)	B	8.45	1.000–1.455	1.147 (0.012)	B	7.60	1.000–1.357	1.169 (0.012)	B	7.12	1.071–1.455	1.216 (0.011)	A	6.26	5.6993	*0.0009*
16	1.000–1.500	1.196 (0.015)	A	9.00	1.071–1.462	1.194 (0.010)	A	5.75	0.929–1.500	1.171 (0.020)	AB	11.84	1.000–1.462	1.137 (0.013)	B	8.08	3.4600	*0.0174*
17	1.000–1.417	1.206 (0.013)	AB	7.54	1.000–1.500	1.250 (0.017)	A	9.57	1.071–1.333	1.207 (0.009)	AB	5.56	1.000–1.417	1.183 (0.013)	B	7.60	4.4618	*0.0047*
18	0.933–1.462	1.215 (0.012)	B	7.20	1.071–1.308	1.194 (0.008)	B	4.85	1.067–1.500	1.275 (0.014)	A	7.80	1.067–1.667	1.275 (0.020)	A	11.34	8.3525	*<0.0001*

Table 4. Range (min-max), coefficient of variation (CV) and mean values (±SE) of thickness of exine along polar axis (Exp) of *R. canina* pollen grains studied. One way ANOVA's were performed separately for each individual shrub to determine the differences among flowers (A, B, C and D). Same letters indicate a lack of statistically significant differences between analyzed species according to Tukey's a posteriori test ($p<0.05$)

Shrub No.	Flower A				Flower B				Flower C				Flower D				ANOVA	
	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	F	P
1	0.6–1.6	0.808 (0.036)	A	31.61	0.6–1.6	0.796 (0.039)	A	34.98	0.6–1.0	0.712 (0.026)	A	25.48	0.6–1.0	0.696 (0.024)	A	24.79	3.1810	*0.0251*
2	0.2–2.0	0.836 (0.065)	AB	55.25	0.6–1.6	0.820 (0.039)	AB	33.87	0.4–1.0	0.664 (0.025)	B	26.83	0.4–2.0	0.932 (0.065)	A	49.55	4.5989	*0.0039*
3	0.4–1.0	0.548 (0.027)	B	35.22	0.4–1.0	0.544 (0.027)	B	35.65	0.4–1.0	0.572 (0.030)	B	36.71	0.4–1.6	0.692 (0.042)	A	43.39	4.6563	*0.0036*
4	0.4–1.0	0.664 (0.030)	B	32.45	0.4–1.6	0.816 (0.055)	A	47.97	0.4–1.0	0.580 (0.028)	BC	33.59	0.4–1.0	0.480 (0.022)	C	32.60	15.4278	*<0.0001*
5	0.2–1.0	0.432 (0.020)	AB	32.88	0.2–1.0	0.444 (0.022)	A	34.40	0.2–1.0	0.480 (0.023)	A	33.67	0.2-0.6	0.368 (0.017)	B	31.75	5.2352	*0.0017*
6	0.2–1.0	0.424 (0.020)	C	33.88	0.4–2.0	0.708 (0.041)	A	41.24	0.2–1.0	0.516 (0.028)	BC	38.43	0.2–2.0	0.652 (0.057)	AB	61.60	10.8526	*<0.0001*
7	0.2–1.0	0.500 (0.024)	B	34.52	0.4–1.6	0.668 (0.043)	A	45.91	0.4-0.6	0.472 (0.014)	B	20.55	0.4–1.0	0.472 (0.020)	B	29.36	11.6562	*<0.0001*
8	0.2–1.0	0.392 (0.025)	C	44.88	0.4–2.0	0.892 (0.065)	A	51.89	0.2–2.0	0.952 (0.055)	A	41.05	0.6-0.6	0.600 (0.000)	B	0.00	34.3293	*<0.0001*
9	0.2–1.0	0.576 (0.030)	B	36.88	0.2–1.0	0.436 (0.020)	C	31.69	0.2–1.0	0.580 (0.031)	B	38.32	0.4–2.0	0.816 (0.050)	A	43.41	20.7798	*<0.0001*
10	0.4–2.0	1.304 (0.071)		38.36	0.4–2.0	1.296 (0.071)		38.72	0.2–2.0	1.204 (0.067)		39.35	0.6–2.0	1.180 (0.069)		41.55	0.8263	*0.4808*
11	0.2–1.6	0.780 (0.053)	B	47.83	0.4–2.0	1.084 (0.074)	A	48.47	0.2–2.0	0.992 (0.063)	AB	44.80	0.6–1.6	0.968 (0.052)	AB	37.88	4.3540	*0.0054*
12	0.2–1.6	0.828 (0.041)	A	34.85	0.2–1.6	0.828 (0.041)	A	34.85	0.2–1.0	0.480 (0.026)	B	38.58	0.4–1.6	0.772 (0.042)	A	38.11	19.3545	*<0.0001*
13	0.4–1.6	0.900 (0.050)	AB	39.46	0.6–1.6	0.768 (0.036)	B	33.01	0.4–1.6	0.776 (0.033)	B	30.20	0.6–1.6	1.004 (0.047)	A	32.82	7.1290	*0.0001*
14	0.2–1.0	0.444 (0.025)	B	39.97	0.6–1.6	0.960 (0.042)	A	30.93	0.6–1.6	0.940 (0.046)	A	34.72	0.6–1.6	0.864 (0.035)	A	28.62	40.8417	*<0.0001*
15	0.4–1.0	0.800 (0.030)		26.73	0.4–1.6	0.860 (0.036)		29.44	0.4–1.6	0.804 (0.041)		35.71	0.6–1.0	0.760 (0.028)		26.05	1.4612	*0.2264*
16	0.2–1.0	0.496 (0.029)	C	41.73	0.4–1.6	0.780 (0.037)	AB	33.27	0.2–1.6	0.684 (0.043)	B	44.63	0.6–1.6	0.880 (0.041)	A	32.79	18.6683	*<0.0001*
17	0.2–0.6	0.384 (0.019)	B	34.64	0.2–0.6	0.372 (0.020)	B	37.64	0.2–1.6	0.576 (0.036)	A	44.17	0.2–0.6	0.352 (0.015)	B	29.40	19.2523	*<0.0001*
18	0.2–0.6	0.400 (0.015)	B	26.73	0.2–0.6	0.424 (0.019)	AB	32.51	0.2–1.0	0.468 (0.018)	A	26.76	0.2–0.6	0.368 (0.016)	B	29.79	6.1049	*0.0005*

Table 5. Range (min-max), coefficient of variation (CV) and mean values (±SE) of Exp/P ratio of *R. canina* pollen grains studied. One way ANOVA's were performed separately for each individual shrub to determine the differences among flowers (A, B, C and D). Same letters indicate a lack of statistically significant differences between analyzed species according to Tukey's a posteriori test ($p<0.05$)

Shrub No.	Flower A				Flower B				Flower C				Flower D				ANOVA	
	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	F	P
1	0.018–0.057	0.0257 (0.0012)	A	34.00	0.016–0.050	0.0244 (0.0012)	A	33.94	0.017–0.033	0.0222 (0.0008)	A	25.39	0.017–0.036	0.0221 (0.0008)	A	26.26	2.9146	*0.0355*
2	0.006–0.063	0.0247 (0.0019)	AB	53.28	0.017–0.047	0.0237 (0.0011)	AB	32.48	0.012–0.031	0.0200 (0.0007)	B	25.86	0.011–0.059	0.0274 (0.0020)	A	50.81	4.1230	*0.0073*
3	0.011–0.033	0.0168 (0.0008)	B	34.33	0.011–0.033	0.0177 (0.0008)	B	33.19	0.011–0.033	0.0175 (0.0009)	B	36.81	0.012–0.050	0.0216 (0.0013)	A	42.66	4.9377	*0.0025*
4	0.011–0.036	0.0213 (0.0010)	AB	33.65	0.011–0.047	0.0243 (0.0015)	A	44.81	0.011–0.033	0.0183 (0.0009)	BC	32.78	0.011–0.031	0.0153 (0.0007)	C	30.69	13.0596	*<0.0001*
5	0.007–0.028	0.0146 (0.0007)	AB	32.69	0.007–0.038	0.0157 (0.0008)	A	35.69	0.007–0.038	0.0170 (0.0008)	A	33.55	0.006–0.023	0.0126 (0.0006)	B	33.08	6.5961	*0.0003*
6	0.006–0.033	0.0142 (0.0007)	B	33.05	0.011–0.067	0.0225 (0.0014)	A	43.32	0.006–0.036	0.0165 (0.0009)	B	38.57	0.007–0.077	0.0217 (0.0020)	A	64.50	9.1572	*<0.0001*
7	0.006–0.029	0.0154 (0.0007)	B	30.61	0.013–0.050	0.0219 (0.0013)	A	42.91	0.011–0.021	0.0147 (0.0004)	B	20.55	0.011–0.031	0.0148 (0.0006)	B	29.19	17.5607	*<0.0001*
8	0.006–0.033	0.0129 (0.0008)	C	44.76	0.012–0.063	0.0293 (0.0021)	A	50.71	0.006–0.077	0.0314 (0.0019)	A	42.61	0.017–0.023	0.0198 (0.0002)	B	6.46	33.7877	*<0.0001*
9	0.008–0.036	0.0195 (0.0010)	B	36.27	0.006–0.031	0.0138 (0.0006)	C	31.84	0.007–0.036	0.0183 (0.0010)	B	38.81	0.012–0.063	0.0267 (0.0016)	A	42.39	23.0409	*<0.0001*
10	0.012–0.077	0.0414 (0.0023)		39.02	0.013–0.071	0.0431 (0.0025)		40.36	0.006–0.067	0.0392 (0.0021)		37.86	0.019–0.077	0.0399 (0.0024)		42.10	0.5703	*0.6352*
11	0.006–0.050	0.0240 (0.0016)	B	46.05	0.013–0.067	0.0354 (0.0024)	A	48.03	0.006–0.059	0.0325 (0.0021)	A	44.78	0.019–0.062	0.0322 (0.0017)	A	37.52	6.1704	*0.0005*
12	0.007–0.057	0.0268 (0.0014)	A	35.91	0.006–0.053	0.0263 (0.0013)	A	35.63	0.006–0.033	0.0150 (0.0008)	B	37.29	0.011–0.050	0.0243 (0.0013)	A	36.73	20.6244	*<0.0001*
13	0.013–0.057	0.0308 (0.0017)	AB	39.69	0.018–0.050	0.0254 (0.0011)	C	31.88	0.013–0.057	0.0257 (0.0011)	BC	31.35	0.019–0.053	0.0329 (0.0015)	A	31.88	7.1346	*0.0001*
14	0.006–0.036	0.0146 (0.0008)	C	40.24	0.019–0.053	0.0308 (0.0013)	AB	29.83	0.019–0.057	0.0309 (0.0014)	A	32.77	0.017–0.047	0.0265 (0.0010)	B	27.67	42.7782	*<0.0001*
15	0.013–0.036	0.0256 (0.0009)		25.95	0.013–0.057	0.0282 (0.0012)		31.13	0.013–0.047	0.0249 (0.0013)		35.82	0.018–0.036	0.0245 (0.0009)		25.58	2.2856	*0.0801*
16	0.005–0.033	0.0153 (0.0009)	C	42.24	0.012–0.050	0.0239 (0.0011)	B	33.16	0.006–0.062	0.0222 (0.0016)	B	51.36	0.016–0.062	0.0291 (0.0015)	A	36.43	18.8101	*<0.0001*
17	0.006–0.021	0.0123 (0.0006)	B	34.00	0.006–0.020	0.0114 (0.0006)	B	37.59	0.006–0.042	0.0176 (0.0010)	A	41.13	0.006–0.021	0.0115 (0.0005)	B	31.72	17.2872	*<0.0001*
18	0.006–0.021	0.0125 (0.0005)	AB	28.23	0.006–0.021	0.0134 (0.0006)	A	33.07	0.007–0.028	0.0139 (0.0005)	A	27.32	0.005–0.019	0.0108 (0.0005)	B	31.83	6.5617	*0.0003*

Table 6. Range (min-max), coefficient of variation (CV) and mean values (±SE) of length of ectocolpi (Le) of *R. canina* pollen grains studied. One way ANOVA's were performed separately for each individual shrub to determine the differences among flowers (A, B, C and D). Same letters indicate a lack of statistically significant differences between analyzed species according to Tukey's a posteriori test ($p<0.05$)

Shrub No.	Flower A				Flower B				Flower C				Flower D				ANOVA	
	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	F	P
1	24–32	28.12 (0.30)	AB	7.52	24–32	28.72 (0.28)	A	7.00	24–32	28.52 (0.25)	A	6.14	22–30	27.48 (0.30)	B	7.61	3.7464	*0.0120*
2	26–36	30.40 (0.28)		6.51	26–36	30.92 (0.31)		7.18	24–36	30.32 (0.32)		7.46	26–36	30.72 (0.32)		7.41	0.8172	*0.4858*
3	22–34	29.44 (0.37)	A	8.79	22–32	27.64 (0.32)	B	8.10	24–36	29.68 (0.38)	A	9.07	20–34	29.00 (0.39)	A	9.48	6.2661	*0.0004*
4	24–34	27.84 (0.33)	B	8.44	24–36	29.64 (0.34)	A	8.14	22–34	28.04 (0.36)	B	9.06	22–32	27.80 (0.32)	B	8.25	6.7093	*0.0002*
5	20–32	26.12 (0.44)		11.92	20–32	25.52 (0.44)		12.32	20–30	26.00 (0.37)		10.07	20–32	26.68 (0.35)		9.16	1.4016	*0.2436*
6	24–30	27.24 (0.30)	AB	7.69	22–34	28.52 (0.39)	A	9.59	24–34	28.20 (0.32)	A	8.01	18–32	26.20 (0.50)	B	13.47	7.4361	*<0.0001*
7	22–34	28.80 (0.40)	A	9.82	20–32	26.96 (0.31)	B	8.24	22–34	28.36 (0.42)	A	10.44	22–36	28.96 (0.36)	A	8.85	5.8506	*0.0008*
8	22–32	27.92 (0.30)	A	7.51	24–32	27.96 (0.31)	A	7.71	22–30	26.68 (0.34)	B	8.91	22–32	27.24 (0.32)	AB	8.24	3.7744	*0.0155*
9	20–30	26.68 (0.36)	C	9.65	24–32	29.24 (0.29)	A	6.89	24–36	28.44 (0.35)	AB	8.68	20–32	27.36 (0.33)	BC	8.41	11.6663	*<0.0001*
10	24–32	28.44 (0.28)	A	7.00	22–32	27.40 (0.30)	AB	7.70	24–32	27.52 (0.33)	AB	8.38	20–30	26.92 (0.30)	B	7.83	4.4417	*0.0048*
11	24–36	29.24 (0.37)	A	8.94	22–34	27.84 (0.35)	B	8.93	20–34	27.72 (0.43)	B	11.01	22–32	27.08 (0.33)	B	8.60	5.9692	*0.0006*
12	22–34	28.52 (0.36)		8.94	22–34	28.52 (0.36)		8.94	24–34	28.84 (0.35)		8.65	24–34	28.64 (0.35)		8.75	0.1789	*0.9106*
13	20–30	26.40 (0.33)		8.92	22–32	27.56 (0.30)		7.66	22–32	27.20 (0.33)		8.53	24–32	27.36 (0.32)		8.15	2.5391	*0.0578*
14	22–30	27.04 (0.27)	B	7.05	24–32	28.08 (0.27)	B	6.90	22–32	27.16 (0.37)	B	9.65	26–34	29.48 (0.26)	A	6.25	14.3903	*<0.0001*
15	24–32	28.08 (0.28)	AB	7.04	24–30	27.00 (0.30)	B	7.95	24–32	28.80 (0.30)	A	7.29	22–32	27.80 (0.30)	AB	7.73	6.3108	*0.0004*
16	24–34	29.52 (0.32)	A	7.69	26–34	29.72 (0.33)	A	7.93	22–34	28.12 (0.47)	B	11.88	22–34	26.80 (0.36)	B	9.42	13.1173	*<0.0001*
17	24–32	28.88 (0.29)	A	7.16	24–36	29.40 (0.35)	A	8.39	26–36	29.36 (0.28)	A	6.80	24–30	27.76 (0.25)	B	6.28	6.7165	*0.0002*
18	24–34	29.48 (0.37)	BC	8.86	24–32	28.96 (0.28)	C	6.73	24–36	30.64 (0.37)	AB	8.49	26–38	30.92 (0.41)	A	9.44	6.7104	*0.0002*

Table 7. Range (min-max), coefficient of variation (CV) and mean values (±SE) of Le/P ratio of *R. canina* pollen grains studied. One way ANOVA's were performed separately for each individual shrub to determine the differences among flowers (A, B, C and D). Same letters indicate a lack of statistically significant differences between analyzed species according to Tukey's a posteriori test (p<0.05)

Shrub No.	Flower A				Flower B				Flower C				Flower D				ANOVA	
	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	min-max	mean (±SE)		CV (%)	F	P
1	0.765–1.000	0.890 (0.007)		5.59	0.765–0.941	0.881 (0.007)		5.46	0.789–0.941	0.889 (0.006)		4.79	0.733–0.938	0.870 (0.007)		5.60	1.8673	*0.1364*
2	0.833–0.947	0.902 (0.005)		3.83	0.824–0.947	0.898 (0.005)		4.22	0.737–1.000	0.913 (0.006)		4.53	0.813–0.947	0.896 (0.006)		4.42	1.9542	*0.1222*
3	0.786–1.000	0.900 (0.006)		4.96	0.786–1.000	0.901 (0.006)		4.98	0.800–1.000	0.903 (0.006)		5.01	0.714–1.000	0.906 (0.007)		5.72	0.1332	*0.9402*
4	0.765–1.000	0.890 (0.007)		5.56	0.800–0.944	0.890 (0.006)		4.61	0.800–1.000	0.884 (0.007)		5.33	0.786–0.941	0.886 (0.006)		4.77	0.2204	*0.8822*
5	0.667–1.231	0.885 (0.018)		14.10	0.625–1.250	0.898 (0.017)		13.69	0.769–1.000	0.917 (0.009)		7.06	0.714–1.000	0.909 (0.008)		6.36	0.9619	*0.4118*
6	0.800–1.071	0.911 (0.008)	A	5.92	0.813–0.944	0.899 (0.005)	A	3.97	0.824–0.944	0.903 (0.005)	A	3.85	0.600–1.000	0.865 (0.011)	B	9.25	6.9150	*0.0002*
7	0.786–0.941	0.893 (0.006)	AB	4.67	0.778–0.938	0.889 (0.006)	AB	4.82	0.765–0.941	0.876 (0.005)	B	4.09	0.786–1.455	0.909 (0.012)	A	9.72	2.9720	*0.0329*
8	0.800–1.000	0.921 (0.005)	A	3.75	0.800–0.941	0.916 (0.005)	AB	3.86	0.765–0.938	0.872 (0.008)	C	6.35	0.786–0.941	0.897 (0.006)	B	4.76	13.1564	*<0.0001*
9	0.786–0.938	0.903 (0.006)	AB	4.55	0.813–1.000	0.920 (0.006)	A	4.57	0.800–1.000	0.897 (0.006)	B	4.97	0.769–0.941	0.894 (0.006)	B	5.01	3.6247	*0.0140*
10	0.750–0.941	0.901 (0.006)		4.71	0.733–0.941	0.904 (0.006)		4.50	0.765–1.000	0.899 (0.008)		6.01	0.769–1.000	0.909 (0.008)		6.36	0.3666	*0.7772*
11	0.800–1.000	0.905 (0.007)		5.61	0.800–1.000	0.908 (0.008)		6.27	0.769–1.000	0.899 (0.008)		6.32	0.813–1.000	0.900 (0.006)		5.06	0.3065	*0.8207*
12	0.722–1.133	0.921 (0.014)		10.69	0.786–1.000	0.898 (0.007)		5.42	0.813–1.000	0.906 (0.005)		4.28	0.778–1.000	0.900 (0.007)		5.86	1.3886	*0.2475*
13	0.769–1.077	0.900 (0.008)		6.63	0.786–1.000	0.911 (0.007)		5.16	0.786–1.000	0.899 (0.007)		5.61	0.800–1.000	0.896 (0.006)		4.82	0.8086	*0.4905*
14	0.786–0.938	0.888 (0.006)		5.00	0.800–0.941	0.902 (0.006)		4.59	0.786–1.000	0.894 (0.007)		5.78	0.824–1.000	0.906 (0.006)		4.77	1.5614	*0.2001*
15	0.800–1.000	0.899 (0.006)		4.82	0.800–0.938	0.880 (0.006)		5.05	0.813–0.941	0.887 (0.005)		4.09	0.786–1.000	0.895 (0.007)		5.34	2.0252	*0.1117*
16	0.813-0.944	0.903 (0.005)	AB	3.80	0.765-0.944	0.908 (0.006)	A	4.75	0.733–1.133	0.889 (0.012)	AB	9.59	0.786-0.933	0.877 (0.006)	B	4.99	3.2374	*0.0233*
17	0.750–1.071	0.922 (0.009)		7.08	0.813–1.000	0.902 (0.006)		4.46	0.813-0.947	0.903 (0.005)		4.08	0.800–1.000	0.905 (0.007)		5.69	1.9317	*0.1257*
18	0.813–1.000	0.910 (0.006)		4.60	0.800–1.000	0.913 (0.006)		4.54	0.765–1.000	0.907 (0.006)		4.80	0.813–1.000	0.896 (0.006)		4.54	1.5804	*0.1954*

Table 8. Range (min-max), coefficient of variation (CV) and mean values (±SE) of all pollen grain morphological features of *R. canina* studied. One way ANOVA's were performed separately for individual features to determine the differences among individual shrubs

Shrub No.	No. of pollen grains	P		E		P/E		Exp		Exp/P		Le		Le/P	
		Mean (±SE)	CV (%)	Mean (±SE)	CV (%)	Mean (±SE)	CV (%)	Mean (±SE)	CV (%)	Mean (±SE)	CV (%)	Mean (±SE)	CV (%)	Mean (±SE)	CV (%)
1	200	31.99	5.90	26.25	7.56	1.224	8.37	0.753	30.59	0.0236	31.17	28.21	7.23	0.882	5.40
		(0.13)		(0.14)		(0.007)		(0.016)		(0.0005)		(0.14)		(0.003)	
2	200	33.92	6.31	28.90	7.01	1.178	8.20	0.813	46.21	0.0240	45.45	30.59	7.14	0.902	4.29
		(0.15)		(0.14)		(0.007)		(0.027)		(0.0008)		(0.15)		(0.003)	
3	200	32.06	7.16	27.67	6.83	1.163	8.79	0.589	39.87	0.0184	38.99	28.94	9.24	0.902	5.15
		(0.16)		(0.13)		(0.007)		(0.017)		(0.0005)		(0.19)		(0.003)	
4	200	31.92	7.18	27.25	8.24	1.178	9.64	0.635	44.48	0.0198	41.42	28.33	8.83	0.887	5.05
		(0.16)		(0.16)		(0.008)		(0.020)		(0.0006)		(0.18)		(0.003)	
5	200	29.00	8.24	27.51	9.18	1.061	10.23	0.431	34.53	0.0150	35.47	26.08	10.95	0.902	10.83
		(0.17)		(0.18)		(0.008)		(0.011)		(0.0004)		(0.20)		(0.007)	
6	200	30.77	7.78	25.86	10.65	1.202	12.34	0.575	51.60	0.0187	53.17	27.54	10.31	0.895	6.34
		(0.17)		(0.19)		(0.010)		(0.021)		(0.0007)		(0.20)		(0.004)	
7	200	31.72	8.62	26.58	10.44	1.203	11.34	0.528	39.83	0.0167	39.38	28.27	9.74	0.892	6.40
		(0.19)		(0.20)		(0.010)		(0.015)		(0.0005)		(0.19)		(0.004)	
8	200	30.45	6.63	28.69	7.18	1.065	8.29	0.709	54.53	0.0233	54.53	27.45	8.26	0.902	5.17
		(0.14)		(0.15)		(0.006)		(0.027)		(0.0009)		(0.16)		(0.003)	
9	200	30.91	7.22	25.73	11.99	1.214	10.53	0.602	46.27	0.0196	46.42	27.93	9.06	0.903	4.87
		(0.16)		(0.22)		(0.009)		(0.020)		(0.0006)		(0.18)		(0.003)	
10	200	30.55	6.56	25.07	11.70	1.232	11.24	1.246	39.41	0.0409	39.78	27.57	7.93	0.903	5.43
		(0.14)		(0.21)		(0.010)		(0.035)		(0.0012)		(0.15)		(0.003)	
11	200	30.97	7.38	24.90	10.55	1.253	9.64	0.956	46.34	0.0310	46.40	27.97	9.76	0.903	5.81
		(0.16)		(0.19)		(0.009)		(0.031)		(0.0010)		(0.19)		(0.004)	
12	200	31.63	7.03	26.04	10.16	1.224	10.29	0.727	41.66	0.0231	42.05	28.63	8.76	0.906	7.08
		(0.16)		(0.19)		(0.009)		(0.021)		(0.0007)		(0.18)		(0.005)	
13	200	30.08	5.88	25.18	9.59	1.204	10.18	0.862	36.06	0.0287	35.95	27.13	8.41	0.902	5.59
		(0.13)		(0.17)		(0.009)		(0.022)		(0.0007)		(0.16)		(0.004)	
14	200	31.12	6.03	26.44	8.28	1.183	8.02	0.802	42.28	0.0257	41.20	27.94	8.24	0.898	5.07
		(0.13)		(0.15)		(0.007)		(0.024)		(0.0007)		(0.16)		(0.003)	
15	200	31.36	6.16	26.81	7.52	1.174	7.63	0.806	29.95	0.0258	30.31	27.92	7.80	0.890	4.89
		(0.14)		(0.14)		(0.006)		(0.017)		(0.0006)		(0.15)		(0.003)	
16	200	31.92	8.38	27.24	5.87	1.174	9.09	0.71	42.43	0.0226	46.36	28.54	10.12	0.894	6.28
		(0.19)		(0.11)		(0.008)		(0.021)		(0.0007)		(0.20)		(0.004)	
17	200	31.80	6.40	26.33	6.41	1.212	7.94	0.421	45.03	0.0132	42.61	28.85	7.53	0.908	5.51
		(0.14)		(0.12)		(0.007)		(0.013)		(0.0004)		(0.15)		(0.004)	
18	200	33.09	7.73	26.77	6.89	1.240	8.69	0.415	30.15	0.0126	31.43	30.00	8.84	0.907	4.64
		(0.18)		(0.13)		(0.008)		(0.009)		(0.0003)		(0.19)		(0.003)	
ANOVA	F	49.6473		46.6268		41.6308		93.6859		98.5657		33.2968		3.5516	
P>F	P	*<0.0001*		*<0.0001*		*<0.0001*		*<0.0001*		*<0.0001*		*<0.0001*		*<0.0001*	

The mean length of polar axis (P, calculated for each individual based on 200 pollen grains) ranged from 29 μm (shrub No. 5) to 33.92 μm (shrub No. 2), thus the highest mean value is ca. 17% higher then the minimal mean value. The mean length of equatorial diameter (E) ranged from 24.9 μm (shrub No. 11) to 28.9 μm (shrub No. 2), with the highest 16% higher than the lowest. The difference among minimal and maximal mean value of P/E ratio for individual shrubs was 18% and the values ranged from 1.061 (shrub No. 5) to 1.253 (shrub No. 11). The thickness of exine along polar axis (Exp) ranged among shrubs from 0.415 μm (shrub No. 18) to 1.246 μm (shrub No. 10); the highest mean value of Exp was three times the minimal mean value. The range of mean values of Exp/P ratio was from 0.0126 (shrub No. 18) to 0.0409 (shrub No. 10), and the difference among the outermost values obtained for individual shrubs was ca. threefold. Mean length of ectocolpi (Le) calculated for particular individuals ranged from 26.08 μm (shrub No. 5) to 30.59 μm (shrub No. 2) (difference among values – 17%), whereas Le/P ranged from 0.882 (shrub No. 1) to 0.908 (shrub No. 17) (difference among values – 3%).

Based on particular shrub individuals, the pollen grain features studied may be ordered from less to most variable as follows: Le/P (mean CV = 5.8%), P (CV = 7%), E (CV = 8.7%), Le (CV = 8.8%), P/E (CV = 9.5%), Exp (CV = 41.2%) and Exp/P (CV = 41.2%).

Discussion

In taxonomical studies on the Rosaceae family and *Rosa* genus, pollen grains are considered as organism characterised by very low variability and, consequently, they are often ascribed diagnostic importance (Hutchinson 1964; Klaštersky 1968; Henker 2000; Kalkman 2004; Potter et al. 2007). On the basis of this assumption, only small numbers of pollen samples are commonly examined for individual species in morphological investigations. In the case of the Rosaceae family, the sample size ranges from 10 to 20 pollen grains (e.g. Hebda and Chinnappa 1990, 1994; Li et al. 2001; Nazeri Joneghani 2008; Wang et al. 2009), while in many of the most recent studies, a sample consists of 30 pollen grains (e.g. Tomlik-Wyremblewska et al. 2004; Bednorz et al. 2005; 2008; Wrońska-Pilarek and Jagodziński 2009, 2011; Wrońska-Pilarek 2011) and samples made up of 50 (e.g. Monasterio-Huelin and Pardo 1995; Wrońska-Pilarek and Boratyńska 2005) or 50–100 pollen grains (and 2008) are much rarer.

In papers cited above concerning pollen grains of Rosaceae, variability of individual pollen grain features was not examined; only mean values or, less frequently, range of values, were presented. These data refer only to selected features, usually it is length of polar axis (P), sometimes – length of equatorial diameter (E). Although these data are based on measurements of few pollen grains and are not subjected to statistical analysis, nevertheless they give a general picture of variability of a given feature. For instance, according to some palynologists, the value of *R. canina* P feature falls within the following intervals: 15.4–36.9 μm (Eide 1981), 26.0–35.0 μm (Gonzalez Romano and Candau 1989), 25.0–32.0 μm (Reitsma, 1966), and 27.0–30.5 μm (Stachurska et al. 1974–1975). According to the most recent studies, the range of this feature ranged from 22–42 μm (Wrońska-Pilarek and Jagodziński 2009), 23.4–28.8 μm (Wrońska-Pilarek 2011) and 28.0–38.0 μm (Wrońska-Pilarek and Jagodziński 2011).

The only study which describes size variation of pollen grains in Rosaceae concerns the genus *Rubus*. Naruhashi and Takano (1980) describe size variation of pollen grains of four *Rubus* species (*R. hirsutus, R. palmatus, R. parvifolius, R. trifidus*) on the basis of measurements of a very large sample (from 780 to 900 pollen grains). They studied pollen grains collected from 4 natural localities for each species mentioned. Three species (*R. hirsutus, R. parvifolius* and *R. trifidus*) were similar in mean pollen size and mean coefficients of variation (from 4.9 to 6.6%). However, *R. palmatus* possesses a considerably different trend in the variability (CV – from 5.7 to 9.0%) of pollen grains from the remaining species. and (2008) did not perform a statistical analysis but gave coefficient of variation (CV) for five quantitative features of pollen grains of 12 species of the genus *Spirea*. For example, for length of polar axis (P), they obtained CV from 6.7% (in *S. trilobata*) to 18.1% (in *S. media*).

As demonstrated, the degree of Rosaceae pollen grain variability at its various levels (i.e. intra- and interspecific or intra- and interindividual variability) is poorly assessed. In our opinion, this new trend of palynological investigations will allow verification of views regarding the full range of pollen grain variability and, consequently, may help determine to what extent the hypothesis about small variability of these organisms is justified and indicate how to collect material for palynological studies in order to reliably describe pollen grain morphological structure of a given species.

The authors began their investigations of pollen grain variability by analysing intra- and interspecific variability of 16 *Rosa* species (including *R. canina*) from 107 natural localities on a sample consisting of 3510 pollen grains and based on 8 quantitative features (Wrońska-Pilarek and Jagodziński 2009). The results obtained indicate considerable variability between individual rose species. Differences in mean values of coefficients of variability (CV) exceed 11%. The smallest mean variability was recorded in the case of *R. pendulina* (CV=7.4%, n=300), *R. villosa* (CV=9.0%, n=30) and *R. jundzillii* (CV=10.8%, n=150) pollen grains, while the highest – in *R. mollis* (CV=18.6%, n=120), *R. kostrakiewiczii* (CV=17.1%, n=30) and *R. majalis* (CV=16.6%, n=150).

It is evident from investigations carried out so far that pollen grain features may differ from one another quite considerably both at inter- as well as intraspecific levels. For example, the range of length (feature P) of 510 *R. canina* pollen grains from 17 natural localities in Poland ranged from 22 to 42 μm, while for all the rose species examined (16) the range of differences of the P feature was greater and ranged from 20 to 50 μm (Wrońska-Pilarek and Jagodziński 2009).

Differences of mean values of the same P feature for 3510 pollen grains of the rose species examined were smaller and fluctuated from 27 μm (in *R. majalis*) to 35.9 μm (in *R. gallica*). The observed fairly large variability of pollen grains features may indicate the need to study greater numbers of pollen grain samples. It turns out that a large sample size of pollen grains is not synonymous with greater accuracy of morphological results determined. It seems that the optimum size of pollen grain sample for morphological study is 30 pollen grains, as for example, the range of values of the P feature for *R. canina* based on the measurement of 30 pollen grains is similar to measurement of 510 pollen grains and amounts consecutively to: 28.0–38.0 μm; mean – 32.5 μm (Wrońska-Pilarek and Jagodziński 2011) and 22.0–42.0 μm; mean 31.0 μm (Wrońska-Pilarek and Jagodziński 2009).

A very interesting result was obtained by Wrońska-Pilarek and Jagodziński (2009), collating all pollen grain features collected from shrubs growing in 42 natural localities of *R. canina*, *R. gallica* and *R. pendulina*. Based on agglomeration grouping using Ward's hierarchical method for the 42 localities, it was found that the localities clustered into three groups that largely represented localities of the particular species. At the same time, no clear correlations between pollen features collected from shrubs growing in the same regions of the country were observed. For example, in the case of *R. canina*, similar features were found in pollen grains collected from sites situated in the lowland, in central Poland as well as in the south of the country, high in the mountains but, simultaneously, pollen grains similar to one another were collected from areas neighbouring with each other. Therefore, it seems that for pollen grain biometric analyses site geographic location of a given shrub is not very important (this refers, in particular, to apomictic species e.g. species from the genus *Rubus*; Wrońska-Pilarek et al. 2006, Wrońska-Pilarek et al. 2012). Therefore, it is reasonable that for reliable analysis of pollen grain morphology of the species, it is more important to collect pollen grains from numerous individuals of the species than from a small number of individuals, even if the number of pollen grains analysed is similar.

This study presents a continuation of the above-described studies, but this time conducted at the level of a *R. canina* population. These are the first, as far as Rosaceae is concerned, investigations in which variability of pollen grain features were analysed both at the level of the flower, among flowers from each shrub and among shrubs of the same species.

Variability of pollen grain morphological features within one flower deriving from one shrub (e.g. shrub No. 1) was relatively small (e.g. for the P feature for flower A, CV=5.27%; see: Table 1). Variability of the same feature in four flowers collected from the same shrub (shrub No. 1) was slightly higher and, on average, amounted to CV=5.90% (Table 8). However, more pronounced differences occurred in some shrubs, e.g. for shrub No. 6 a single A flower was characterized by CV = 4.36% and the mean coefficient for all four flowers from this shrub was higher, reaching 7.78%. This indicates that biometric analyses should not be based on material from a single flower but rather on several flowers. Recapitulating, the determination of the full range of variability of pollen grain biometric features within one individual (shrub) is more reliable if several pollen grains from several flowers are examined rather than for the same number of pollen grains derived from a single flower.

In the *R. canina* population examined separately, significant interindividual variability was found between individual shrubs with regard to all pollen grain features examined (Table 8), which proves that in order to characterize a population of a given species well palynologically, the plant material examined should be derived from a reasonably large number of individuals (shrubs).

On the other hand, when analyzing values of coefficients of variation (CV) of pollen grains derived from individual shrubs, we can see similar, low or medium CV values for P, E, Le, P/E and Le/P features (from 4.29 to 11.99 %; Table 8). On the other hand, the variability of Exp and Exp/P features is very high (CV value; from 30.15 to 54.53%; Table 8). These high differences probably resulted from the natural variability of the exine thickness as well as from the fact that, from among pollen grain quantitative features, this feature is the most difficult one for accurate measurement under the light microscope, and hence has the highest measurement error (Wrońska-Pilarek and Jagodziński 2009, 2011; Wrońska-Pilarek 2011).

The research results presented here fully corroborate suggestions made by Naruhashi and Takano (1980), who emphasized that in studies on pollen grain quantitative features, it is not sufficient to provide only mean values of these features but also to investigate their variability. It is also very important to pay attention to the method of collection of pollen grains intended for biometric analyses, i.e. whether the analysis of morphological variability of pollen grains is based on grain measurements derived from a single flower, several flowers of a single shrub or from several flowers collected from a few to several shrubs of the same species. The method of material collection for biometric analyses may exert a significant impact on obtaining information regarding full pollen grain morphological variability of the species examined.

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