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POLLEN MORPHOLOGICAL STUDIES ON *ARABIS ALPINA* L. (BRASSICACEAE) POPULATIONS FROM THE ALPS AND THE RILA MOUNTAINS

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Summary: Pollen morphology of *Arabis alpina* L. populations growing in the French Alps (high mountain station of Lautaret, near Col de Galibier) as well as in the mountain of Vercors and in the region of the Seven Rila Lakes in Bulgaria was studied. The aims of the research were to examine the pollen morphological characteristics of plants grown on different substrates and altitudes, to discuss and compare similarities/differences as well as the relation between the size of flower and the size of pollen. The observations were done using scanning electron (SEM) and light (LM) microscopy. The pollen grains of *A. alpina* can be characterized as 3-colpate, oblate spheroidal in pollar view and elliptic in equatorial view, with reticulate ornamentation. Although most of the pollen grains are 3-colpate, 4-colpate pollen grains were also found in all populations studied. The ratio sterile/fertile pollen in all populations was also calculated. The similarities and relationship between *A. alpina* populations support previous taxonomical decisions.

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Keywords: *Arabis alpina*, Brassicaceae, pollen grains, sterility/fertility, variations.

Abbreviations: A – apocolpium; ColpL - colpus length; ColpS - colpus width; E - equatorial diameter; LM - light microscopy; M – mesocolpium; P - polar diameter; PCA - Principal Component Analysis; SEM - scanning electron microscopy.

INTRODUCTION

Alpine rock-cress, *Arabis alpina* L., is a widespread plant species distributed throughout the alpine habitats in Europe, in the arctic zone of Greenland and North America, in the high mountains of northern and eastern Africa and Anatolia, and in the ranges extending into the Caucasus and the Near East (Meusel et al., 1965). In Europe

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it is distributed in the arctic areas of Iceland and Scandinavia, and the alpine regions of the Pyrenees, Alps, Apennines, Balkan, Carpathian and Tatras mountain systems (Jalas and Suominen, 1994). The species is variable in the mountainous areas of Europe, southwest Asia and northern Africa (Tan, 2002) and this variation was taxonomically differently treated in some floristic editions (Greuter et al., 1986; Akeroyd, 1997; Marhold, 2011). In Bulgaria this species is presented by the subspecies *flavescens* (Griseb.) Hayek (Ančev, 2001) accepted as a synonym of subsp. *caucasica* (Willd.) Briq. (Akeroyd, 1997; Marhold, 2011). The typical subspecies is not distributed in Bulgaria but is found in the arctic and alpine regions of the European mountains. Both subspecies differ in their corolla length, siliqua length (both longer in subsp. *caucasica*) and the color of the petals (yellowish in subsp. *caucasica* and white in subsp. *alpina*). *Arabis alpina* is distributed in subalpine meadows, rock crevices and scree slopes mainly on limestone (Tan, 2002). Some of the populations in Bulgaria are distributed on serpentine and silicate between 1000 and 2900 m a.s.l.

Information about pollen morphology of *Arabis* species is available from several regional pollen morphological studies and from general surveys of the family (Erdtman et al., 1963; Moore and Webb 1978; Moore et al., 1991; Faegri and Iversen, 1989; Anchev and Deneva, 1997; Perveen et al., 2004; Beug, 2004; Mutlu and Erik, 2012). The pollen grains from different species of Arabis are related to two different pollen types: *Hornungia* type (Beug, 2004) and *Sinapis* type (Moore and Webb 1978; Moore et al. 1991). Pollen morphology of *A. alpina* was studied previously by Moore et al. (1991), Anchev and Deneva (1997), Beug (2004), and Mutlu and Erik (2012). The main pollen characters used for determination of *A. alpina* were pollen size, aperture, structure of the exine and ornamentation. *A. alpina* pollen grains are characterized as 3-colpate, oblate spheroidal in pollar view, elliptic in equatorial view, with reticulate ornamentation. Pollen of this species was included in *Sinapis* type (Moore and Webb 1978; Moore et al., 1991) altogether with more than 20 taxa characterized by a reticulate ornamentation, thick exine (<4 μm), grains <40 μm in size, circular or elliptic outline in equatorial view, long colpi without margo and thin muri. Mutlu and Erik (2012) studied the pollen morphology of the genus *Arabis* in Turkey and its taxonomical significance. They divided it into three pollen types, including *A. alpina* as *Arabis* type characterized by polar axis equal or longer than 25 μm, equatorial axis longer than 23 μm, outline of a pollen grain as viewed from directly above one of the poles - 23 μm.

To our knowledge, there is no detailed study on the variation of pollen morphological characters of the widely distributed in the Northern hemisphere *A. alpina* species (Al-Shehbaz, 1988). Also, there is not enough information about pollen sterility/fertility, pollen morphology in relation to pollen size and flower characters in *Arabis*. The aims of the present research were to estimate: 1) pollen morphological characteristics of *A. alpina*; 2) variation of pollen characters in plant populations found on different substrates and altitudes, and compare the similarities/differences between them; 3) the ratio of viable pollen for each population; 4) the relation between flower size and pollen size.

MATERIAL AND METHODS

Plant sampling

Pollen from seven populations of *A. alpina* distributed at different altitudes and mountain areas (French Alps, Massive des Ecrins and Massive du Vercors in France, and Rila Mountains in Bulgaria) was collected and the voucher specimens were deposited in the Herbarium of the University of Sofia (SO) and Joseph Fourier University, Grenoble, France (Table 1). The French localities were based on previous results using genome scan of regional population samples of *Arabis alpina* (Poncet et al., 2010).

Mixed samples from minimum five flowers of at least five individuals for each population were prepared for LM and SEM analyses. The pollen samples were acetolyzed according to Erdtman (1966) and Skvarla (1966). The measurements and pollen descriptions were made on acetolyzed pollen. For LM analyses slides were prepared by mounting the pollen in glycerol gelly and observations were made with an OLYMPUS BX-51 microscope under E40, 0.65 and oil immersion (E100, 1.25) magnification, using 10x eye piece. For each population 30 pollen grains were measured for 6 features: polar diameter (P), equatorial diameter (E), colpus length (ColpL), colpus width (ColpS) measured in the equatorial area, apocolpium (A) and mesocolpium (M). The P/E ratio was also calculated in order to determine pollen shape. The minimal, maximal and average values for each morphological feature are shown in Table 2.

Pollen grains for scanning electron microscopy (SEM) were suspended in a drop of 95% ethanol coated with gold and examined with a microscope JEOL-JSM-5510. Selected SEM and LM photographs, prepared using ZEISS Axiocam ERc5s camera were used to

Table 1. List of the localities of the studied populations of *Arabis alpina*.

Locality	Site code	Geographical coordinates	Altitude [m]	Substrate
Bulgaria, Northern Rila Mt., near lake “Bliznaka”	BG1	42° 12’15.79″ N 23° 18′ 55.56″ E	2250	Silicate
Bulgaria, Northern Rila Mt. near lake “Trilistnika”	BG2	42° 12′ 30.48″ N 23° 18′ 59.37″ E	2230	Serpentine
France, Gal Massive des Ecrins, French Alps, Col de Galibier	FR3	45° 03′ 50.4″ N 06° 24′ 28.8″ E	2600	Schist
France-004 Massive du Vercors, Villard de Lens	FR4	45° 04′ 15″ N 05° 33′ 05″ E	1500	Calcareus
France-005 Massive du Vercors, Côte 2000II, Villard de Lens	FR5	45° 04′ 07″ N 05° 32′ 36″ E	1842	Calcareus
France-006 Massive du Vercors, Villard de Lens	FR6	45° 04′ 41″ N 05° 35′ 36″ E	1300	Calcareus
France-107 Massive du Vercors, Villard de Lens	FR7	45° 04′ 50″ N 05° 33′ 12″ E	1600	Calcareus

Table 2. Pollen morphological data of the examined populations of *A. alpina* with measurements (μm) with mean (in brackets) ± standard deviation and ranges of the pollen characters: polar (P) and equatorial (E) diameter, colpus length (ColpL), colpus width (ColpS), apocolpium (A), mesocolpium (M), shape index P/E and pollen shape in percentages. Abb.: subS (subspheroidal), OS (oblate spheroidal), PS (prolate spheroidal), subP (subprolate), and Pr (prolate). Site codes correspond to data in Table 1.

Site code	P	E	ColpL	ColpS	A	M	P/E	subS	OS	PS	subP	Pr
BG1	27-36 (31.44±2.2)	22.5-34.5 (27.72±2.6)	22.5-31.5 (28.56±2.3)	4.5-7.5 (6.06±1.1)	4.5-7.5 (6.36±0.9)	13.5-21 (17.82±2.2)	1.0-1.4 (1.13±0.1)		16	40	40	4
BG2	25.5-34.5 (29.82±1.9)	25.5-31.5 (28.92±1.5)	25.5-33 (28.5±1.7)	4.5-9 (6.96±1.1)	4.5-9 (5.82±1.1)	15-19.5 (18.42±1.3)	0.85-1.35 (1.03±0.1)	4	28	60	8	
FR3	24-36 (27.9±2.6)	21-36 (27.18±2.8)	24.5-34 (27.12±2.4)	4.5-7.5 (6.06±0.8)	3-7.5 (5.7±1.2)	12-21 (17.94±1.7)	0.94-1.36 (1.03±0.09)		60	32	4	4
FR4	22.5-33 (27.66±2.9)	19.5-30 (26.52±2.7)	21-31.5 (26.16±2.8)	3-7.5 (5.58±1.3)	4.5-7.5 (6.06±1.1)	13.5-19.5 (16.62±2.0)	0.79-1.33 (1.04±0.1)	12	28	36	24	
FR5	21-34.5 (28.14±3.4)	21-31.5 (26.58±2.4)	21-33 (26.76±3.3)	4.5-7.5 (6.12±1.0)	4.5-7.5 (5.76±0.9)	13.5-21 (17.34±2.3)	0.88-1.16 (1.06±0.1)	4	36	44	8	8
FR6	24-33 (27.48±2.4)	22.5-30 (26.76±1.9)	24-31.5 (26.22±2.1)	4.5-7.5 (5.82±1.0)	3-7.5 (5.52±1.2)	6.5-21 (18±1.2)	0.94-1.22 (1.03±0.09)		44	52	4	
FR7	24-33 (28.62±2.4)	21-34.5 (27.96±3.0)	22.5-31.5 (27.12±2.2)	4.5-9 (5.7±1.1)	3.0-7.5 (4.98±0.9)	12-21 (18.12±2.2)	0.84-1.44 (1.04±0.2)	4	60	20	8	8

demonstrate pollen morphology of the species. The pollen terminology in general follows Faegri and Iversen (1989), Punt et al. (2007) and Hesse et al. (2009).

To estimate the percentage ratio of fertile/sterile pollen in all populations 3 anthers per flower and 20 flowers per population were taken. The mean values for each population were presented (Fig. 1). Each anther was broken onto microscope slides under cover slips, after adding a drop of Alexander stain (Alexander 1969). Pollen grains were counted under the microscope at 20x magnification; those stained in red were considered fertile.

The fresh or rehydrated flower parts of the selected flowers for pollen morphological studies were measured using STEMI - 2000 stereoscope (magnification 0.65 and 10x eye piece) as an indicator of flower size. The following parameters were measured: calyx length (CaL) for outlier leaves, calyx width (CaS), corolla length (CoL), corolla width, length of the longer stamen (StL), length of the shorter stamen (StS), style (Sty), stigma (Stg), and pistil length (PistL).

Data Analysis

Univariate and multivariate statistical procedures were applied to examine variation among the populations on the basis of pollen-morphological measurements. Cluster analysis using Euclidean distance and Unweighted Pair Group Means Average (UPGMA) was used as computational criteria to determine the similarities between populations using mean values of the pollen features (Fig. 2).

Ordination by Principal Component Analysis (PCA) was also used to show the loadings for each character and which character contributed most for differences between populations, to display the results in a non-hierarchical way and to show the relationships between variables (Fig. 3 and 4). Eigenvalues were extracted from the correlation matrix. PCA assumes linearity which simplifies the mathematical problem by restricting the set of potential bases and formalizing the assumption of continuity in a data set (Shlens 2003). All statistics were performed using StatSoft – Statistica 7 program.

RESULTS

Pollen morphology

The pollen grains of *A. alpina* are 3 (4)-zonocolpate (Plate 1). Both, polar (P) and equatorial diameter (E) were in the range from 22.5 µm to 36 µm. The highest mean value for the polar (31.44 µm) and equatorial diameter (28.92 µm) was calculated for pollen grains from the Bulgarian populations while the smallest value (22.14 µm) was found for the population from the French Apls (FR5). Pollen shape, calculated as a ratio between polar and equatorial diameter (P/E), in all populations was quite variable (Table 2, Plate 1, Figs. 1-5). Most often the grains were prolate spheroidal (P/E 1.0-1.14), oblate spheroidal (P/E 0.88-1.0) or subprolate (P/E 1.14-1.33) (Plate 1, Figs. 2-4). The calculated percentage for these types from all studied pollen grains was 40.57%, 39.43% and 13.71%, respectively. The number of oblate spheroidal pollen grains in the populations from France was higher than in the Bulgarian populations. The last ones were characterized mainly by prolate spheroidal grains. The highest number of subprolate pollen was found in one of the Bulgarian populations (BG1) in equal proportion to prolate spheroidal

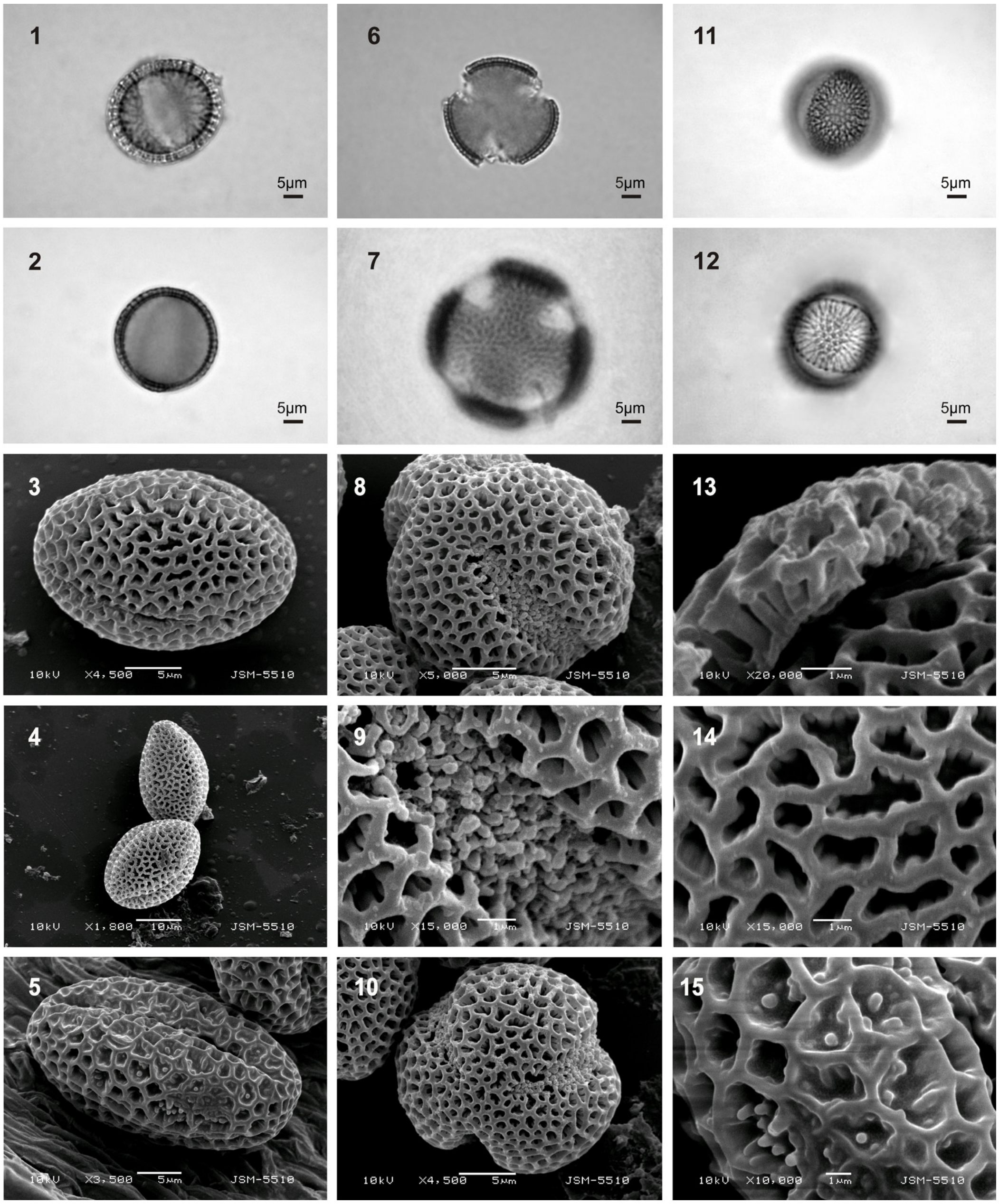

Plate 1. LM and SEM micrographs of pollen grains of *Arabis alpina*, Fig.1 - Oblate spheroidal pollen grain (LM); Fig. 2 – Prolate spheroidal pollen (LM); Fig. 3 and 4 – Subprolate pollen (SEM); Fig. 5 – Prolate (SEM); Fig. 6 – Polar view, 3-Colpate pollen (LM); Fig. 7 - Polar view, 4-Colpate pollen (LM); Fig. 8 – Colpus and ornamentation (SEM); Fig.9 – Colpus membrane (SEM); Fig. 10 – Apocolpium ornamentation (SEM); Fig. 11 and 12- Mesocolpium (LM); Fig. 13 - SEM section of the exine; Fig. 14 – Ornamentation in the mesocolpium (SEM); Fig. 15 - Free-standing columellae in the lumina.

pollen. Rarely, subspheroidal and prolate pollen gains also appeared (Plate 1, Figs. 1, 5). In relation to the pollen shape index equatorial view varied from spheroidal to elliptic. In polar view the grains were circular or lobate (Plate 1, Fig. 6).

Generally, pollen is 3-zonocolpate but 4- zonocolpate pollen was also found more often in the populations from France (Plate 1, Fig. 7). The ectocolpi are long, straight, pointing at the poles and gradually narrowing to them. The highest mean value (ca. 28.5 µm) was calculated for the pollen from the Bulgarian populations. The mean colpus length for the French populations varied between 26.22 and 27.12 µm. Approximately uniform was the width of the colpi in the equatorial region for all populations. The colpus margin was uneven without a margo. The colpus membrane was covered by different in size granules (Plate 1, Figs. 8-9). The apocolpia (Plate 1, Fig. 10) and the mesocolpia (Plate 1, Figs. 11-12) also varied between the populations. More variable was the mesocolpium width which is obviously related to the variation of the pollen equatorial diameter.

The exine thickness varied between 1.5 and 3 µm, thicker in the mesocolpia. In the aperture area it was ca. 1.5 µm. The exine structure involved a thin endexine, a foot layer, an infratectum with long (0.6-1.2 µm), straight, unbranched columellae, and ca. 0.3-0.5 µm thick tectum (Plate 1, Fig. 13).

The ornamentation was reticulate, varying from reticulate in the mesocolpium (Plate 1, Fig. 14) with free-standing columellae in the lumina (Plate 1, Fig. 15) to reticulate-microreticulate in the apocolpia. The reticulum was homobrochate. The largest lumina (1.5- 3.0 µm) were observed in the equatorial area. Their size was smaller in the apocolpia. The number of lumina across the mesocolpium varied between 9 and 12.

Pollen viability

The percentage of fertile/sterile pollen for the populations is presented in Fig. 1. Both populations (BG1) from Bulgaria and France (FR5) showed a very high

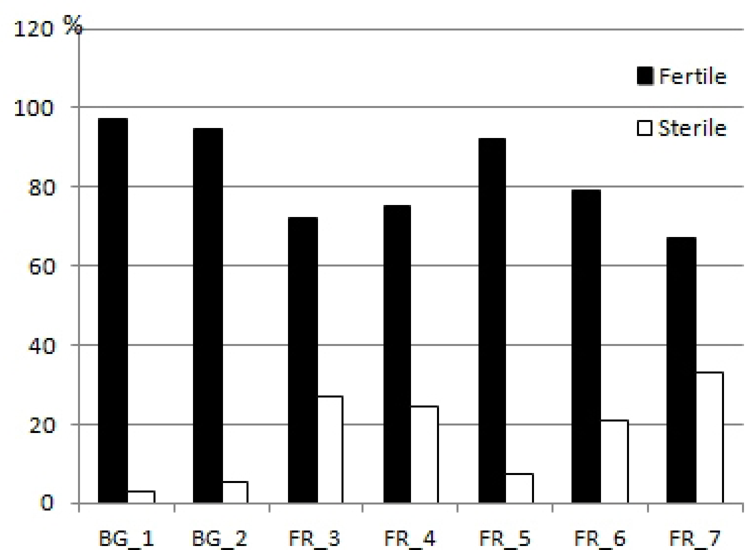

Figure 1. Fertile/sterile pollen ratio in all studied populations. Site codes correspond to data in Table 1.

percentage of fertile pollen (above 90%). The sterile pollen in these populations was below or a little above 5%, a limit considered as a normal abortion (Mičieta and Murin, 1996). The rest of the studied French populations had sterile pollen higher than 20%. The highest percentage of sterile pollen (32.76%) was calculated for population FR7. Significant differences were not found between the percentage of sterile pollen in the anthers of the short and long stamens in the flowers.

Correlations between populations

The dendrogram obtained by the hierarchical cluster analysis (Fig. 2) reveals two groups (clusters) of populations formed at a linkage distance around 3.9. In cluster (A) all populations of the species originating from France are arranged. All they have pollen grains smaller than the two Bulgarian populations combined in cluster (B). The main difference between the groups refers to the pollen diameter (P) which is below 30 μm for pollen grains from cluster (A) and above 30 μm for those in cluster (B). A very high degree of similarity (lowest Euclidian distances) was established for the pollen grains of populations from Gal 3 (Fr3) and Fr5. On the opposite, low degree of similarity was established between these populations and the population from the mountain of Vercors (Fr7). These results were confirmed by the PCA when species populations (cases) were plotted.

The principal component analysis carried out with all pollen traits used as variables demonstrates the loadings for each variable and their contribution to each of the PCs (Fig. 3). Most of the pollen traits (P, ColpL, A and P/E) had positive coordinates and correlations with factor 2. There was no variable with positive coordinates with factor 1. The most variable trait was the polar diameter while apocolpium was

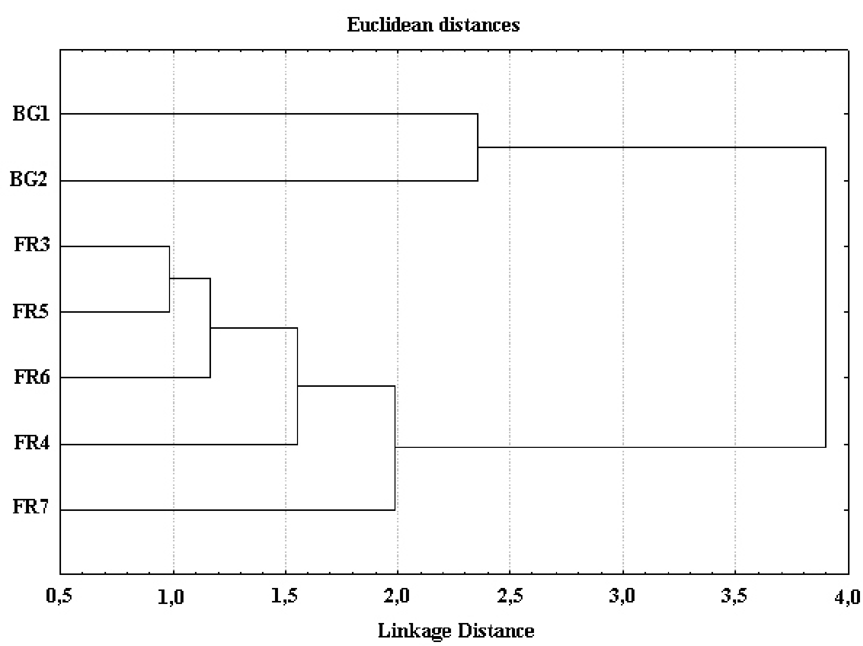

Figure 2. Similarity dendrogram obtained by cluster analysis (Euclidean distances) applied to data of the measured pollen characters.

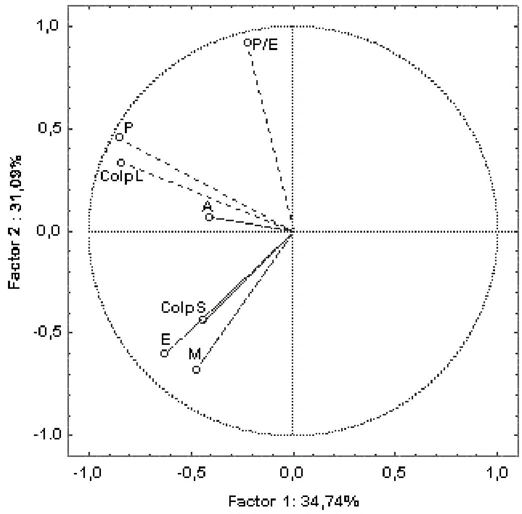

Figure 3. Principal component analysis plot pollen traits (vectors). Length of the vectors is proportional to strength of the correlations between variables and one of the PCs (factors). Variable codes as in the text.

more conservative. Equatorial diameter (E) and colpus width (ColpS) as well as polar diameter (P) and colpus length (ColpL) were very closely correlated.

Flower size has been used as one of the criteria to establish infraspecific limits in *A. alpina*. The mean flower size represented by the corolla length (CoL), corolla width (CoS), calyx length (CaL), calyx width (CaS), length of the longer stamen (StL), length of the shorter stamen (StS), style (Sty), stigma (Stg), and pistil

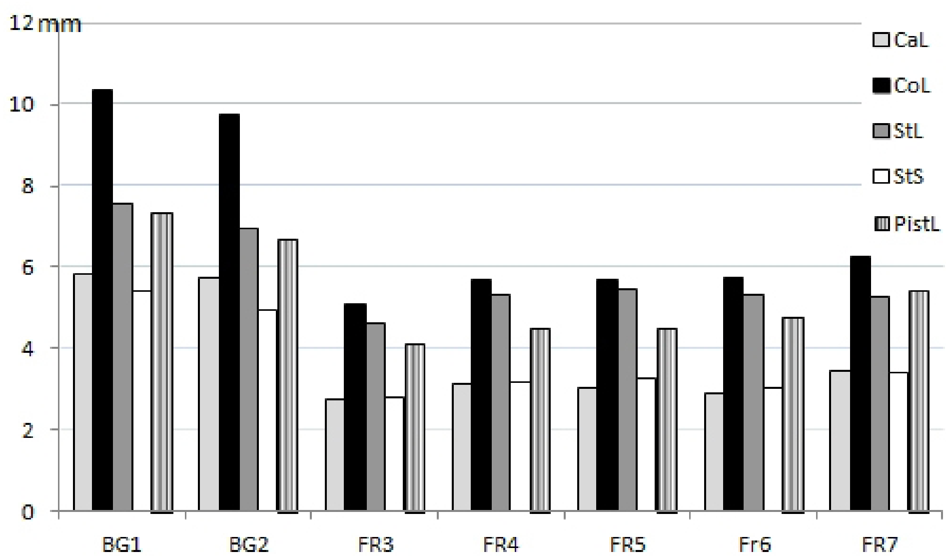

Figure 4. Mean values of the flower size represented by the corolla length (CoL), calyx length (CaL), length of the longer stamen (StL), length of the shorter stamen (StS), and pistil length (PistL).

length (PistL) are shown in Table 4. The mean values (except for the style and stigma) for the populations are presented in Fig. 4. As shown in the diagram, the extremes of the flower length variation are found in both Bulgarian populations.

The principal component analysis performed with all pollen traits and floral characters (Fig. 5) demonstrated that the length of the long (StL) and short stamen (StS), pistil length (PistL), calyx (CaL) and corolla length (CoL) as well as polar

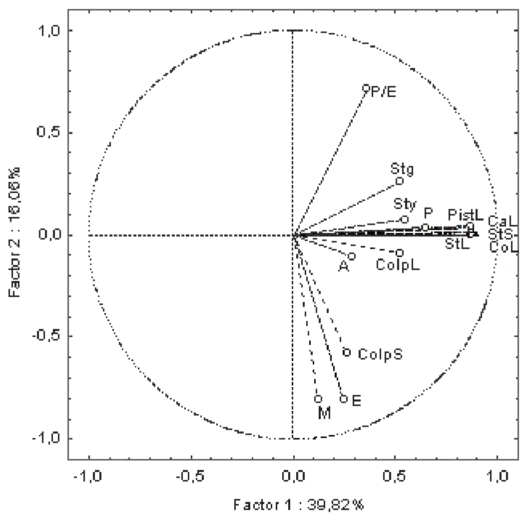

Figure 5. Principal component analysis performed with all pollen and floral traits (vectors). Variable codes as in the text.

Table 3. Summary of the statistics for the Principal Component Analysis (PCA) using pollen morphological characters as variables. Percentage eigenvalues, variance explained by first three components, and factor loadings of the variables.

	Variable code	Factor 1	Factor 2	Factor 3
Eigenvalues (%)		34.74	31.09	13.04
Cumulative (%)		34.74	65.83	78.87
Variable				
Pollen diameter	P	-0.85	0.45	0.09
Equatorial diameter	E	-0.63	-0.61	-0.06
Colpus length	ColpL	-0.84	0.33	0.12
Colpus width	ColpS	-0.44	-0.44	0.35
Apocolpium	A	-0.40	0.06	-0.86
Mesocolpium	M	-0.47	-0.68	0.06
Pollen diameter : equatorial diameter	P/E	-0.22	0.92	0.15

Table 4. Floral morphological data of the examined populations of *A. alpina* with measurements (mm) with mean (in brackets) ± standard deviation and ranges of the characters: calyx length (CaL), corolla length (CoL), length of the long stamen (mean value) (StL), length of the short stamen (mean value) (StS), style length (Sty), stigma length (Stg), pistil length (PistL). Site codes correspond to data in Table 1.

Site code	CaL	CoL	StL	StS	Sty	Stg	PistL
BG1	4-7 (5.85±0.78)	8-12 (10.35±1.3)	5.5-9 (7.55±0.9)	3.5-7 (5.53±0.9)	0.2-0.3 (0.25±0.05)	0.2-0.4 (0.27±0.06)	6-9 (7.35±0.76)
BG2	4-7 (5.76±0.8)	7-11 (9.73±1.1)	5.5-8 (6.95±0.76)	3.5-6 (4.93±0.59)	0.2-0.3 (0.24±0.05)	0.2-0.3 (0.24±0.05)	5.5-8 (6.7±0.68)
FR3	2-3.5 (2.75±0.48)	3.5-7.5 (5.09±1.0)	3-5.5 (4.6±0.73)	1.5-3.5 (2.81±0.63)	0.1-0.2 (0.13±0.4)	0.1-0.2 (0.12±0.4)	2.5-5.4 (4.12±0.72)
FR4	2.5-4.2 (3.13±0.45)	4.5-6.5 (5.7±0.7)	3.5-6 (5.33±0.6)	2.4-4 (3.19±0.5)	0.1-0.3 (0.2±0.05)	0.1-0.3 (0.21±0.05)	3-5 (4.5±0.5)
FR5	2.5-4.2 (3.02±0.42)	5-6.5 (5.7±0.7)	4.7-6 (5.46±0.38)	2.4-4 (3.25±0.48)	0.1-0.3 (0.21±0.04)	0.1-0.3 (0.21±0.04)	3.8-5 (4.5±0.3)
FR6	2.5-4.2 (2.9±0.32)	5-7 (5.75±0.5)	4.5-6.5 (5.34±0.43)	2.5-4 (3.04±0.43)	0.1-0.3 (0.21±0.04)	0.1-0.3 (0.24±0.06)	4.2-6 (4.78±0.46)
FR7	2.5-4 (3.46±0.39)	5-7.3 (6.28±0.72)	3.5-6.5 (5.28±1)	2-4.4 (3.43±0.7)	0.2-0.3 (0.23±0.04)	0.1-0.3 (0.2±0.08)	3-6.7 (5.42±1.14)

diameter (P) and the P/E ratio were the most important traits for separation of the populations. They had positive coordinates with both factors. Among the studied pollen traits the polar diameter was very closely correlated with the generative trait (pistil length).

In both principal component analyses the first component accounts for most of the variation (34.74 and 42.78%, respectively), and the eigenvectors of the variables have similar weights (Table 3 and 5).

The analysis using only floral characters showed that all characters except pistil and stigma length correlated positively with factor 2. They correlated closely in between and quite variable.

DISCUSSION

The pollen morphological data for *A. alpina* confirm previous information about the species (Anchev and Deneva, 1997; Beug, 2004; Mutlu and Erik; 2012). The species should be related to *Sinapis*

Table 5. Summary of the statistics for the Principal Component Analysis (PCA) using pollen morphological characters and measured floral parts as variables. Percentage eigenvalues, variance explained by first three components, and factor loadings of the variables.

	Variable code	Factor 1	Factor 2	Factor 3
Eigenvalues (%)		39.82	16.06	12.18
Cumulative (%)		39.82	55.88	68.06
Variable				
Pollen diameter	P	0.65	0.03	-0.70
Equatorial diameter	E	0.25	-0.81	-0.16
Colpus length	ColpL	0.52	-0.09	-0.74
Colpus width	ColpS	0.27	-0.58	-0.01
Apocolpium	A	0.29	-0.11	-0.22
Mesocolpium	M	0.12	-0.81	-0.03
Pollen diameter : equatorial diameter	P/E	0.36	0.71	-0.49
Calyx length	CaL	0.90	0.04	0.14
Corolla length	CoL	0.90	0.00	0.19
Long Stamen length	StL	0.87	-0.00	0.24
Short Stamen length	StS	0.90	0.01	0.13
Style	Sty	0.55	0.07	0.27
Stigma	Stg	0.52	0.26	0.33
Pistil length	PistL	0.87	0.04	0.21

pollen type according to Moore et al. (1991) which is characterized by an exine < 4 μm, grains size < 40 μm, long colpi, wide to narrow slit-like without visible granulate membrane, circular to elliptic in equatorial view, edge of the colpi reticulate not tectate, muri simplicolumellate and narrower than lumina. This pollen type was previously suggested by Erdtman at al. (1963) where more than 20 genera and species were included. This description of the pollen grains of *A. alpina* almost completely fits to our observations. The only difference that was clearly visible even under a light microscope is the granulate colpus membrane (Plate 1). Such a granulate colpus membrane was previously described by Anchev and Deneva (1997).

Pollen grains in the studied populations demonstrate differences mainly in the pollen size and shape. Although the higher pollen size is often considered to be in positive relation with ploidy level and has been confirmed for many plant taxa from different families (Guinet and Ferguson 1989; Cubas and Pardo, 1994; Anchev and Deneva, 1997) our results do not support such a relation and confirm the diploid level for the studied populations (unpublished data). Similar results were also presented by Mutlu and Erik (2012) for other species of the genus *Arabis*. Obviously, the factors influencing the pollen size are not only genetical. Infraspecific pollen variation is frequently linked to the size of the species area and than in turn, to the ecological diversity (Guinet and Ferguson, 1989). The growth under unusual ecological conditions and changes in soil mineral nutritive elements increases also the amplitude in the variation of pollen characters (Guinet and Ferguson, 1989). It could be suggested that the reason for the smaller pollen size in one of the Bulgarian populations (BG2) is the stress of the environment provided by the serpentine rock. A combination of physical, chemical and biotic factors can limit plant growth and change morphology referred to as the "serpentine syndrome" (Kruckeberg, 1984). However, such a relation should be proved in precise experimental conditions.

Pollen grains are quite variable in size even in one and the same individual and in the same anther. There were no clear differences between pollen from anthers of long and small stamens in the same flower of *A. alpina*. Often the smallest grains in the anthers were sterile. Our results demonstrated a higher proportion of sterile pollen in the French populations which could be a result of different factors, both genetical and environmental. The species *A. alpina* demonstrates divers reproductive systems, reproducing either sexually or asexually via stoloniferous growth (Hegi, 1986). It has been also shown that *A. alpina* is self-compatible (Tedder et al., 2011) and highly selfing in populations studied from the European Alps (Ansell et al., 2008).

Environmental factors such as light and temperature are considered to regulate various events during flower formation like fertility, sex expression and floral induction (Vince-Prue, 1975). In our study, a very high percentage of sterile pollen was found in flowers where the pistil was longer than the stamens. Such flowers were frequently found in the studied French populations. This result confirms the data provided by Heberle-Bors (1982) who considers that sterile pollen grains are produced in high frequency in flowers which exhibit a shift in sex balance (measured as a ratio between pistil length and mean stamen length without anthers) towards femaleness. The habitats occupied by *A. alpina* are characterized by temperature extremes. The low temperature is also a reason for high quality sterile pollen or completely sterile anthers containing few and mostly collapsed pollen grains (Heberle-Bors, 1982). Based on palynological studies on the *Arabis* species in Turkey Mutlu and Erik (2012) conclude that variation of pollen morphology, muri, and exine thickness are changed by ecological and climatic differences and altitude as well. We agree that environmental factors can influence pollen size and shape of different species but these are also factors causing variation in the populations of the same species which was confirmed in this study. Pollen ornamentation in *A. alpina* populations was found to be not so variable such as pollen size and shape. This fact should be related to adaptation to specific pollinators as it was previously pointed out for different genera from Fabaceae (Guinet and Ferguson, 1989).

The number of colpi in *Arabis* pollen is mainly 3 but pollen with 4 colpi has been also found. In this study, such pollen grains prevailed in the populations from France. Although Mutlu and Erik (2012) observed 2-colpate pollen in *A. alpina* in Turkey, such grains were not found in our study.

The flower size in *A. alpina* has been used in different floras (Akeroy, 1997; Asenov, 1970; Tan, 2002) as one of the criteria to establish infraspecific rank. The results of this study and the Principal Component Analysis confirm that variation in pollen size can be related to differences in flower size. The correlation between bigger flower and bigger pollen grains was positive. The variation in flower vegetative traits was higher compared to the generative one. The correlations among vegetative floral traits and the correlations among generative flower traits were significantly greater than the correlations across these two groups of traits in all studied populations. The strong and significant correlation found between polar diameter and pistil length supports previous data (Plitmann and Levin, 1983) and is synchronized with the roles and functions of the pistil and pollen during fertilization.

Pollen morphological data correlate well with the current infraspecific limits established within *A. alpina*. The pollen characters of different populations support the taxonomical decision for the separation of the Central European populations of the species as subsp. *alpina*, which is different from the Balkan populations, separated as a distinct subspecies, named differently in the Floras of the Balkan countries (Tan, 2002; Asenov, 1970).

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