Pollen morphological studies on *Arabis alpina* L. (Brassicaceae) populations from the Alps and the Rila mountains

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Introduction

Alpine rock-cress, *Arabis alpina* L., is a widespread plant 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). 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 (Anchev, 2001) accepted as a synonym of subsp. *caucasica* (Willd.) Briq. (Akeroyd, 1997; Marhold, 2011). *Arabis alpina* is distributed in subalpine meadows, rock crevices and scree slopes mainly on limestone (Tan, 2002), serpentine and silicate between 1000 and 2900 m a.s.l.

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 pollen grains of *A. alpina* were characterized as 3-colpate, oblate spheroidal in pollar view, elliptic in equatorial view, with reticulate ornamentation.

The aims of the present research were to estimate: 1) the 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.

Fig. 1. Map of Mediterranean Europe showing the location of the studied populations: Bulgaria Rila Mt. - non serpentine (BG 1), Bulgaria Rila Mt. - serpentine (BG 2), France (FR 3), France (FR 4) , France (FR 5), France (FR 6), and France (FR 7).

Results

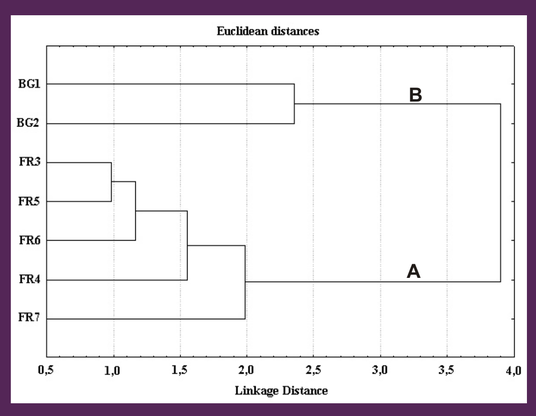
Pollen morphology

The pollen grains are 3 (4)-zonocolpate (Plate 1). Both, polar (P) and equatorial diameter (E) were in the range from 22.5 µm to 36 µm. Pollen shape in all populations is quite variable (Table 2, Plate 1, Figs. 1-5). More often the grains are 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 number of oblate spheroidal pollen grains in the populations from France is higher than in the Bulgarian populations where prolate spheroidal grains prevail. Equatorial view varies from spheroidal to elliptic. Polar view circular or lobate (Plate 1, Fig. 6). 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 varies between 26.22 and 27.12 µm. The colpus margin is uneven without a margo. The colpus membrane is 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) are also variable between the populations.

The exine thickness is variable between 1.5 and 3 µm, thicker in the mesocolpia. 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 is 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.

Fig. 2



The dendrogram obtained by the hierarchical cluster analysis (Fig. 2) reveals two groups (clusters) of populations formed at linkage distance around 3.9. Cluster (A) arrange all populations of the species originated from France. All they have pollen grains smaller than the two Bulgarian populations combined in cluster (B). The main difference between groups is 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 Fr3 and Fr5.

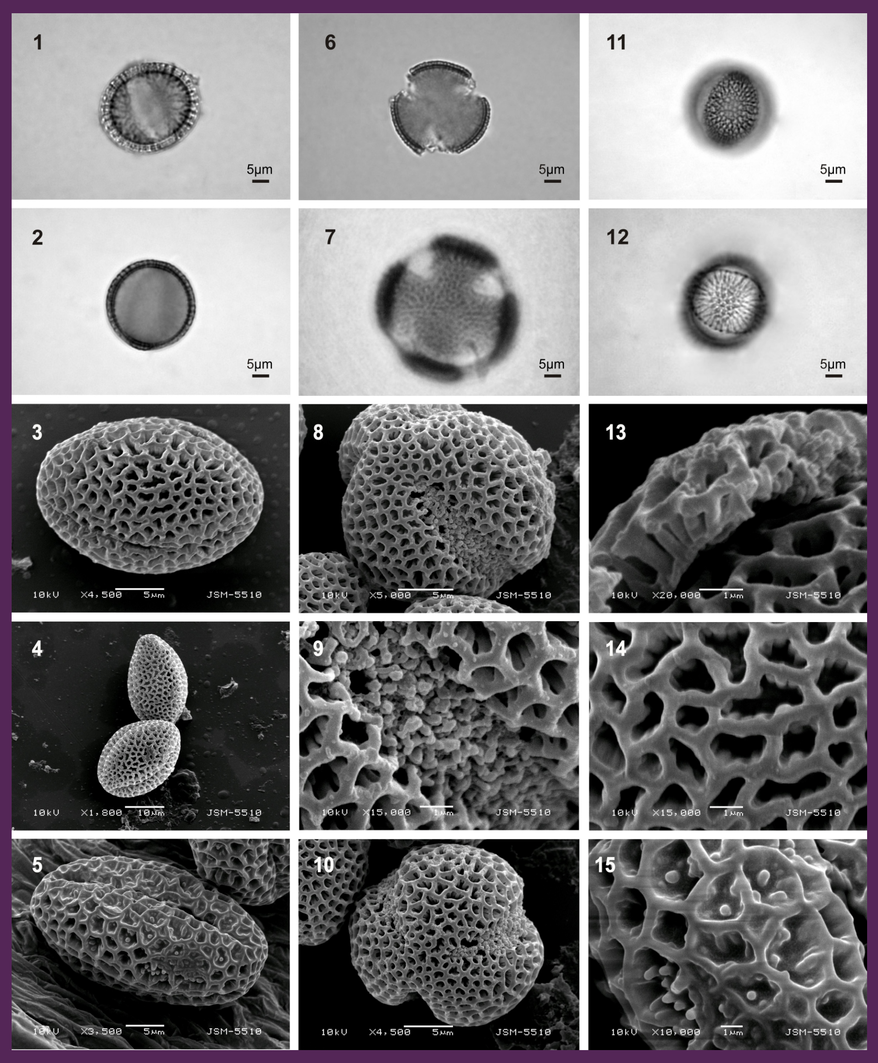
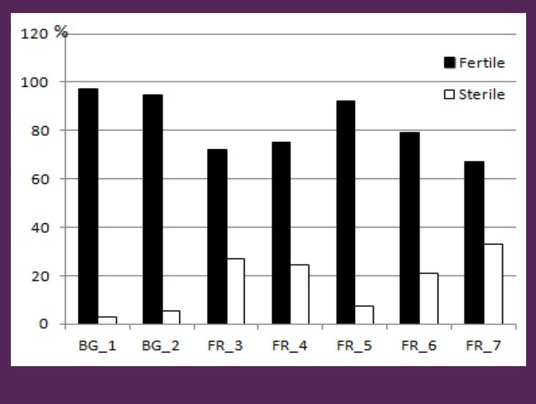
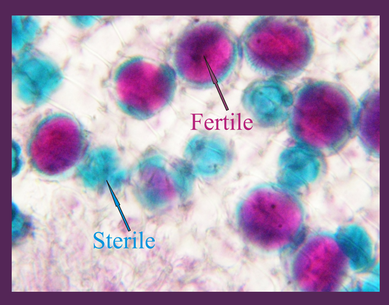


Plate 1. LO and SEM micrographs of pollen grains of *Arabis alpina*, Fig.1 - oblate spheroidal pollen grain (LO); Fig. 2 prolate spheroidal pollen (LO); Fig. 3 and 4 subprolate pollen (SEM); Fig. 5 prolate (SEM); Fig. 6 Polar view, 3-colpate pollen (LO); Fig. 7 - Polar view, 4-colpate pollen (LO); Fig. 8 colpus and ornamentation (SEM); Fig.9 colpus membrane (SEM); Fig. 10 apocolpium ornamentation (SEM); Fig. 11 and 12- mesocolpium (LO); Fig. 13 - SEM section of the exine; Fig. 14 ornamentation in the mesocolpium (SEM); Fig. 15 - free-standing columellae in the lumina.

Fig. 3





Pollen viability

The percentage of fertile/sterile pollen for the populations is presented on Fig. 3. The populations (BG_1) from Bulgaria and from France (FR_5) show a very high percentage of fertile pollen (above 90%). The rest of the studied French populations have 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.

Material and methods

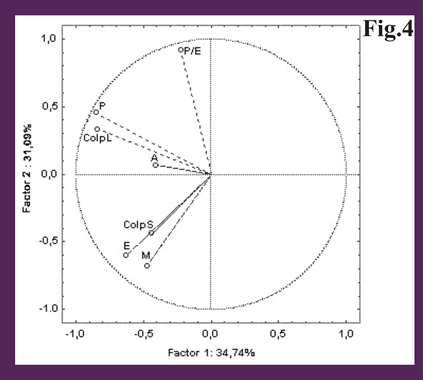
The material for investigation (pollen and flowers) was obtained from natural populations of *A. alpina* (Fig. 1). The measurements and pollen descriptions were made on acetolyzed pollen prepared in the standard procedure (Erdtman 1966). Thirty measurements of pollen features: P (polar diameter), E (equatorial diameter), ColpL (colpus length), ColpS (colpus width), A (apocolpium), M (mesocolpium), and P/E ratio were done for each population. For scanning electron microscopy (SEM) pollen suspended in a drop of 95% ethanol was coated with gold and examined. Anthers were squashed and the pollen grains stained (Alexander 1969) in order to estimate percentage pollen viability. The viable and inviable pollen grains were counted and the mean values for 3 anthers per flower and 20 flowers per population were presented (Fig. 3).

Seven parameters (calyx length (CaL) for outlier leaves, corolla length (CoL), length of the longer stamen (StL), length of the shorter stamen (StS), style (Sty), stigma (Stg), and pistil length (PistL)) from 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.

Cluster analysis using Euclidean distance and Unweighted Pair Group Means Average (UPGMA) was used to determine the similarities between populations (Fig. 2). Ordination by Principal Component Analysis (PCA) was used to show the loadings for each pollen and flower character and their contribution for differentiation of the populations (Fig. 4 and 5).

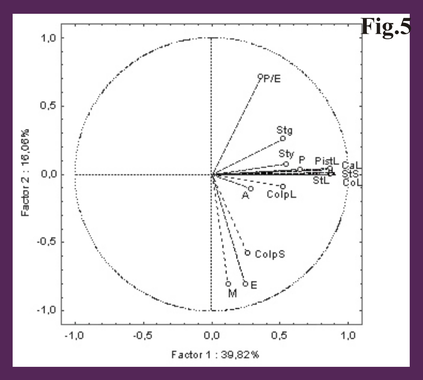
The pollen terminology in general follows Faegri and Iversen (1989) and Punt et al. (2007).

Fig.4



The PCA carried out with all pollen traits used as variables (Fig. 4) demonstrates that P, ColpL, A and P/E have positive coordinates and correlations with factor 2. The most variable was polar diameter while apocolpium was more conservative trait. The equatorial diameter (E) and colpus width (ColpS) as well as polar diameter (P) and colpus length (ColpL) were very closely correlated.

Fig.5



The PCA performed with pollen traits and floral characters 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 diameter (P) and the ratio P/E were the most importance traits for separation of the populations (Fig. 5).They have positive coordinates with both factors. The polar dia-meter was very closely correlated with pistil length.

Conclusions

- The pollen morphological data for *A. alpina* confirm previous known information about the species. The species should be related to *Sinapis* pollen type according to Moore et al. (1991).
- Pollen grains are 3 (4)-zonocolpate. The colpi are long, shallow with granulate membrane. The exine is 1.5-3µm thick and the ornamentation is reticulate with different in shape and size lumina and free-standing columellae in them.
- Pollen shape in all populations is quite variable, more often spheroidal, oblate spheroidal or subprolate.
- Pollen size is variable even in one and the same individual and in the same anther. There are no clear differences between pollen from anthers of long and small stamens in the same flower. Often the smallest grains in the anthers were sterile. A higher proportion of sterile pollen was in the French populations which could be a result of different factors, both genetical and environmental..
- Pollen ornamentation was found to be not so variable such as pollen size and shape probably related to adaptation to specific pollinators.
- The PCA confirm that variation in pollen size can be related to differences in flower size. The strong and significant correlation found between polar diameter and pistil length supports previous data and is synchronized with the roles and functions of the pistil and pollen during fertilization.
- Pollen morphological data correlate with the current infraspecific limits established within *A. alpina* and support the taxonomical decision accepted in different floristic editions.

References

Akeroyd, J. 1997. *Arabis* L., In: Tutin, T. G., et al. (eds.), Flora Europaea Vol. 1: 352-356, Cambridge.
Alexander M.P. 1969. Differential staining of aborted and non-aborted pollen. Stain Technology 44: 117-122.
Ančev ME. 2001. Family Brassicaceae Burnett (Cruciferae Jussieu) in Bulgarian Flora. Taxonomical structure, diversity, phytogeography, speciation process and evolutionary trends. DSc Thesis, Institute of Botany, Bulgarian Academy of Sciences, Sofia.
Anchev M. & Deneva B. 1997. Pollen morphology of seventeen species from the family Brassicaceae (Cruciferae). Phytol Balcan 3(2-3): 75-82.
Beug H-J., 2004. Leitfaden der pollenbestimmung fur Mitteleuropa und angrenzende Gebiete. Munchen, pp.542
Erdtman G. 1966. Pollen Morphology and Plant Taxonomy. - 458p. New York.
Faegri K. & Iversen J.1989. Textbook of pollen analysis. - 328pp. John Wilry & Sones, Chichester.
Greuter, W., Burdet, H.M., Long, G. 1986. Med checklist. Dicotyledones (Convolvulaceae Labiatae), vol. 3, Conservatoire et Jardin botaniques de la Ville de Genève, Genève.
Marhold K. 2011. Brassicaceae. In: Euro+Med Plantbase the information resource for Euro-Mediterranean plant diversity, http://www.emplantbase.org/home.html.
Meusel H, Jäger E, Weinert E. 1965. Vergleichende Chorologie der zentraleuropäischen Flora. Jena, 190.
Moore P., Webb J. & Collinson M. 1991. Pollen analysis (second edition). - 216pp. Blackwell Sci. Publication.
Mutlu, B., Erik, S. 2012. Pollen morphology and its taxonomic significance of the genus *Arabis* (Brassicaceae) in Turkey, Plant Syst Evol (2012) 298: 19311946.
Punt W., Blackmore, Nilsson S. & Le Thomas A. 1994: Glossary of pollen and spore terminology. - 71pp. Lab. Palaeobot. and Palynol., Utrecht.
Tan, K. 2002. *Arabis* L.. In : Strid, A. and Tan, K (eds.) Flora Hellenica, vol. 2: 184-192.

Acknowledgements

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