

Pollen Heteromorphism in *Panax ginseng* C.A. Meyer (Araliaceae) Anthers

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Like in animals, the development of plant male gametes is a lasting process of a gradual structural conversion of early gametes into late ones [1, 2]. The stages of *P. ginseng* pollen grain development from archesporium to the stages of microspore germination, generative cell division, and sperm formation were described using light microscopy [3, 4]. These studies established that mature ginseng pollen grains have exine with three pores and contain a vegetative and two generative cells. In research using light and scanning and transmission electron microscopy, exine morphology and ultrastructure were examined for pollen of *P. ginseng* and other species of the genus *Panax* [5]. Morphological characteristics of pollen grains and ultrastructure of exine are traditionally used in taxonomic and phylogenetic plant studies [5–8]. Wen and Nowicke [5] described and illustrated exine ultrastructure for ten *Panax* species and concluded that the palynological analysis does not confirm the monophyly of the group *Panax* species including *P. ginseng*, *P. notoginseng*, and *P. quinquefolius*, although close relationship between them was stated on the basis of plant morphology and the triterpenoid qualitative analysis [9]. Similarly, close sister-pair relationship between *P. ginseng* and *P. quinquefolius* suggested by Li [10] was not supported.

As a rule, in such palynological studies, a structural diversity of pollen grains within anthers is not taken into account, and the morphs are compared, which possibly differ in their developmental stages. Using light microscopy, it was earlier demonstrated that anthers from open *P. ginseng* flowers contain pollen grains differing in their morphology [11].

In this work, we used scanning electron microscopy to study pollen morphogenesis in early and reproductively mature ginseng anthers.

Inflorescences of *P. ginseng* C.A. Meyer were collected in summer 2004–2005 from plants transferred for further growth from wild populations of Primorsk krai to the nursery collection. Anthers were sampled from both closed flower buds and open flowers.

Five flower buds and five open flowers were used for morphometry. One anther was taken from each bud or flower. Anthers were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.5) for 24 h. Anthers were cut in a drop of buffer, and their contents were squeezed. After liquid drying, pollen grains were examined with a light Polyvar microscope. The maximum and minimum grain diameters were measured with the ocular micrometer. The mean values represented the average diameters of pollen grains, and the average values for 150 pollen grains from flower buds or open flowers represented the average pollen grain size of early and late anthers, respectively. The results obtained were statistically processed using a Microsoft XL program using a Student's test.

For scanning electron microscopy, anthers were also fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.5) for 24 h. Thereafter, buffer drops saturated with released pollen grains were air-dried on polylysine-coated Thermanox coverslips, which were then rinsed with buffer and placed in the 2% osmium tetroxide in cacodylate buffer for post-fixation and in a series of alcohols and acetone for dehydration; then they were subjected to critical-point drying. Thermanox coverslips with dried pollen were coated with gold and mounted on aluminum stubs. Pollen grains were examined with a Leo-340 scanning electron microscope.

It was established that both early and late anthers contained pollen grains differing in size and shape (Fig. 1). The average diameter of the largest pollen grains was 27 μm , whereas the smallest grains were 16 μm in diameter. In each of anthers examined, along with the largest and smallest pollen grains, a set of grains intermediate in size and morphology was present. It was found that, during anther maturation, the proportion of small grains increased and the proportion of large grains decreased (the table). Thus, the average pollen

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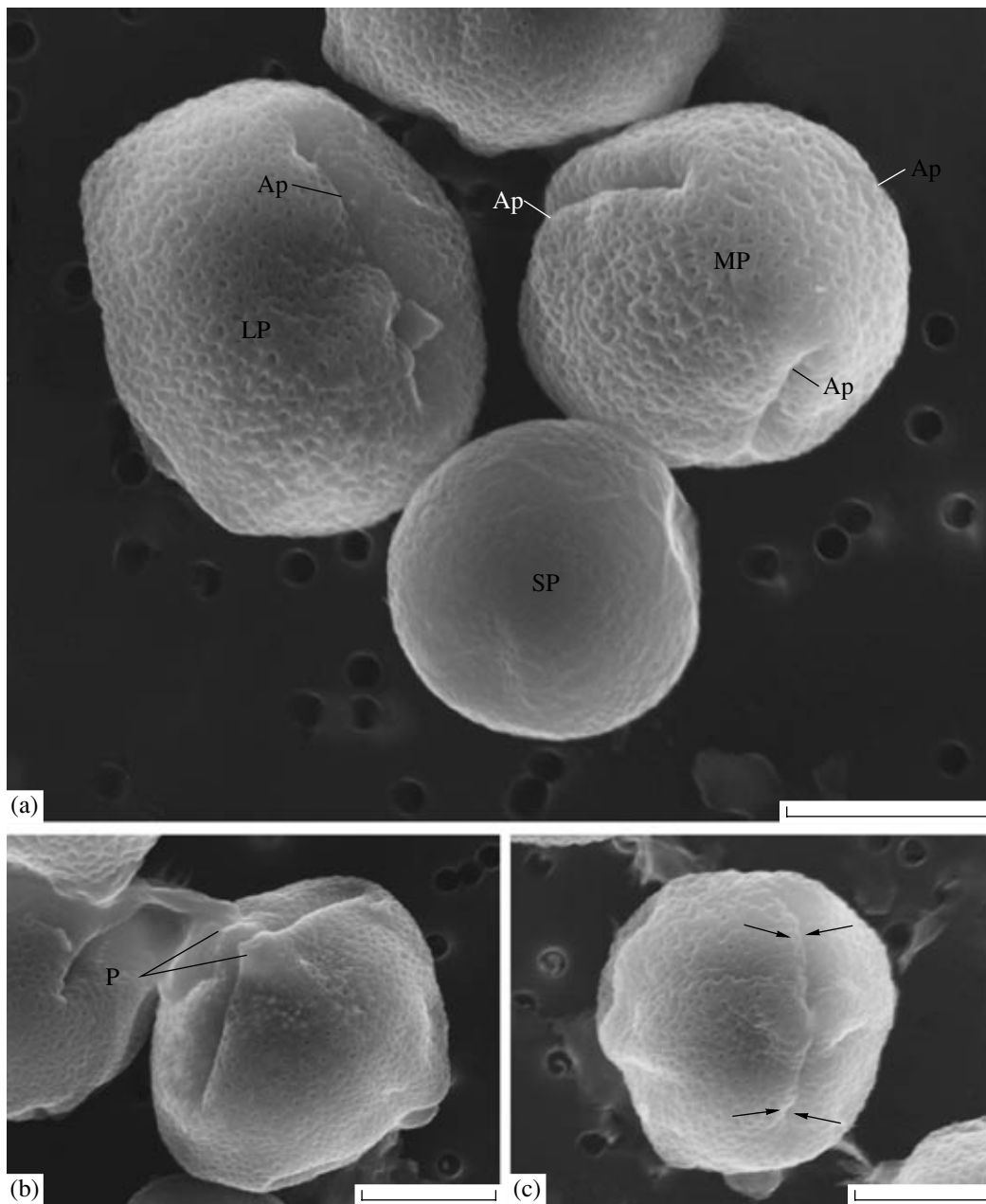


Fig. 1. Morphological varieties of differentiating *P. ginseng* pollen grains. (a) Large pollen grains (LP), medium-sized pollen grains (MP), and the smallest pollen grains (SP); (b) pollen grain with fused margins of apertures. Ap—aperture, P—projection. Scale bar is 10 μm .

grain diameter was 24 μm in early and about 18 μm in late anthers. These results indicate that the process of large pollen grain transformation into small ones occurred in developing anthers.

It was also observed that, along with reduction of pollen grain size, their morphology changed as well. Thus, the largest pollen grains (LP) had oval shape, were covered with a porous wall with shallow meridional apertures (Fig. 1a). Medium-sized pollen grains (MP) had deeper apertures (Fig. 1a). During subsequent pollen grain reduction, clearly visible projections

appeared in the middle of apertures (Fig. 1b). Later, during successive morphogenetic stages, the margins of these protrusions came together and fused (Fig. 1c). As a result, the smallest pollen grains (SP) had no apertures visible (Fig. 1a). It seems likely that aperture deepening and their margin fusion are the mechanisms for the reduction of pollen grain sizes during morphogenesis. The smallest pollen grains (SP), which are the final stage of *P. ginseng* pollen differentiation, are spherical in shape and have a smooth nonporous surface (Fig. 1a).

Proportion of small, medium-sized, and large pollen grains in the anthers of *P. ginseng* C.A. Meyer, %

Average diameter of pollen grains, μm	Stage of anther development	
	flower buds	open flowers
16–18	6.9	81
20–23	75.8	16.3
25–27	17.3	2.7

Thus, gradual pollen grain transformation from the LP with the porous surface to SP with almost smooth surface is characteristic of *P. ginseng* anthers. As a result of pollen grain morphogenesis directed to the reduction of their sizes, diverse morphs differing in size, shape, and surface structure are present within the anthers.

Other researchers also observed the intraspecies pollen variability in other plant species [5, 6, 12]. Wen and Nowicke [5] showed that the sizes of pollen grains in each of the *Panax* species tested varied in a wide range. According to the data of these authors, *P. ginseng* pollen grains from different samplings differed in the ultrastructure of their exine. In addition, the exine structure of the pollen grains of this species varied in different time of the day [5]. Earlier, it was shown by light microscopy that *P. ginseng* pollen grains are characterized by a high variability in size, shape, and the number of apertures [11]. Morphogenesis of gametes directed from large morphs with the porous surface toward small ones with the almost smooth surface was observed by scanning microscopy in the anthers of *Aralia elata*, *Aralia continentalis*, *Aralia cordata*, and *Oplopanax elatus* (unpublished data). So far, it is not clear the functional meaning of pollen heteromorphism arising during its morphogenesis. However, we agree with the idea [13] that a male gamete morphological diversity could be necessary to grant a species reproductive success.

Taking into account the results obtained, it seems evident that only completely developed male gametes should be compared in taxonomic or phylogenetic studies. Just such approach was applied in the phylogeny of multicellular animals [14]. Certainly, early pollen morphs with developed sculpture of the surface could be also used in comparative palynology of the genus *Panax* and other members of the Araliaceae family. However, preliminary investigations of pollen morphogenesis should be performed for compared species to select morphs suitable for comparison.

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