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GENERAL BIOLOGY

Morphological Study of Pollen Grains in Mature Anthers of *Aralia elata, A. continentalis*, and *A. cordata* (Araliaceae)

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The genus *Aralia* is one of the large taxa of the family Araliaceae. According to different sources, it includes from 35 [1, 2] to 50 and more [3, 4] species. Most *Aralia* species inhabit South-Eastern Asia, others live in North, Central, and South America [5, 6]. Only three species are met in Russia: *Aralia elata* (Miq.) Seem., *A. continentalis* Kitag., and *A. cordata* Thunb. [1].

A. elata is distributed in North-Eastern China, Korea, and Japan; in Russia, it occurs in the south of the continental part of the Far East, in the south of Sakhalin, and Kuril islands [5, 6]. *A. continentalis* is met in China and on Korean peninsula. In Russia, this species was found only in extreme south of Primorskii krai [5, 6]. *A. cordata* is not met on the continent; it inhabits Sakhalin, South Kurils, and Japan islands [5, 6].

As distinct from deciduous *Aralia elata*, *A. continentalis* and *A. cordata* are herbaceous perennials. They are so close in appearance that their identification after herbarium samples is very difficult [5]. *A. cordata* was described in 1784 by Tunberg on the basis of samples collected in Japan [6]. Herbaceous *Aralia* growing on the continent was firstly mentioned by Komarov in 1905 as *A. cordata* Thunb. [7]. Only in 1935, the Japan botanist Kitagawa [8] described it as a separate species *A. continentalis* Kitagawa. However, Chinese botanist Li did not accept this classification: he believed that continental populations belong to *A. cordata* [9].

In plant taxonomy and the establishment of their phylogenetic relations, not only macroscopic features but also morphological characteristics of their pollen grains are used: pollen grain shape, exine structure, the number of apertures and pores, and the pattern of the surface [10–13]. *Aralia* pollen is poorly studied. The morphology and exine structure was studied under electron microscope only in the pollen of six *Aralia* species, and none of them is met in Russia [11].

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In recent literature devoted to spermatogenesis in multicellular animals, the notion appeared that studying not only mature gametes but also specific features of their differentiation could be used in the taxonomy and phylogenetic analysis [14]. In plants, some peculiarities of male gametophyte development, such as the type of microspore development (successive or simultaneous) and the type of mature gametophyte (two- or three-celled) could be considered as taxonomic traits. Earlier, when we studied ginseng (*Panax ginseng* C.A. Meyer) pollen, we have observed a high morphologic diversity of pollen grains in this member of the Araliaceae family and showed that this diversity resulted from the process occurring in mature anthers, e.g. the conversion of large morphotypes into morphologically diverse small ones. It was of interest to elucidate whether this phenomenon observed for ginseng is also characteristic of other members of the Araliaceae family, in particular of Far East *Aralia.* We also would like to reveal morphological traits of pollen grains important for taxonomy and phylogenetic analysis.

Inflorescences of *Aralia elata, A. continentalis*, and *A. cordata* were collected from the plants grown in the Botanical Garden of the Far East Division of RAS. We examined pollen grains from mature anthers in the stage preceding pollen release.

To perform morphometry, pollen grains were fixed in the 2.5% glutaraldehyde solution in 0.1 cocadylate buffer, pH 7.5, for a day. The anthers were broken mechanically in a drop of buffer, and thus their contents was released. After liquid drying, the highest and lowest diameters of pollen grains were measured for 150 grains under a Polyvar light microscope. The mean of these two measurements was taken as the size of the pollen grain. Three anthers of each species were examined. For scanning microscopy, anthers were fixed similarly as for light microscopy. Buffer drops filled with pollen grains released from anthers were placed on the Thermanox slides covered with polylysine, and then liquid was dried. Slides with pollen were washed several times in buffer and placed in the 2% solution of osmium tetraoxide prepared in cocadylate buffer and then in the series of alcohols and acetone. After liquid drying, slides were powdered with carbon, positioned

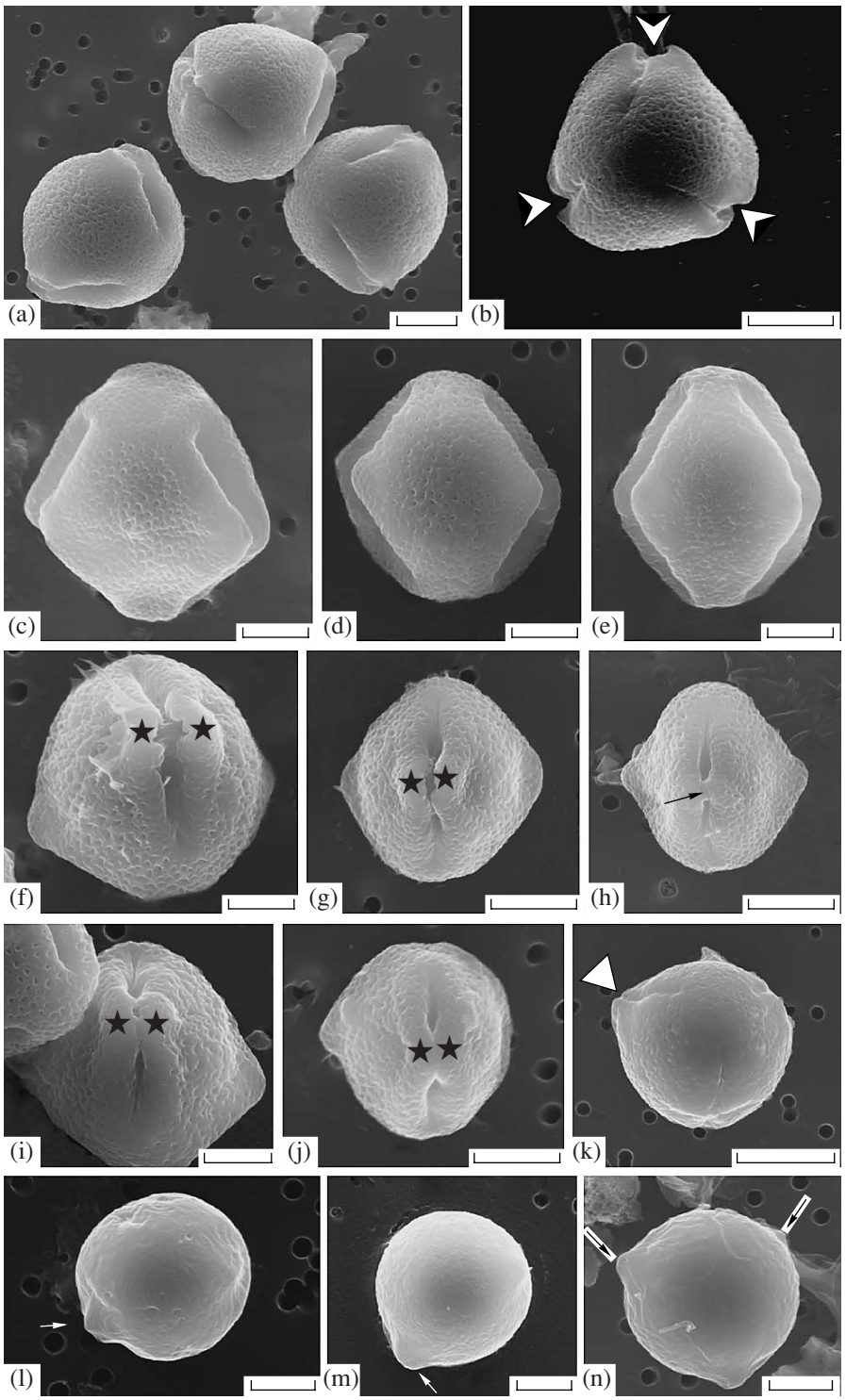

Fig. 1. Morphological types of pollen grains differentiating within the anthers of *Aralia elata, A. continentalis*, and *A. cordata.* (a, b) Large pollen grains of *Aralia elata*; furrows are designated with arrowheads. Scale bar is 10 μm. (c–e) Pollen grains of *Aralia elata, A. continentalis*, and *A. cordata,* respectively. Scale bar is 5 μm. (f–k) Pollen grains of *Aralia elata* with fusing margins of furrows. Asterisks designate furrow margins; the black arrow indicates the bridge between fusing furrow margins; triangular designates protuberance produced by fusing furrow margins. Scale bars are 5 (f, i, j), 10 μm (g, h, k). (l–n) Small pollen morphotypes of *Aralia elata, A. continentalis*, and *A. cordata*, respectively. White arrows indicate protuberances on pollen grains produced after fusion of furrow margins. Scale bar is 5 μm.

on the aluminum support, and examined under the Leo-340 scanning electron microscope.

We demonstrated that pollen grains of *Aralia elata, A. continentalis*, and *A. cordata* differed in their sizes and shapes. Pollen grain size varied from 25 to 15 μm. In this work, pollen grains were compared in the order of a decrease in their size.

It was established that large pollen grains (Fig. 1a) (with the average diameter of about 25 μm) of three species were similar: all of them were spherical with three longitudinal apertures and were covered with porous wall (Fig. 1b).

Smaller pollen grains (with the average diameter of about 19 μm) manifest species-specificity in their contour shape. Thus, the lateral contour of the male gametophyte of *Aralia elata* of this size appears as an irregular rhomb (Fig. 1c), of *A. continentalis*, as a regular rhomb (Fig. 1d), and of *A. cordata,* as an elongated rhomb (Fig. 1e).

A further decrease in the pollen size in three species tested resulted in pronounced morphological diversity of pollen grains, which seems chaotic at the first glance. Nevertheless, when to examine male gametophytes in the order of a decrease in their average diameter, it becomes evident that pollen morphological diversity at this stage is determined by the sharp change of their shape related to the fusion of the margins of longitudinal furrows and subsequent disappearance of the latter. When initially a rather wide and deep furrows were seen between the aperture margins (Fig. 1f), during subsequent differentiation, these margins approached each other (Fig. 1g). Between the margins, the bridge was formed (Fig. 1h) or these margins were drawn close together (Fig. 1i). As a result of both processes furrow margins fused (Fig. 1j).

As a result of gradual furrow disappearance, pollen grains became almost spherical and their walls became less porous. It is of interest that, in *Aralia elata* and *A. continentalis*, only two furrows disappeared completely, whereas protuberances on the margins of the third furrow remained quite pronounced (Fig. 1k). During further differentiation of pollen grains and a decrease in their size down to 15 μm, two apertures disappeared completely and the fusing margins of the third one created a characteristic protuberance, which is a specific morphological trait of the smallest pollen grains of *Aralia elata* and *A. continentalis* (Figs. 1l, 1m). For the smallest *A. cordata* pollen grains, the presence of not a single but three protuberances produced by fusing aperture margins is characteristic (Fig. 1n).

Thus, the approach used in this work for examination of pollen grains in the anthers of three *Aralia* species in the order of a decrease in their size permitted observation of pollen differentiation directed from large to smaller morphotypes. This process determines pollen morphological diversity in anthers. Earlier, we have observed similar phenomenon in *P. ginseng*; it seems likely that it could be also found in other members of the Araliaceae family. We believe that the occurrence of this phenomenon should be taken into the account by researchers describing pollen in terms of taxonomy.

The analysis performed showed that mature pollen differentiation in various *Aralia* species had common features. Large pollen grains representing the early stage of differentiation displayed no species-specificity: they were similar morphologically. However, species-specificity appeared in the following stage of differentiation in pollen grains of smaller size. Indeed, the lateral contours of these grains in three plant species were irregular rhombs, regular rhombs, or elongated rhombs. This phenomenon of morphological pollen grain specificity at the medium stage of differentiation of mature male gametophytes is described for plants for the first time and evidently deserves further investigation.

The morphology of the smallest pollen grains is also species-specific. After completion of differentiation, small gametophytes differed sharply from preceding morphological forms. They acquired a compact shape; their walls became smoother and devoid of pores; they had no furrows, which disappeared due to the fusion of their margins. Morphotypes of small grains were most alike in *Aralia elata* and *A. continentalis.* Both had only a single protuberance. Nevertheless, the shapes of these morphotypes had some differences. In *Aralia elata*, grains were spherical, whereas in *A. continentalis*, oval-rounded. The smallest pollen grains of *A. cordata* were spherical, but they differed from two other *Aralia* species by the presence of three protuberances. On the basis of morphology of the smallest male gametophytes, we can suppose that *Aralia elata* and *A. continentalis* are most close phylogenetically, whereas *A. cordata* is a more distant species. These results confirm data we obtained earlier using RAPD marker DNA [15].

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