Pollen Germination in Gymnosperms

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Gymnosperms are amazing representatives of the flora. On the one hand, they are ancient plants with primitive characteristics of anatomical structure; on the other hand, they are perfectly adapted to their habitat and are the dominant species in many ecosystems due to their impressive size and longevity, with their reproductive system being of particular interest. It has progressive features, because in this group the reduced male gametophyte—pollen grain—first appeared, as well as the ability to form seeds. In addition, this group still represents a wide variety of reproductive patterns, strategies, and relationships. For example, the degree of gametophyte reduction varies, there are both zooidogamy and siphonogamy, and the reproductive process can both be relatively fast and last over several years.

plant reproduction   pollen germination   pollen tube growth   gymnosperms   conifers

male gametophyte   polar growth

1. Introduction

The transition from zooidogamy to siphonogamy, that is, to fertilization by immobile gametes instead of motile spermatozoa, occurred during the evolution of gymnosperms, and nowadays both options are found in this taxon [1]. Extant gymnosperms include 12 main families and 83 genera. The term "conifers" refers to a group of gymnosperms that make up the division Pinophyta. To date, physiological studies of gymnosperms were carried out almost exclusively on conifers (mainly pine and spruce, rarely cypress, fir, and others), and all of them are characterized by typical siphonogamy, that is, fertilization with immotile gametes. The physiology of conifer pollen is discussed in the following sections.

The male gametophyte of gymnosperms is generally more complex than that of flowering plants; it develops and germinates more slowly; and it is formed through three to five mitoses, which in different groups taxa occur at different stages—before or after pollination [2]. In this regard, pollen released from microsporangia consists of different number of cells (**Figure 1**): four cells in pine and ginkgo (two prothallial cells, antheridial cell and tube cell), two in sequoia, and one in juniper [2][3][4]. Male gametes in all cases are formed after pollination.

Figure 1. Cellular composition of some gymnosperm pollen grains at dehiscence.
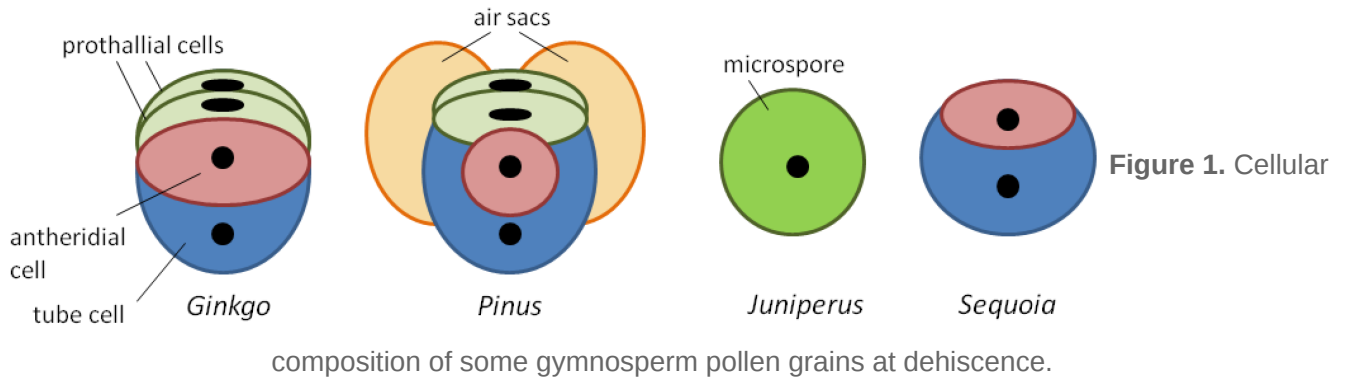
Pollen grain morphology in gymnosperms is very diverse, as well as their structure, but several key patterns can be distinguished that are typical for cycads, ginkgo, gnetales, and conifers (**Figure 2**).
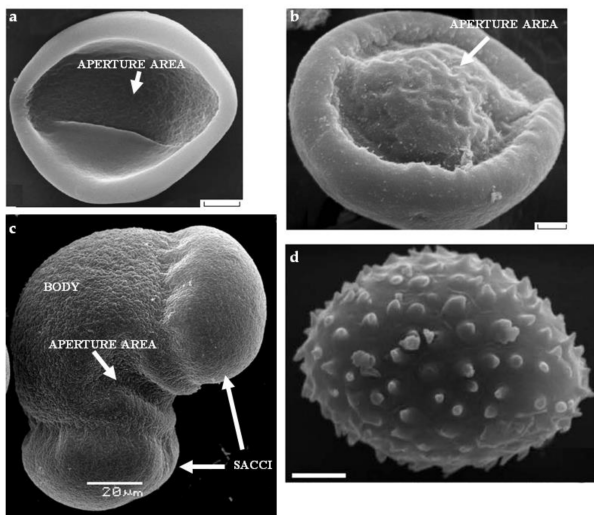

Figure 2. SEM of gymnosperm pollen: (**a**) *Cycas micholitzii* Dyer (Cycadaceae); (**b**) *Ginkgo biloba* L. with a bulge in the aperture area; (**c**) *Picea pungens* Engelm. with typical saccate morphology; (**d**) *Gnetum macrostachyum* Hook with microechinate sculpture. Scale bar: (**a**,**b**,**d**)—3 μm. Pictures are from the following articles: (**a**,**b**) [5], (**d**) [6].

Gametophyte cells are separated by thin walls, and from outside, as in flowering plants, are protected by inner cellulosic wall (the intine) and massive outer sporopollenin wall (exine) [7]. Apertures are specialized areas, where the exine is thinner and the intine is usually thickened, channeled, or forms multiple layers [8]. Pollen wall in many cases has polar structure, and the polarity of microspores of gymnosperms, such as angiosperms, is determined during meiosis [4]. The proximal pole (the one closer to the tetrad center) later becomes more convex, and the distal pole becomes flatter, for example, in *Ginkgo biloba* (**Figure 2**b); exine is thicker on the proximal side, and the large aperture is located on the distal side. Cytological polarity is manifested in the cell arrangement: prothallial cells are located at the proximal end, and the large tube cell is located at the distal end [9][10][11]. In cycads, the aperture occupies almost half of the pollen grain surface, exine is the thickest in the proximal region and the thinnest in the distal one, intine is relatively thin, and exine is smooth (**Figure 2**a) [12]. The question of the number of apertures and polarity in Gnetales has not yet been fully clarified. In *Gnetum*, pollen is apertureless with internal polarity, and exine is embossed (**Figure 2**d) [6][13][14].

In most Pinaceae plants, pollen is saccate, which means, it has *sacci* (air sacs)—large hollow outgrowths formed by the exine (**Figure 1** and **Figure 2**c). The sacs are located at the distal end of the pollen grain, and the exine of the proximal part is thickened. The presence of air sacs is associated with the pollination mechanism in many conifers: pollen landing on a pollination drop. This is the liquid secretion of the receptive ovule, which forms a large drop and rises to the micropyle surface. The drop holds pollen and pulls it into the ovule, where it germinates [15] [16]. As it was shown in a model experiment, the floating of pollen grains in pollinating droplets (due to the air sacs) is an effective mechanism for their retraction into micropyle; therefore, air sacs serve not only for pollen transfer by the wind [17]. The number of air sacs, as well as the size of pollen grains and the number of mitoses during the gametophyte development, are variable [4]. Pollen of gymnosperms, unlike angiosperms, are not covered with a sticky lipid-rich pollenkitt [2].

2. Pollination and Pollen Germination in Gymnosperm Species

Approximately 98% of gymnosperm plant species are wind-pollinated [18], which largely determines their structural features (for example, specific shape of pollen grains and air sacs) and physiology. It is known that pollen of almost all modern gymnosperms, with the exception of some species of Araucariaceae and *Gnetum*, is characterized by a high degree of dehydration [19] and can cover great distances. Pollination with insects (mainly beetles) has been described in some Cycadales and Gnetales species [13][20], including fossils, which became possible due to the discovery of coprolites in the cones of ancient cycads [21]. Extant pollen grains have significant differences in wall structure, depending on whether they are carried by the wind or by insects, even within the same genus, which was shown for *Ephedra* [13].

When cultivated in vitro, pollen of many coniferous plants germinates during 1–2 days. Protocols have been developed for many species [22][23][24], allowing efficient pollen germination and monitoring of its behavior for a long time. The studies of conifer pollen tubes in vitro revealed their differences from the tubes of angiosperms, including cytoskeleton organization, regulation of organelle movement, and endo/exocytosis [25], which we will discuss in the next section.

The place on the grain surface where the pollen tube appears in gymnosperms is not predetermined to the same extent as in most flowering plants. The latter in most cases have one of several apertures, intended for the fast tube outlet [26]. According to data obtained on *Arabidopsis*, the optimal number of apertures is three [27]. In saccate conifer pollen grains, the pollen tube emerges between the sacs, at the pole opposite to prothallial cells. Aperture area in this case is a furrow with relatively thin exine, and in its distal areas the appearance of exine ruptures is most likely [28]. However, since there are two such areas, some of the pollen grains produce not one but two tubes, which could be considered as a feature that reduces the germination rate, or as a potential adaptation. In a study conducted on pollen grains of blue spruce germinating in vitro, this phenomenon was described as "bipolar germination", and it was found to occur only in case of optimal germination medium, and, apparently, it is not an adaptation for obtaining additional nutrients [28]. If there are no air sacs in the pollen grain, but it is polarized, then the place where the tube exits is a rather wide zone opposite to prothallial cells (*Ephedra* or *Welwitschia* pollen)

[29]. No signs of polarity were found in unicellular pollen (juniper), and it is believed that the place of tube appearance is not determined [2][30][31].

In most gymnosperms, a pollen grain upon reaching the female cone lands on a pollination drop. Droplet volume ranges from 10 nL to 1 μL [33]; large drops are visible to the naked eye [13]. The list of species that do not have a pollination drop reduces as pollination is being studied closely, including through phylogenetic mapping [34]. A small or rapidly disappearing drop is often found. A pollination drop is, first of all, an apoplastic liquid. It contains inorganic substances, carbohydrates, and proteins, including enzymes [16][30][33][35]. Comparison of the pollination drop with the more studied apoplastic fluids of angiosperms (stigmatic and ovular exudates, nectar) revealed a significant similarity in their composition [16]. On this basis, it was suggested that the droplet functions are not limited to delivering pollen to the ovule [16]. Droplet enzymes, in particular chitinases, may be involved in protecting the ovule from pathogens. Possible regulatory functions of droplet components during pollen germination are also discussed. However, all these assumptions have not yet been verified experimentally.

Different pollination strategies are found in conifers [36][37]. Saccate pollen of *Pinus* and *Picea* floats on the surface of the pollination drop and with it is transferred to the nucellus, where it germinates. In *Abies*, an analogue of a pollination drop is formed from moisture collected after rain or dew. Pollen often germinates in the micropilar canal and the tube grows towards the nucellus. In *Larix*, the pollination drop is absent, but the micropilar canal is filled with ovular secretion. Surrounded by this secretion, pollen hydrates, swells, and sheds exine. In this form, it floats to the nucellus, where forms a pollen tube. This usually happens a few weeks after pollination. In some *Tsuga* species and all Araucariaceae species, there is no pollination drop [34]—pollen lands on the conical surface next to the ovule and may remain there for several weeks. It then germinates and forms a long tube that grows through the micropyle into the ovule and reaches the nucellus. The variety of patterns of pollen behavior in the female cone is extensively discussed in the literature, since it can provide important information about the evolution of pollination [36][16][18].

Comparing angiosperms and gymnosperms, we find that the male gametophyte of the latter often has to cover a shorter distance after pollination, but it does it for a much longer time. Male gametophyte germination and growth occur slowly at all stages: the hydration of conifer pollen usually occurs in the first day after pollination, and pollen tube appears within a few days, while in flowering plants these processes take minutes and hours [25][38]. Thus, growth rate of *Picea abies* pollen tube is about 20 μm/h, which is a striking contrast compared to 300–1500 μm/h in angiosperms. In addition, conifers are characterized by a period of long dormancy when the pollen tube does not grow. In particular, the tube growth can stop for the time required to complete female gametophyte development— up to a year. In this case, a few days before fertilization, the pollen tube resumes its growth and delivers sperms to the ovule. For example, in pine and some Araucariaceae, pollen germinates shortly after pollination and the tube enters the nucellus. The dormant period lasts from mid-summer to the next spring. In *Pinus taeda*, the dormant period of the tube starts when meiosis begins in the ovule and ends a few days before fertilization [4]. Thus, for different plant groups, the time from pollination to fertilization varies from several weeks (most species of Cupressaceae and Pinaceae) to a year (*Pinus* and some Araucariaceae) [4].

The review of physiological data obtained for pollen germination in gymnosperm species can be found in the review article on which this topic is based.

3. Future Perspectives

One of the promising directions for this topic is the deepening understanding of the regulatory mechanisms that differ in gymnosperms and angiosperms. In this sense, pine or spruce pollen suspension is a very convenient model system, as it is rather well studied in comparison to the representatives of other taxa, and, accordingly, the integration of new additional knowledge will help build an adequate model of the origin, development, and transformation of regulatory systems on the way from conifers to flowering plants.

Another interesting direction is specialization, that is, clarification of the features of pollen physiology in special groups of gymnosperms, and in this sense, cycads, Gnetales, and ginkgo are of great interest, since the pollination process in these plants has been studied from the point of view of morphology and anatomy, but the data on pollen physiology are missing, and very little is known about pollen tube growth regulation and the peculiarities of its functioning as a haploid organism.

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