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Pollen embryogenesis: atavism or totipotency?

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Summary. The origins of pollen embryogenesis are still in doubt. Totipotency of plant cells has traditionally been put forward as an explanation for this phenomenon but we have found this interpretation to involve some shortcomings. The pollen grain is a highly differentiated structure which should have a very reduced capability of regenerating a whole plant, whereas in some species the induction of androgenesis appears to occur with greater facility than somatic embryogenesis. Furthermore, some microspores seem to have a tendency to morphogenesis and organogenesis; spontaneous androgenesis occurs naturally in various species and many examples also occur of pollen dimorphism. Totipotency would seem to be insufficient to explain androgenesis and we propose that its origin might be found in the phenomenon of atavism. According to studies published on ancestral precursors of pollen, these structures appear to have had high proliferation capacity. The ability to form a multicellular structure from a single haploid cell is shared by the meiocytes of ancestral algae, of the first land plants, and of present-day ferns, which are evolutionarily related to pollen. Atavism is only expressed under certain circumstances, as indeed is androgenesis, normally as a consequence of an environmental stress. Our conclusion is that there is evidence enough to suggest that androgenesis may well be the expression of archaic genes of meiocytes with morphogenic capacity which were naturally expressed in the ancestors of flowering plants.

Keywords: Androgenesis; Atavism; Embryogenesis; Plant totipotency; Pollen.

A full understanding of the ontogeny of multicellular organisms is still some way off and many questions remain to be answered about the process which leads to the formation of an animal or a plant from a single cell. Within this context androgenesis, or pollen embryogenesis, is an interesting phenomenon, the study of which might well go some way to bringing about a greater understanding of some of the general principles involved in plant embryogenesis. Although pollen grains of flowering plants are programmed for terminal differentiation into gametes, a small number of pollen grains have been found to divide in an essentially immortal way when they are cultured at an appropriate stage of their development in a suitable medium. This phenomenon is known as androgenesis, or pollen embryogenesis (Raghavan 1986, 1990). Androgenesis implies a deviation from gametophytic development to an organogenic one, leading to the production of a whole haploid plant.

Completely homozygous plants obtained after androgenesis are especially suitable for plant breeding and many papers have been published on the subject of optimum culture conditions to induce pollen embryogenesis in the greatest number of species. Nevertheless, we are unaware of any studies concerning the origin of the androgenic capability of pollen grains. Efforts have been concentrated on characterizing the genes expressed during androgenesis or on searching for the cytological (Sangwan and Sangwan-Norreel 1996) and molecular (Cordewener et al. 1996) markers of androgenesis. However, it would be useful to be able to find some sort of answer to the following questions: Can we provide a satisfactory biological explanation for androgenesis, and why are only some microspores responsive to androgenic induction? We hope that the results of our initial studies may serve to shed some light on the workings of this process, which is initiated in the pollen grain, a very special-

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ized cell within the whole plant. Raghavan (1986, p. 116), commenting upon totipotency, says that "... a cell is defined as totipotent if it can regenerate in full multicellularity, sexuality and structure the phenotype of the organism of which it was a part." Bearing in mind the definition of androgenesis as we have outlined it above and Raghavan's definition of totipotency, androgenesis might be considered as a typical example of totipotency in the plant kingdom. And in fact, this definition, originally applied to somatic embryogenesis, has also been used by several authors to explain androgenesis. Reynolds (1997, p. 1), for example, claims that androgenesis is "one of the most striking examples of cellular totipotency". In our opinion, however, this interpretation of pollen embryogenesis has certain shortcomings and we believe that there are other factors to be taken into consideration apart from that of totipotency when explaining the phenomenon of androgenesis.

The pollen grain is a very specialized and differentiated structure within the whole plant. These characteristics are revealed in its morphology and in its physiology, both of which are specially adapted for pollination. Examples of these adaptations are its resistant cell wall and cytosolic dehydration. The fact that the germinal tissues appear at the last moment of the life of the meristem (Lyndon 1990) reinforces the specialized character of the pollen grain. In accordance with this, and with the definition of totipotency we have already seen, it should be more difficult to culture such differentiated cells than meristems or other less differentiated tissues. That is to say, somatic embryogenesis, which is indeed a typical example of plant totipotency, should be much easier than pollen embryogenesis. This in fact seems to be true in most plants studied, but not all of them. Since 1964, when androgenesis was first described by Guha and Maheshwari (1964), a significant number of species have been found in which pollen embryogenesis occurs with relative facility, despite the high degree of specialization of the original tissue. We intend to point to some of these responsive examples as we continue with our hypothesis.

Within the angiosperms, the Solanaceae are particularly responsive to androgenesis. Pollen embryogenesis in *Nicotiana tabacum*, for example, requires no more than a culture medium containing mineral salts and sucrose (Sunderland 1971); similarly in *Datura innoxia* (Nitsch 1972, Raina et al. 1982) and certain species belonging to other families, such as *Hyosciamus niger* (Raghavan 1976, 1978), *Brassica napus* (Palmer et al. 1996), the addition of hormones to the medium is not required in order to induce androgenesis. In somatic embryogenesis, on the other hand, it is essential to add phytohormones to the medium to obtain calluses and somatic embryos (Zimmerman 1993). In the above-mentioned species at least it would seem to be easier to induce morphogenesis in pollen than in somatic tissues, a fact that contradicts the accepted premises of totipotency, which affirm that the more differentiated a tissue, the smaller its capability of regenerating the whole plant (Meins 1986).

Whilst hormones are not an important factor in androgenic induction, stress treatment before culture, whether thermal, chemical, or nutritional, would indeed seem to be important (Touraev et al. 1996a). Touraev et al. (1996a) maintain that an understanding of the role of shock as a key factor in the induction of androgenesis may provide a key step towards obtaining a unified culture medium to be used with various species. It has been shown, for example, that a combination of starvation and mild heat shock contributes significantly to inducing androgenesis in species such as *Triticum* spp. (Touraev et al. 1996b), *Nicotiana tabacum* (Touraev et al. 1996a), and *Quercus suber* (Bueno et al. 1997).

A comparison of androgenesis with somatic embryogenesis reveals that, contrary to the assertions of the law of totipotency, in certain species androgenesis seems to occur more easily than does somatic embryogenesis. Microspores in these plants seem to have a tendency to morphogenesis and organogenesis. We suggest from this that there must be biological factors other than totipotency involved in confering this morphogenic capability upon the microspores.

Further support for our hypothesis would come from the existence of spontaneous androgenesis in nature. The phenomenon has in fact been described in several hybrids of the genus Solanum (Ramanna 1974, Ramanna and Hermsen 1974) and in Narcissus (Koul and Karihaloo 1977). In these hybrids the spontaneous formation of polynuclear pollen grains has been seen to occur in the absence of either stress treatment, hormones, or indeed anther culture. According to these examples, we tend to the conclusion that, at least in some species, androgenesis and somatic embryogenesis are examples of two different biological processes.

Another interesting phenomenon which tends to separate androgenesis from the totipotency is pollen dimorphism. Pollen dimorphism is the single-anther

occurrence of pollens of two different morphologies, which are predisposed to different ways of development. The largest grains develop gametophytically, giving rise to mature pollen grains, whilst the smallest ones show an inherent tendency towards morphogenic development, giving rise to multicellular structures (Rashid 1983). This phenomenon has been described in several species (Dale 1975, Horner and Street 1978, Kaltchuk-Santos et al. 1993, Rashid 1983). In some species such as *Nicotiana tabacum* the frequency of embryogenic pollen grains varies according to the culture conditions of the donor plant, which may indicate that there is a certain predetermination involved in pollen dimorphism (Heberle-Bors and Reinert 1981, Heberle-Bors 1982). Or to put it another way, the microspores show their androgenic tendency within the donor plant before microspore culture. The existence of pollen dimorphism also runs contrary to the idea that androgenesis is an expression of totipotency because pollen dimorphism implies a predetermination in the morphogenic capability of pollen grains.

We have tried to show so far that totipotency is not enough to explain the phenomenon of androgenesis in that the biological premises for androgenesis are not equivalent to those of somatic embryogenesis. But if pollen embryogenesis is not just an example of plant totipotency, what is the origin of the sporophytic development of the pollen grain?

The answer to this question may lie in the evolutionary history of pollen itself. We propose that androgenesis could be an example of atavism in the plant kingdom, atavism being definable as the "... reappearance of a lost character (morphology or behavior) typical of remote ancestors and not seen in the parents or recent ancestors of the organisms displaying the atavistic character" (Verhulst 1996, p. 59). This means that during the normal development of an organ or tissue, certain characteristics may appear that are not habitual in the species in question. These characteristics were usually manifested in the ancestors of such species. We can apply this concept to androgenesis, the morphogenic capability of pollen being an archaic characteristic expressed in a natural way in its ancestral structures, which nowadays has lost its original function, but remains latent in pollen grains. The display of this atavistic characteristic (morphogenic potential) only comes to the fore under certain stress conditions, such as a thermal shock and/or starvation.

To support this hypothesis we shall first try to trace the evolutionary history of the pollen grain, searching for ancestral structures in which the morphogenic capacity is determinant. We shall also compare pollen embryogenesis, which we propose to be an atavism, with some other well recognized atavistic characteristics.

Although the phylogeny of flowering plants is not completely clear, they seem to have evolved from some tree-like pteridophytes which lived in the mid-Devonian (Cronquist 1988, Stewart and Rothwell 1993). Whatever the exact structure of these extinct plants was, it is generally accepted that they must have produced spores for reproduction, in a similar way to present-day ferns. It has been fairly conclusively shown that these primitive spores are evolutionarily related to the pollen grain; that is to say, the spores of the ancient "proto flowering plants" are the evolutionary predecessors of the present-day pollen grain (Blackmore and Knox 1990, Crane 1990). It is assumed that these primitive pteridophytes produced meiospores, the morphology of which was similar to the pollen grain. The structure and composition of the cell wall and the production of callose support this hypothesis (Blackmore and Knox 1990, Crane 1990). These meiospores are presumed to have had a high proliferation capacity, leading to a haploid multicellular gametophyte. This characteristic is shared by most of the present-day ferns. It is assumed that the male gamete was a multiflagellate cell. Fecundation gave rise to a diploid zygote that regenerated the sporophyte and closed the life cycle. The resemblance between the primitive pteridophyte spores and the pollen grain is so conspicuous that the life cycle of the flowering plants is considered to be a drastic simplification of the pteridophytes' life cycle. This simplification involves a reduction in the number of cells in the male gametophyte, which went from being a multicellular structure in the pteridophytes to the simple pollen grain of today's flowering plants.

On comparing the life cycle of the primitive pteridophytes with that of the flowering plants, we have found some aspects to support our hypothesis. The meiospore of the primitive pteridophytes evidently had morphogenic capacity since they were able to produce multicellular gametophytes. Our proposal is that the flowering plants inherited from these primitive ferns the genes responsible for the organogenic development of their meiospores. In ferns the spore gives rise to the multicellular gametophyte, whilst in the androgenic pollen the microspore is able to produce an embryo. The developmental pattern

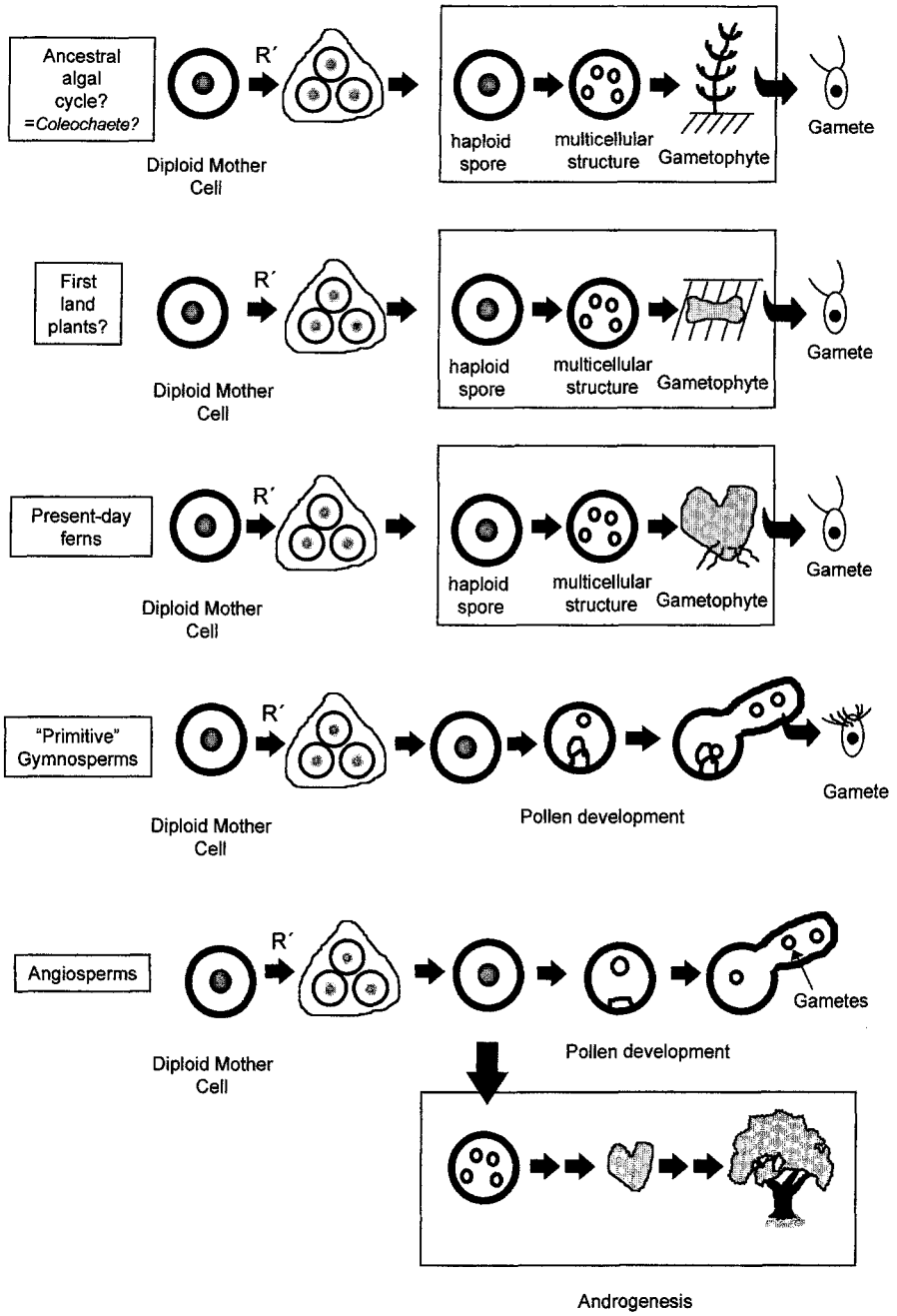

Fig. 1. Proposed model for the evolution of pollen from the algal ancestor to present-day angiosperms. The processes contained within the frames represent the morphogenic capacity of the meiocytes, i.e., the formation of a multicellular structure from a haploid cell. It can be seen how this supposed capacity is evident in the ancestral algae, in the first land plants, and in the present-day ferns, in which the meiocyte gives rise to a multicellular gametophyte. Nevertheless, the importance of this gametophyte gradually lessened in land plants during their evolution from ferns to angiosperms. In the most "primitive" gymnosperms, such as *Gingko biloba*, the gametophyte is still multicellular and the gamete still flagellate, whereas in the angiosperms the meiocyte has only two or three cells and the gametes are no longer flagellate. When androgenic development occurs, however, it involves the "memory" of the ancient morphogenic capacity, the microspore under stress conditions "remembering" the "forgotten" genes inherited from its ancestors. It has been shown that one of the optimum stages of pollen development at which androgenesis is most effectively induced is the unicellular one (Reynolds and Kitto 1992). This idea is supported by the finding that new sets of genes are expressed at the moment of initial haploid mitosis. This means that this stage may well represent an important developmental switch (Reynolds and Kitto 1992). It so happens that the spores of ferns and algae begin their morphogenic development (germination) at this unicellular stage. This apparent coincidence goes further to support our hypothesis that androgenesis is an expression of atavism

expressed during the first embryogenic divisions of a microspore seems to be equivalent to that expressed when a fern spore germinates to produce the prothallus (Fernández et al. 1993). In the former case the proliferative behavior occurs in response to stress upon the microspore, resulting in atavism, whereas in the latter it is a normal event in the life cycle of the fern, although stress may also play a part in the process (Touraev et al. 1996).

The primitive pteridophytes, ancestors of flowering plants, evolved from algae that colonized the land 450 million years ago (Stewart and Rothwell 1993), although the scanty fossil record left by these algae makes it difficult to identify them precisely according to present-day algal taxonomy (Graham 1990, 1993). Nevertheless, it is generally accepted that those algae, from which the flowering plants eventually evolved, are closely related to the present-day group known as "green algae" (Graham 1990, 1993). Flowering plants share with these modern algae some biochemical, cytological and molecular characteristics, which have been used as proof of their cognation (Graham 1990, 1993).

Several authors have studied these algae to discover more about the origin of flowering plants (Graham 1990, 1993). Pollen grains are assumed to have evolved from green-algal meiospores (Blackmore and Knox 1990, Crane 1990). Sporopollenin and callose has been shown to be present in the microsporogenesis of certain green algae, such as the genus Coleochaete, belonging to the subgroup Charophytes (Graham 1990, 1993). These and other characteristics may indicate that the microsporogenesis of flowering plants and that of green algae are equivalent from an evolutionary point of view (Crane 1990, Graham 1990). This leads us to conclude that the meiospores of the green algae and pollen grains are evolutionarily related.

Thus, the pollen grain seems to have evolved from a meiospore produced by a fresh-water alga (Graham 1993), and the most interesting aspect of this relationship lies in the morphogenic capacity of the algal meiospore. In most multicellular algae that reproduce sexually, the meiospores have a high organogenic capacity. Which means that these meiospores are structures that can produce a multicellular organism via a more or less complex process of morphogenesis. In short, both the pteridophytes and later the flowering plants inherited the morphogenic capacity of the male meiospores from their common green-algal ancestors. Stress can promote the expression of this morphogenic capability in the form of atavistic development in flowering plants.

As defined above, atavism is the phenomenon by which an organ during its ontogeny acquires some aspect of morphology reminiscent of one belonging to its ancestors or another related species. A typical example of atavism is found in the white mulberry tree, *Morus alba*. The leaves of the white mulberry tree have serrated edges, but in some circumstances clearly lobulate leaves can be seen. Interestingly enough these lobulate leaves are very similar to the leaves of the fig tree, *Ficus carica*, and both species are phylogenetically related within the family Moraceae. We might be led to believe then that in some cases, the white mulberry tree shows characteristics belonging to an ancestral species which had leaves similar to those of the present-day fig tree (Sebánek 1991, Sladky 1991). It is also interesting to note that, according to our observations so far during our studies, atavistic characteristics seem to be more frequently expressed in ornamental varieties of the mulberry tree than in wild ones although we have not as yet had the opportunity to apply any strict statistical analysis to this observation. Nevertheless, we suspect that intense culture of a plant may distort its ontogenic program, producing organs or tissues that differ slightly from those of wild individuals of the same species. In this case the culture of the plant may provide the shock necessary to induce the expression of atavistic growth.

Atavism can be found not only in somatic tissues but also in the germinal cell line. Some authors have found, for example, an interesting similarity between the sperm cells of some angiosperms such as *Asclepias* and *Monotropa*, and the spermatozoid of certain ferns (Knox and Ducker 1991). This curious similarity could be considered as being the expression of an ancestral characteristic within the pollen grain, i.e., an atavism.

In the examples mentioned so far atavism appears to be expressed spontaneously in the life cycle of the plant. However, in some cases the atavistic character is only expressed under certain conditions. A good example of this is the horse-chestnut tree, *Aeusculus hippocastanum*, belonging to the family Hippocastanaceae. This tree normally shows the large webbed leaves characteristic of the genus. Nevertheless, as a consequence of certain environmental stresses, such as freezing, mechanical wounding, or being subject to depredation, the tree produces pinnate leaves. These pinnate leaves are a normal to the family Sapin-

daceae, which is phylogenetically related to the Hippocastanaceae (Sebánek 1991, Sladky 1991).

Genetically, atavism may imply that the more highly "evolved" organisms such as flowering plants conserve certain genes deriving from more "primitive" taxonomical groups (e.g., some ancient pteridophytes or green algae). These "archaic" genes may remain in the genome of the present-day species, although their routine expression is suppressed by more recently acquired genes, which are responsible of the maturation of the pollen grain. The expression of these "old" genes under certain circumstances is the atavism.

In conclusion we suggest that androgenesis is the process via which a microspore, when subject to a certain stress, changes its pattern of genetic expression and takes on morphogenic capacity. According to our hypothesis this new pattern of development implies the expression of archaic genes, which were naturally expressed in the ancestors of the flowering plants. These androgenic genes may play a regulatory role, controlling the induction of pollen embryogenesis.

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