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SHORT COMMUNICATION

Key innovations in transition from homospory to heterospory

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ABSTRACT

Heterospory (i.e. dimorphic spores) is a long-lasting topic discussed in plant biology. It is observed in many of ferns, fern allies, and seed plants. The rise of heterospory and the mechanisms underlying its success in plant evolution are not clearly elucidated. In this short communication, an attempt is made to shed some light on these two questions.

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Background

Recently, Fu et al. reported the earliest fossil flower from the Early Jurassic.[1] This finding brought forward the time of angiosperm origination to the Early Jurassic, almost 50 million y before the previous record. Some questions regarding to key evolutionary innovations such as how the earliest flower looks like can be solved by discovery of fossils. But others such as how heterospory originated may not be solved by direct fossil evidence. To the questions of latter kind, other approaches may help to find answers.

In the history of life, a novel biological program, named sexual reproduction cycle (SRC), emerged in most eukaryotes, and is punctuated by three key events: meiosis, heterogametogenesis, and fertilization. Unlike in mitotic cell cycle, the daughter cells (cells produced after fertilization) in the SRC have genome arrangement different from the parental cell due to recombination of independent assortment and other genetic variations introduced during meiosis and selection through competition in fertilization. The difference in genetic composition between parental and daughter cells allows SRC to define "generation" for the first time. These biological variations allow the organisms to integrate to various unpredictable extracellular environment, making SRC a unique strategy adopted by almost all eukaryotes. It is not surprising that SRC becomes the backbone for the diversification of life cycle in multicellular eukaryotes and consequent evolution of these organisms.[2]

In unicellular eukaryotes, two intervals of cell proliferation are interpolated between zygotes and meiotic cells (diploid) and between meiotically produced cells and gametogenic cells (haploid), respectively, during SRC.[3] If the free-living cells proliferated during the intervals are organized, multicellular organisms emerge. In plants, multicellular structures in various forms are produced and interpolated into both the intervals. Such a morphogenetic mode is designated as "double-ring mode".[2,4] The multicellular structures are integrated with the SRC through two types of germ cells: diploid germ cells that lead to meiosis and haploid germ cells that lead to heterogametogenesis[5] (Figure 1).

A spore is an indispensable cell type in plant life cycle and morphogenesis. It is a specially differentiated haploid cell type derived from meiosis, resisting tough environments and hinging the two "rings", i.e. diploid and haploid multicellular structures or alternation of generations. In bryophytes and majority of ferns, only one type of diploid germ cells in capsules or sporangia enters meiosis and produces morphologically indistinguishable spores. This phenomenon is called "homospory" or "isospory". In other ferns and seed plants, two types of diploid germ cells in macro- and micro-sporangia, respectively, enter meiosis and produce two types of spores of different sizes (called megaspores and microspores, respectively). This phenomenon is called heterospory.[6,7]

For most unicellular eukaryotes, either meiosis or heterogametogenesis can be induced by harsh environmental stresses.[3] In multicellular plants, germ cells, both diploid and haploid, differentiate under protection of multicellular structures, such as sporangia or stamen/ovule (angiosperms) in diploid phase and antheridium/archegonium in haploid phase. Since there are two interpolations of multicellular structures (sporophyte/gametophyte) in both the intervals of SRC in plants, the differentiation ensuring heterogametogenesis exhibits diversified and complicated patterns. In bryophytes and some of ferns with homospory, the pattern of development ensuring heterogametogenesis occurs on gametophytes as differentiation of antheridium/archegonium. In other ferns/fern allies and seed plants, in addition to differentiation of antheridium and archegonium to various extents, sporangia in diploid phase diverged into macrosporangia and microsporangia, and enforce the following gametophytes, i.e. haploid multicellular structures being canalized into one of

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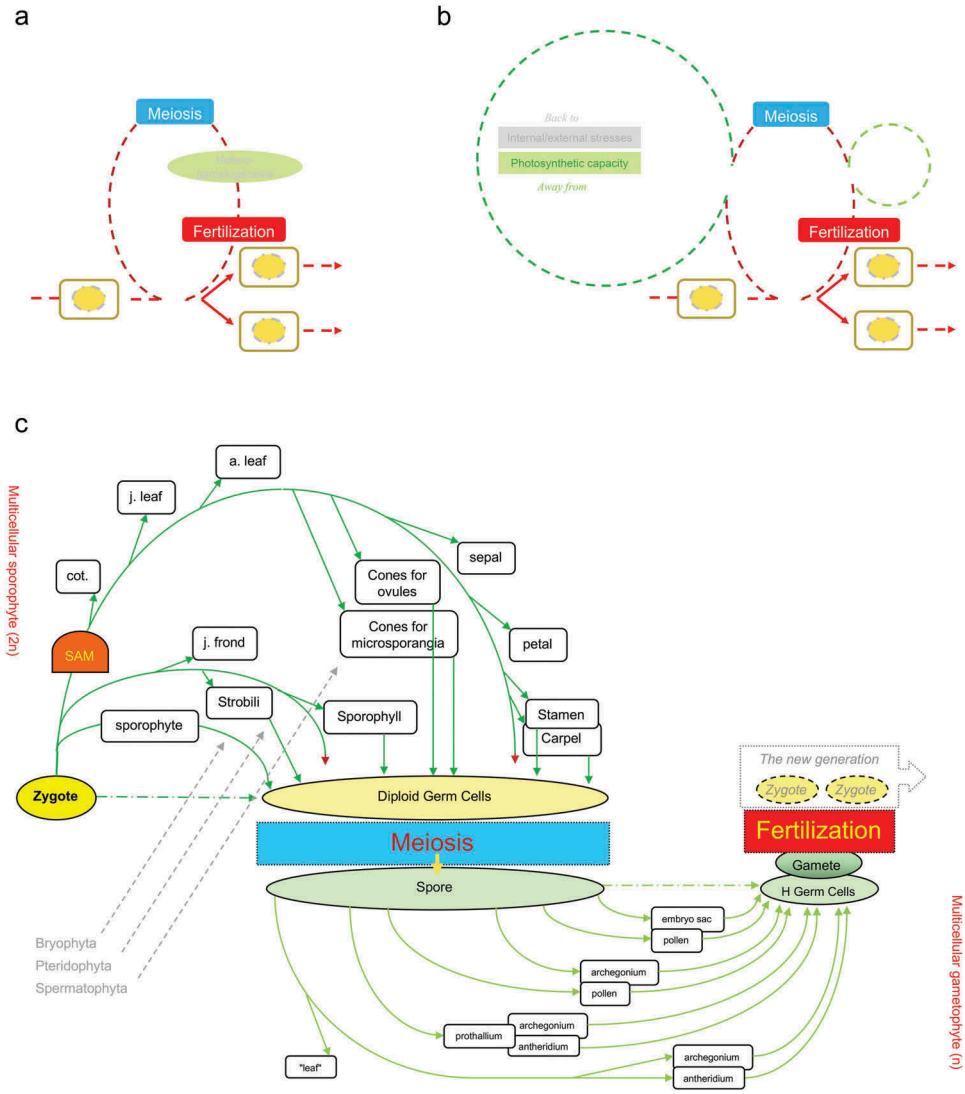

Figure 1. Schematic elaboration of a SRC-derived "double-ring mode" of plant morphogenetic strategy and its application to the three major plant groups.
(a) A diagram of the modified cell cycle called "sexual reproduction cycle, SRC". The three rounded rectangles containing yellow ovals represent diploid cells. The red dashed line and arrows represent one diploid cell becomes two (a cell cycle). Dark red dashed curve represents a process, in which three biological events, i.e. meiosis, fertilization, and heterogametogenesis, were integrated and inserted into the cell cycle represented by the rounded rectangles. Through the SRC, a diploid eukaryote can autonomously generate genetic variations and increase fitness to the unpredictably changed environment. (b) A diagram of the SRC-derived double-ring mode of plant morphogenetic strategy. Into the two intervals in the SRC, two multicellular structures are interpolated, i.e. sporophytes (green dashed circle) and gametophytes (light green dashed circle). In the either ring, the multicellular structures increase the size from a single cell (such as a zygote or a spore) driven by photoautotroph, and reduce the size compelled by internal and external stress, ultimately back to the unicellular SRC through induction of germ cells. (c) A transformation of the double rings by stretching the circle into a linear version, thus to position the diploid ring above the core process of SRC and the haploid ring below. In such a transformation, the major morphological structures, the lateral organs derived from growth tips, of all three groups of land plants, i.e. bryophyta, pteridophyta and spermatophyta, can be aligned for comparison (reprinted from the Figure 1 in reference 5).

the two tracks to produce either sperms or eggs. How the divergence point leading to heterogametogenesis shift from gametophyte to sporophyte occurred? This is an interesting question.

Key innovations in transition from homospory to heterospory

For a long time, the transition from homospory to heterospory has been a popular topic in plant biology.[6–8] According to Bateman and DiMichele and Qiu et al, there is a general consensus that heterospory is derived from homospory[7,8]. Homosporous bryophytes are gametophyte dominant and monosporangiate, i.e. with single type of capsule in their sporophytes. In contrast, heterosporous plants are sporophyte dominant and multisporangiate, *i.e.* with multiple sporangia in their elaborated sporophytes. As sporangia of heterosporous plants are initiated in diploid sporophytes, rise of sporophyte dominance needs to be explained before discussing transition from homospory to heterospory.

Gifford and Foster have pointed out that the polymer lignin is of paramount importance for vascular plant (dominant sporophyte) evolution.[6] Such an opinion was further elaborated by Chen et al., who reported that secondary cell wall (SCW) thickening is a rapid process that requires

dramatic increase of protein synthesis.[9] This finding suggests that diploidy is more affordable of the acute need of RNA through the extra set of chromosome. While more evidences are necessary to explain the emergence of sporophyte dominance, the vascular equipped diploid sporophytes at least partially explain their higher efficiency in SCW material supply and their unprecedented complex robust morphology against diverse environmental stresses. Now the questions are what mechanisms underlie the heterospory and how the ensuing heterogametogenesis is correlated with heterospory?

The survey on fossil and extant plants indicates that the earliest trace of heterospory (intrasporangial heterospory) is seen in *Chaleuria cirrosa* (aneurophytalean progymnospermopsid of the Eifelian, Middle Devonian, >388 million y), in which spores of different sizes are seen within a single sporangium.[7] Similar situation is also seen in Zosterophyllopsida (Late Devonian), *Archaeopteris* (Late Devonian), Sphenopsida (Early Carbonifers), and living *Isoetes*.[7] Difference in rate of mega- and microsporangia enlargement around the time of sporangia initiation and early end of the increase of sporogeneous cell (diploid germ cell) numbers in megasporangia have been reported.[10] More interestingly, the differentiation of mega- vs. microsporangia can be affected by exogenous application of Ethyphon.[11] All these observations suggest that dimorphism of megasporangia as well as megaspores and their microcounterparts can be induced by various internal and external environmental conditions. The differences in size and morphology of spores are here called the first key innovation for heterospory. Despite long existence of intrasporangial heterospory and intersporangial heterosporangy, little is known about the relationship between them, namely whether intersporangial heterosporangy is correlated with intrasporangial heterospory, how one type of spores exclude the other completely from the sporangia, how and why the originally similar sporangia evolve different morphology. Studying *Isoetes* with isospory and comparing it with heterospory in others, especially at molecular level, may shed some light or at least eliminating some of the alternatives.

While lycophytes and some ferns of heterospory release heterospores for freely living, all seed plants evolve further with more sophisticated innovations. Along with the increase of complexity of the multicellular structures in sporophyte, the second key innovation came: retention of megaspores or endomegasporangy. This feature symbolizes the initiation of a key trend in plant evolution leading to the success of seed plants, because with endomegasporangy, megaspores are better protected against harsh environment and ensured with better nutrition supply.[7,12] Although its ecological success is self-evident when the prosperity of seed plants is taken into consideration and such megaspore retaining may be dated back to as far as the Middle Devonian (385 million y ago),[13] effort is still necessary before the causes and process of such retaining are known at molecular levels.

Since the completion of SRC requires meeting of gametes, the retention of megaspores within sporangia raises a critical challenge: how can the sperms from the antheridia of free-living gametophytes reach the eggs in the megagametophytes that are retained on the aerial terminus? To solve this problem, the microspore has to remain portable (which means little space for male gametogenesis) and simple to be easily moved to the megagametophyte, then the third key innovation came: retention of microgametophyte (i.e. gametophyte development within pollen grains), parallel to endomegaspory. Bateman and DiMichele had correctly pointed out a reliable pollination mechanism as a key for reproduction breakthrough.[7] Although successful pollination by pollen grains with retained microgametophytes can at least be dated back to the Late Carbonifers,[14] it appears rather bizarre that the retention of microgameophyte was rarely discussed in the literature.

After these innovations, there is no room for free-living gametophyte differentiation and therefore no chance for antheridia and archegonia differentiation to occur in gametophytes. Only those species with coupled microgametogenesis and megagametogenesis completed within separated micro- and mega-spores, respectively, can survive to the evolution. Such a coupling is related with the former disarticulated heterogametogenesis and heterospory. And divergence point leading to heterogametogenesis formerly restricted to the haploid gametophyte phase (more controlled by environmental and/or endogenous factors) is now shifted onto the diploid multicellular structures of sporophytes.

Conclusions

In animal kingdom, the embryogenesis for most of species follows similar principles and therefore renders a comparison of developmental processes among diversified species possible. In plant kingdom, although the concept of alternation of generation provides a common reference framework for comparison of life cycles of various taxa,[15] emphasis was traditionally focused on differences in morphogenetic processes, such as dominance of gametophyte or sporophyte, being vascular or non-vascular. Homospory vs. heterospory appears puzzling and downplayed for long time. However, if we look at life cycle of plants from the perspective of "SRC", the comparison of developmental process of diversified plant taxa can be easily aligned.[2,3] From such a perspective, multicellular structures are interpolated into the two intervals between the three core cells in SRC, exhibiting a "double-ring" mode.[4] The multicellular "rings" are formed under two driving forces, namely photoautotroph and stress-response, for acquiring energy, providing nutrition for growth, and protecting new-born structures from harsh and enduring environmental stresses. Bower has suggested the multicellular structures emerged as a result of delayed meiosis.[16] Now under the concept of SRC and "double-ring mode" of plant developmental program, multicellular structures can be taken as blown-up bubbles inserted between the three core cells that can accommodate unprecedentedly increasing morphological variations. Based on such a conceptual framework, we provide a new perspective on heterospory and its success in view of the plant evolution. Also, we explained why somatic differentiations ensuring heterogametogenesis shifted from gametophytes in homosporous plants to sporophytes in heterosporous plants. While more empirical evidences are

necessary to test this explanation, such a paradigm shift would inspire and guide new explorations before reaching a more clear solution.

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