Double Fertilization in Flowering Plants: 1898–2008

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Abstract—The present article gives a brief survey of results of studies in the area of plant embryology directly associated with the discovery made by S.G. Navashin in 1898 of double fertilization in vivo and in vitro. These studies utilized methods of electronic and fluorescence microscopy, cytophotometry, and cultures of isolated ovules, sperm, and the embryo sac central cell. Questions related to the origin of the female gametophyte of flowering plants, double fertilization, and the endosperm are considered. It is emphasized that progress in this field is associated chiefly with the study of molecular processes that regulate the development and functioning of the female gametophyte and the sporophyte on early stages of ontogenesis.

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“New Observations on Fertilization in Fritillaria tenella and Lilium martagon,” a report submitted by Sergei Gavrilovich Navashin, professor at Kiev University, on August 24 (old calendar), September 6 (old calendar), 1898 to the X Congress of Russian Naturalists and Physicians held in Kiev has immortalized the name of Navashin. In the form of brief summaries Navashin set forth the following conclusions: (1) not one, but both generative nuclei enter the embryo sac from the pollen tube; (2) the generative nuclei are worm-like in form and are apparently capable of engaging in worm-like movement; (3) one of the nuclei penetrates into the egg, while the other copulates with the upper polar nucleus of the embryo sac; (4) only afterwards do the upper and lower polar nuclei merge together, the product of the fusion of these three nuclei then dividing, producing, as is customary, the primary endosperm; (5) thus, like the embryo, the endosperm emerges by means of a fusion of the sex nuclei: one male nucleus with the generative nucleus of the egg, i.e., with the other female nucleus; (6) as for the sexual origin of the endosperm, Navashin tried to explain this phenomenon by deriving it from polyembryony, i.e., by considering the endosperm as a second, indirectly developing embryo. The report was published in German in the journal, Izvestia Akademii Nauk (Bull. Acad. Sci. St.-Petersburg, November, 1898, vol. 9, no. 4, pp. 377–382), under the title, “Resultate einer Revision der Befruchtungsvorgange bei Lilium martagon und Fritillaria tenella.” Navashin’s discovery was soon confirmed in Lilium martagon and L. pyrenaicum [1, 2]. In English, the term, “double fertilization,” first appeared in the title of an article by Thomas [3] and in the text of an article by Sargant [4]. The results of Navashin’s subsequent investigations of the process of fertilization in dicotyledonous and monocotyledonous plants were published in his studies, On Fertilization in Asters and Orchids, On the Processes of Fertilization in Certain Dicotyledonous Plants, On the Independent Mobility of the Male Sexual Nuclei in Certain Angiospermous Plants, A Detailed Study of the Formation of Male Sexual Nuclei in Lilium martagon, On the History of the Development of the Chalazogam Juglans nigra and Juglans regia (with V. V. Finn), and An Experiment at Structural Representation of the Properties of the Sexual Nuclei [5]. These studies considered questions related to the nature of the fusion of the secondary sperm with the embryo sac central cell, the nature of the endosperm, the movement of the sperm, and the structure of the sexual elements in connection with their function.

Navashin’s discovery of double fertilization and his subsequent finding that it exists in almost all of the species of angiospermous plants that had been studied in this regard demonstrated the commonality and uniqueness of the phenomenon of double fertilization for flowering plants [6–13]. His discovery exerted a profound influence on the science of botany as a whole and promoted a wide-ranging development of plant embryology and cytology. We need only recall that the uniqueness of the phenomenon of double fertilization for the angiospermous plants became one of the basic arguments in support of the theory of the monophyletic origin of this most highly organized division of the plant world.

The use of methods of electronic and fluorescence microscopy, immunocytochemistry, cytophotometry, and, especially, methods involving culturing of isolated ovules in recent years for the purpose of investigating this process should especially be noted. More recently, methods involving cultures of isolated sperm, eggs, and even the central cells of the embryo sac that have led to successful fertilization in vitro have been used. It became possible to implement the latter methods due
to the development of corresponding media and techniques of isolating male and female structures [14–16]. The first experiments in an in vitro culture were conducted with isolated *Papaver somniferum* ovules [17]. In these experiments pollen germinated, the pollen tubes penetrated the embryo sac, and double fertilization and the formation of embryo and endosperm occurred. Fertilization of the central cell in a culture of isolated male and female structures in *Zea mays* and *Nicotiana tabacum* has been successfully achieved recently [18]. The improved technique of “test tube fertilization” is widely used to overcome nonhybridizability in remote hybridization and self-incompatibility [19, 20]. This approach in particular has opened up new opportunities for studying the role of recognition molecules in fusion of sperm and egg and in the fusion of polar nuclei and sperm, a mechanism that prevents polyspermy as well as helping us gain an understanding of the molecular biology of double fertilization and the formation of the ovule and endosperm on early stages [14, 21].

Below, we will briefly discuss results of studies that were inspired by the discovery of double fertilization and have been carried in recent years, in an effort to find answers to questions that had been raised by Navashin.

**TYPES OF DOUBLE FERTILIZATION**

It has been established from the results of numerous observations conducted on different species of angiospermous plants that double fertilization in the norm is realized in all types of embryo sacs, independently of the number of polar nuclei that fuse with the secondary sperm of the polar nuclei or the ploidy of the secondary nucleus of the central cell of the embryo sac, for example, eight polar nuclei in the embryo sac of *Peperomia magnolifolia*, four in *Euphorbia procera*, and one in *Oenothera biennis*. Only in certain species of orchids does the sperm just come into close contact with the polar nuclei without fusing with them (endosperm does not form in these species).

Three types of fertilization are now distinguished, pre-mitotic, post-mitotic, and intermediate on the basis of light optical investigations of the behavioral features of the nuclei of sperm after they have penetrated into the egg [22–24]. In the *pre-mitotic* type of fertilization, fusion of the sperm nucleus with the nucleus of the egg occurs immediately following penetration of the sperm into the egg. The sperm chromosomes despiralize in the egg nucleus and a nucleolus forms, the latter usually fusing with the nucleolus of the egg nucleus prior to the start of mitosis. In the *post-mitotic* type of fertilization, the sperm nucleus does not fuse with the nucleus of the egg before the start of mitosis. Despiralization of the chromosomes occurs in the sperm nucleus, which is in close contact with the egg nucleus. The volume of the sperm nucleus increases, reaching that of the egg nucleus, and a nucleolus separates out. Unification of the chromosomes of the spermium and egg occurs following disintegration of the nuclear envelope in the later prophase or mitosis metaphase. The *intermediate* type constitutes, as is indicated by its name, a transitional type between the two basic types, the pre- and post-mitotic types. Here the fusion of the sperm nucleus with the egg nucleus does not conclude prior to the start of mitosis, and instead terminates in the prophase. This type of fertilization should probably be considered a pre-mitotic type, though one that occurs somewhat more slowly, possibly under the influence of a change in the surrounding conditions, for example, temperature. In artificially obtained tetraploids, for example, in species of the genus *Crepis* [24], the process of syngamy is realized according to the intermediate type, i.e., slowed down by comparison with the initial diploid forms, for which the pre-mitotic type is characteristic.

Three mechanisms of karyogamy in seed-bearing plants were proposed in the course of the development of these conceptions, based on data on DNA content in the nuclei of the fusing cells and zygote. The mechanisms are also formally associated with a particular phase of the cell cycle [25–27]: (1) karyogamy realized in the *G*1 phase of the cell cycle, i.e., the nuclei of the gametes fuse immediately after coming into contact and the subsequent phases of the cell cycle, i.e., *S* and *G*2, are then realized in the zygote nucleus; (2) DNA synthesis (*S* phase) occurs in synchrony in the nuclei of the egg and sperm once the nuclei come in contact, i.e., it precedes fusion of the gametes; (3) DNA synthesis occurs in the gamete nuclei even before they come into contact (the nuclei fuse in the *G*2 phase). Thus, in the first case the gamete nuclei fuse in the haploid state, while in the second and third cases, in the diploid state. It has been shown that in *Nicotiana tabacum*, DNA synthesis begins in the sperm nuclei following outflow of the contents of the pollen tube into the synergid, while at the same time the DNA contents increase in the egg nucleus and 48 h after pollination the contents are found between 1C and 2C, which points to synchronicity of the phases of the cell cycle in the gametes prior to their fusion [28].

**ULTRASTRUCTURE OF SPERM AND EGGS**

The sexual cells, or gametes (eggs and sperm) of angiosperms constitute highly differentiated cells and possess a number of morphological and physiological features that are typical of these plants, in particular, specific exchange of matter by comparison with somatic cells, haploid chromosome set, variable nucleus-cytoplasm relationships, etc.

**Sperm.** Navashin’s description in the early twentieth century of the nuclei of sperm immersed in the cytoplasm of the pollen tube and lacking their own cytoplasm in *Lilium martagon* [29] laid the foundations for discussions of the structure of the sperm of angiospermous plants which have continued over the course of several decades. In 1912 Navashin, in a discussion