Fertilization in Flowering Plants

2. Selection of the Male Partner is the Prerogative of the Maternal Parent

K R Shivanna

After the pollen grain reaches the stigma through outsourced agents (pollinators), the next step before fertilization is to select the right type of pollen. Similar to a marriage in human beings, flowering plants also have evolved elaborate screening process to select the right pollen grains and to reject the wrong ones. Even after initial screening for the right pollen, the pistil imposes a tough competition amongst them, comparable to a swayamvara of Indian mythology, to select the best available pollen. Flowering plants have evolved into a matriarchal society. The selection of the male partner is totally the prerogative of the mother (pistil); the boy (pollen grain) and the girl (ovule) has no say in this selection.

Introduction

The fascinating adaptations the flowering plants have evolved to achieve pollination, the process that brings the pollen grain to the stigma are described in an earlier article[1]. Being the seat of all post-pollination activities, the structure of the female sexual organ (pistil) is more elaborate. The pistil can be compared to the residence of the female partner (ovule) differentiated into various compartments. The stigma acts as the main door to receive the pollen grain, the style is the long corridor through which the pollen tube has to grow and the ovary in which the female partner, the ovule, is located, can be compared to the room of the ovule. For successful fertilization, the pollen tube has to enter the ovule and eventually the embryo sac (female gametophyte) before delivering the male gametes for fertilization (*Figure* 1a). Although there is considerable variation in the composition of the embryo sac, the most common type is made up of seven cells (*Figure* 1b).

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The egg apparatus, located at the micropylar[2] end, is made up of the central egg and the two surrounding synergids[3]. The three antipodal cells are located at the opposite end. The two sets are separated by a large central cell with two nuclei; the nuclei fuse to form the polar nucleus before fertilization. Pollen tube enters one of the synergids and discharges the two male gametes. One of them fuses with the egg to give rise to the zygote and the other fuses with the polar nucleus of the central cell to give rise to the primary endosperm cell. These two fusion events, termed double fertilization are unique to flowering plants. The zygote develops into the embryo and the primary endosperm cell develops into the endosperm which nourishes the embryo. The ovule develops into the seed (*Figure* 1c) and the ovary into the fruit. This completes sexual reproduction in flowering plants.

Figure 1. **(a)** Diagrams depicting the details of pollen-pistil interaction and fertilization. Following pollination, pollen grain germinates on the stigma and the pollen tube grows through the tissues of the stigma and style and enters the ovule by making a 90° turn. **(b)** Embryo sac. **(c)** Seed.
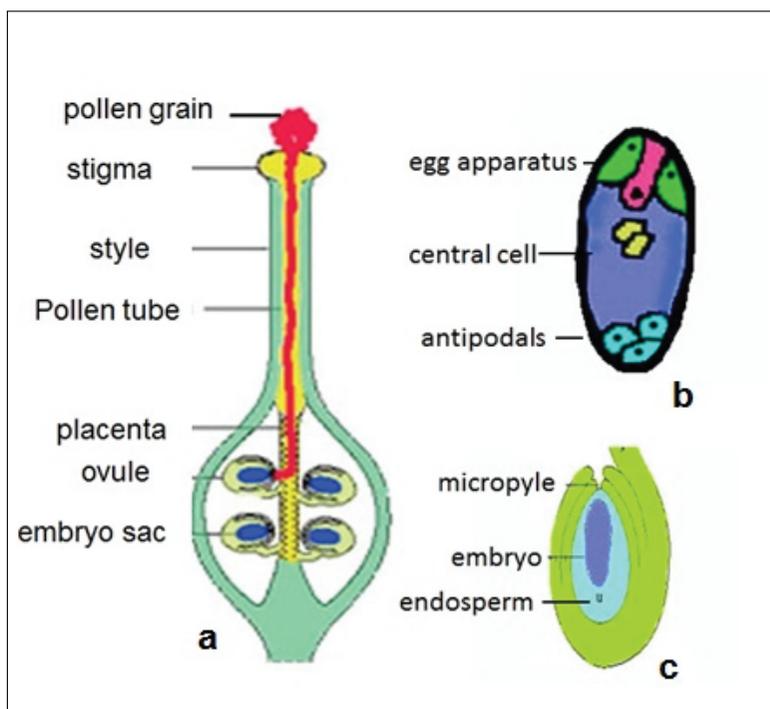
Most of the pollen grains deposited on the stigma during pollination are homospecific (of the same species). However, some of the pollen grains may be heterospecific (of other species) as the pollinator often visits flowers of other species also. Homospecific pollen is generally a mixture coming from the same flower (autogamous), other flowers of the same plant (gytonogamous) and other flowers of a different plant but of the same species (cross-pollen). The parents of heterospecific pollen may be closely related or distantly related to the pistillate (female) parent.

Fertilization has to be between the male and female gametes of the same species to maintain species identity. This requires identification of heterospecific pollen from homospecific pollen and prevention of heterospecific pollen from achieving fertilization. Flowering plants have evolved into a matriarchal society. Selection of the male partner (pollen) is an elaborate process and is the prerogative of the maternal parent (pistil); the pollen grain or the ovule has no role in this selection. This article highlights this elaborate screening event, more complex compared to the screening of the bridegroom in human society.

Structure of Pollen Grain and Pistil

It is necessary to give a brief account of the structural details of the pollen grain and the pistil (*Figure* 2) which are relevant to pollen screening. Pollen grains, when shed, are made up of two cells – a vegetative cell and a generative cell or three cells – a vegetative cell and the two male gametes (*Figures* 2a, b). In two-celled pollen, the generative cell divides to form the two male gametes during pollen tube growth in the pistil. Pollen wall is very complex; it is made up of two layers (*Figure* 2c). The inner intine, comparable to the cellulosic wall of any other plant cell and the outer exine made up of highly resistant material, the sporopollenin. Both intine and exine contain extracellular components which are highly heterogeneous; they contain proteins, glycoproteins, carbohydrates, lipids and few other compounds in small quantities.

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Figure 2. (a–c). Structural details of 2- and 3-celled pollen grains. A part of the pollen wall is enlarged to show the structure of the pollen wall and the location of extracellular components in the intine and exine. The components are present in radially elongated tubules in the intine and on the surface of the exine depressions (ecc = extracellular components). (c, d). ecc on the surface of the stigma localized by cytochemical method. (c). Dry stigma of *Zephyranthes*. One of the papillae is focussed to show ecc as a dark thin lining (arrow) on the surface of the papilla. (d). Wet stigma of *Vigna*. Dark coloured products in the exudate represent ecc (arrow).
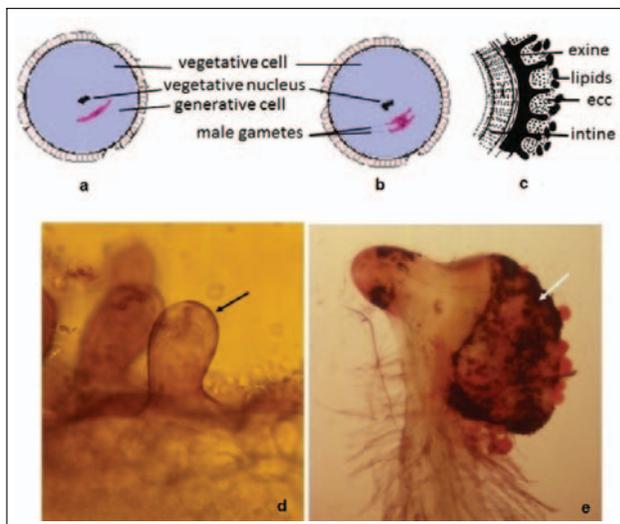
The stigma shows great variation in its morphology. Stigmas of different species can be grouped into two categories; the dry type in which the stigma surface is free from any visible exudate and the wet type in which the surface is covered with exudate of varying quantity. Each of them can be divided into several groups. Irrespective of its morphological variations, stigma surface invariably contains extracellular material, similar to those present in the pollen wall. In the dry type of stigma, they form a thin lining on the stigmatic papillae (*Figure* 2d) and in the wet type, they are present as a part of the exudate (*Figure* 2e).

The style is also basically of two types, solid and hollow (*Figures* 3a–f). In the former, a solid strand of transmitting tissue with large intercellular spaces connects the stigma with the ovary. The intercellular spaces contain extracellular components secreted by the cells of the transmitting tissue. In hollow style, a canal bordered by one or a few layers of glandular cells – canal cells, connects the stigma with the ovary. Here also, extracellular components fill up the whole canal or the inner lining of the canal cells. Pollen tubes invariably grow through the intercellular spaces of the transmitting tissue (solid styles) or through the stylar canal on the surface of the canal cells (in hollow styles) (*Figures* 3b, c, e, f).
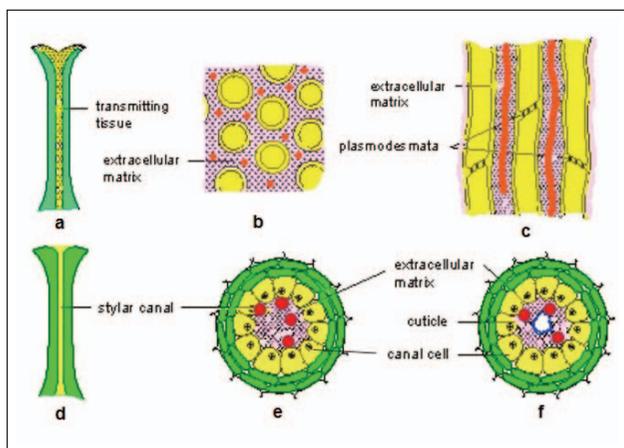
Figure 3. Diagrams to show cellular details of the solid (**a–c**) and hollow (**d–f**) styles. (**a**) and (**d**). Longitudinal sections of the stigma and style. (**b**). Transverse section of solid style to show cells of a part of the transmitting tissue (yellow) with large intercellular spaces filled with extracellular matrix. Red dots in the matrix represent transversely cut pollen tubes. (**c**). Longitudinal section of a part of the transmitting tissue of a solid style to show elongated cells with plasmodesmatal connections at their tips and extracellular matrix making continuous channels through which pollen tubes (red coloured) grow. (**e**) and (**f**). Transverse sections of hollow styles with extracellular matrix filling the canal (**e**) and the inner surface of canal cells (**f**). Red dots in the matrix represent transversely cut pollen tubes.

Pollen-Pistil Communication

The events that occur from pollen germination until its entry into the embryo sac are referred to as pollen-pistil interaction and play a significant role in pollen selection and their screening. Any selection has to involve extensive communication between the partners. Plants have evolved an effective communication system through chemicals between the pollen grains and later the pollen tube, and the tissues of the pistil. There is a continuous dialogue between the partners throughout pollen-pistil interaction. The chemicals involved in the initial communication are present in the pollen grain wall and on the surface of the stigma. During pollen tube growth, the communication takes place between the chemicals released from the tips of the growing pollen tubes and those present in the intercellular spaces of the transmitting tissue (in solid styles) or in the stylar canal (in hollow styles).

Screening for Heterospecific and Homospecific Pollen

As pointed out earlier, pollen grains deposited on the stigma may be homospecific or heterospecific depending on the visits by the pollinator species. Therefore, the pistil has to distinguish heterospecific pollen from homospecific pollen; it should inhibit heterospecific pollen from reaching the ovules and facilitate homo-

specific pollen to enter the ovule and embryo sac. The pistil can be visualized as the residence of the female parent with a series of locked doors, one at the level of the stigma and others along the corridor in the style. For successful completion of post-pollination events, the pollen has to have the matching key to open each of these locks.

Evolutionary divergence is the basis of speciation. To begin with, any two diverging species being closely related, continue to have several matching keys to open the locks of the pistil. As evolution progresses, they become more and more distantly related and lose more and more keys to the locks present in the pistil of the parent species. Depending on the extent of divergence, heterospecific pollen grains are inhibited at the level of the stigma or at some level in the style depending on the extent of loss of the keys. Thus, the rejection of heterospecific pollen is largely passive. However, when the pollen grain is from a very closely related species, the pollen may still have all the matching keys to the locks of the pistil. Such a pollen grain may germinate and reach the ovule. Some of them may even achieve fertilization but their embryos generally abort, thus preventing the formation of interspecific hybrids.

Homospecific pollen grains have all the keys for the locks present in the pistil. They get hydrated on the stigma and release pollen wall components on to the stigma surface. The pollen wall components act as visiting cards. The surface components of stigma are able to read the visiting cards and recognize the pollen grain as of the same species. The stigma and style then activate all the processes that facilitate pollen germination and pollen tube growth in the pistil without any hindrance (*Figures* 4a, b).

Screening for Self-Pollen

Unlike animals, majority of flowering plants are bisexual[4]. There are ample opportunities for the pollen of the same plant landing on the stigma. This results in inbreeding depression as a result of accumulation of lethal genes. It also reduces genetic variabil-

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[4]Each flower produces both male (stamens) and female (pistil) partners.
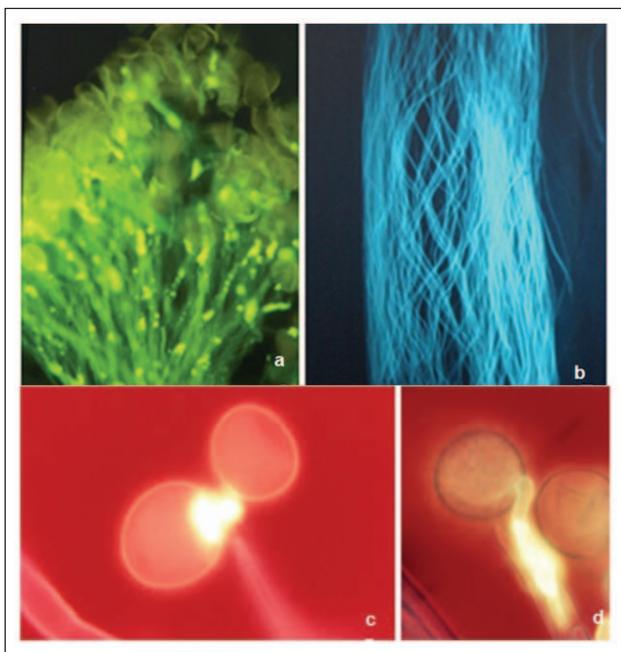
Figure 4. Fluorescence micrographs of stigma **(a)** and style **(b)** of tobacco pollinated with the right type of pollen (compatible pollen) to show profuse pollen germination on the stigma and growth of a large number of pollen tubes in the style. **(c)**, **(d)**. Fluorescent micrographs of the stigma pollinated with self-pollen in a self-incompatible grass species. Pollen grains are recognized as incompatible and inhibited at the level of germination itself **(c)** or soon after entering the stigmatic lobe **(d)**.

ity resulting in the loss of adaptability to changing environment. Therefore, plants have evolved a number of devices, explained below, to discourage or even prevent inbreeding and encourage outbreeding.

1. Herkogamy: The anthers and the stigma are located at different levels in the flower so that the chances of self-pollination is minimized.

2. Dichogamy: Shedding of the pollen grains and receptivity of the stigma (ability to facilitate pollen germination) is not synchronous; pollen grains are shed before the stigma becomes receptive or alternately the stigma becomes receptive before the pollen grains are shed. Even if some self-pollen grains land on the stigma, they cannot germinate as the stigma is not receptive.

3. Self-incompatibility (SI): This is a genetic mechanism in which self-pollen grains are effectively recognized and ac-

tively inhibited before they reach the ovules. Many plant species, particularly cultivated species have become self-compatible as a result of human selection for assured fruit set; in such species even self-pollen can function. SI is considered as the primary breeding system and self-compatibility as a derived condition. SI is controlled by multiple alleles termed S_1, S_2, S_3... S_n at one locus, in most of the plants. When the allele present in the pollen matches with one or both the alleles present in the pistil, as it happens in self-pollination, the pollen grains are inhibited at the level of the stigma or at some level in the style before they reach the ovules (*Figures* 4c, d).

4. Unisexuality: Here the male and female flowers are seperate. When the flowers are unisexual, male and female flowers may be located on the same plant (monoecy) or on different plants (dioecy). In dioecy male and female plants are separate as in a majority of animals. Unlike other outbreeding devices in which there is a possibility of self-pollen landing on the stigma, there is no possibility of inbreeding in dioecy.

Amongst the above outbreeding devices, herkogamy and dichogamy are effective in preventing autogamy (pollination with pollen of the same flower) but not very effective in preventing geitonogamy[5] as the pollinator can bring pollen from other flower of the same plant which is in the male phase and deposit on the receptive stigma. Self-incompatibility and dioecy prevents inbreeding completely.

The analogy given to visualize inhibition of heterospecific pollen can be extended to self-pollen also. Self-pollen grains do have all the matching keys to the locks present in the pistil at the level of the stigma as well as the style. However, once the pistil recognizes that they are self-pollen grains, it activates physiological responses to bolt these doors from behind. When the door present in the stigma is bolted, germination or entry of pollen tubes into the stigma is inhibited. When the doors present at the level of the

[5]Pollination with pollen of another flower of the same plant.

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style are bolted, pollen grains may germinate, but pollen tubes are inhibited in the style. Often, self-incompatible plant becomes self-compatible as a result of mutation of the *S* allele in the pollen or the pistil. When this happens, the pistil is unable to recognize self-pollen and hence it does not activate the processes to bolt these doors thus allowing the pollen to pass through. Also, under certain conditions of environmental stress, screening of self-pollen becomes weak allowing them to get through. There has been a lot of progress in recent years in identifying genes that determine SI, their products and their location in the pollen and in the pistil. In some species, the mechanism of recognition and inhibition of self-pollen is also known.

Once the right type of pollen grains are recognized by the stigma, a signal is transmitted to the ovary to initiate the processes to facilitate pollen tube reception in the embryo sac. In some of the orchids even the initiation of ovule differentiation in the ovary requires pollination signal (see *Box* 1).

Another aspect that has attracted the attention of reproductive biologist since long is the controlling mechanism that determines the direction of pollen tube growth in the pistil from the stigma to the ovary (see *Box* 2).

Box 1. Pollination Stimulus is Needed for Ovule Differentiation in Some Orchids

In a majority of flowering plants, the ovules are fully differentiated by the time the flowers open. Interestingly, in few orchids, ovule development is not even initiated at the time of pollination; in such species ovules differentiate only after pollination. Although pollen tubes reach the ovary in about two weeks after pollination, fully differentiated ovules are formed only by about 10 weeks after pollination. Until then, pollen tubes cease growth and hibernate in the ovary. Once the ovules are fully differentiated, pollen tubes initiate growth again, enter the ovules, and release the male gametes for fertilization. The mechanisms responsible for cessation of pollen tube growth in the ovary and re-initiation of their growth are not yet known.

Box 2. Directional Growth of Pollen Tubes in the Pistil and their Entry into the Ovule

Flowers may be erect or bent to different degrees or completely inverted. Irrespective of the orientation of the flowers, pollen tube growth always follows the same pathway, from the stigma to the ovary. Since long, botanists thought that there is a chemotropic gradient in the style from the stigma to the ovary and pollen tube growth follows this gradient. During 1960s and 1970s some observations indicated that calcium is the chemotropic gradient. Subsequent studies, however, did not support this concept. The consensus now is that the orientation of the cells of the transmitting tissue (in solid styles) with large intercellular spaces forming continuous channels (see *Figure* 3) and the morphology of the canal cells (in hollow styles) guide the pollen tubes towards the ovary.

However, when once they reach the ovary, they have to turn by about 90° to enter the ovules (see *Figure* 1a) A number of elegant studies in recent years have shown that the embryo sac is necessary for changing the direction of the pollen tubes to enter the ovule. The ovules in some mutants where some of the ovules do not have embryo sac, fail to receive pollen tubes. The synergids surrounding the egg are the source of the signal for the pollen tubes. The genes involved in this process and their products have also been identified in a few species.

Competition Amongst the Right Pollen

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Pollen grains which are most vigorous, that is, those which germinate early and those in which pollen tubes can grow fast, reach the ovary early and fertilize all available ovules.

Even after selecting the right type of homospecific pollen, the pistil does additional screening to select only the best amongst them to fertilize the ovules. The number of ovules in the ovary of different species are highly variable from one to hundreds. Under optimal pollination conditions, the number of the right pollen grains landing on the stigma is many times more than the number of ovules available for fertilization. This is especially true in species with one or a few ovules. Therefore, not all the right pollen grains are able to find a partner. The pistil imposes competition even amongst the right type of pollen to select the best available pollen for fertilization. Pollen grains which are most vigorous, that is, those which germinate early and those in which pollen tubes can grow fast, reach the ovary early and fertilize all available ovules. Less vigorous pollen grains may be able to reach the ovary later but by the time all the ovules present in the ovary are fertilized. There are several evidences to indicate that such competition improves the fitness of the progeny. There is no possibility of such competition in lower groups of plants. Any male

gamete coming in contact with the female gamete will succeed in fertilization, irrespective of its vigour. There are many animals in which males have to prove their vigour by succeeding in the fight amongst themselves to gain access to the females.

Conclusions

The pistil is able to recognize and select the right type of pollen grain and facilitate its passage through the tissues of the stigma and style. It rejects wrong pollen grains at the level of the stigma or the style. Further, even amongst the right type of pollen, the pistil creates competition and selects the most vigorous pollen grains for fertilization. Thus, the selection of the male partner in

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Box 3. *In vitro* Pollination and Fertilization

In flowering plants, fertilization is not readily accessible for experimentation as it takes place deep inside the embryo sac covered by the tissues of the ovule and ovary. Because of this inaccessibility, information on fertilization in flowering plants has lagged far behind when compared to that in animals and some of the lower groups of plants. Realization of *in vitro* fertilization similar to that achieved in animals has been a dream of experimental embryologists since long. In 1960s, a beginning was made in this direction at the University of Delhi by late Prof P Maheshwari and his associates. They were able to achieve fertilization following pollination of cultured ovules. They also showed that through ovule-pollination, fertilization can be achieved by both self-pollen and interspecific pollen, which are inhibited in the pistil following pollination on the stigma. Further progress to accomplish *in vitro* fertilization, using isolated eggs and sperms was hampered at that time for lack of effective techniques for isolating male and female gametes.

Following rapid progress in protoplast technology and somatic hybridization during the 1970s and 1980s, isolation of sperms and egg cells became a reality in several plant species. By 1990, all the basic requirements needed to attempt *in vitro* fertilization were available. The pioneering success in achieving *in vitro* fertilization using isolated egg and sperm cells was achieved by Kranz and his associates at the University of Hamburg, Germany. They were able to achieve *in vitro* fertilization in maize and raise plantlets from *in vitro* fertilized zygotes. When transplanted to the soil, these plantlets grew into fertile plants. Subsequently the technique has been extended to several other species. Using *in vitro* fertilization, considerable progress is being made towards understanding several fundamental aspects of fertilization and initial growth of the embryo. It has been possible to achieve fertilization *in vitro* between species and even genera with considerable ease. These studies also support the concept that the egg has no say in rejecting the male partner when once the pistil permits it to reach the embryo sac.

The selection of the male partner in flowering plants is the monopoly of the maternal parent.

flowering plants is the monopoly of the maternal parent. Male (pollen grain) and female (ovule) partners have no say in the selection. The ovule has to accept whatever pollen tube the pistil allows to reach the ovule. This conclusion is also supported by a number of experiments related to *in vitro* fertilization (see *Box 3*). Such rigorous screening of male partners has not evolved in any other group of plants. Evolution of biotic pollination and of the pistil, which imposes competition even among the right type of pollen grains are considered to be the main reasons for the evolutionary success of flowering plants when compared to other groups of plants.

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