Magnolia virginiana: Ephemeral Courting for Millions of Years

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The Arnold Arboretum's magnolia collection currently holds 157 accessions of native and non-native magnolias.

At the end of the seventeenth century, Henry Compton, the Bishop of London and a man known for his passionate love of gardening, sent the Reverend John Banister on a missionary trip to the New World. Banister arrived in Virginia in 1678 and, in addition to his clerical work, collected many new plant species for Bishop Compton. Among these was a tree species never before seen in Europe, specimens of which were planted and flourished near Fulham Palace, the Bishop's residence. After observing these specimens, botanist Philip Miller recorded the first written reference to this species in his book, *The Gardeners Dictionary.* Miller was not only the chief gardener of the Chelsea Physic botanic garden, the second oldest in Britain, but also a plant collector and conservationist who cultivated many exotic species. Interestingly, the garden was visited by Linnaeus during his trip to England in 1736. Miller was influenced by the new system of classification that Linnaeus proposed, to the extent that he organized the garden following the Linnaean system. In 1753, Linnaeus included for the first time in his world renowned work *Species Plantarum* the specimens that Miller observed at Fulham Palace, with the name *Magnolia virginiana.*

Magnolia virginiana is a woody flowering plant native to the east coast of the United States, growing from Florida and Texas to Pennsylvania, New Jersey, and Massachusetts, the northernmost point in its range. Because of its preference for marshes and moist areas, *M. virginiana* was called swamp bay magnolia or swamp laurel. It is most commonly known as sweetbay magnolia and has been integrated into the local lore and culture where it grows. For example, early inhabitants made a tincture from its bark to use in chronic rheumatism and a medicine to treat coughs, colds, and fevers.

The Arnold Arboretum hosts an important collection of both native and non-native magnolias. In 1919, Charles Sargent studied the distribution of *Magnolia virginiana* from North Carolina to Florida and Texas, noticing for the first time the southern form, *M. virginiana* var. *australis* (note that current taxonomy lumps this variety back into *M. virginiana*). In the *Bulletin of Popular Information,* Sargent described the flowering of *M. virginiana,* then also listed as *M. glauca,* in this way: "… the flowers are small, cup-shaped, creamy-white and delightfully fragrant, and continue to open in succession from the middle of June until August. In all North America there is not a more delightful shrub to plant in the garden, or one that will give larger returns in beauty and fragrance.…" Its form, flowers, and attractive leaves (glossy dark green with silvery white undersides) make this species valuable as an ornamental tree in gardens, parks, and other areas. *Magnolia virginiana* specimens at the Arboretum bear flowers continuously throughout summer, pervading the evening air with their marvelous sweet, lemony fragrance.

Magnolias and the Evolution of Flowers

All magnolias belong to the large plant family Magnoliaceae, within the relatively ancient order Magnoliales. Until the end of the last century, plant biologists considered Magnoliales to be among the oldest flowering plants, based on the morphology of the flowers, the characteristics of the pollination process, and some aspects of the internal anatomy of the wood. However, with the addition of research using molecular markers, and a vastly improved fossil record of the earliest flowering plants, it is

NANCY ROSE

Magnolias bear conelike aggregate fruits. At maturity, the individual follicles open, revealing seeds covered with scarlet arils. Seen here is a fruit of *M. virginiana* × *virginiana* 'Milton', accession 779-87.

now known that the first flowering plants were mainly shrubs, lianas, and aquatic plants such as water lilies.

While Magnoliales diversified early compared to more derived angiosperms (the latest estimations date the emergence of the order around 95.5 million years ago), they are now considered ancient but distinctly specialized flowering plants. The fossil record establishes that magnolias have remained relatively unaltered for millions of years, and have been widespread in tropical and subtropical climates. The early expansion and diversification of flowering plants led to the colonization of all types of environments. The biological forces behind this rapid diversification have posed a challenge to plant biologists for decades. However, it appears that the development of novel reproductive structures—flowers—played a significant part.

Before the emergence of flowering plants, gymnosperms (conifers, ginkgos, etc.) domi-

nated terrestrial ecosystems. Both gymnosperms and angiosperms are seed plants, and seeds are the product of fertilized ovules. Herein lies the main difference between both plant groups: while gymnosperms have their ovules exposed or "naked," flowering plants developed maternal tissues to shelter their ovules. Despite the more complicated new arrangement of the ovules, flowers increased the efficiency of sexual reproduction and opened up many new opportunities for coevolutionary relationships between flowering plants and insect pollinators. Flowers became key evolutionary innovations, opening a door for innumerable new reproductive strategies that can be seen throughout the great diversity of flowering plants.

Sexual Reproduction and Flower Receptivity

In 1694, Rudolf Jakob Camerarius published his discovery that plants undergo sexual reproduction. Flowers are the reproductive parts of angiosperms, performing two main functions: they act as a showy display to attract pollinators, and they bear the germ lineages (gametes). The germ lineages are housed inside of a number of tissues specialized for either dispersal (for the male gametes) or protection (for the female gametes). The contact of both male and female gametes in most flowering plants involves the transfer of pollen between individuals, which is a task often carried out by insect pollinators. The first major studies on plant pollination were done by Kölreuter (1733–1806), but Darwin was also interested in pollination and breeding systems, writing two books on the the topic: one on insect pollination of orchids (*Fertilisation of orchids*, 1862) and another on selfing and outcrossing in plants (*The effects of cross and self fertilisation in the vegetable kingdom*, 1876). The end of the nineteenth century and the beginning of the twentieth century saw an upsurge of studies on the reproductive biology of flowering plants, showing that the amazing diversity of flower morphologies are directly tied to the myriad of pollination and fertilization processes that flowers undergo to produce offspring.

A bisexual flower bears both male and female germ lineages in its reproductive organs. The pollen grains that will produce male gametes

are formed within the anthers. The female gametes are housed within special structures called female gametophytes within multi-layered structures (the ovules), which are further enclosed by the pistil tissues. The whole of the female reproductive structure is known as a gynoecium. The typical gynoecium is composed of three contiguous reproductive tissues, from the apical part to the base of the gynoecium: the stigma, the style, and the ovary. The first contact between male pollen grains and female flower tissues occurs on the stigma, a specialized receptive tissue exposed at the tip of the gynoecium. On the stigma, pollen grains hydrate and then germinate, producing a pollen tube containing the two sperm cells that elongates in a tip-oriented growth within the pistil tissues to reach the ovules. Once a tube penetrates an ovule, the two male gametes are discharged into the female gametophyte where a process known as double fertilization takes place. One of the sperm cells fuses with the egg cell, while the other one fuses with another female gamete. The former fusion will produce the embryo while the latter will give rise to the endosperm, which becomes the tissue that nourishes the developing embryo. In general terms this double fertilization process to form a new generation is shared by all flowering plants.

As might be imagined, coordinating all of the events between mothers and fathers in flower-

Bees visiting a flower of *Magnolia grandiflora* at the Arnold Arboretum. Nitidulidae beetles are considered the natural pollinators of magnolias, but bees are also possible pollinators, though their effectiveness as magnolia pollinators is still under debate.

ing plants is a highly sophisticated and complex process. In each species, a dialog between male and female components of the reproductive equation is carried out by species-specific molecular interactions. The gynoecium of flowers, far from being a passive actor during the pollination process, plays an active role in the recognition and regulation of pollen tube growth on its journey through the pistil. On one side, the pistil tissues have the ability to distinguish between pollen grains from different species and impede their germination, in a mechanism known as interspecific incompatibility. On the other hand, germination of very similar pollen grains is also blocked in many species, and this is called self-incompatibility, which prevents self-fertilization and promotes a mixture of different genetic material from individuals of the same species (remember, Darwin wrote a whole book about this topic!).

The recognition of pollen grains/tubes by maternal tissues of flowers has been revealed at the molecular level. Pollen grains/tubes bear proteins that are unique to the species, acting as molecular fingerprints. Those proteins can be recognized by counterparts in the gynoecial tissues. Depending on whether they can interact or not (and thus whether or not the pollen grain is acceptable), it allows a maternal flower tissue to allow or deny pollen tube elongation. Therefore, the reception of pollen grains is decisive in the fertilization process. However, the stigma is not always ready, and pollen grains have to reach the stigma at the right time—when this tissue is mature. If a pollen grain lands on a stigma before or after the surface is receptive, it is not likely to germinate and thus fertilization is not achieved (no seed is formed). The time frame in which a stigma allows pollen germination is referred to as stigmatic receptivity. This parameter varies between different plant groups and acts as an important filter during plant evolution—and as will be seen in *Magnolia virginiana,* the dance between male and female requires some remarkably interesting dialog.

Flower Receptivity in *Magnolia virginiana*

Magnolia virginiana flowers provide an excellent arena to study both the process and evolution of sexual reproduction in plants. As a member of an ancient lineage of flowering plants, *Magnolia virginiana* has many characteristics that are thought to be relatively ancient in flowers. At the time of pollination, the central and most distal part of the flower looks conelike. This is the female part of the flower and is made up of numerous carpels, each of which terminates in a stigma which will ultimately receive pollen. Each stigma connects directly with a single ovary. Below the female portion of the flower are a very large number of colorful and showy stamens, the organs that produce pollen.

The presence of male (stamens) and female (gynoecia) organs in a single flower can lead to a very high probability of pollen moving

Magnolia virginiana **protogynous flowering cycle. (A) The first flower opening (the female phase) occurs the first evening, and the stigmas (maternal tissues) are exposed to receive pollen grains (containing male gametophytes). (B) Four to five hours later, the inner tepals close and form a chamber enclosing the stigmas; this lasts for around 24 hours. (C) The cycle is completed the second evening with the male phase; the flower reopens at the same time that anthers open and shed pollen. This protogynous cycle (the female function precedes the male function) is a temporal separation that prevents the flower from self-pollinating. However, flowers opened at both male and female phases occur on the same plant, so cross-pollination between flowers of the same plant is possible.**

within a flower—self pollination (the equivalent of marrying a very close relative). But in *Magnolia virginiana* (and in other *Magnolia* species), a temporal separation of the activities of the male and female parts of individual flowers acts to diminish the possibility of inbreeding. The temporal separation of both sexes is manifested as a protogynous flowering cycle (proto = first, gynoecium = female parts, or "ladies first"), and is delimited by floral movements. As a result, the female phase precludes the male phase and they do not overlap, thus creating a two day flowering cycle. Flowers open the first day at dusk (opening takes around 20 minutes and can be observed by just staring patiently at the right flower) as females with wet, sticky stigmas that receive pollen grains, and then close when night falls. They remain closed until the evening of the following day, when flowers reopen in the male phase, at which point stamens shed pollen. During the stage in which the flower remains closed, the flowers generate heat in order to give shelter to their main pollinator, beetles. The ability for flowers to produce heat is common to all magnolias (and other members of the family), and so is thought to be an ancestral character for the lineage. Other pollinators, such as bees, have been observed to act as pollinators for these plants, but little is known about how effectively they transfer pollen from flower to flower.

The timing of flower movements affects reproductive performance and points to the importance of a rhythm. This rhythm could be associated with pollinator behavior, in our case mainly bees and bumble bees, and possibly beetles. Our research project with *M. virginiana* at the Arnold Arboretum started with the observation of this cycle and pollinator inter-
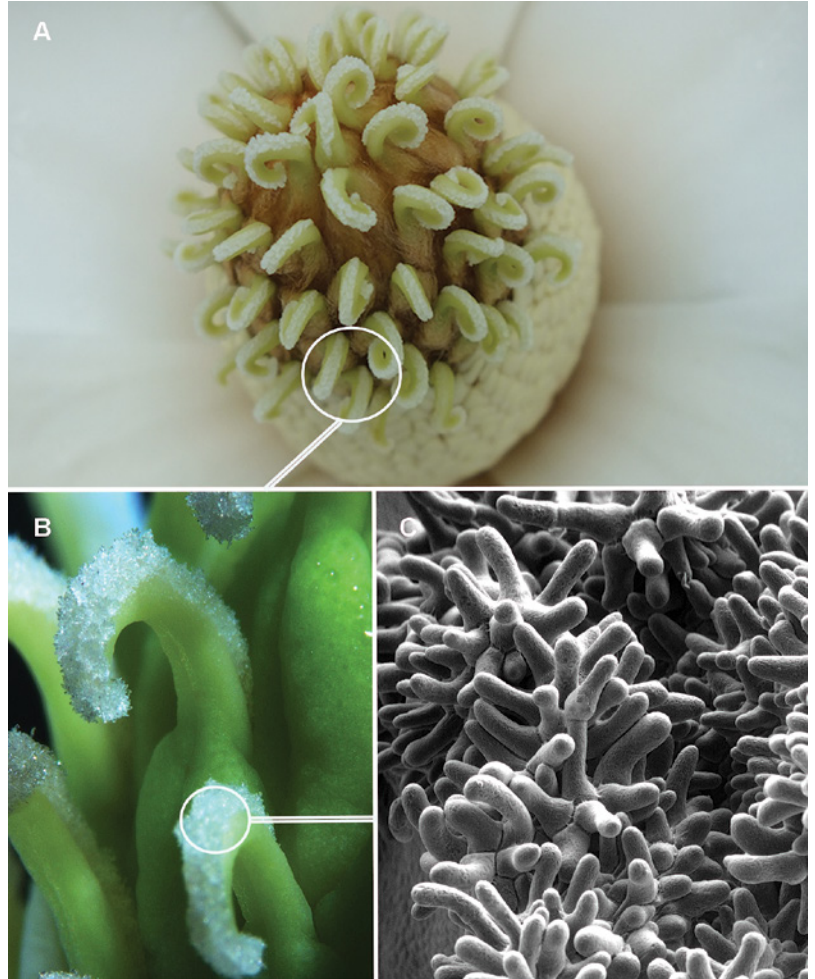
Female phase of *Magnolia* flowers. **(A)** A *Magnolia* flower shows multiple gynoecia at the first flower opening. **(B)** Detailed view of the hooked stigmas that have bright, sticky surfaces ready to receive pollen grains. **(C)** Scanning electron micrograph of the stigma surface in *Magnolia virginiana*, showing the fingerlike cells (papillae) that form an intricate network for pollen grain gathering.

actions, recorded with time-lapse photography under controlled conditions The resulting video is available online:

http://www.youtube.com/
watch?v=Ja3GJyJ98uI

A few studies in the reproductive biology of the genus *Magnolia* suggested that the period of female receptivity was connected to these flower movements, but exact timing was unknown. Our investigations in the Arboretum with controlled pollinations in the laboratory confirmed those suggestions, and showed that stigmatic receptivity is remarkably short.

Stigmas of *M. virginiana* are only ready to allow pollen grain germination for a few hours following the first flower opening. As soon as the flowers close, the stigmas lose the capacity to allow pollen grain germination.

With a better understanding of the time frame of stigma receptivity in *M. virginiana*, the question remained as to what molecules are involved in the communication between the male pollen grains and the stigmatic tissue. Our previous work with apple (*Malus*) flowers established what factors are involved in the reception and acceptance of compatible pollen (pollen that is not being rejected), and what their effect was on fruit production. We found that a group of glycoproteins (complex molecules
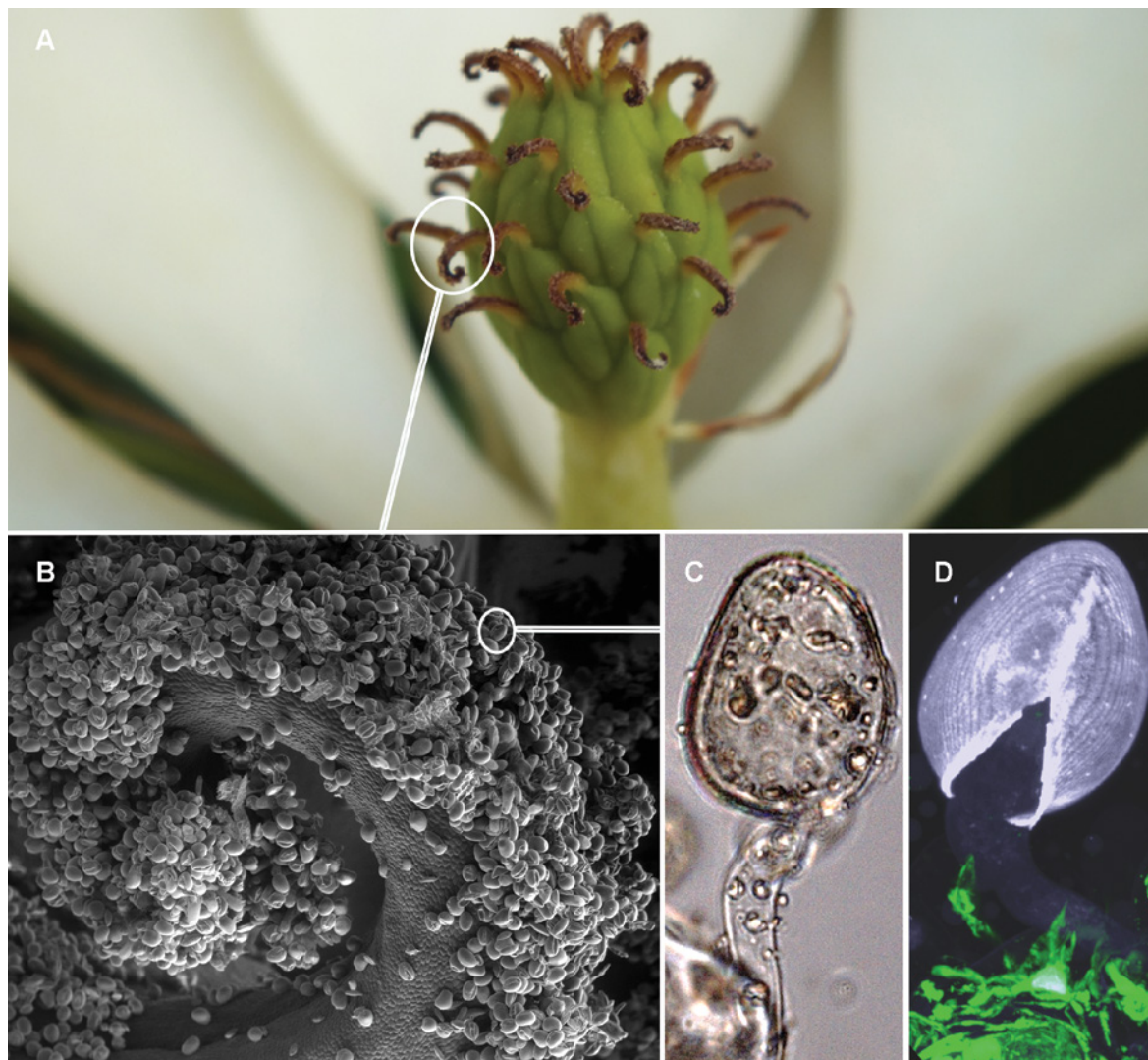
Male phase of *Magnolia virginiana* flowers. **(A)** Stigmas showing a brown coloration the second day of flower opening. **(B)** Scanning electron microscopy of a hand-pollinated stigma of *Magnolia virginiana*: many pollen grains are seen between cells of the stigma surface before germination. **(C)** Light micrograph of a single germinated pollen grain on the *Magnolia* stigma showing the pollen tube and other associated materials on its surface. **(D)** Laser confocal microscope view of a germinated pollen grain on the stigma of *Magnolia virginiana*: the white part is the pollen grain coat (exine), opening at the bottom to allow pollen tube emergence. The fluorescent green shows glycoprotein revealed by using the immunolocalization technique.

composed of two organic units: small amino acid backbones, and large sugar moieties where the functional capacity resides), which have numerous functions in plants such as acting as mediators in cell-to-cell communication, were secreted towards the apple stigma surface precisely at the time of receptivity. Furthermore, these glycoproteins are known to control plant cell elongation processes, and could be involved in pollen tube elongation. Their conspicuous presence in female tissues of apple flowers prompted us to wonder whether ancient lineages of angiosperms (flowering plants) would use similar molecular mechanisms.

Microscopy evaluation of *Magnolia virginiana* stigmas showed that the nutrient movements in stigmatic tissues followed a precisely defined cycle, and that the secretory products on the stigma surface were mainly saccharides (short chains of sugars, based on the binding of individual units such as glucose or fructose). Furthermore, by using antibodies (immunolocalization) specific for the glycoproteins that were also present in apple stigmas, we detected these molecules during the short period of stigmatic receptivity in *M. virginiana.* This suggests that in *M. virginiana,* as in apple, specific glycoproteins mark the short time frame that flowers are able to allow pollen grain germination on the stigmatic surface. This work showed for the first time in a member of the Magnoliaceae that maternal tissues bear glycoproteins during pollen reception, and hinted at their involvement in pollen tube elongation towards the ovules.

Combined, all this data offers new perspectives on how different flowering plants control the production of offspring. The presence of common nutritive factors secreted from the female tissues at times of pollen reception in very distantly related species points to a possible conserved mechanism across all angiosperms. But also, it sheds light on the molecular crosstalk during initial stages of male–female interactions in seed plants. The stigma appears to be a unique tissue with a crucial function during the reproductive process.

Yet our results point to unresolved questions on the stigmatic behavior in other primitive flowering plants, where few studies have been performed. Understanding the molecules that
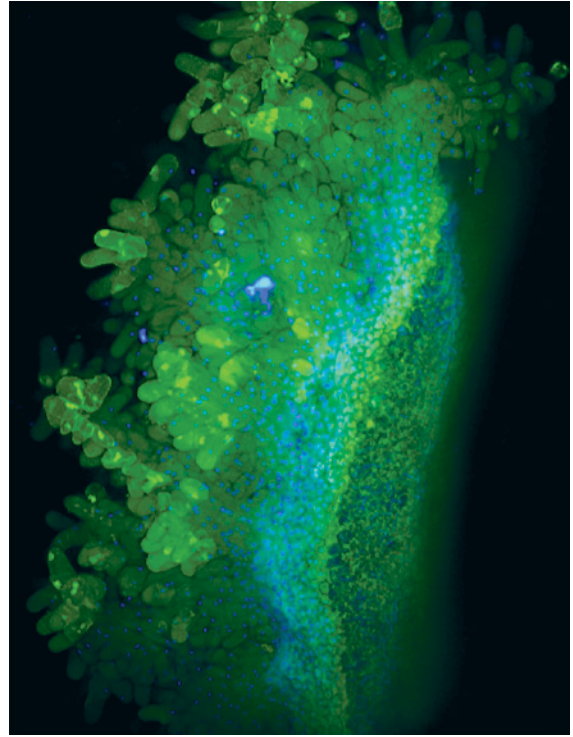
In this image of a *Magnolia virginiana* stigma surface the cell nuclei appear in blue and glycoproteins in fluorescent green. The fluorescence of glycoproteins is achieved by immunolocalization, a technique that tags specific parts of proteins with monoclonal antibodies that specifically bind them. A secondary antibody bearing a fluorescent label is then added to bind the primary one, thus allowing observation of the protein location in plant tissues under a fluorescent light/laser microscope.

mark receptivity can give insight into the complex mechanisms that flowers have to recognize the male counterparts and promote their growth. In order to figure out how these mechanisms may have influenced the evolution of this lineage, we plan to compare how different female tissues of the style and ovary can control pollen tube growth, and we plan to include a wider range of taxa in this study. The finely-tuned mechanism of flower receptivity in *Magnolia virginiana* displays the amazing capacity for precision during angiosperm reproduction. The coordination of pollinator activity, flowering cycle, and molecular performance offer an effective system in the time frame of only a few hours for possible interaction.

Long ago, Bishop Compton and many royal European families recognized the beauty and pleasant scent of *Magnolia virginiana* flowers

The Evolution of Pollen Receiving Structures in Seed Plants

SHOWN HERE are illustrations of longitudinal median sections of different maternal tissues receiving pollen grains in seed plants. The associated cladogram shows the estimated time of emergence for general seed plant lineages (mya=millions of years ago).

The earliest group shown is the gymnosperms, which arose around 290 million years ago, and are characterized by naked ovules that have a liquid secretion at their ovule tips (the pollination droplet) directly catching pollen grains. Those pollen grains germinate following contact with ovule tissues. In contrast, angiosperms evolved around 243 million years ago, and most basal flowering plants had already developed maternal tissues surrounding their ovules. Among them, the apical part (the stigma) establishes the first contact between maternal tissues and paternal pollen grains. In the basal angiosperm lineages (Amborellales, Nymphaeales, and Austrobaileyales), the stigmas produce a copious secretion at their surface for pollen reception. More evolved but still relatively early divergent angiosperms show large stigmatic surfaces and a wet appearance, but lack a copious secretion. Pollen grains can develop different pollen tube lengths depending on the area of the stigma where they are deposited. Finally, in most evolved angiosperms (in a broad sense), stigmas tend to reduce their area, whereas larger styles developed, and a specialized central transmitting tissue is the arena for pollen tube elongation towards the ovules.

These illustrations emphasize the importance of the stigma during the first male–female recognition in flowering plants, but also the gradual physical separation between ovules and stigmatic tissues during flower evolution.
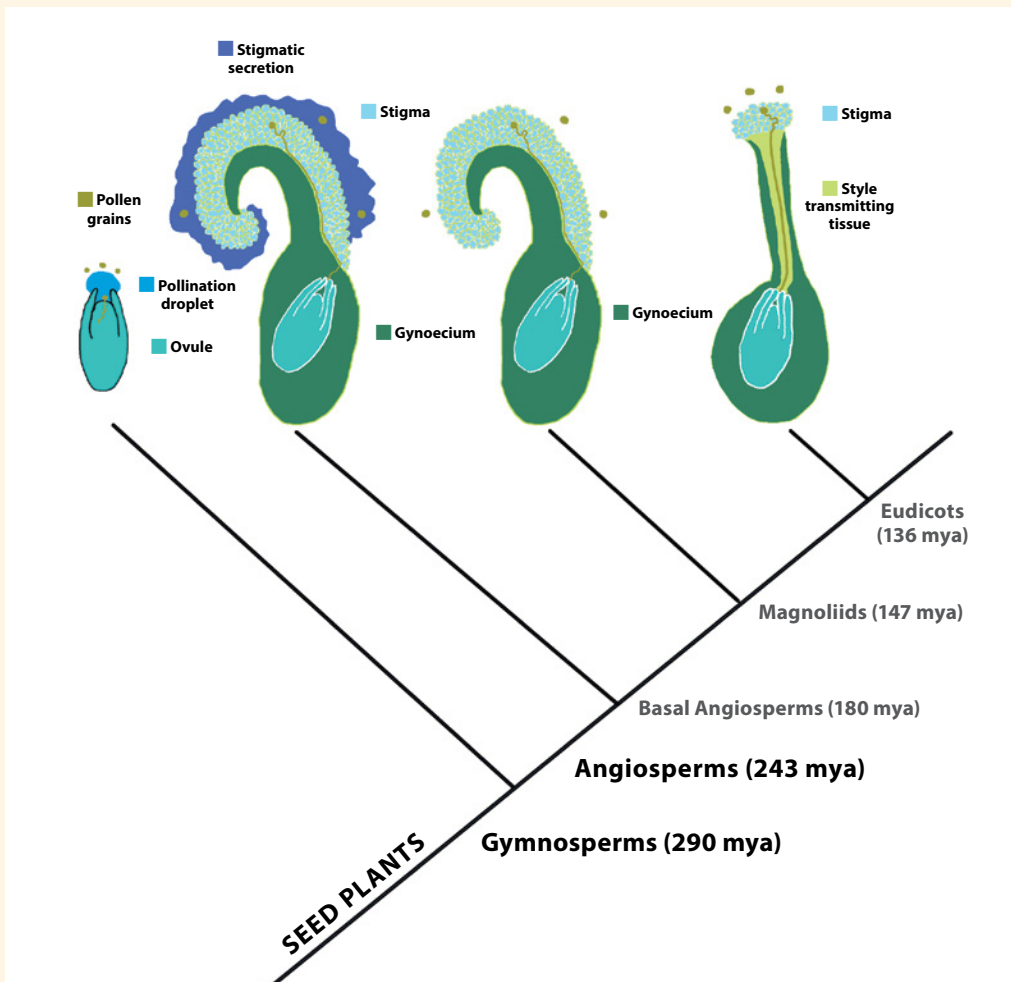
when they included this species in their palace gardens. However, they missed the equally remarkable story behind what was happening within those flowers: the impressive coordination of floral movements and molecular interactions that created the ephemeral female phase, a short time for a courtship repeated every blooming period for millions of years.

Acknowledgements

References

Camerarius, R. J. 1694. *De sexu plantarum epistola.* Tubingen.

Darwin, C. R. 1862. *Fertilisation of orchids.* London: John Murray.

Darwin, C. R. 1876. *The effects of cross and self fertilisation in the vegetable kingdom.* London: John Murray.

Friedman, W. E. 2006. Embryological evidence for developmental lability during early angiosperm evolution. *Nature* 44: 337–340.

Friis, E. M., P. R. Crane, K. R. Pedersen. 2011. *Early flowers and angiosperm evolution.* Cambridge: Cambridge University Press.

Hunt, D. (ed.). 1998. *Magnolias and their allies.* International Dendrology Society and the Magnolia Society. David Hunt: Milborne Port.

Linnaeus, C. 1753. *Species Plantarum.* Stockholm.

Losada, J. M. and M. Herrero . 2012. Arabinogalactan protein secretion is associated with the acquisition of stigmatic receptivity in the apple flower. *Annals of Botany* 110: 573–584.

Maheshwari, P. 1950. *An introduction to the embryology of angiosperms.* London: McGraw-Hill.

Millais, J. G. 1927. *Magnolias.* London: Longmans and Green.

Qiu, Y. L., B. Wang, J. Y. Xue, T. A. Hendry, R. Q. Li, J. W. Brown, Y. Liu, G. T. Hudson, Z. D. Chen. 2010. Angiosperm phylogeny inferred from sequences of four mitochondrial genes. *Journal of Systematics and Evolution* 48: 391–425.

Rankin, G. 1999. *Magnolia: a Hamlyn care manual.* London: Hamlyn.

Sargent, C. S. 1919. *Bulletin of Popular Information.* 5(9): 35.

Soltis, P. S., D. E. Soltis, M. W. Chase. 1999. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature* 402: 402–404.

Treseder, N. G. 1978. *Magnolias.* London: Faber and Faber.

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