

ON THE NATURE OF THINGS: ESSAYS
New Ideas and Directions in Botany

Illuminating the incredible journey of pollen

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Humans have a strange fascination with flowers. From the multi-billion dollar cut-flower industry where flowers are bred, grown, admired, and then thrown away to the houses of Christian Dior and Yves Saint Laurent, where floral extracts are used to make us smell less like mammals and more like plants, our flower obsession makes it easy to forget that they evolved not to beguile us, but primarily to facilitate pollen movement from anthers to stigmas. Despite a torrent of pollination studies since Darwin (1862), pollen movement, one of the most important aspects of pollination, remains one of its hardest to quantify (Minnaar et al., 2019a). The pollen journey from anther to ovule is like an obstacle course, with barriers, hurdles, twists, and turns (Inouye et al., 1994). With so many possible outcomes, it is difficult to determine why some pollen grains make it through the course but others get held up or lost along the way (Minnaar et al., 2019a). However, if biologists could label and distinguish cohorts of identical-looking pollen grains from separate flowers, and then recapture those labelled grains at different points along the obstacle course, we could begin to trace the complex pathway leading to pollen success or failure. Although molecular tools can determine the pollen parent of seeds, difficulties in sequencing pollen (but see Matsuki et al., 2007) and applying labels to many of them, mean that the fates of most pollen grains (>98% of which do not fertilize seeds; Harder and Thomson, 1989) remain mysterious.

Two breakthroughs provide us with new tools to follow grains. The first is the ability to determine the genotypes of individual pollen grains (Matsuki et al., 2007; Hasegawa et al., 2015). The second is the application of quantum dot nanotechnology as fluorescent

pollen markers (Minnaar and Anderson, 2019). Quantum dots (Q-dots) are crystals of semiconductor metals, so small (10,000 Q-dots span the width of a human hair) that they behave somewhat like atoms (Gammon, 2000). When exposed to ultraviolet light, their electrons jump excitedly between states, emitting super-bright photons of light (Ekimov, 1991). By fine-tuning Q-dot size, the color of the emitted light can be altered (Yoffe, 2001). Q-dots covered in a fatty-acid coating can then be attached to pollen grains simply by suspending them in solution and applying the solution to anthers using a pipette. UV illumination can then be used to distinguish pollen grains labelled with different colors after they have been deposited on stigmas or pollinators (Fig. 1). Q-dots have advantages over traditional dyes because unlike dyes, they adhere to individual pollen grains, allowing quantitative estimates of pollen movement. In contrast, dye particles are qualitative surrogates for pollen movement. Unlike other pollen-marking techniques (reviewed by Minnaar and Anderson, 2019), the simple application of quantum dots can be used in most flowers (except plants with very restricted anther openings), they seldom change the transfer characteristics of pollen grains, they can be immediately visualized, and they can be applied in the field.

WHY IS IT IMPORTANT TO TRACK POLLEN?

From an ecological and economic perspective, fruit production, seed production, and demography are often closely linked to pollen receipt and to the relative effectiveness of different pollinators.

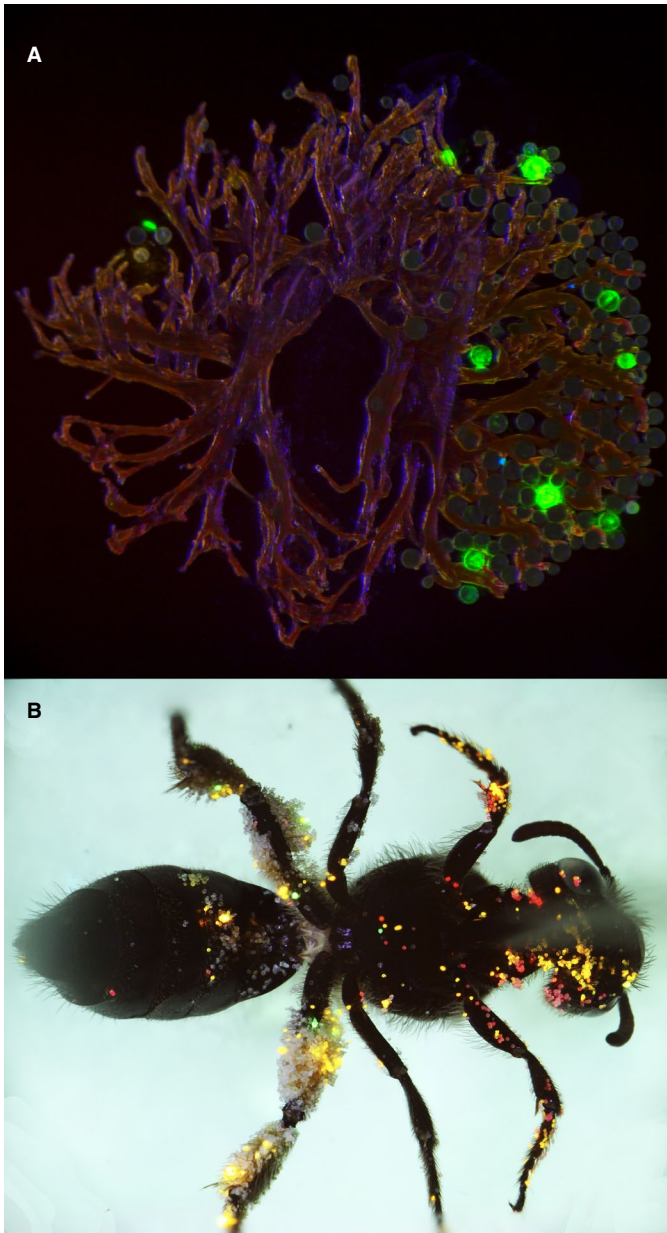


FIGURE 1. Q-dot labelled pollen as viewed in a fluorescence box. (A) Q-dot labelled pollen grains fluoresce green on a stigma harvested from an *Oxalis purpurea* flower after being visited by a sweat bee (B) that had previously visited several *O. purpurea* flowers with pollen labelled using different-colored Q-dots. The various-colored Q-dots can be seen on the bee's body. Photos: Corneile Minnaar.

Q-dots may help us directly determine the importance of different pollinators for wild and crop plants in terms of pollen grains deposited per visit, proportions of pollen deposited to pollen lost (efficiency), and how far pollen is moved.

FIGURE 2. Theoretical depiction of pollen export, receipt, and ovule fertilization for a small population of plants. (A) Illustration of how individual plants may export and receive pollen, resulting in differences in their fitness through male and female pathways. In this population, plants are assumed to have only 12 ovules each and are self-incompatible. (B) The realized fitness (seeds parented) is compared between male and female fitness pathways. A typical pattern is presented where female fitness is mostly maximized and limited to the number of ovules present in the plant, while male fitness varies widely. This variation in male fitness lays the platform for strong selection on traits that promote mating success. Illustrations: Corneile Minnaar.

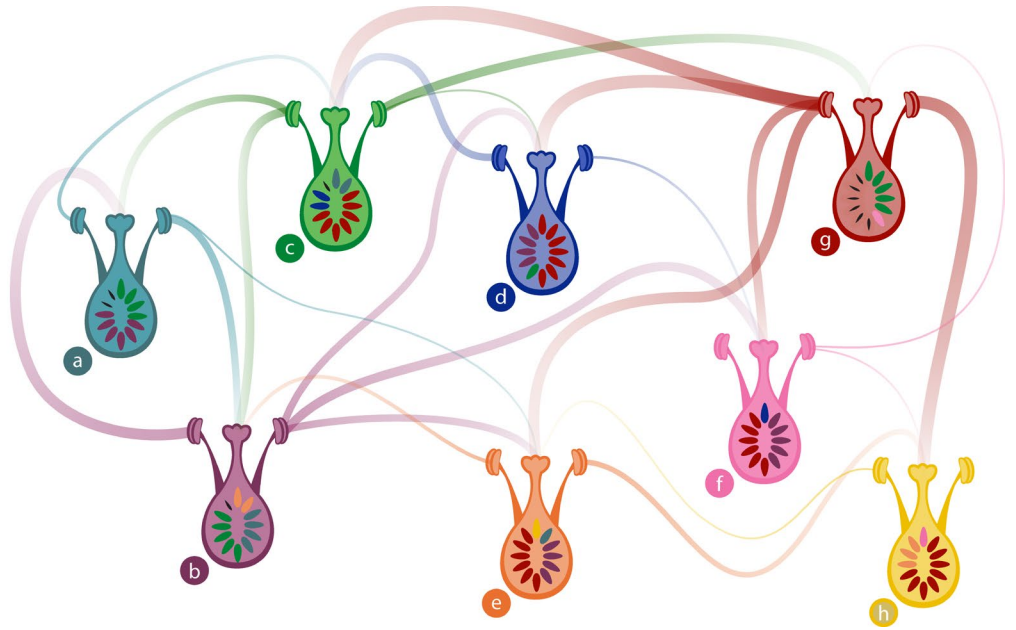
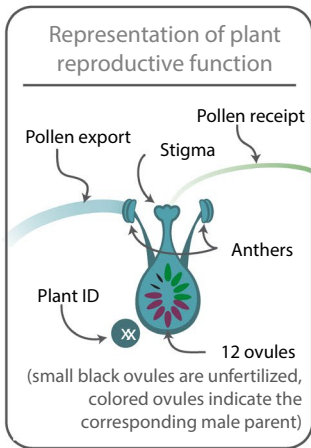
From an evolutionary perspective, hermaphroditic plants have two fitness pathways: male and female (Fig. 2). Both can potentially influence the evolution of flowers (Willson, 1979). The male pathway has been selected for mating success (mate quantity), primarily through mass export of tiny pollen grains (Bateman, 1948). On the female side, mate quantity is traded for offspring survival, and ovules are given more resources to increase germination success and survival. This extra investment means that fewer ovules are produced than pollen grains. In populations where flower visits are frequent, most ovules end up being fertilized (i.e., female mating success is similarly high for all individuals) because it takes only a few pollen grains to do so (Fig. 2). With so many pollen grains and so few ovules, the battle for mating success on the male side is much fiercer. The domination of these few ovules by a small number of individuals make male fitness more variable than female fitness (Bateman, 1948; Tonnabel et al., 2019) where some individuals have high male success and others have much lower male success (Fig. 2H). Consequently, an individual plant can have poor female fitness, but still have high overall fitness if it is a good pollen exporter (Fig. 2G).

But there are many factors that may influence the success or failure of pollen export. We may ask: Were flowers visited? Did pollen grains adhere to the flower visitors? Did floral visitors deposit pollen grains, and if not, why? Did deposited pollen grains make it to the stigma of a conspecific/receptive flower? Did they grow pollen tubes, and did they win pollen tube races? These are just some of the twists, turns, and barriers of a typical pollen journey, and each represents a unique opportunity for selection to act on floral or pollen traits (Minnaar et al., 2019a). Despite the relevance of the male pathway, difficulty in estimating siring success (the currency of male fitness) versus the comparative ease of counting seeds (the currency of female fitness) means that floral evolution has most often been studied through the lens of the female fitness pathway. The complex pollen export pathway remains a metaphorical black box, housing many unseen mechanistic components of selection, which we hope to illuminate.

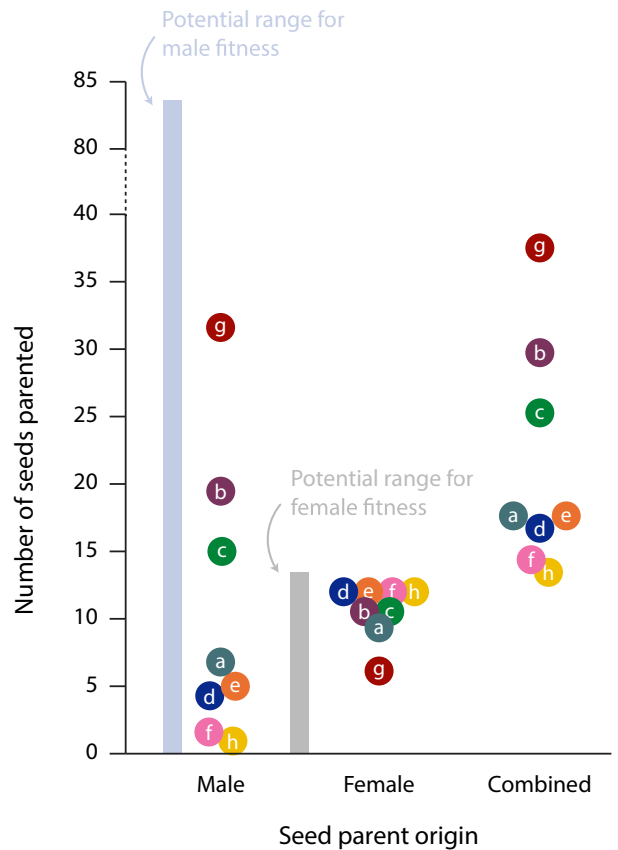
FUTURE DIRECTIONS

The darkest corners of this box relate to the fates of pollen grains after deposition on pollinators. We like to think of pollinators as buses that transport pollen grain passengers between stigmatic stations. However, these buses do not follow set schedules, and there are far more passengers than the buses can accommodate. If buses are limited and often full, we contemplate whether pollen grain passengers would wait politely for an empty bus to arrive. We do not think so. We imagine a scene of much pushing, shoving, jostling, and perhaps even some killing—just to make it onto the bus. And then there is further carnage to get the prime seats—those close to the exits—to be first off at the next stigma-stop. We imagine plants loading pollen grain teams onto buses, teams that work together smothering or displacing other teams from rival plants. We even envisage the use of chemical weapons and strange floral structures that scrape and push to help clear the bus

A A theoretical small population of plants showing typical pollen export, import, and ovule fertilisation



B Realized fitness (in terms of seeds parented) achieved by individual plants



of rival teams. On the basis of animal studies, we may expect plant equivalents of scraper-adorned penises to remove rival male sperm or chemicals to block female reproductive tracts after mating. Two studies suggest similar battles in plants: The pollinaria of some orchids compete with one another for limited attachment space on pollinator proboscides (Duffy and Johnson, 2014), and “physical struggles” between asclepiad pollinaria appear to have led to the evolution of strange horn-like structures that prevent interference from rival males (Cocucci et al., 2014). It could be a war out there for plants, too, with similar weaponry and strategies to animals. Others have suggested this (Willson, 1979; Janzen, 1977), but few have looked.

Pollen marking also enables us to distinguish pollen grains from within the same flower, something that sequencing cannot do. Color labelling may allow us to distinguish whether pollen grains from different anthers within a flower suffer different fates. Consequently, color labelling may allow us to ask questions about the functions of individual anthers in a flower. Like the castes of social insects, anthers may also serve specialized roles, such as the reproductive anthers versus the feeding anthers of buzz-pollinated plants. Could some anthers on a flower be adapted to one pollinator, while other anthers are adapted to another pollinator? Or could anther positioning be a mating strategy where some anthers within a flower are adapted for outcrossing and others for selfing. Perhaps some anthers may provide the soldiers to smother the pollen grains of rivals, while others may function to provide the potential princes.

So far, Q-dots have been used in two ecological/evolutionary studies. The first examines pollen movement within a population of *Lapierousia anceps* (Iridaceae) with highly variable corolla-tube lengths (Minnaar et al., 2019b). Tube-length variation resulted in nonrandom pollen movement and reproductive barriers between plants of different tube lengths. This study suggests that pollinators can be important agents of ecological speciation because they drive allopatry allopatric, which also contributes to reproductive isolation in sympatry. The second study assessed the pollination efficacy of a leaf cutter bee, which is a specialized Asteraceae visitor (Konzmann et al., 2019). These bees drum their hairy abdomens on the pollen presenters of daisies to collect pollen. This method of pollen collection appears to be so effective that the bees hardly transfer any outcrossed pollen between flowers. These are only the first little gems to emerge from what is probably a treasure trove of new possibilities.

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