

# Pollination: Cooperation or Arms Race?

## An analysis of competition in biotic pollination

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Bachelor thesis

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### Abstract:

On the surface biotic pollination may appear to be a cooperative effort between pollinator and plant. We present an in depth analysis of the pollination process and several related adaptations in plants and pollinators. We show a high degree of competition between plants and pollinators. The primary focus is on honeybees as pollinators. Honeybees tend to collect and consume large amounts of pollen for its protein, which then cannot be used for pollination. This signifies conflicting interests. It is found that both flowering plants and bees show adaptations that increase their benefit from pollination. Flowering plants take advantage of electrostatic forces to transfer pollen from anther to pollinator, and from pollinator to stigma. Some plants produce pollen with morphological features that interfere in the pollen collection by bees, meaning these pollen can be saved for pollinators that do not consume them. Bees are shown to generate positive charge during flight, aiding in electrostatic transfer. Bees are covered in hairs that aid in pollen collection and subsequent removal through grooming behaviour, plus their hind legs feature pollen storing organs. Since the adaptations of bees and flowering plants are in conflict, it is concluded that pollination is not a cooperative effort, but rather an evolutionary arms race.

## Introduction:

Plant pollination is the sexual reproduction of vascular plants. This process happens by abiotic and biotic means. 98 percent of abiotic pollination happens through wind pollination, by plants simply releasing their pollen into the air, with only a small fraction of the pollen finding its way to conspecific female flowers (Shukla *et al.*, 1998). Biotic pollination happens with an animal as carrier of the pollen, usually offering the animal a reward for doing so. This reward comes in two primary forms, nectar and pollen (Nicholls & Ibarra, 2016). This pollination type can be seen as an example of mutualism between different species.

Inefficient pollen transfer can negatively impact a plants' fitness through loss of reproductive potential (Nicholls & Ibarra, 2016) however if a plant were to increase pollen production to compensate, more resources would be required. Another way for a plant to compensate, would be to increase the efficiency of pollen transfer.

Very few pollen actually make it to female flowers. There are many ways for them to be lost along the way. Minnaar *et al.*, (2018) document 16 ways for pollen to be lost, most of which are during transportation. Several of these are relevant to this thesis. The first being that not all pollen is collected during the blooming period, meaning this pollen cannot be used for the purpose of pollination. Another important way for pollen to be lost is consumption by visitors. For example, honeybees use pollen as an important protein source for larvae growth and adult maintenance (Haydak, 1970). The primary focus of this thesis will be on honeybees as pollinators, but when appropriate, reference other important ones like bumblebees. A lack of adhesion to either pollinators or stigmatic surfaces can also contribute to pollen loss (Minnaar *et al.*, 2018). For a plant to increase its reproductive potential, measures are expected that decrease the afore mentioned losses.

Even though pollination is an example of mutualism, there can be conflicting interests involved. Consumption of pollen is beneficial to the pollinator, whereas it has a negative effect on the reproductive potential of the plant. In this thesis we will take a deeper look at pollination related adaptations in honeybees and biotically pollinated plants, after which we determine to what degree they compete with each other. For the plants, we expect adaptations that limit pollen loss and improve their transfer, thereby limiting loss of reproductive potential. For the pollinators, we expect morphological and behavioural adaptations that increase their collection rate of pollen, since pollen are an important source of protein. For the purpose of this thesis, the following research question was formulated:

“To what degree do pollinators and animal pollinated plants compete in the pollination process?”

Answering this question will require the answering of the following subquestions:

- (1) “What mechanisms determine adhesion of pollen to pollinator bodies?” Several mechanisms of adhesion have been studied thus far, these are: electrostatic forces, pollen morphology, bee morphology and bee behaviour.
- (2) “What adaptations are causing the mechanisms that could explain the degree of competition between plants and pollinators?” Here we examine how plants and honeybees have adapted to increase their respective benefits from the pollination process. After mapping out these adaptations, it is possible to determine the degree of competition that is involved in bee induced pollination.

## What mechanisms determine adhesion of pollen to pollinator bodies?

Knowing that pollen are costly to produce and flowering plants are highly dependent on pollen transfer for reproduction (Nicholls & Ibarra, 2016), it is in the plant's best interest to make pollen transfer is as efficient as possible. There are multiple factors that help in this regard. Important and well-investigated aspects are: electrostatic forces, pollen morphology, bee behaviour and bee morphology.

### Electrostatic forces:

Electrostatic forces provide aid in the transmission of pollen from flower to pollinator and the other way around. Minnaar *et al.* (2018) show that one of the ways for pollen to be wasted is never being picked up in the first place, as some pollen can remain inside a flower. Therefore, ways that improve the initial adhesion to pollinators can be beneficial for fitness. Electrostatic forces have been shown to help the initial adhesion. In physics it is well known that opposite charges attract each other, due to a law known as Coulomb's Law (Ganatra *et al.*, 1995). Clarke *et al.* (2017) have shown that plants and their flowers carry a small negative electrostatic charge. The atmosphere is positively charged. The higher up, the greater the charge, this is called the atmospheric potential gradient (APG). This is shown in Figure 1. Negative charge accumulates at the ground's surface through electrostatic induction, this process causes negative charge to move closer to a positively charged object, in

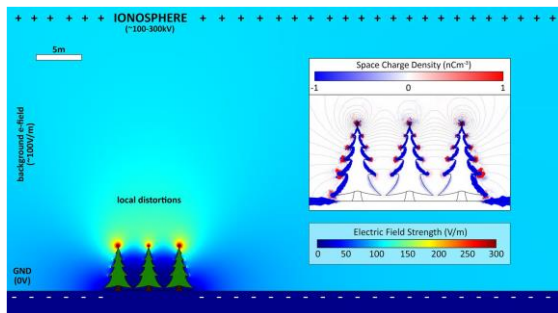


Figure 1. An overview of the atmospheric potential gradient (APG), with field strength in V/m. Plants serve as an extension of the grounds' negative charge. (Clarke *et al.*, 2017)

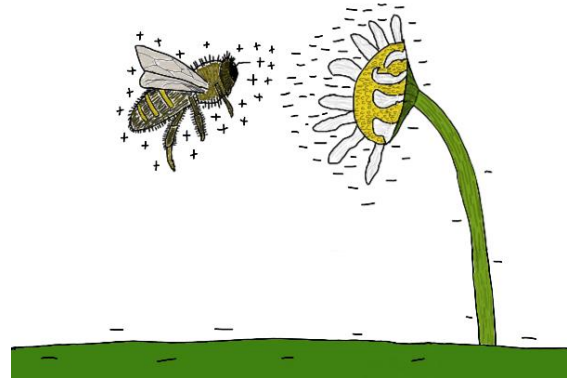


Figure 2. Inductive charging of flowers by insects. The earth's negative charge moves towards a positively charged body, causing negative charge to accumulate in the flower.

this case the atmosphere (Clarke *et al.*, 2017). Plants, including their flowers, take on some of this negative charge (Clarke *et al.*, 2017). Negative charge has also been measured in pollen (Vercoulen *et al.*, 1992), suggesting the pollen take on the charge of the flower. In order for electrostatic attraction to occur the pollinators are expected to carry a positive charge, and there are multiple studies that confirm this indeed to be the case. It has been known for decades that honeybees can carry electrostatic charges (Erickson, 1975; Yes'Kov & Sapozhnikov, 1976). The positive charge has been shown to accumulate during flight caused by their wing movement (Gan-Mor *et al.*, 1995), as passive bees do not accumulate any charge. The same effect has also been shown to occur in bumblebees, *Bombus terrestris* (Montgomery *et al.*, 2019). This would allow electrostatic induction to occur, as can be seen in Figure 2.

Thorp suggested that these forces could help in the pollen transfer from anther to pollinator (Thorp, 1979). In later experiments it was proven that electrical charge can indeed detach pollen from anthers without physical contact and transfer them across a small gap of around 0.5mm (Corbet *et al.*, 1982) or 0.6mm (Gan-Mor *et al.*, 1995) (Figure 3.).

This is however only a part of the story, as pollen transfer from the pollinator to a flower's stigma is also necessary for successful pollination. Dai and Law used computer simulations to show that the floral electric field

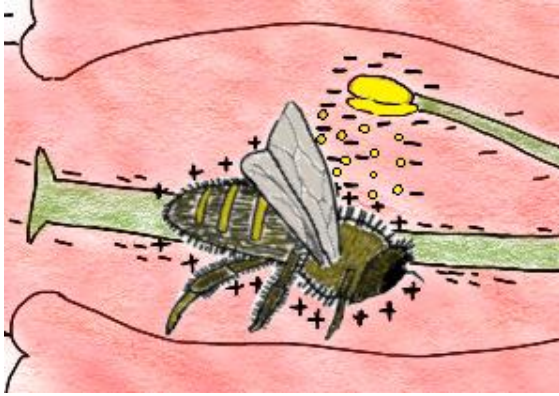


Figure 3. Pollen transfer can happen across a small gap. The positive charge of this honeybee attracts the negatively charged pollen during a visit.

is the strongest right above the stigma (Dai & Law, 1995), which could suggest that the electric field draws the pollen to the stigma, resulting in more efficient pollen transfer. This was later proven in an experiment where clouds of charged and uncharged pollen were released near almond flowers. The charged pollen concentrated at the stigma and the edge of the corolla, whereas the uncharged pollen were distributed evenly on the entire flower, including the petals (Vaknin *et al.*, 2001). This study also examined the effects of flower shape. It was found that the corolla angle plays an important role in guiding the pollen to the stigma (Vaknin *et al.*, 2001). They found a 90° angle lead to the highest amount of pollen on the stigma and corolla edges. Longer stigmas were also associated with higher pollen deposition on the stigma (Vaknin *et al.*, 2001). There is no empirical evidence that flowers evolved to take advantage of electrostatic forces yet, but it seems likely, since pollen transfer can greatly improve if these forces are used. This points to electrostatic forces between pollen and pollinator being a relatively unknown selective driver that certainly warrants more study.

### **Pollen morphology:**

Three main factors of pollen morphology have thus far been suggested to influence the adhesion of pollen to pollinators. The first factor is the presence of a substance called pollenkitt. It covers the outer layer of the pollen and has several important functions, most importantly providing stickiness and protection (Halbritter

*et al.*, 2019, p24). It also plays a part in keeping the pollen together and keeping it from prematurely falling from the anthers (Pacini, 2000). Research has shown this substance is important for adhesion to pollinators, one study showed that pollen accumulation can drop to half the normal rate when pollenkitt is removed (Amador *et al.*, 2017). Another study concluded that pollen adhesion is between three and six times higher with pollenkitt than without (Lin *et al.*, 2013). Lin *et al.* also found that more pollenkitt volume increases adhesion. The second factor is the shape of the outer pollen wall. The outer wall, or exine, is a protective layer made up of a material called sporopollenin, a material that is highly resistant to degradation (Domínguez *et al.*, 1999). The morphology of this outer layer varies widely between different plant species, from smooth to highly echinate, meaning covered in spines (Lin *et al.*, 2013; Konzmann *et al.*, 2019; Vaissière & Vinson, 1994). The third and last potential factor is pollen size. Size of pollen can vary between 10- and 100 µm (Halbritter *et al.*, 2019, p57). The size of cotton pollen was believed in the past to prevent bees from gathering them, however Vaissière & Vinson (1994) found that size alone could not have a significant impact on adhesion. In their experiment the large spines of cotton pollen, *Gossypium hirsutum* L., were proven to hinder pollen collection by honeybees (Vaissière & Vinson, 1994). The pollenkitt free spines present on *Alcea rosea* pollen were shown to protect the pollen from being collected by corbiculate bees (Lunau *et al.*, 2014). The study by Lin *et al.* (2013) showed that echinate pollen surfaces have an enhanced effect on adhesion from pollenkitt, as well as higher substrate dependency. Konzmann *et al.* (2019) concluded that size and spines do matter in collectability, but they alone do not entirely explain it. They showed that pollen that were uncollectable for bumblebees were both large and echinate, yet other species' pollen with similar shapes could be collected (Konzmann *et al.*, 2019). This suggests that there are other factors at play like pollenkitt and its distribution.

There is currently no clear consensus on the way adhesion is influenced by the afore mentioned factors. This suggests that the adhesion of pollen to pollinators is a highly complex process, involving an interaction of the three described factors and potentially more, making it difficult to isolate individual factors during these studies.

### **Bee behaviour and morphology:**

Bees show a wide variety of morphological features and behaviours that increase their collection of pollen. As we've established in the introduction, pollen is used as an important protein source for both the larvae and adults (Haydak, 1970). It was recently discovered that larvae from pollen stressed colonies grow up to be much less efficient foragers with higher mortality (Scofield & Matilla, 2015). This means that there is an important incentive for bees to actively collect pollen.

Portman *et al.* (2019) define pollen collection to be 'active' when pollen are the primary objective of the bee. Passive pollen collection is the incidental collection that happens when bees are after a flowers' nectar. Using videos of flower visiting bees, Portman *et al.* documented six different types of active pollen collecting behaviour: scraping the anthers with their legs and mouthparts, buzzing, rubbing with their bodies, tapping the anthers with their abdomen, rubbing the anthers with their face and rasping the anthers with the thorax (Portman *et al.*, 2019). The variety of behaviours used in the pollen collecting process shows that the collection by bees is more than incidental collection that happens in their search for nectar. This suggests that passive collection alone does not provide the colony with enough protein to optimally. Bees have also been shown to be selective in which types of pollen they collect, but the actual mechanisms behind their preferences are still a highly debated subject (Nicholls *et al.*, 2016).

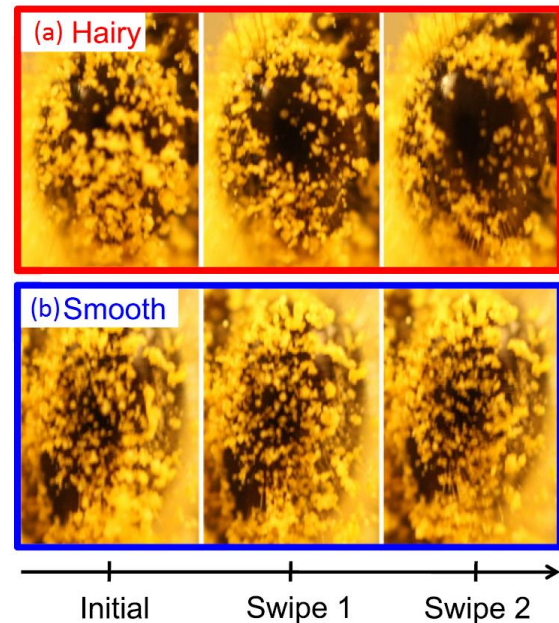


Figure 4. The difference in grooming between hairy (a) and smooth (b) bee legs. Edited. (Amador *et al.*, 2017)

After being covered in pollen through active and passive collection, bees perform grooming behaviour with their legs to gather the collected pollen. Amador *et al.* (2017) showed that bee hairs are vital for this grooming process, as the hairs that cover the bee, including their eyes, suspend the pollen above the body and eyes. The space in between these hairs is small enough to keep the pollen suspended and keep them from penetrating the fur too deeply. This research showed that bees failed to clean themselves from corn starch, partly because the smaller grains were too small to be suspended by the bees' hairs (Amador *et al.*, 2017). The spacing of the hairs on bee legs is also an important factor in grooming, as the hairs are spaced in such a way that they allow pollen to be wedged between them for optimal removal (Amador *et al.*, 2017). The smaller corn starch grains cannot be wedged between these hairs, further complicating grooming. If the bees' legs are 'smoothed' by dipping them in wax, thereby filling in the space between the hairs, the removal rate of regular pollen drops significantly. This is shown in Figure 4. (Amador *et al.*, 2017).

During the grooming process, the pollen are moved to structures that facilitate temporary pollen storage. In most bees this is on the hind

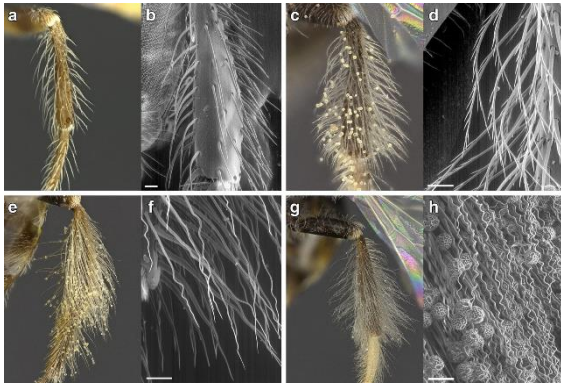


Figure 5. Multiple hair types found on bee scopa. These types are simple (a,b), branched (c,d), wavy (e,f) and corkscrew shaped (g,h) (Portman & Tepedino, 2017)

legs on either a structure known as a ‘pollen basket’, also called ‘corbicula’, or on a collection of long hairs called a ‘scopa’. Corbiculae are present in four tribes within the *Apidea* family: bumblebees, honeybees, stingless bees and orchid bees (Cane, 2008). Cane (2008) describes this as a “slightly concave surface surrounded by guard hairs” and this is where the pollen are gathered in a droplet. Bees using scopa for pollen transport exhibit a wide variety of hair types and densities (Figure 5.) and can transport the pollen either wet, glazed or dry (Portman & Tepedino, 2017). While not confirmed, this variety may indicate specialisation in the collection of specific pollen types. After collection, the pollen can be delivered to the hive along with the collected nectar, serving as food for the colony and its larvae.

### **What adaptations are causing the mechanisms that could explain the degree of competition between plants and pollinators?**

Now that we have an idea of the mechanisms behind pollination, it is time to uncover how this fascinating process emerged in the evolutionary history and what selective forces there are currently. There is right now no consensus on when in the earths’ history the angiosperms first appeared, as there is much conflicting evidence on the subject. Few angiosperm fossils are dated earlier than the Cretaceous period (Mandel, 2019; van der Kooi & Ollerton, 2020). Studies

using molecular clock dating indicate an origin in the late to mid Jurassic (Zeng *et al.*, 2014). More recently a large study reconstructed the phylogenic tree of the angiosperms using 80 genes from 2,881 angiosperm genomes and cross referenced them to fossil calibrations. This study dated the origin of angiosperms back to the late Triassic period, which is over 200 million years ago (Li *et al.*, 2019). This is significantly earlier than most fossil records of angiosperms, with a difference of roughly 70 million years. Li *et al.* call this unexplained difference the “Jurassic angiosperm gap”. They also show that there were two major radiations in the angiosperm evolution, the first in the Jurassic period and the second in the early Cretaceous (Li *et al.*, 2019). Mandel (2019) points out that the origin of angiosperms and the radiations afterward coincide with several radiations within the animal kingdom. This suggests that the explosive increase of angiosperm species supported many new niches and therefore led to more animal species.

Bees evolved from spheciform wasps (Grimaldi & Engel, 2005, p454-463), as they turned to floral pollen for their dietary protein. From there they evolved some impressive adaptations, like those discussed previously that helped them increase pollen collection: the hairs that suspend the pollen above their bodies, combined with the hairs on their legs with optimal spacing for pollen grooming, the variety of methods of active pollen collection during flower visits, the presence of pollen transporting organs like scopa and corbiculae, their tendency to accumulate positive charge while in flight and their grooming behaviour. All of these adaptations suggest that pollen collection plays a significant role in the evolution of bees.

It is possible several plant species have evolved to be selective in which pollinators can collect their pollen. We know now that bees tend to collect, and take home, high amounts of pollen when visiting flowers. The pollen that bees consume is no longer available for pollination, while the plant has to invest resources into the

production of those pollen (Nicholls & Ibarra, 2016). At the same time we have seen examples of pollen that are difficult for bees to collect, due to a combination of spines, size and/or pollenkitt distribution. This begs the question, have the plants carrying these pollen evolved to resist pollen collection from bees and other pollen consuming pollinators, thereby saving them for other pollinators that feed primarily on nectar and leave the pollen undisturbed?

## Discussion:

There clearly are conflicting interests at play in the pollination process. Whereas on the surface it can be seen as an example of mutualism, it is far from an example of cooperation. With plants evolving to limit pollen collection by bees and bees evolving to collect more, it is more akin to an evolutionary arms race. Hopefully the readers of this thesis have gained new insights in the workings of pollination and ideas for future research.

Pollination is a highly complicated process and our understanding of it is still very limited. It is recommended that more research is done into this topic to fill in the gaps in our knowledge. A good place to start would be more research on the adhesion of pollen to pollinators. It is still unclear how the discussed morphological features interact with each other to determine adhesion. While it will prove difficult due to the complexity of this subject, it can provide interesting new insights that may have applications in our daily lives, like new ways of adhering materials together. The same applies to the morphology of bees. More insight in the way their hairs seem optimized for pollen capture and removal could lead to new filtering techniques.

Another suggestion for more research would be the floral usage of electrostatic forces and the optimization there of. There are very few studies into the subject of floral shape in relation to those forces. This means that the evidence behind a conclusion on whether or not flowers

evolved to take advantage of these can only remain thin. More knowledge on this subject might lead to innovations in the technical and agricultural sectors, like artificial pollination of crops.

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