Biology is the study of complicated things…

Observing Pollination in Flowering Crops

It's a complicated thing. There are plants that do not require pollination of any kind to produce fruit and seeds. There are some that require the stimulus of pollination, but not actual fertilisation, to fruit. Where pollination *is* required a plant may use pollen that it has produced (in the same or a different flower), or may have to use pollen from another, distant, plant of the same species. Unfortunately too, there are plants that have a bet each way, both 'cross-pollinating' and 'self-pollinating'.

Pollen is passively dispersed by currents of air and water but animals can be induced to visit flowers for rewards like pollen itself, nectar, resins, and oils, or even by deception, in order to carry pollen to another plant. A pollen vector has to transport a quantity of viable pollen to a receptive part of the plant (the flower stigma), and we look for direct (pollen grains) and indirect (fruit or seed set) evidence that this has happened. That's pollination. We have to understand the possible plant reproductive arrangements to work out what contribution any given pollinator might be making, and we have to understand a lot about possible pollinators, their morphology, seasonality, nutrition, and behaviour, for example.

Many of our fruit and seed crops benefit from being assisted in this way, and for some it is essential. In these cases the important visitors are almost always insects. We call this '*entomophilus'* pollination, and improving the circumstances in which insects (and a few other small 'bugs!) operate can result in quantitative and qualitative improvements in yield, and better financial and nutritive outcomes. Observing pollination in order to make such improvements is not however straightforward. We have to be able to look at the biology of both the plant and the animal in unaccustomed detail and understand how each has to respond to fluctuating environmental conditions. Not only that, but consider that 'crops' are a social construction and a part of a market economy. 'Crops' are much more than their biology.

Studying pollination

Agents capable of pollinating flowers can be abiotic (wind, water, gravity, electrostatic forces, rain) or biotic (such as birds, bats, insects, mammals). Any one of these may be capable of meeting all or part of a plant's requirement. Biotic pollinators are different in that the relationship is built on some form of exchange (even if a fraudulent one) that is advertised, desirable, rewarding, social, and constrained. There are then questions to be asked about how the potential exchange is communicated and to who, the costs and benefits of the 'reward' to each party, choice and competition between the interests of the individuals and the populations they are part of, and the 'rules' that affect the trade (physical, chemical, and biological), things like pollen presentation, anther dehiscence, flower duration, stigma receptivity, and the physical ability of particular pollinators. Much of the 'mechanics' of observing pollen transfer is tried and tested and standard methods have been worked out. Often, pollination studies tend to stop short of including fertilisation; the conditions for good pollen viability, germination, pollen tube growth, and pollen (in)compatibility have to be found elsewhere but actually form part of the 'whole picture'. Rarely do studies give much thought to the actual transportation; how does the pollinator find, collect, and carry pollen?

In the case of honeybees the COLOSS Beebook describes protocols for identifying and evaluating pollen quantity and quality transported by bees, evaluating the same deposited on stigmas, estimating the proportion of foragers from a colony visiting a crop, observing bee densities in field plots, appraising the effect of competition between plants for pollination, and conducting pollination research in unusual environments like greenhouses. DNA meta-barcoding is beginning to be used to identify pollen loads, sigma deposits, and track pollen flow. These kinds of protocols are generally equally applicable to most pollinators and are intended to ensure studies are robust, comparable, and repeatable, but beyond collecting observations about the process of transportation, pollination studies have a long row to hoe.

The 'Nature' of advertising

Plants, and pollinators, have options, some more than others. Before any kind of exchange can take place plants have to prompt pollinators to come visiting (plants are, literally, rooted in place), but leaving food rewards out for all and sundry will hardly achieve the required outcome. Successful pollination has come to rely on signals, simple, elaborate, and occasionally dishonest, for communicating necessary information. From one perspective, plants 'advertise' in a marketplace for service, and the most obvious representation of that advertising are flowers. In markets, supply and demand fluctuate. At any point in time there may be a surplus or deficit of flowers relative to the number of pollinators. Increasing competition between flowers might result in an increased investment in rewards and display; reduced competition might have the opposite effect. Advertising comes at a cost and in the end a plant's pollination and reproduction will depend not only on the efficacy and 'value for money' of its own signals but also on the signals of co-flowering species, their distribution, and relative abundance.

We do not see the world as others see it. Signals that plants can produce that can be perceived from a distance fall into two types. There are those that make use of the electromagnetic spectrum, (that we refer to as colour – a limited part of the spectrum), and the emission of volatile chemicals (some of which we smell). Such signals can be tailored so that they are detected by a broad range of pollinators, or only by a few specific pollinators, perhaps one, and not necessarily by us. They may indicate the reward is available, exhausted, or located in a specific place. Comparatively well known, colour and ultra-violet 'nectar-guide' patterns can be accompanied or supplanted by far more important but cryptic 'odour-guide' patterns. Olfactory signals appear to be especially easy to learn and remember, at least for bees. Some recent studies suggest 'odour plumes' sometimes used by pollinators can be disrupted by anthropogenic pollution, such as ozone and diesel. Signals are easily overlooked or misunderstood in pollination studies because it's necessary to recognise if and when a specific animal will sense a particular signal, and how that will be understood, prioritised, and remembered.

Temporal change in food networks

Nectar is essentially a modified phloem exudate available as a carbohydrate food reward for pollinators, mostly a source of energy. Plants can control nectar secretion and can adjust it according to rate of consumption, temperature, or humidity. In some plants there is a daily rhythm to production, in others it is clearly under the control of a hormone. The 'transience' of the secretion serves to conserve an expensive product; in some examples nectar secretion was estimated to account for nearly 40% of the total carbon absorbed by the plant. The main carbohydrate is sucrose which is metabolised in a time/temperature-dependant way, by enzymes ('invertase') on the cell walls of the nectary tissue, to hexose sugars. Some invertase remains in the nectar, so the nectar sugars continue to change composition with time. Sugar solutions are also hygroscopic to varying degrees, so their concentration is not fixed, nor is the viscosity. The environment has an effect on the nectar itself, humidity and heat changing the physical properties of the solution. Besides sugars, there are other constituents that matter. Minerals, amino acids, enzymes, alkaloids, volatiles, and antimicrobials are selectively attractive and unattractive to various flower visitors, by design. Microbes (like yeasts) come to inhabit the nectar and are also responsible for significant changes to its composition, and its allure.

Pollen is foremost in the mind looking at pollination of course, but besides the obvious function for the plant it is also a food reward for the pollinator, but, unlike nectar, directly linked to a plant's reproductive success. It, and its compliment of microbes, is the major (perhaps the only) source of food required for pollinator activity, growth and repair, comprising amino acids, fats, oils, and minerals. It comes 'packaged' in a huge variety of perishable forms, sometimes 'labelled' with aromatic or gustatory compounds that try to protect it from or limit herbivory (for example, alcohols and phenols like linalool or perseitol). Bees' aversion to chemically defended pollen depends on what else is available, and they can be put off the whole patch by a few unpleasant flowers. As a valuable product pollen supply can be constrained by things like morphology, by environmental factors (for example, temperature and humidity restraining antithesis), by its longevity, and by competition or theft. There is an obvious tension between pollen being both food and the object to be transported. Some pollinators specialise in one, or one or two, types of pollen, while others require a great variety. Lately there is a suggestion that stoichiometry may be a guiding principle when it comes to what pollen suits a particular pollinator. While there is scant evidence that pollinators are able to assess its food value, there is plenty of evidence that they select on the basis of its physical form, its presentation or accessibility, odour, or taste, and that their appetite or tolerance for different pollens changes depending on, for example, scarcity or abundance.

In practice then, nectar or pollen can be harvested by a parade of visitors as time and circumstance change; visitors with different mouthparts, different appetites, and different priorities. Pollination studies consequently have several questions to think about. How, and when, is the supply of nectar and pollen regulated? How do we account for depletion by visitors, by wind, rain, heat, and humidity, or by the plant conserving it? How can we determine if and when the temporal changes in its chemical and physical properties affect its attraction and utility for different visitors? Last, how well does a given pollinator compete with the other flower visitors, and do they need to?

The importance of memory

In recent times navigation and memory have become topical and the subject of some studies that suggest impairment of these functions can occur following exposure to agricultural chemicals, although so far it is hard to see how the comparison is being made with only a rudimentary understanding of what the 'baseline' is. Pollinators are thought to acquire information about the location of their nest-site and food sources in ways that are common to most if not all insect orders, the hymenoptera being the most closely studied. An insect monitors and stores distance and compass metrics derived from its own movement and is able to compare its current sensory experience with a memory of the desired sensory experience (in one expression, by matching 'images'). Various guidance strategies allow pollinators to travel at a distance from their nest site, and with experience, to embark on, and return from, complicated journeys. With increasing experience accuracy emerges automatically as more precise or reliable cues have more successful outcomes and information from the various strategies is evaluated and refined. While these strategies work together one may be prioritised over the others at any particular time to resolve conflicts or deficiencies, and importantly each can be used train or 'calibrate' the other guidance systems. It's clear that memory is a crucial part of the system, and that different memories are recalled 'on-cue' and depending for example on whether an animal is seeking food, or has gathered food; what has been referred to as 'motivational state'. We also know that memories are 'layered', and time dependent – they are acquired, reinforced, and extinguished in ways yet to be understood. There is a complex relationship between memory acquisition and extinction, with physiological age being a factor. Older foragers are often more likely to show evidence of the progressive loss of some types of brain function, particularly spatial memory. On the other hand younger foragers are thought to have a shorter memory. Forgetting what they knew yesterday encourages them to try new locations and food sources and so builds experience. This might be particularly useful for pollinators that forage on much more transient and distributed flora. We should not expect all pollinators to be the same.

Bumble bees and solitary bees are masters of the local search and have progressed from simple Levy search patterns to develop very effective routes (like 'trap-lining'). Honey bees are not as efficient in the same way, however, their ability to recruit nest-mates to exploit the same resource, and their ability to navigate more effectively over large distances, ensures efficiency at the colony level. Many pollinators, and particularly honeybees, also display a learned preference for particular flowers based on memory of a previous experience. They become especially adept at recognising and manipulating a type of flower and remain loyal to it for a significant period of time, a phenomenon now known as 'floral constancy'. It's possible this is more important for foragers that use image matching navigational strategies. In observing pollination, a forager's motivational state, its sensory and locomotive abilities, and how and when it stores, manages, and retrieves memories is of fundamental importance if we are to understand how pollinators make decisions. Just as much as varying nutritional demands or sensitivity to signals, varying aptitude for using the same basic neurological tool-set is responsible for stratifying pollinators within a landscape and across time.

Business practices and Commerce

Flowering crops are unlike plants that exist in a natural ecosystem in that they are significant aggregations of selected plants purposely planted and cultivated for food or commerce. These plantings can take many forms such as pasture, field crops, agroforestry, and greenhouses, and are generally monocultures with different degrees of human intervention. The planted area can be sufficiently large that it can test the foraging range of some pollinators, modify their diet, and reduce their density to a point that limits full pollination. This can be aggravated by the displacement of local nesting sites through shading, soil tillage, and overzealous 'horticultural hygiene'. The cyclic overabundance and famine that characterises a flowering crop is not necessarily an advantage over the lifetime of an embedded pollinator. These simplified environments are less resilient to changes in weather, season, and climate, when a single adverse event can produce unsuitable conditions that curtail the activity of insects and disturb the pollination of the entire crop. Pollinators will respond to all manner of changes, like flower abundance and diversity, climate change, land use change and intensification, and introduced species and pathogens. These responses change their distribution, physiology or seasonal phenology and so synchrony between visiting behaviour and the timing and duration of flowering. The consequences of fluctuation and change in the environment can be buffered or offset by the presence of a variety of flower visitors that can assume complimentary roles under changing circumstances.

Besides one of scale, the pollination of these plants has extra dependencies that stem from sociocultural or horticultural practice and pollinator management. In horticulture the organisation of things superficially quite peripheral to pollination, for example, crop-load, rootstocks, pruning, irrigation, windbreaks, pest management, and the use of dormancy breakers or growth regulators, can have a significant impact on pollination success. Likewise, pollinators can be 'managed' but this only applies to a quite limited subset of possible pollinators, nearly all of them bees. Honeybees have become the most popular managed crop pollinator largely because of their persistent, perennial life-cycle, and portability. A few species of bumblebees can be managed, but like the few solitary bees that are used their colonies are fundamentally seasonal and year-round availability requires specialized controlled environments. The supply, placement, and timing of introduced pollination units, or the maintenance of pasture grazing, 'set-aside', headland, and field margins, are activities with their own financial costs, benefits, and risks that relate to the economy they are part of.

A social enterprise

The 'outcome' required from a flowering crop is may not be full pollination nor, necessarily, is there a long-term plan. The prominence of financial drivers and assumptions about 'value' quite possibly mean that what biology might see as sub-optimal may remain a preferred state when the costs of labour and horticultural management are considered. Value is at the core of many pollination studies and, as such, they need to be quite explicit about the time and spatial scales the data relate to for it to support future planning. There are useful examples that provide a social context for the relative importance of pollination compared to other interventions, and that illustrate the interplay between ‘social’ issues and crop pollination. Here are three.

In New Zealand a significant number of kiwifruit blocks are now covered by a canopy. These canopies consist of a hail resistant netting supported by cables attached to rammed posts, and can cover a considerable area, thousands of square meters. Many, but not all, are fully enclosed with netting down to ground level along the sides. From a grower's perspective these provide some substantial benefits. Obviously, one is protection from the elements. Even unnoticed hail or wind damage can cause a significant fall in the return a grower gets for their fruit. Another benefit is a significant reduction in bird damage to buds and fruit, and any waste due to bird lime. The factor that may have pushed these expensive constructions 'over the line' was an introduced bacterial disease that can rapidly destroy an orchard. The canopy provides an element of phytosanitary security and protects the plants from wind damage, reducing broken shoots in the spring that are an important point of entry for the bacteria. Considering the effect these covers would have on the honeybees that are universally used to pollinate the flowers had not been a priority; unless the disease could be managed there would be no flowers. Under the covers large numbers of foraging bees failed to return to their colony and died. Those that did return delivered pollen that is mono-floral and without the proper balance of amino acids the bees require for adequate protein nutrition. A hive starved by both quantity and quality of pollen deteriorates very quickly. The loss of bees also affects the colony's ability to regulate temperature and care for brood, young bees that normally care for brood begin foraging prematurely, protein deficiency encourages brood cannibalisation, the queen will stop laying eggs that produce new bees, and the hive enters a spiral of decline that takes months to correct but is very quickly an ineffective pollinating unit. Many beekeepers refuse to place their hives under covers, and were in a position to send them to honey crops instead. The industry is trying a range of 'work-arounds', including alternative pollinators, aids to navigation, supplementary feed, and even supplemental artificial pollination (mechanical hand pollination if you like!). The introduced native bumblebee *Bombus terrestris* is a possible alternative, although not without ecological risk. Commercial supply of these is available, and it is it is possible to rear colonies locally albeit on a seasonal basis. However, where local annual production has been tried it has not been able to out-compete the supply from continuous, year round, and sometimes counter-seasonal production of large multi-national suppliers, increasing the risk to local ecosystems, and local pollinators.

Further afield, in Maoxian County, Sichuan, China, by the 1980s land-tenure customs had produced quite small holdings from which farmers were being incentivised to maximise a cash return from a crop to market (rather than as food). The rules and standards in a commodity market are entirely different. The apple orchards are located in cool, mountainous regions with differences in sub-climate and elevation altering the flower phase of every orchard. Pollinators struggled. To make the most of their land-use growers reduced or eliminated polliniser trees, maximised fruiting trees, and focused on high yields, confident people could pollinate the trees reliably. Pollen could be sourced from the few remaining pollinisers however distant, shared, and people could apply it in the frequent conditions that were not favourable for bees. Relatives or neighbours might get pollen free of charge. The different flowering times enabled neighbours to help each other pollinate and close community bonds kept the labour costs down. Polliniser trees were often planted in home gardens so that flowers would not be stolen and where it was convenient for flower harvesting and pollen extraction. Pesticides could be used extensively, a disincentive for beekeepers who anyway preferred to use their bees for honey crops elsewhere. Nor was it ever clear how a beekeeper would be paid for a pollination service. Faced with a multitude of 1/5th ha land-owners, whose trees was he responsible for pollinating? Whose pollen was being used? A decade later the economy had changed again. By 2011, apple growing had considerably reduced in the most marginal areas. Climate change had increased the amount of cool, rainy, cloudy weather, the farm gate price of apples had dropped substantially, and production costs had risen, exacerbated by young people drifting to work in the cities. The famers had adapted by shifting away from apples, a crop that requires cross-pollination, to fruit and vegetable crops that were not cross-pollinated, like lettuce, Chinese cabbage, tomato, celery, onion, along with other fruit trees, such as plum, loquat, and walnut. What was the value of pollinators now?

An almost identical story comes from south of Chengdu where Hanyuan County is the biggest pear producer in Sichuan. As in Maoxian, since the 1980s the area has been undergoing a transition from subsistence cereal crops to cash crops, especially pears. Most pear varieties are self-incompatible and need cross pollination to set fruit and keeping one to two colonies of honeybees (*Apis cerana cerana*, the Chinese honeybee) was common for some households. Until about 1985 wild insect pollinators were mostly common, and erratic fruit yield and quality were not a concern. Traditional land tenure was fragmented, land owners were often absent engaged in off-farm activities, while the small plots were managed season-to-season, the insecurity stalling investment-led change. As pear trees became more abundant insect pests (mainly fruit moths and *Psylla chinensis* aphids) flourished and entrenched extensive broad-spectrum spray programmes that also killed off the potential pollinators. This established hand pollination, but also ensured that pear trees were always over-pollinated, producing unsustainable fruit-sets and heavy, labour-intensive, fruit thinning. Returning to bee pollination is not proving to be easy, even if pesticides are controlled. Native pollinators were not scalable. Pears do not provide a nectar surplus and the bees cannot produce honey, so beekeepers in pear orchards end up incurring a cost for feeding bees with sugar syrup that they argue compounds the lost income from honey.

These days China's government agricultural officers spend their time trying to unravel these archaic interdependencies, while the rest of world uses them as poster-children for 'bee-mageddon'. In both these cases from China some commentary framed them in terms of 'pollination failure' and its mitigation by (low waged – exploited) human labourers. An alternative framing might see farmers managing a family business driven by financial decisions and choosing cost effective pollinators.

Looking ahead

Observing pollination is an absorbing, multi-disciplinary challenge at every level of study, and one with real, tangible consequences. It can only become more important as climate change rapidly introduces yet more uncertainty about the conditions pollinators will operate under in the future. The speed of change appears to be such that some pre-emptive management is preferable to a conservative or even reactionary, response; relationships formed over millennia might alter in decades. Understanding what these are and how they operate now is vital to sustain these processes, and to construct a flexible, responsive, diversity of pollinating mechanisms that will work whatever the circumstances. We will be unable to predict the specifics of our changing climate with any certainty, and continuing a dependency on any single species of pollinator seems unwise.

****"Biology is the study of complicated things that have the appearance of having been designed with a purpose".*** **Richard Dawkins.**