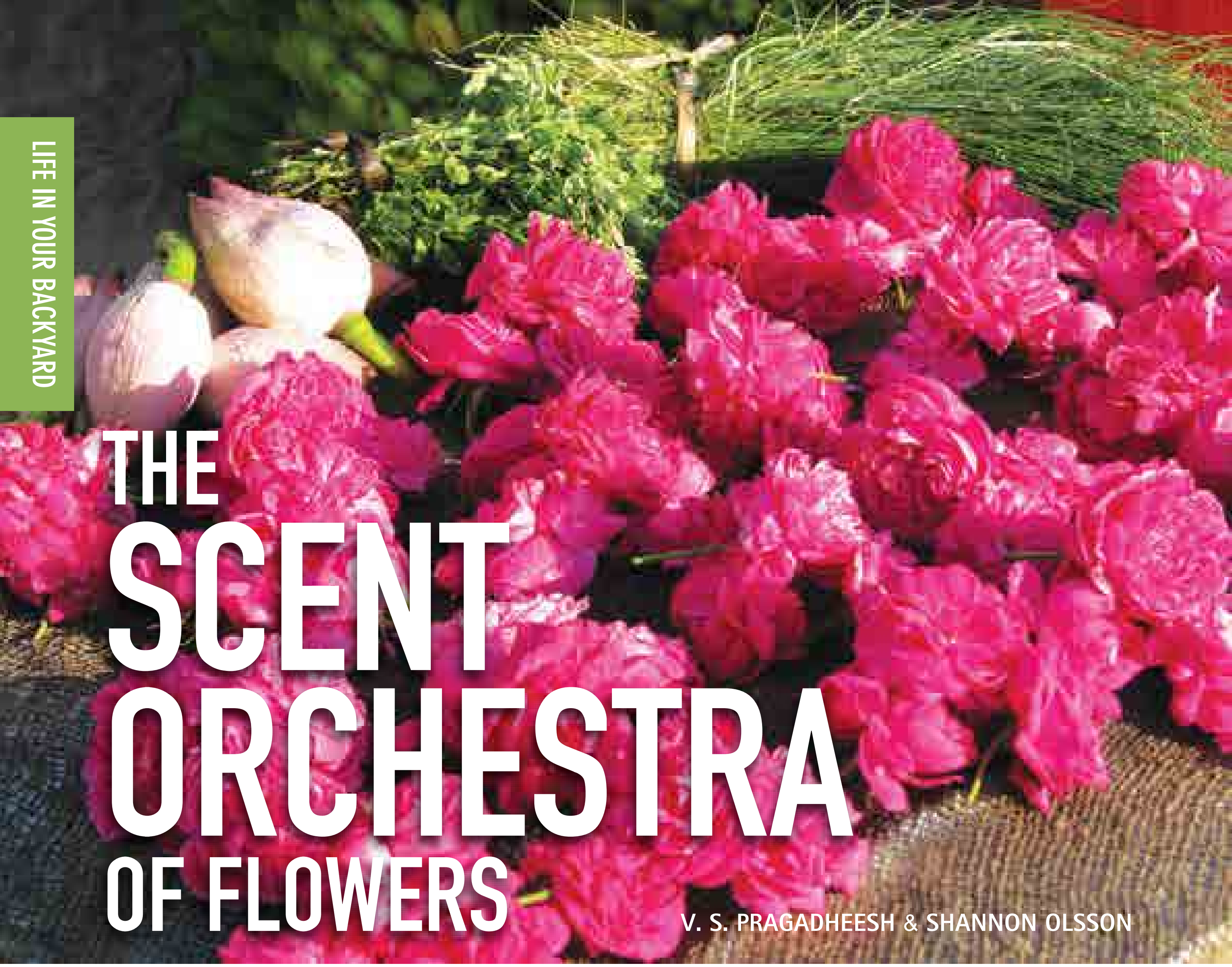

THE SCENT ORCHESTRA OF FLOWERS

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While walking through a beautiful garden, you are met with a lovely bouquet of fragrances from its many flowers. These smells are so pleasing that we use them in our homes, food, and even on our bodies. Have you ever considered what makes these flowers smell sweet, and how plants might benefit from these scents?

Shakespeare once said that *"...A rose by any other name would smell as sweet..."* – (Romeo and Juliet, Act II, Scene II).

A flower is the reproductive organ of a flowering plant, and contains its male and/or female gametes (refer **Fig. 1**). The male gametes are the pollen grains. Pollen is found on the anther of the flower. These grains need to be transferred to the stigma, the female organ, for fertilization to take place. After fertilization, the plant produces seeds that can germinate into new plants. The process by which pollen is transferred from the male to female organs is called pollination.

The transfer of pollen within the same flower is called self-pollination, and between different flowers is cross-pollination. Pollination can occur abiotically by wind, water, or even gravitational force; and, biotically through insects, bats, birds and many other animals. Around 90% of flowering plants are pollinated by animals[1] (refer **Fig. 2**). The colorful butterflies, buzzing bees, hovering flies, moths, bats, and hummingbirds that you see in your garden are performing this vital job of transferring pollen, day and night (refer **Fig. 3**). Thus, pollination is what we call an essential 'ecosystem service' or, in other words, a natural process within ecosystems that benefits humankind. Without pollination, there would be no flowering plants. No more fruits and vegetables. Life, as we know it, would not exist! But, have you ever considered how plants attract these animals for pollination in the first place?

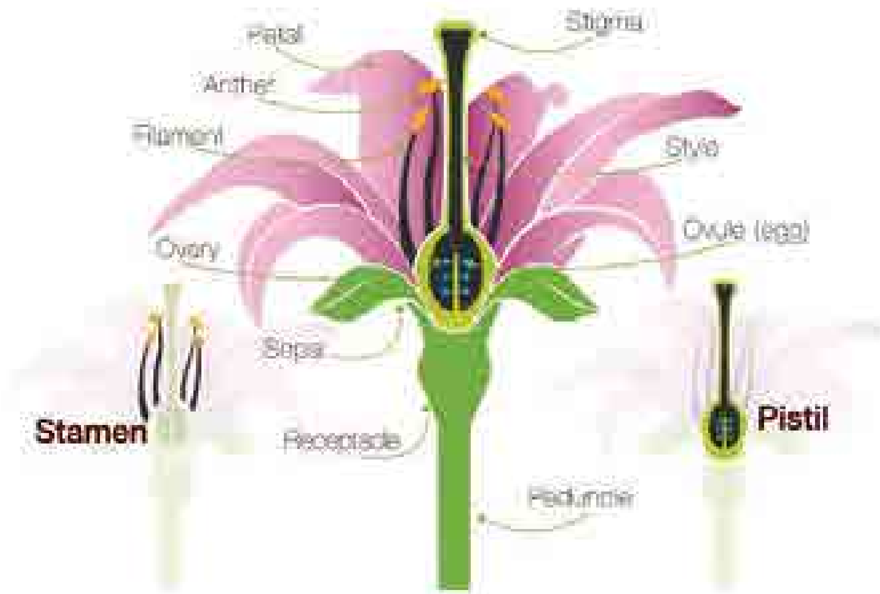

Fig. 1. The reproductive parts of a flower.

Flower scent

Plants attract pollinators using the fragrance and visual characteristics of their flowers, such as color and shape. Odors can travel in the air over several meters. As such, scent is a particularly important cue to attract pollinators to flowers from a distance.

All chemical compounds produced by plants can be divided into two categories — primary and secondary metabolites. Glucose, proteins, and lipids that are necessary for the growth and development of the plant are classified as primary metabolites. Compounds produced as byproducts of these metabolites, including terpenoids, flavonoids, steroids, alkaloids, and the many other chemicals not related to growth and development, are known as secondary metabolites. Secondary metabolites can also benefit the plant by protecting them from disease, drought, sun damage, and plant-eating animals (herbivores) through pigments, noxious taste, and water-retaining waxes, among other mechanisms.

Floral scents are produced by plants as byproducts of glucose synthesis and are, therefore, considered to be secondary metabolites. The remarkable variation of floral scents in plants is produced mainly from three groups of chemical compounds — terpenoids, phenylpropanoids, and fatty acid derivatives[2]. Lavender and rosemary release odours from mostly the terpenoid group; whereas rose, jasmine, and lily have scents produced by all three floral scent groups. Phenylpropanoids are also involved in the production of pigmentation (color) in flowers, whereas terpenoids act as a deterrent against herbivores in other plant parts, such as the leaves and stem[3].

Floral scents also serve other functions that include warding off non-pollinating flower visitors such as nectar robbers, and inviting natural enemies of the herbivores that feed on the plant. For example, leaves, stems, and flowers of plants release several chemicals when

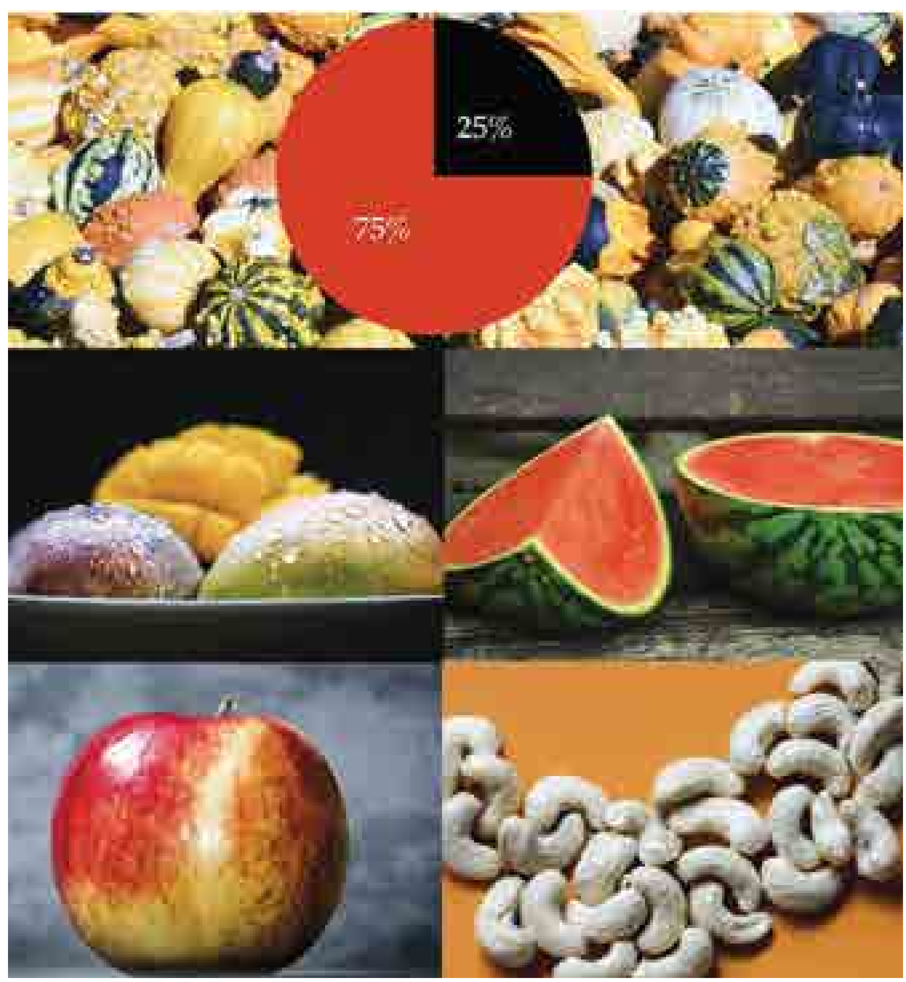

Fig. 2. Common crops for which animal pollination is vital. A pie chart showing percentage of crops (75%) that rely in part on animal pollination (FAO).

Credits: Shannon Olsson.

chewed or broken. You will be familiar with this if you enjoy the smell of fresh-cut leaves and grasses. Crab spiders use chemicals like β-ocimene, released by flowers when they are chewed on by insects, to locate and feed on these insects. Indeed, flowers may even emit larger amounts of β-ocimene when attacked by florivores (refer **Fig. 4**)[4]. In another example, the presence of methyl anthranilate, a bird repelling compound, in flowers may help repel birds that feed on pollinating insects. The chemicals responsible for floral scents can also possess anti-microbial properties that inhibit microbial growth in the flower.

Flower constancy and specialisation

Each plant species uses a small number of chemical synthesis pathways to generate a large variety of low molecular weight volatile organic compounds (VOCs). It is the unique blend of its VOC's that gives the flowers of each species their specific fragrance.

VOCs can attract a variety of pollinators — ranging from insects to birds to small animals. However, the cacophony of many plants, many pollinators, and many scents can create a problem — if pollinators visit flowers of different species, how can a plant ensure that its pollen gets transferred to another flower of the same species?

Pollinators like honey bees visit a number of plant species, and are called generalists. However, many of these generalist pollinators show a certain degree of flower constancy. Flower constancy is the tendency of a pollinator to visit several flowers of the same species and transfer pollen within that species. The specific composition of the flower scent of each species plays a key role in maintaining this flower constancy by allowing pollinators to identify and learn the flower species.

Alternatively, some pollinators only visit a single species of flowering plant throughout their lifetime. Such pollinators are termed as specialists. In

Fig. 3. An insect pollinating a flower.

Fig. 4. A florivore feeding on a Hibiscus flower.

turn, the flowers of such plant species are often visited only by that pollinator species. For example, *Vanilla planifolia*, the plant responsible for producing the vanilla bean, is pollinated only by a single species of *Melipona* bee. Thus, when this plant is cultivated outside the range of these bees, it cannot survive[5] unless it is hand pollinated — as is done, here, in India to allow for the production of vanilla flavoring from its beans.

Diurnal and nocturnal flower scents

Many plants tend to emit scents rhythmically over the length of a day and night. The floral scent of diurnal (day-blooming) flowers is different from that of nocturnal (night-blooming) flowers.

The time of the day when the maximum scent is emitted from a flower is associated with its nectar availability, pollen maturation, and the activity of its pollinators. For example, diurnal flowers, like the rose, emit maximum concentrations of odour during the day and attract bees, beetles, butterflies, flies etc. In contrast, nocturnal flowers, like jasmine, release their maximum scent in the night to attract pollinators such as bats, mice, and nocturnal moths. The scents of night-blooming flowers are composed of chemicals such as linalool, nerolidol, certain aromatic esters, and nitrogenous indole and oximes. These chemicals are collectively referred to as the 'white floral-olfactory image'[6].

Pseudocopulation

Flowers can sometimes go to great lengths to attract pollinators. One of the ways to help ensure pollination is to evolve a specialized relationship so that you attract only one species of pollinator — increasing the likelihood that the pollinator will visit only your species. One of the most ingenious methods flowers can employ is to "pretend" they are pollinators themselves!

Some orchids, like *Ophrys sphegodes*, mimic the appearance and volatile pheromones of the females of the bee species *Andrena nigroaenea* to attract males of that species to visit and transfer their pollen. The process by which the male confuses the flower with a female is known as 'pseudocopulation' (refer **Fig. 5**). Interestingly, the scent of the flower changes after pollination — the chemical farnesyl hexanoate increases in concentration and inhibits further visitations by male bees[7]. Since the biosynthesis of flower scent is a costly effort, plants like the *Orphys* orchid use these chemical odours judiciously to maximize pollination by reducing the attraction of insects to already pollinated flowers.

Fig. 5. A mining bee *(Andrena nigroaenea)* pollinating an early spider orchid *(Ophrys sphegodes).*

Credits: Keith Wilson. URL: https://www.flickr.com/photos/wislonhk/27042427467/in/photostream/.

Teaching Tips

Guess the Pollinator: Take a trip outside and observe several flowers. Have the students guess what types of pollinators could be attracted to these flowers (e.g. Are they night-blooming? Are they insect or bird-sized?). Then, using the internet, try to identify the flowers and their pollinators.

The Perfumery: Obtain several essential oil samples from flowers. Have the students guess which plants they come from. Which scents are similar? Which are different? Is there something about the flower biology that makes some flower scents similar — for example, do they have similar pollinators (do they bloom in the same areas at the same times of day)? If possible, try to observe the real flowers and test your hypotheses!

Make a Flower: Create your own artificial flower experiment! Using paper, crayons or pencils, and toothpicks, create artificial flowers to test outside in the morning. Did you trick any pollinators to visit your artificial flowers? Add a drop of essential oil from a flower or some perfume and see if this enhances the attraction of pollinators to your artificial flower. Were you successful? Why or why not?

Importance of Pollinators: Have the students use reputed websites such as FAO.org to research the importance of pollinators to our crops. See if they can guess which fruits and vegetables are 100% reliant on animal pollination. How many of these crops grow here in India?

Studying flower scents

Flower scents are studied by collecting gaseous compounds emitted from flowers and analyzing them in the laboratory. Several techniques are available for the collection of volatiles. Floral odours can be collected from the air around the plant, known as headspace collection; or by directly extracting compounds from the floral tissue using solvents (much like we make tea). Plant volatiles can also be collected by trapping them on adsorbent surfaces, like charcoal or polymers, through a process known as solid-phase extraction (refer **Fig. 6**). To analyze floral volatiles using solid-phase extraction, they must then be removed from the adsorbent surface using either solvents or heat and separated from the mixture by fractionation. After collection, individual floral scent compounds are further isolated using a technique known as gas chromatography and identified using techniques such as mass spectrometry.

To understand the function of individual floral volatiles in nature, we can create artificial 'dummies' or 'flower lures' with the colours, shapes, and odours of real flowers, and observe how pollinators respond to them (refer **Fig. 7**). These artificial flower lures can mimic a flower with or without its scent, with a different colour, or with different combinations of floral scents. Scientists

Fig. 6. Examples of solid-phase extraction of plant volatiles.

(a) Solid-phase extraction of volatiles using Polydimethylsiloxane (PDMS) tubes from an inflorescence of *Pentas lanceolata*.

(b) Solid-phase micro extraction of volatiles in the inflorescence of *Hedychium* species.

Fig. 7. An insect visiting an artificial flower made of paper and artificial odour.
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can then use these lures to test how different colours, shapes, and scents change the preference of pollinators in different ecologies[8, 9].

Conclusion

"Perfumes are the feelings of the flowers" — Heinrich Heine

The next time you walk in a garden, don't just smell the beautiful fragrances of flowers. Take a moment to stop and think how these lovely odours are the plant's means to interact with its ecology. This is the language of nature. At any given moment, there are thousands of chemically-mediated interactions happening in your own backyard — in each plant, animal, and microbe you see. If you have any questions or observe any of these interactions, do write to us.

Glossary

Ecosystem: The interactions between a community of living organisms and the non-living components in a particular area.

Terpenoids: A class of organic compounds formed by the combination of five carbon units, linked together in a head-to-tail fashion.

Flavonoids: A group of organic compounds with two phenyl groups attached to a ring of carbon and oxygen atoms. Most color pigments in plants belong to this group.

Steroids: Another group of organic compounds with three six membered carbon rings and a five membered carbon ring attached to each other in a specific arrangement.

Alkaloids: A group of nitrogenous organic compounds that have significant physiological effects on humans.

Phenylpropanoids: A class of organic compounds having a phenyl group attached to a propane (three carbons) side chain. Phenylpropanoids are derived from the amino acids phenylalanine and tyrosine.

Fatty acid derivatives: Volatile organic compounds derived from fatty acids present in the cell wall, and released during cell wall rupture.

Nectar: A sugar-rich solution produced in plant glands called nectaries. Plants secrete this solution in flowers to attract pollinators, and in vegetative parts like stems and leaves to attract animals, such as ants, that can deter herbivores.

Microbes: Unicellular or multicellular organisms such as bacteria, fungi, etc. that are too small to be seen with the naked eye.

Volatility: The relative tendency of a substance to evaporate into the air.

Linalool: A terpenoid alcohol present in most plant volatiles.

Orchids: Members of the family *Orchidaceae*, with flowers that are often colourful and fragrant.

Pheromone: Chemical compounds released by animals as signaling compounds to another member of the same species.

Extract: A process of separating chemical compounds from any material (mainly biological) using solvents.

Solvents: A liquid, solid, or gas used to dissolve a substance (called a solute) without changing the nature of the solute.

Gas chromatography: A separation technique mainly used to separate gaseous compounds based on the partition between a stationary liquid (or solid) phase and a mobile gaseous phase.

Mass spectrometry (MS): An analytical technique that ionizes a chemical compound, separates its ions, and quantifies them, to obtain a chemical fingerprint that allows identification of the compound.

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