

Right Under Our Noses: The Role of Floral Scent in Host Detection for a Solitary Specialist Bee

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The Boyce Thompson Arboretum manages to balance a hands-on laboratory for the scientifically inclined with an aesthetic studio for admirers of the beauty inherent to the natural world. On any given spring day at the Arboretum, leguminous bushes present flowers with wings, banner, and keel carefully folded around troves of pollen. Narrow *Penstemon* flowers coyly curl fused petals around heart-shaped anthers and copious drops of nectar. Easter Cacti (like *Echinopsis candicans*), demurely drooped during daylight hours, are boldly unfurled and ethereally white at night when moths and bats might peruse the gardens. Boyce Thompson Arboretum displays the spectacular beauty of flowers, a mosaic of delicate orange and red-hued blossoms contrasted with a palette of blues and purples, while simultaneously preserving botanical rarities and varieties scientists might otherwise have to leave the country to observe.

The amazing diversity of colors seen among the flowering plants at Boyce Thompson Arboretum are in large part a manifestation of a flower's need to advertise to potential pollinators. Pollinators learn to associate flower color, shape, and size with pollen and nectar rewards (Dafni, 1992) and, as they move between conspecific plants, induce fertilization. In addition to the vibrant and obvious colors and shapes that flowers use to attract bees, there is the more subtle attractant that is floral

scent. Though oft-noted by those who enjoy flowers, floral scent is one of the least studied aspects of pollination ecology, and the specifics of its role in attracting pollinators are not well understood (Raguso, 2008).

Bees are one of the primary pollinators of flowering plants, responsible for seed-set in the majority of wild flowers in temperate regions, as well as for many agriculturally important plants (Axelrod, 1960, Ricklefs and Renner, 1994, Klein et al., 2007). Nearly all of the flowers on display at the Boyce Thompson Arboretum are visited by one to many of a wide array of bee species.

Worldwide there are over 20,000 species of bees; here in the U.S. there are nearly 4,000 species, and in Arizona, where the Boyce Thompson Arboretum resides, there are over 1,000 species (Ascher and Pickering, 2013). These bees are as diverse in form and color as the flowers they visit. Blonde *Perdita* are only a few millimeters from antennae to sting. In contrast are tar black *Xylocopa*, looking and sounding like miniature helicopters. In between are jeweled gems like *Osmia*, some of which are emerald green or bright metallic blue. *Nomada* are red and yellow striped. *Diadasia* look just like flying fuzzy bears. *Eucera* have antenna that stretch the length of their bodies. And there are of course the well-known honey bees and bumblebees. As well-known as they are, they make up less than 1% of all bee species, and their life history is hardly representative of bees at large.

Most bees are solitary, not social like honeybees and bumblebees. For solitary bees, there is no queen bee, there are no workers, and though there are of course male bees, there are no drones. There is therefore no division of labor between hive members for solitary bees (for there is no hive). Instead, each female builds her own nest which she provisions with pollen and nectar that she collects to provide for eggs that she lays. What's more, most bee nests are dug in the ground (Cane, 1991), though they are also commonly found inside the pithy stems of twigs and dead wood (Westrich, 1996). Some are mud constructs cobbled together with rocks and plastered to the side of some vertical surface (Kuhlmann and Timmermann, 2009). Half a dozen to several dozen eggs are laid in each nest, but each one gets its own nest cell (nursery) and its own "loaf" of pollen. Nest cells are separated from each other by carefully constructed partitions built by the female. These nests are often elaborately prepared before eggs are laid inside. For example, *Colletes* species "paint" the walls, using their specially flattened tongues, with a sort of cellophane that makes the nest waterproof (Hefetz et al., 1979). Some species collect flower petals or leaves and wall-paper the insides of their nests, wrapping each egg in an envelope of herbaceous material. Still other species scrape the pubescence off of plant leaves and line the insides of their nest with this soft material.



Figure 1.
A cactus-specializing
Diadasia on
Cylindropuntia (cholla).

All figures by Olivia
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Bees rely on flowers for all their nutritional needs; they drink nectar for their own energy and carry both nectar and pollen back to the nest. Pollen is collected from flowers and packed into special hairs (called scopa) on their legs or abdomen for transport back to their nests. The pollen is there removed, mixed with nectar and left for the larvae to find when it hatches from its egg. Pollen is energy-rich, and contains many amino acids and other “essential nutrients” needed for proper larval development (Roulston and Cane, 2000). Without flowers, bees would be... well, wasps.

Many bees are floral specialists (called oligoleges); they collect pollen from only certain flowering plants (Linsley and MacSwain 1958, Linsley 1961) for provisioning nest cells. This specialization persists between non-overlapping generations, and throughout the geographic range of the bee. While specialist bees usually forage from a range of closely related plant species (called hosts), the breadth of this specialization varies between species. For example, several bees specialize on species in the genus *Sphaeralcea* (globe mallows). Other species are more broadly oligolegetic, visiting only genera in Malvaceae (e.g., *Sphaeralcea*, *Malacothamnus*, and *Sidalcea*). Still other species are known as “eclectic oligoleges”; they visit only a select few plants, but these plants are not closely related. *Osmia ribifloris* for example, visits only *Cercis*, *Berberis*, and *Arctostaphylos*, three genera in three different plant families (Fabaceae, Berberidaceae, and Ericaceae respectively; Sipes and Cane, 2006). Scientists estimate that 30-70% of the world’s bees are oligolegetic (Robertson 1914, Linsley 1958, Moldenke 1979), and that this dietary restriction is especially common in arid regions (Pekkarinen 1997, Minckley 2008).

An evolutionary pattern commonly seen in specialist bee taxa is the switching of closely related bee species to distantly related floral hosts. For example, the bee genus *Ptilothrix* includes species that visit Convolvulaceae, Malvaceae, Onagraceae, or Cactaceae (Sipes and Tepedino, 2005). *Lithurgus* specialize on

Cactaceae, Malvaceae, or Asteraceae (Michener 2000). *Chelostoma* are nearly all specialists, with different species specializing on Asteraceae, Campanulaceae, Hydrophyllaceae, or Ranunculaceae (Sedivy et al., 2008). Note that none of these transitions represents an increase in the dietary breadth of the bee, but merely a switch in host preference. Presumably at some point in the past, some individuals of the common ancestor to today’s extant species switched to a new host.

Scientists hypothesize that the first bees were specialists, expressly cued in to the visual and olfactory signals presented by their host flowers. Early specialists, it is thought, likely lacked the receptors necessary to sense flowers other than their own. For example, bees smell flowers using their antennae. Receptors on the antennae correspond to particular scent compounds; if a bee doesn’t have the receptor for a particular compound, it can’t smell it. Honeybees, who are among the best studied bees in terms of their ability to smell, have hundreds of receptors and can distinguish between complex scent bouquets (Laska and Galizia, 2001). Honeybees are generalists, and this receptiveness to the odors of diverse flowers likely helps guide them to the many resources they require. A specialist, on the other hand, may have fewer antennal receptors because they only need those that help them distinguish their host plant from any co-blooming non-hosts. Over evolutionary time scales, generalist bees arose from specialists as they developed the receptors for more and more compounds. Put another way, specialists may be physiologically limited in their ability to recognize the majority of floral scent compounds produced.

I set out to test this theory with a specialist bee genus, *Diadasia*. There are 45 species of *Diadasia*, 28 of which are found in the western (especially southwestern) United States. While all *Diadasia* are specialists, the plant families on which they specialize differ. Six species will only visit Cactaceae. One species each visits only Asteraceae (specifically *Helianthus*), Convolvulaceae (specifically *Calyptegia*), or Onagraceae (specifically *Clark-*

ii). The rest specialize to various extremes on Malvaceae, with some species visiting one genus exclusively, and others dining on many genera within the family. Malvaceae is the most likely host plant for the ancestor of all *Diadasia*, so at some point host-switching to these alternative families occurred.

If it is true that specialist bees are limited in their ability to recognize the chemical or visual signals put out by any host plants but their own, how could this host-switching happen? How could a bee that can only smell one type of flower recognize another at all? I hypothesized that:

1. Malvaceae, Cactaceae and other host flowers that are visited by *Diadasia* must smell remarkably similar, thus explaining the ability of this specialist bee to make forays onto new host plants.

2. *Diadasia* are unable to recognize compounds produced by other plants that bloom in the deserts at the same time as their hosts, explaining why they do not visit these flowers.

Materials and Methods

To test these hypotheses, I analyzed the floral scent of host plants for *Diadasia*, including mallows that bloom in the desert southwest, focusing on *Sphaeralcea* (globe mallow), and several genera of cactus, focusing on species of *Opuntia* (prickly pear). *Sphaeralcea* and *Opuntia* are common in the southwest, and are major hosts for many species of *Diadasia*. The Boyce Thompson Arboretum has a diverse cactus collection, and I was able to collect scent from many cactus flowers that I would have otherwise been unable to include in my study. Outside the arboretum, the desert southwest is replete with mallows that are visited by *Diadasia*, including *Sphaeralcea*, *Malacothamnus fasciculatus* (Bush Mallow), *Hibiscus denudatus* (Rock Hibiscus), *Malvella leprosa* (Alkali Mallow), and *Eremalche rotundifolia* (Desert Five-Spot). I also collected scent from a number of co-blooming non-host plants. I focused on plants that are attractive to other bees, including other specialists, but are never visited by *Diadasia*: *Baileya multiradiata* (Desert Marigold), *Wyethia scabra* (Mule's ears), *Gutierrezia sarothrae* (Broom Snakeweed), *Larrea tridentata* (creosote), *Argemone corymbosa* (Prickly Poppy), and *Cirsium arvense* (thistle).

To collect the scent from each of these flowers, I used a miniature 'vacuum cleaner'. Each flower was enclosed in a plastic bag, from which the headspace was sampled. Scent from this enclosed space passed through a glass vial filled with Porapak Q (80-100 mesh, Supelco)—adsorbent crystals, similar to charcoal. I collected scent from flowers in this way for a standard one hour time period. I washed the scent compounds from the adsorbent with hexane, and used a gas-chromatograph coupled to a mass-spectrometer to determine which compounds were present in each flower. A gas chromatograph separates compounds based on their chemical properties, so



Figure 2. Sampling the “headspace” around a *Sphaeralcea* flower using a miniature personal air sampler and a glass vial containing the adsorbent called Porapak Q.

that each passes from the instrument individually. Its identity can be determined by examining both the time it takes it to pass through the gas chromatograph (its retention time) and the spectra of the compound based on the masses of the molecular fragments comprising the original compound.

With a list of the compounds produced by each plant in hand, I next determined whether *Diadasia* are capable of recognizing those compounds. Without the appropriate antennal receptors, *Diadasia* are incapable of recognizing specific compounds. I attached electrodes to the ends of a freshly removed *Diadasia* antenna, and then exposed those antennae to individual compounds. If the antennae had receptors for a particular compound, an electrical signal was generated, indicating that the bee would be capable of smelling that compound.

Finally, I tested whether the ability of *Diadasia* to recognize a compound was the equivalent of being attracted to it. I used a cotton swab to apply a miniscule amount of different scent

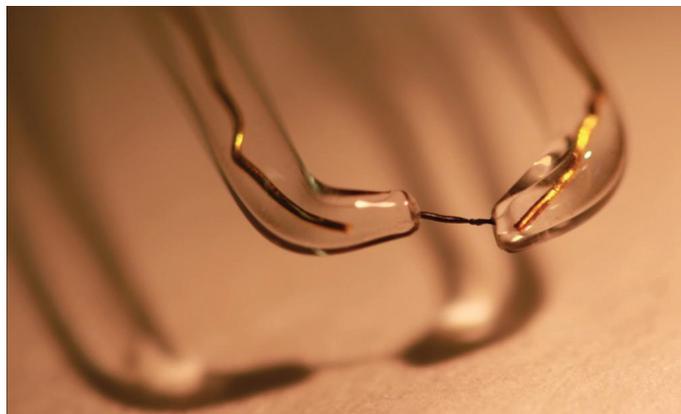


Figure 3. A *Diadasia* antenna placed between the electrodes used to measure electrical stimulus when the antenna is exposed to isolated scent compounds.

compounds to host-flowers (*Sphaeralcea* and *Opuntia*) in the field and watched for changes in visitation rates. Flowers were only treated with scent compounds that I had found to be recognized by *Diadasia*.

Results

Mallow (Malvaceae) and cactus (Cactaceae) flowers visited by *Diadasia* produce a wide array of compounds. In general, cacti produce fewer compounds than mallows, with *Sphaeralcea* flowers producing the most (between 50 and 80 compounds, with any one flower producing between 35 and 50). A handful of compounds are shared between mallows and cacti, with nonanal, trans- β -ocimene, benzyl alcohol, and benzaldehyde being the most predictable in both plant families. One notable difference between *Opuntia* and *Sphaeralcea* was that *Sphaeralcea* contained more methyl salicylate than *Opuntia* flowers. Many of the individual compounds identified are strong-smelling (nonanal is very common in *Diadasia* visited plants, and smells—to me—like warm cooking oil, benzaldehyde smells like almond extract, methyl salicylate smells like wintergreen, and isoeugenol, which was found in some non-host flowers, smells like cloves); together, and in specific amounts, these and other compounds produce unique scents.

Interestingly, none of the compounds produced by *Diadasia*-visited mallows and cacti were unique to them. All of the compounds found in, for example, *Sphaeralcea*, are also found in other plants that co-bloom in the desert southwest. The most abundant compounds (i.e., those that most contribute to the smell of *Sphaeralcea*) are in fact the most abundant in any floral scent. For example, trans- β -ocimene is found in over 70% of all plants that have been studied in terms of their floral scent around the world.

So while the presence of these compounds certainly united *Diadasia* host plants, it did not separate them from the other



Figure 4. A mallow-specializing *Diadasia* on *Sphaeralcea*.

plants that also bloom in the desert at the same time. In other words, it did not explain why a *Diadasia* would switch from Malvaceae to Cactaceae, and not, for example, to *L. tridentata*, or *A. corymbosa*—all contain trans- β -ocimene (among other common compounds).

For non-host flowers, I found a much greater diversity of compounds than in *Diadasia* host plants. *Larrea*, for example, contains nearly 100 different compounds. Other non-hosts are similarly rich in compounds, containing all of those found in host flowers as well as many others. Put simply, *Diadasia* host plant flowers are particularly bland when it comes to floral scent.

I next assessed which of the compounds produced by host-flowers were detectable to *Diadasia*. I found that *Diadasia* are not capable of sensing every compound produced by their host flowers. In fact, of the 35 to 50 compounds produced by *Sphaeralcea*, *Diadasia* are only able to recognize 12 to 15 compounds. None of the compounds that *Diadasia* can recognize are unique to their host flowers, and all are commonly found in non-hosts. Interestingly, all of the compounds found in Malvaceae that elicited an antennal response in *Diadasia* are also found in the cactus flowers that host *Diadasia*. Even methyl salicylate, the

	Diadasia australis (Cactaceae)	Diadasia vallicola (Sphaeralcea)
Benzaldehyde	0/5	3/3
Benzyl alcohol	2/5	1/3
Trans- β -ocimene	3/5	7/7
Linalool	5/5 (-) ($t_6=2.82$, $p=0.03$)	4/4 (-) ($t_{22}=2.582$, $p=0.017$)
Nonanal	3/3	6/6
Methyl salicylate	5/5 (-) ($t_{22}=2.34$, $p=0.029$)	6/6
Isoeugenol	4/4 (-) ($t_{22}=2.541$, $p=0.0186$)	4/4 (-) ($t_{22}=4.665$, $p<0.001$)
Trans- β -caryophyllene	1/6	3/5
Trans-trans- α -farnesene	6/6	5/5

Table 1. *Diadasia australis* specializes on Cactaceae, and *Diadasia vallicola* specializes on *Sphaeralcea*. These are nine of the compounds that elicited antennal responses from these bees; the numerator indicates the number of antennal responses and the denominator indicates the number of trials. Fractions in bold indicate that the compound naturally occurs in the host plant (e.g. methyl salicylate was found in *Sphaeralcea* but not in *Opuntia*, linalool occurs in neither). All compounds were tested in the field, but only those compounds not naturally occurring in the floral scent changed the visitation rates of these specialist bees to their hosts. A (-) next to the fraction indicates that adding the compound to a host flower (either *Opuntia* or *Sphaeralcea*) significantly decreased the number of visits, compared to an untreated flower (thus, the pairwise t-test values listed).

compound that occurred in greater abundance in *Sphaeralcea* than in *Opuntia* flowers, was detected by the antennae of both mallow-specializing and cactus-specializing *Diadasia*.

In addition to those 12 to 15 compounds from host plants, I also found that *Diadasia* are capable of recognizing many compounds produced by non-host flowers. For example, trans- β -ocimene and nonanal both generate strong electrical signals in *Diadasia* bee antennae. Both are commonly found in the mallow and cactus species visited by *Diadasia*, as well as in non-host flowers like *L. tridentata* and *A. corymbosa*. Isoeugenol is produced by *A. corymbosa*, and it elicits a strong response from *Diadasia* antennae even though it is not found in their host flowers.

Lastly, I applied miniscule amounts of the compounds recognizable to *Diadasia* to host flowers. For compounds that were already in their host plants, the application of additional scent did not change visitation rates. As an example, adding more trans- β -ocimene to *Sphaeralcea* or cactus flowers that already contained it did not change the number of visits it received from *Diadasia*. For compounds that occurred in non-host flowers, adding the scent to a typical host flower significantly decreased visitation. Adding isoeugenol, which occurs in *A. corymbosa* flowers, to *Sphaeralcea* flowers or *Opuntia* flowers caused a significant decrease in the number of visits by the *Diadasia* that specialize on each of them. Adding methyl salicylate to *Opuntia* flowers caused a decrease in the visitation of cactus-specializing *Diadasia*; adding the same compound to *Sphaeralcea* flowers did not cause a similar decrease in mallow-specializing *Diadasia*.

Discussion

Taken all together, it appears that the hypothesis that specialist bees are limited in their ability to detect any but the compounds found in their host flowers doesn't apply to *Diadasia*. *Diadasia* are capable of detecting many compounds that they do not encounter in their host flowers. They are not attracted to any of these non-host compounds, however, and steer clear of flowers that produce (or have been modified to include) them. This is a novel finding in the field of specialist bees and floral scent. It suggests that not all specialist bees evolved the same way. A group of scientists recently developed a hypothetical pathway by which specialization and generalization could evolve, and suggested that, though all bees started as specialists, some evolved to be generalists, and then branches of those generalist lineages evolved *back* into specialists (Sedivy et al., 2008). Perhaps the ability of *Diadasia* species to detect more compounds than just those in their host plants hints at a period in history when their ancestors were generalists.

Returning to my original quandary about why species of *Diadasia* currently visit such disparate plant families, could it be that *Diadasia* switched from some species of Malvaceae to some species of Cactaceae because they are similar in that they are both remarkably bland? Considering that many pollens are 'poisonous' to bees not adapted to digesting them, a strange compound emanating from a flower may signal to a bee that there is a likelihood of being harmed if that pollen is ingested. *Diadasia* appear to have inherent preferences for certain compounds over others, and these include nonanal, trans- β -ocimene, and several other common plant compounds. Interestingly, it appears that these preferences may be genetically driven, and differ even between species of *Diadasia*, with

mallow-specializing *Diadasia* being more attracted to (or less deterred by) the presence of methyl salicylate than are cactus-specializing *Diadasia*.

My research is an important building block in the field of chemical ecology as it pertains to plant-pollinator interactions. The chemical cues produced by flowers are a way for flowers to communicate to bees, and provide important information about the resources they contain. Whereas some compounds indicate that a floral-visit will be rewarding, others let a bee know that they should seek to fulfill their nutritive needs elsewhere. Right under our noses there are conversations playing out daily. On your next visit to Boyce Thompson Arboretum, consider what the floral displays that you see may mean, and what messages these beauties are sending to their pollinators via floral scent.

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