



SYMPOSIUM

The Neuroecology of a Pollinator's Buffet: Olfactory Preferences and Learning in Insect Pollinators

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Synopsis Plants and their pollinators are excellent examples of mutualistic associations that range in specialization, from obligate to generalized mutualisms, with many pollinators interacting with diverse species of flowers while still maintaining specialized associations. Although floral odors have been implicated in mediating these interactions, identification of the odors—and how the odors are represented in the olfactory system—has been elusive, and the manner in which olfactory learning mediates the generalized plant–insect interactions in the field remains unclear. This review details the composition of floral bouquets that elicit strong attraction in pollinators, demonstrating that for some species of plants the composition of the bouquet plays an important role in exploiting the insect's olfactory system, thereby driving innate attraction, whereas other bouquets can be learned as an associative cue for the nectar reward. By associative learning of nonattractive floral odors with a nectar reward—through octopamine-associated modulation of neurons in the antennal lobe—insects have the ability to exploit alternate floral resources when their preferred flowers are no longer present. Such neural mechanisms, present in specialist and generalist pollinators, provides the means by which pollination associations can range from specialized to generalized while permitting insects to exist within a dynamic floral environment.

Introduction

Pollinators operate as agents of fitness and selection, and play fundamental roles in biodiversity and maintenance of the structure of plant communities (Campbell 1989; Conner et al. 1996; Schemske and Bradshaw 1999; NRC 2007; Gómez et al. 2009). With a range of cognitive abilities and innate preferences, pollinators have the ability to engage in specialized interactions with one, or several, species of flowers, while having the flexibility to learn to exploit alternate resources (Kelber 2002; Rodríguez et al. 2004; Riffell et al. 2008a). Indeed, community-level pollination studies in a variety of different ecosystems have shown that most plant–pollinator interactions are generalized (Waser et al. 1996; Jordano et al. 2003; Waser and Ollerton, 2006), with species typically interacting with a core group of generalists, i.e., keystone species (Bascompte et al. 2003). Yet, a fundamental gap in these studies is a detailed

understanding of the mechanisms mediating these generalized interactions—particularly the role of specific floral traits and the behavioral and learning abilities of the pollinators—and the sensory mechanisms mediating the specialized relationships between specific plants and pollinators (Bascompte and Jordano 2007; Raguso 2008). The composition of the assemblage of pollinators and behaviors mediating interactions may operate as a selective force on a key floral species, but these effects are not well understood.

A fundamental component mediating the generalized interactions in the pollination “network,” as well as the specialized interactions between specific plants and their pollinators, can be broken into two elements: (1) the neural and sensory bases of the pollinators' behaviors, and (2) the floral displays that operate as attractive “signals” to pollinators. The display of the flower, involving scent, color, pattern, morphology, touch and taste all serve to

stimulate the pollinator's sensory system. For example, color is the dominant floral trait mediating visitation by the diurnal butterfly, *Vanessa indica*, and the hawkmoth, *Macroglossum stellatarum* (Ômura et al. 2005; Balkenius et al. 2006), whereas for plasterer bees (*Colletes cunicularius*) the floral odor is more important than color (Vereecken and Schiestl, 2008). These “innate” responses to flowers involve specific preferences towards certain traits, including flowers' color or scent, which maintains the specialized associations. Conversely, the predominance of many generalized interactions in pollination “networks” may lie in the ability for pollinators to learn to exploit alternate floral resources. Honeybees and bumblebees (*Apis mellifera* and *Bombus* spp., respectively) are excellent examples of memory and learning ability; a honeybee, for instance, has the ability to learn a human's face (Dyer et al. 2005) and has the ability to retain a memory for weeks to months (Lindauer 1967; Menzel 1968). Even pollinators that are more specialized can learn (Lewis 1983; Kearns et al. 1998; Weiss 2001; Riffell et al. 2008a). For instance, the pipevine swallowtail butterfly (*Battus philenor*) is innately attracted to yellow flowers of *Lantana camara*, but can switch their preferences based on the presence of a nectar reward in magenta flowers (Weiss 1997). Similarly, the hawkmoth, *Manduca sexta*, is a specialized to flowers in a given location, but has the ability to learn the association of a novel floral odor with a nectar reward (Riffell et al. 2008a). These studies demonstrate that examining pollinators' innate and learned responses to specific floral cues can lead to improved understanding of interactions in the field.

An important floral trait that has until recently been neglected is floral scent. Floral scent mediates many plant–pollinator interactions through the pollinator's innate preferences and the ability of pollinator to learn the association between the floral scent and nectar (Raguso 2008). For example, the moth, *Manduca sexta*, has innate preferences for aromatic-rich floral scents that help maintain the mutualistic associations between these moths and the “moth pollinated” flowers (Riffell et al. 2009a; Reisenman et al. 2010). Floral scents can also exploit an innate olfactory preference of the pollinator. For example, in the deceptive pollination system of the *Arum palaestinum* lily, *Drosophila* spp. are attracted to the vinegar-like smell—an indicator for an oviposition site or food resource—emitted from the lily. The scent lures the fruit fly into the lily's chamber, where they become trapped and exploited as pollinators (Stöckl et al. 2010). Similarly, the orchid,

Chiloglottis trapeziformis, attracts males of its pollinator species, the thynnine wasp *Neozeleboria cryp-toides*, by emitting a scent that is also produced by female wasps as a sex pheromone (Schiestl et al. 2003). Conversely, pollinators have the ability to rapidly learn the association between a floral nectar reward and the scent; generalist pollinators, like bumble bees and honey bees, have been particularly well-studied models for determining the cognitive mechanisms of olfactory learning. Once learned, honeybees have the ability to retain an olfactory memory for weeks to months (reviewed by Menzel 1985; Menzel and Muller 1996), and this cognitive ability by honeybees is thought to be a reason for their success and a major generalized player in pollinator assemblages.

The sometimes incredibly specialized floral displays operating as attractive “signals” to pollinators, and the ability for pollinators to learn other floral resources, make plant–pollinator interactions excellent systems for examining the neural bases of the behaviors with how those behaviors control interactions in the field. In light of the sensory basis of the pollinator (the “receiver”) and the flower (the “signaler”), it is useful to examine these interactions within a framework of sensory communication: (1) Floral odors have evolved as either “honest signals” of the floral rewards (pollen, nectar) or alternately they can operate to “exploit” the pollinator's olfactory system; and (2) The generalized interactions that are common between many plants and pollinators (Waser et al. 1996) may be due to the evolution of floral signals attracting multiple receivers, or alternately, through the flexibility of the pollinator's learning systems.

In the last several years, our understanding of the contribution of floral odors in mediating pollinator behavior has increased (reviewed by Dobson 2006; Raguso 2008) in parallel with our understanding of the molecular mechanisms regulating production of the floral scent (Dudareva and Pickersky 2006). But our understanding of the contribution of those processes and the sensory mechanisms of pollinators in mediating interactions in the field has only now begun to yield fruit (Schiestl et al. 2003; Stöckl et al. 2010), and olfactory and neurobiological bases of these interactions have remained elusive. This review details some of the recent work on floral odor chemistry and the mechanisms by which these odors are perceived by pollinators. I argue that detailed understanding of the neural bases of these behaviors provides outstanding opportunities for predicting how the behavior of the

pollinator controls interactions in the field. Towards this end, this review is organized around three foci:

- A description of the diversity of floral odors that operate as attractive “signals” to pollinators.
- The manner in which floral odors are processed by the pollinator’s olfactory system to mediate attraction to flowers.
- How pollinator plasticity and learning may influence interactions in the field.

Signaling by floral odor: specificity and diversity

The importance of floral odors in mediating plant–pollinator interactions, and the role of specific volatile chemicals in the floral scent, has become an increasingly important research topic in the past 15 years. Part of the difficulty in understanding the contribution(s) of floral scent is the complexity of the emitted bouquet—which can contain more than 300 volatiles (Vainstein et al. 2006)—, and technological limitations associated with the isolation and identification of the scent’s compounds. Compounding the difficulty in understanding the roles of floral scent in pollinator behaviors is identification of the specific compounds that mediates the pollinator visitation. For example, often the most important compounds in the scent can be the present at the lowest concentration (Riffell et al. 2009a). Recent technological advances in analytical techniques (reviewed by Tholl and Röse 2006; Riffell et al. 2008b) have made floral scent chemistry applicable to biologists working both in the laboratory and field. For example, in an elegant series of studies Roy and Raguso used headspace sorption and gas chromatography with mass spectrometric detection to identify the volatile components in the scent of *Puccinia monoica* fungus, a flower mimic (Raguso and Roy 2002), and examined the response of bees to manipulations of the floral scent components in field (Roy and Raguso 1997). The manipulative experiments were able to demonstrate the importance of floral odor in attracting generalist halictid bees (Roy and Raguso 1997). Furthermore, Gabel and coworkers (1992) combined an electrophysiological (antennal) assay with gas chromatographic detection (termed GC-EAD) to examine the responses of the European grapevine moth, *Lobesia botrana*, to floral odors (Gabel et al. 1992). Results from this study showed that the moths responded to only 9 out of the 200 volatiles extracted from the tansy flowers (*Tanacetum vulgare*). More recent work using multi-channel recordings in the antennal lobe (AL;

primary processing center of olfactory information) in the moth, *Manduca sexta*, showed similar results: only 9 out of the 60 odorants in the bouquet of the *Datura wrightii* flower were consistently processed in the AL (Riffell et al. 2009a, 2009b). Importantly, in both studies behavioral experiments confirmed the importance of the volatile subset in attracting the moths (Gabel et al. 1992; Riffell et al. 2009a, 2009b). Together, the advancement in analytical technologies with electrophysiological and behavioral studies have made identifying the floral odors mediating pollinator interactions an exciting and growing field of study.

A basic question about the role of floral scent is: to what degree is the floral scent operating to selectively attract a given pollinator, and is there a trend for the presence of specific components in the scent visited by a given taxon of pollinator? The specialization between plants and pollinators, termed “syndromes,” has been a debated topic in pollination biology during the past 15 years (Waser et al. 1996; Bradshaw and Schemske 2003; Fenster et al. 2004; Waser and Ollerton 2006; Ollerton et al. 2009), and analysis of the floral scent for both specialized and generalized pollination systems suggests that both processes are at play. For example, orchids pollinated by male euglossine bees emit specific fragrances also used by the male bees to attract females (Eltz et al. 1999), and the sexually deceptive orchid, *Chiloglottis trapeziformis*, emits a pheromone that attracts wasps (Schiestl et al. 2003).

In contrast to these examples of specialized floral scents, more generalized floral species that serve a large pollinator assemblage may emit a diverse bouquet likely reflecting the variation and diversity in the effective pollinators (Dobson 2006; Knudsen and Klitgaard 1998; Knudsen and Gershenson 2006). For instance, flowers of the plant *Browneopsis disepala* are visited by both moths and bats and displays a mixed pollination syndrome with a bat pollinated floral display and an odor profile that is dominated by aliphatics and monoterpenes but lacks sulfur compounds (Knudsen and Tollsten 1995). Similarly, *Agave* spp. in the southwestern United States operate as “keystone” species in this semi-arid environment by providing abundant nectar resources to a diverse pollinator assemblage (Alarcón et al. 2008). The agaves emit a diverse fragrance composed of low levels of sulfur compounds (<1%)—a common marker of bat-adapted flowers—, esters (~50%), terpenoids (~30%) and aromatics (~15%). Sulfur compounds are important attractors of bats to flowers (von Helversen et al. 2000) and it has been suggested that shifts from bat to insect

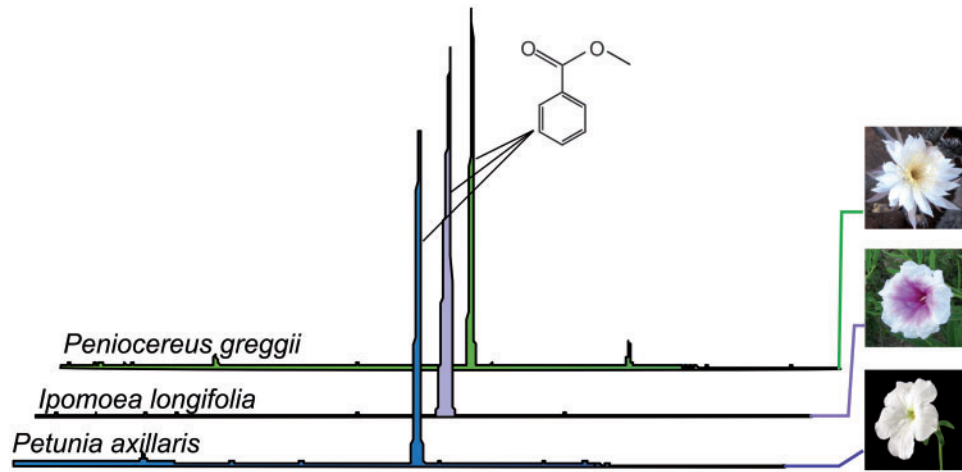


Fig. 1 The composition and similarity of scents emitted by flowers that are pollinated by *Manduca sexta*. (Left) Total ion mass chromatograms of the floral scent. The flowers visited by *Manduca sexta* have converged on similar bouquets dominated by oxygenated aromatic volatiles, in particular, the compound methyl benzoate (pictured). (Right) Flowers are: *Petunia axillaris* (Solanaceae), *Ipomoea longifolia* (Convolvulaceae), and *Peniocereus greggii* (Cactaceae).

pollination reflects the reduction of sulfur compounds and the presence of terpenoids and benzenoids in a floral headspace. Together, these studies suggest that floral species emitting odors that mediate obligate mutualisms may exploit preexisting olfactory biases in pollinators, whereas generalist floral species may attract a diverse pollinator assemblage by emitting a complex scent that is attractive to many different pollinators.

The two extremes of pollination biology, obligate mutualism and extreme generalization, are partly mediated through floral scent. However, the majority of plant–pollinator interactions fall within an intermediate area of facultative mutualisms and diverse interactions. Yet, even in this case floral scents can show a range of characters, from scents composed of a few compounds attractive to a pollinator class, to scents that are more diverse (Knudsen et al. 1993). For example, many hawkmoth-visited flowers emit fragrances dominated by oxygenated aromatic compounds, particularly those with alcohol functional groups such as methyl benzoate (Fig. 1); these compounds are highly attractive to hawkmoths, and mediate their odor-tracking and foraging responses to the floral odors (J. A. Riffell et al., unpublished data). Alternately, volatiles emitted by figs assist in mediating interactions between figs and fig wasps, with emissions consisting of mixtures of a few volatiles that are typically sesquiterpenes (Grison-Pigé et al. 2002). In these two cases and others, however, pollinator taxa have been shown to visit flowers exhibiting diverse floral traits and scents, suggesting that the floral odors are not operating as “private channels” (Raguso 2008). Nonetheless, even in

pollination systems in which interactions are relatively fluid, the floral scent may serve to attract a given pollinator class, thereby maintaining an association.

Last, the diversity of floral scent compounds between closely related species may reflect either shared evolutionary history or selection imposed by certain pollinator taxon. The scent diversity may reflect evolutionary “baggage” from a related species that is pollinated by other taxa, such as in *Ipomopsis aggregata* in which two subspecies exist in close proximity. The two subspecies, *I. aggregata* subsp. *candida* and *I. aggregata* subsp. *colina*, exhibit different floral traits—thin long tubes and whitish corollas for subspecies *candida*, and robust red flowers with shorter corollas for subspecies *colina*—and are pollinated by different species, *Hyles lineata* moths for subspecies *candida* and hummingbirds for *colina*. While the two floral species differ in their scent, with *candida* emitting an aromatic-rich scent and *colina* having lower emissions of scent, the two closely related species overlap heavily in their composition of terpenoid compounds likely reflecting their shared evolutionary history (Kenney et al., unpublished data). An alternate hypothesis for the diversity of floral scent lies in the metabolic pathways associated with the production of certain biologically-relevant components of scent, with the secondary compounds produced along the pathway having little function. The diversity of floral compounds may serve a dual function of fitness redundancy by attracting the “most important pollinator” while also attracting other pollinators when the “best” pollinator is temporally or spatially variable.

Pollinators' olfactory channels and processing

The diversity of floral odor chemistry and how that might mediate interactions in the field has preceded our understanding of the mechanisms by which these odors are perceived by the pollinator. Molecular and cellular studies of the mechanisms by which odors activate sensory neurons in the pollinator's peripheral olfactory system has dramatically increased within the past decade (reviewed by Touhara and Voshall 2009), but the mechanisms by which these complex odors are processed in the pollinator brain remains ill-defined. The antennal (olfactory) lobe is the first stage of processing in the insect brain and previous work has shown that many of the processing mechanisms for odor information occurs in the AL (reviewed by Hildebrand and Shepherd 1997; Wilson 2008), including combinatorial processing of complex odors causing the odors to be represented as unique patterns of spatial and temporal information. Input from the olfactory receptor cells that express a given olfactory receptor protein all converge into a distinct region of neuropil, called a glomerulus, with output neurons from each glomerulus (projection neurons, or PNs) proceeding to the higher centers of the brain involved in learning and memory (Fig. 2). Glomeruli can interact with one another via local interneurons that modulate the glomerular activation patterns through presynaptic inhibition, thus influencing the spatial representation of the olfactory signal (Christensen et al. 2000; Olsen and Wilson 2008).

Behavioral results from diverse taxa of pollinators have all shown that pollinators innately respond to a mixture of certain key odorants in the floral scent, but not to the individual odorants, thus suggesting a perceptual “binding” mechanism in the pollinator's brain. For example, through optical imaging in the honeybee's brain Deisig et al. (2006) showed that beyond three odorants in a mixture the representation of the mixture in the antennal lobe reached a steady-state representation and did not recruit additional glomeruli into the spatial activity pattern. The patterns of neural activity to the mixtures, although different from the responses to a single odorant, were more similar to “salient,” or key, odorants in the mixture suggesting that floral odor processing may follow elemental rules. Alternately, through optical imaging in the AL of the oriental fruit moth, *Cydia molesta*, Piñero et al. (2008) found that a key component in a floral mixture that caused synergy in the neural representation, thereby suggesting unique processing of the mixture. Similarly, through

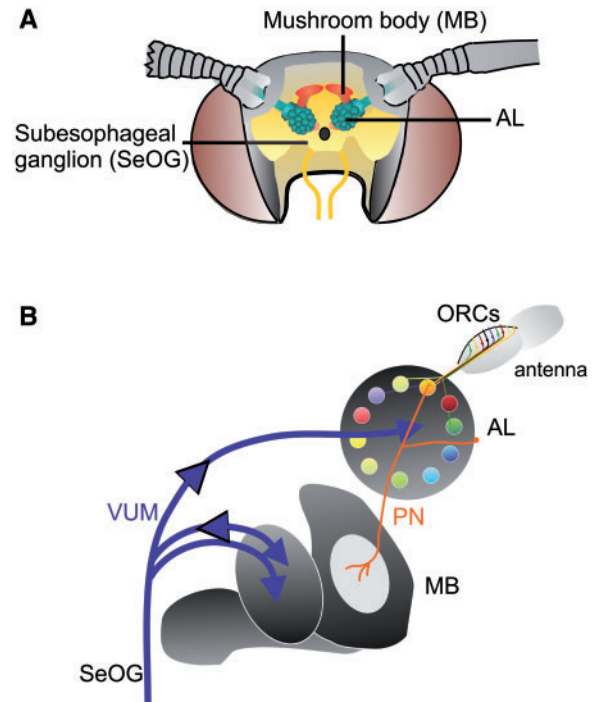


Fig. 2 Organization of the olfactory pathway of the adult moth, *Manduca sexta*. (A) Schematic drawings of the head (left), cut away to reveal the brain and the antennal lobe (AL), the primary processing center of olfactory information; the Mushroom Body (MB), the region of learning and memory; and the Subesophageal ganglion (SeOG) that receives gustatory input. (B) Depiction of the octopaminergic input from the VUM-like neurons to the MB and AL. Olfactory information arrives from the olfactory receptor cells (ORCs) on the antenna. Projection (output) neurons from the AL send axons down to the calyx of the MB, where the MB also receives input from the VUM-like neuron.

multi-channel recordings in the AL of *Manduca sexta* moths, Riffell et al. (2009a, 2009b) found that the floral mixture elicited a unique neural representation compared to the responses to single odorants, and that as long as certain key odorants were maintained in the mixture (linalool, benzaldehyde, and benzyl alcohol) the neural representation remained the same. Importantly, innate behavioral preference also correlated with the neural representation in the *M. sexta* and *C. molesta* work (Piñero et al. 2008; Riffell et al. 2009a, 2009b). Last, in an elegant series of experiments Stökl et al. (2010) showed that key odorants in the floral scent of the lily, *Arum palaestinum*, activates an evolutionary conserved olfactory receptor protein and AL circuit in *Drosophila* spp. The deceptive flower thereby “exploits” an innate sensory preference in the fly (Stökl et al. 2010).

The behavioral and electrophysiological studies described above, and those conducted by others on diverse taxa, have repeatedly demonstrated that

mixtures are discriminated from their single constituents (Hopkins and Young 1990; Dekker et al. 2002) and that certain components of a mixture are more salient than others (Wright and Smith 2004; Deisig et al. 2006). Indeed, the work mentioned above demonstrates that neural responses to the single odorants rarely resemble responses elicited by the complete floral mixture. What processing mechanisms take place in the AL to cause the unique representation of the floral mixture? One means is through interglomerular inhibition. Glomeruli in the AL of *M. sexta* are innervated by diverse local interneurons (LNs) that modulate PN responses (Lei et al. 2002; Reisenman et al. 2008). The inhibition imposed by the GABAergic LNs serves to modulate the activity of PN responses and AL activity, including enhancing the contrast in odor representation (Sachse and Galizia 2002), and mediating intraglomerular and interglomerular synchrony of neural activity (Christensen et al. 2000; Lei et al. 2002). In this manner, the inhibitory networks function to shape AL processing of floral mixtures in a manner not predictable from responses to the single volatile constituents or from the peripheral input from the antennae (Schlieff and Wilson 2007; Silbering and Galizia 2007).

The spatial (glomerular) and temporal (synchronized neuronal firing) patterns of activity in AL neurons provide a means by which floral odors may be efficiently processed. If flowers are pre-dominantly visited by a given class of pollinators, or pollinator genera, then having related floral odors may serve to maintain a constancy of interactions through stimulating a pre-existing olfactory circuit. The fact that fruit flies have an evolutionarily conserved olfactory circuit and behavioral preference towards aliphatic esters—volatile signals of microbe-rich food and oviposition sites—suggests that certain flowers have evolved that exploit the olfactory system (Stöckl et al. 2010; Ayasse et al. 2011). In a somewhat related manner, the convergence of similar floral scents, dominated by aromatic compounds and oxygenated monoterpenes, produced by floral species pollinated by hawkmoths may serve to be encoded similarly and generalized by the moth's olfactory system and activate the same olfactory information channels underlying innate odor preferences (Raguso et al. 2003; Riffell et al. 2008a). In the example of the fruit fly, the floral odor “exploits” the insect olfactory system causing the pollination to occur. In the example of the moth, the floral odor provides an “honest” signal that predicts the nectar reward. The fact that many moth-pollinated flowers have converged on a common scent suggests that the floral signal operates

on a pre-existing circuit in the moth olfactory system. The commonalities in olfactory coding and behavioral responses between many different taxa of pollinators suggests that common processes may control plant–pollinator interactions.

Learning and memory: a mechanism for generalized interactions in the field

Neural substrates and learning protocols

Insect pollinators have outstanding abilities for learning and memory of floral traits, and these processes can shape their flower preferences. The understanding of the neural substrates for learning and memory have benefited from classical conditioning experiments—much of this research coming from work with bees and more recently with fruit flies—, in which the insect is trained to associate a conditioned stimulus (odor) (CS) with an unconditioned stimulus (US), or sugar reward. Alternating the timing of the sugar reward and odor stimulus revealed that the association in the insect's memory forms when the odor precedes the reward within 1–3 s (forward paired). When the odor is presented after the reward (backward pairing), or randomized with respect to the reward, then the insects do not seem to learn the association between the two stimuli (Bitterman et al. 1983; reviewed by Menzel and Müller 1998; Daly et al. 2001). Memory of the association between the CS and US can persist for hours or days, depending on the training regimen and stimuli (Friedrich et al. 2004). Multiple learning trials, spaced over several hours, form long-term memory that persist for days (Tully et al. 1994; reviewed by Menzel and Müller 1998). The temporal association between the odor and reward stimulus for learning and memory suggests that information for the odor and reward stimulus are associated with specific neurons in the olfactory pathway, and that this olfactory circuit is dynamic.

Research on the neural substrates associated with olfactory learning and memory come from work with the honeybee (*Apis mellifera*), and more recently with the fruit fly (*Drosophila melanogaster*). In a classic series of papers, Martin Hammer and coworkers found in honeybees a group of neurons in suboesophageal ganglion (SeOG) of bee (ventral unpaired medial neurons [VUMmx1]) that respond to sucrose stimulation. This neuron innervates the mushroom body—thought to be the location of learning and memory in insects—and the antennal lobe (Kreissl et al. 1994). Hammer found that if he stimulated the neuron after he presented the bee with odor, the VUMmx1 stimulation mimicked the reinforcing

effects of sucrose in one-trial odor-conditioning: bees began responding to the odor stimulus via PER (Hammer 1993). Because VUMmx1 neurons are octopaminergic (OA), this suggested that octopamine mediates the reinforcing effects of VUM stimulation, and potentially also of sucrose reward (Kreissl et al. 1994) (Fig. 2B). Furthermore, this work suggested that output from the mushroom body is necessary for recalling the association between odor and reward, indicating that the olfactory memory is represented in mushroom body neurons and that the octopaminergic US pathway intersects the MB and AL. To determine the connection between the AL and MB, Hammer injected octopamine into the brain (mushroom and AL) and found that he could substitute the release of octopamine in response to the sucrose reward to initiate PER (Hammer and Menzel 1998). More recent studies have found that OA blocking via RNAi impairs appetitive learning in bees (Farooqui et al. 2004), and in flies OA conversion impairs odor learning (Schwaerzel et al. 2003; Schroll et al. 2006).

What are the effects of learning in the primary processing center, the AL, and how does the response of the mushroom body's neurons form the olfactory memory? Research in the past decade has shown that learning modulates the AL's responses to odor. Learning-dependent modulation in the insect's AL was first examined using functional calcium imaging by Faber et al. (1999), who showed that olfactory responses in the AL might change as a result of experience. With an associative learning paradigm, increased odor-driven calcium signals were observed in honeybee's ALs when the odor (CS) was paired with

a sugar reward (US). Subsequent work with multi-channel recording electrodes and optical imaging experiments in the AL of bees, flies, and moths have shown similar modulatory effects induced by appetitive learning or electrical stimulation of the MB (Daly et al. 2004; Yu et al. 2004; Arenas et al. 2009; Denker et al. 2010). Learning increases the levels of odor-evoked firing rates by neurons in the AL, and synchronized activity in the AL (Fig. 3; J. A. Riffell, unpublished data). This learning-evoked plasticity in the AL leads to enhanced discrimination of odors as well as to increase the overall gain of the system (Fernandez et al. 2009; Denker et al. 2010). For a foraging insect, plasticity in the AL may increase the sensitivity to the learned floral odor thereby increasing the constancy of the association.

Strong evidence has suggested that the mushroom body is the site for the CS–US association. These results come from the link between the VUM-like neurons in the AL and MB (Hammer 1993; Dacks et al. 2005; Sinakevitch and Strausfeld 2006), and the inability for insects to associate the US and CS when the neurons in the MB are experimentally ablated or anatomical mutations exist in the MB (Heisenberg et al. 1985; de Belle and Heisenberg 1994). Projection neurons in the AL transmit olfactory information to the calyx of the mushroom body, where the dendrites of Kenyon cells are widely distributed and believed to integrate the olfactory information. How olfactory information is processed by Kenyon cells is not well understood, but electrophysiological and optical recordings have demonstrated that odor-evoked activity by Kenyon cells are relatively “sparse,” with odors activating a few subsets of

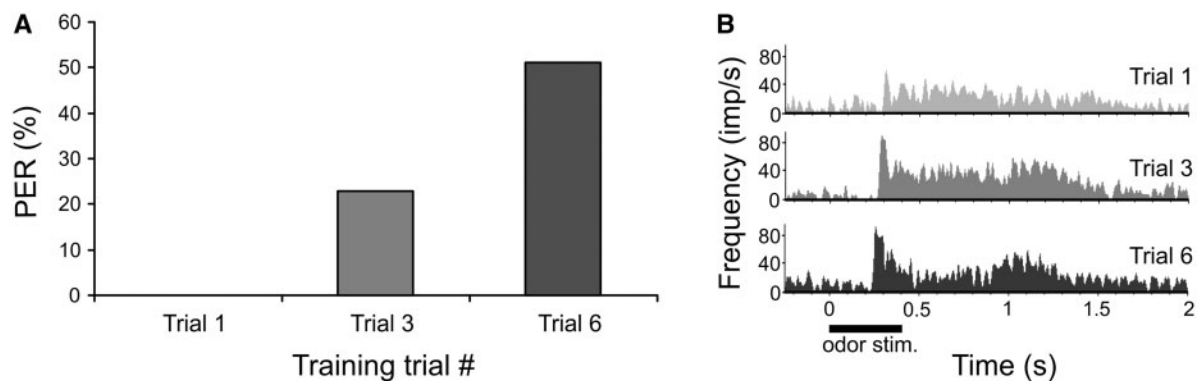


Fig. 3 Olfactory learning modifies the neural representation of the odor in the insect antennal lobe. **(A)** Acquisition of olfactory learning in *Manduca sexta* moths during classical forward-paired conditioning experiments with a floral odor (CS) and sucrose reward (US). Initially moths did not respond to the odor stimulus (trial 1), but by the third trial they learned the association between the odor and reward. **(B)** Peri-event histograms of the response by a single projection neuron to the CS odor through training. The ability to learn the association between odor and reward directly correlated with the change in odor-evoked AL neural responses in the moths. As the moths learned, AL neurons significantly increased their firing rate, decreased their latency of response, and synchrony between neurons increased.

Kenyon cells (Ito et al. 2008). Dubnau et al. (2001) and McGuire et al. (2001) found that blocking the output of Kenyon cells prevented the retrieval of appetitive memories in fruit flies, but not during the acquisition phase of olfactory learning. Nonetheless, how these Kenyon cell ensembles might represent the odors as the insect learns the US–CS association is unclear, but experiments in the future could record from the Kenyon cells while training the insects, thereby examining whether plasticity in the AL correlates with the MB and ascertaining how long the MB ensemble “retains” the olfactory memory represented by the sparse ensemble of Kenyon cells.

Memory effects and interactions in the field

Extensive work with bees (*Apis mellifera*, *Bombus* spp.) and other insects has expanded information about the mechanisms of learning by insect pollinators in the field. For example, young forager bees learn floral odors associated with model bees as well as odors that forager bees bring back into the hive (Free 1987; Seeley 1994; Dornhaus and Chittka 2005; Farina et al. 2005). Bees encountering a flower patch for the first time exhibit “orientation” flights in which the bee is thought to be learning the olfactory, visual, and spatial cues associated with the patch (Collett and Collett, 2002; Collett et al. 2002). The bees develop site fidelity within this restricted location that may allow for learning of specific cues associated with the flowers of individual plants to determine which provide higher rewards (Free 1987; Kulahci et al. 2008). The learning ability of a bee appears to increase before reaching asymptotic values after ~10 days of foraging activity (Dukas and Visscher 1994; Schippers et al. 2006). The change in learning ability of the bees correlates with the volume of the Mushroom body in the bee’s brain, with more experienced bees having a larger Mushroom body (Durst et al. 1994; Mares et al. 2005).

Research on other insects has demonstrated that odor learning can influence plant–pollinator visitations in the field. For example, our research with *Manduca sexta* show that learning an initially nonattractive floral odor can cause the moths to almost exclusively visit the flowers once the association between reward and odor is learned (Riffell et al. 2008a) (Fig. 3A; J. A. Riffell, unpublished data). Compared to bees, which need only one trial to learn an odor–sugar reward association (Bitterman et al. 1983), *M. sexta* moths need approximately five trials to learn the association (Daly et al. 2001). This

is similar to results found in other moths, including *Heliothis virescens* (Jørgensen et al. 2007), and may reflect the smaller Mushroom body relative to that of bees. Interestingly, *M. sexta* moths visit relatively few floral species in a given geographic location, and this may be reflected in the moths’ innate preferences for hawkmoth flowers emitting aromatic-rich floral scents as well as the limited acquisition ability of the moths (Alarcón et al. 2008; Riffell et al. 2008a). Further research examining the learning abilities of more specialized insect pollinators should shed light on how tight the plant–pollinator associations might be and how flexible they are in switching to alternate floral resources.

Prospectus

This review details some of the olfactory and neuroecological mechanisms associated with plant–pollinator interactions. Given that olfactory preferences by pollinators for specific floral scents is now well documented, future research can focus on a variety of topics associated with understanding the ecological basis of learning, plasticity in pollinators’ choices and the neural mechanisms of innate preferences and how those preferences mediate interactions with plants. These research foci are outlined below.

Interfacing plant–pollinator networks, floral scents and pollinators’ olfactory systems

Increasing community-level approaches towards examining plant–pollinator relationships have repeatedly shown complex interactions and networks (Waser et al. 1996; Jordano et al. 2003; Waser and Ollerton 2006). While these networks are amazingly similar in structure across different ecosystems and habitats—suggesting common processes may be at play (Bascompte et al. 2003)—more recent studies have shown that networks can be dynamic both seasonally and yearly (Olesen et al. 2008; Dupont et al. 2009; Alarcón et al. 2010). In all cases, however, detailed understanding of the mechanisms mediating these networks is lacking. In particular, a fundamental gap exists in our understanding of the role of floral traits and the behavioral abilities of the pollinator community, and the fitness-level consequences that certain pollinators have for key floral species.

Can predictions be made regarding pollination associations based on the sensory abilities and preferences of the pollinators and on floral signals (including scent)? The next step will be to link the neural and sensory basis of pollinator’s behaviors

with interactions and processes in the field. The recent incorporation of the behavior of pollinators into network models suggests that behavior can play a key role in altering the linkages between species (Bosch et al. 2009; Kaiser-Bunbary et al. 2010).

Diverse pollinators are vital for crop production (an estimated annual value of \$3 billion due to native pollinators; Gallai et al. 2009) and plant reproduction in natural systems. In addition, many pollinators are in decline due to anthropogenic and disease-related effects (reviewed by Tylianakis et al. 2008; Potts et al. 2010). Therefore, understanding the lability of associations between plants and pollinators and mechanisms involved will be important for determining how these associations change in the decades ahead.

Technologies for studying behavior and plasticity in the field

Interfacing the neural mechanisms of pollinator's behaviors with the behavioral effects on pollen transfer and gene flow between plant populations is a daunting task, but recent technological advances should increase our ability to easily and rigorously quantify these interactions in the field. For instance, use of harmonic radar for honeybees (*Apis mellifera*) and direct radio-tracking of carpenter bees (*Xylocopa flavorufa*) showed that foraging distances were generally shorter than the maximum flight distances (~3–10 km) (Riley et al. 2005; Pasquet et al. 2008). In parallel with field-tracking of pollinators, close range (~1–10 m³) behavioral video-tracking systems and analysis software offer the rare opportunity to directly quantify the behaviors and decisions of pollinators in the field. For instance, Straw et al. (2011) recently developed a video-tracking system that permits the tracking of multiple flying insects in real time. Such systems, when deployed in the field, can manipulate sensory feedback of the pollinator, thus allowing explorations of the neural basis of behavior and plasticity in behavioral response. Pollinators are making moment-to-moment foraging decisions depending upon environmental (flower number, type, nectar amounts) and contextual (physiological, learning and memory) variables. Fruitful avenues of research could thus link direct manipulations of floral signals with quantification of the plasticity of the pollinator's behavior. Such future studies will be necessary for determining the effects of behavior on pollen transfer and gene flow, and how pollinator networks are temporally dynamic.

Evolution of floral traits and pollinators' sensory systems

Determination of the evolution of pollinators' communication systems and the evolution of floral scents may help determine the ecological links and selective pressures involved in pollinator–plant associations. Towards this goal, recent phylogenetic analyses of floral volatiles show striking differences in the composition of floral scent between related plants (Schiestl 2010). Distinct volatiles can be produced by enzymes in a metabolic pathway, thereby providing the potential for only a few mutations to alter the scent's composition. For example, mutation in the linalool synthase gene pathway increases the geranyl-diphosphate precursor for other monoterpenes, including myrcene and ocimene via the myrcene and ocimene synthase pathways (Dudareva and Pickersky 2006). Since linalool is a component attractive to hawkmoths (Riffell et al. 2009a), and myrcene and ocimene are attractive to bees (Granero et al. 2005), a single mutation may cause a change in the floral scent and an alteration of the pollinators visiting the flower. Alternately, understanding how the olfactory system of the pollinator (receiver) may evolve in response to olfactory stimuli will provide information on the timing by which the selection takes place. One mechanism for selection of key floral volatiles is through modifications of the insect's periphery, through the expression of the olfactory receptors (ORs). The insect OR consists of 62 OR genes in fruit flies (Robertson et al. 2003), approximately 79 in *Anopheles gambiae* (Hill et al. 2002), and 162 in *Apis mellifera* (Robertson and Wanner 2006). Many of these ORs are broadly tuned, responding to certain functional groups on the volatile chemical. Thus modifying the expression of specific ORs, or the tuning of a given OR, may influence the pollinator's perception of a floral scent. For example, fruitflies (*Drosophila melanogaster*) and mosquitoes (*Anopheles gambiae*) respond both to aromatics and esters, but expression of ORs that elicit responses is higher for nitrogen-bearing aromatics in mosquitoes and higher for esters in fruitflies (Carey et al. 2010). Another mechanism whereby preference for floral signals may be altered is through changes of the olfactory circuits in the brain (Ibba et al. 2010), but it remains uncertain whether selection for certain floral volatiles occur more readily in the periphery or in the central nervous system. Nonetheless, the olfactory system of pollinators appears to be flexible enough, evolutionarily, to adapt to different floral cues, but the mechanisms involved and the dynamics of these changes for both the signaler

(flower) and receiver (pollinator) have yet to be fully elucidated.

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References

- Alarcón R. 2010. Congruence between visitation and pollen-transport networks in a California plant–pollinator community. *Oikos* 119:35–44.
- Alarcón R, Davidowitz G, Bronstein JL. 2008. Nectar usage in a southern Arizona hawkmoth community. *Ecol Entomol* 33:503–09.
- Arenas A, Giurfa M, Farina WM, Sandoz JC. 2009. Early olfactory experience modifies neural activity in the antennal lobe of a social insect at the adult stage. *Euro J Neurosci* 30:1498–508.
- Ayasse M, Stöckl J, Francke W. 2011. Chemical ecology and pollinator-driven speciation in sexually deceptive orchids. *Phytochemistry*, published online (doi:10.1016/j.phytochem.2011.03.023).
- Balkenius A, Rosen W, Kelber A. 2006. The relative importance of olfaction and vision in a diurnal and a nocturnal hawkmoth. *J Comp Phys A* 192:431–7.
- Bascompte J, Jordano P. 2007. Plant–animal mutualistic networks: the architecture of biodiversity. *Annu Rev Ecol Evol Syst* 38:567–93.
- Bascompte J, Jordano P, Melián CJ, Olesen JM. 2003. The nested assembly of plant–animal mutualistic networks. *Proc Natl Acad Sci USA* 100:9383–7.
- Bitterman ME, Menzel R, Fietz A, Schäfer S. 1983. Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). *J Comp Psychol* 97:107–19.
- Bosch J, Martín González AM, Rodrigo A, Navarro D. 2009. Plant–pollinator networks: adding the pollinator’s perspective. *Ecol Lett* 12:409–19.
- Bradshaw HD, Schemske DW. 2003. Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* 426:1768.
- Campbell DR. 1989. Measurements of selection in a hermaphroditic plant: variation in male and female pollination success. *Evolution* 43:318–34.
- Carey AF, Wang G, Su C-Y, Zwiebel LJ, Carlson JR. 2010. Odorant reception in the malaria mosquito *Anopheles gambiae*. *Nature* 464:66–71.
- Christensen TA, Pawlowski VM, Lei H, Hildebrand JG. 2000. Multi-unit recordings reveal context-dependent modulation of synchrony in odor-specific neural ensembles. *Nat Neurosci* 3:927–31.
- Collett M, Harland D, Collett TS. 2002. The use of landmarks and panoramic context in the performance of local vectors by navigating honeybees. *J Exp Biol* 205:807–14.
- Collett TS, Collett M. 2002. Memory use in insect visual navigation. *Nat Rev Neurosci* 3:542–52.
- Conner JK, Rush S, Jennetten P. 1996. Measurements of natural selection on floral Traits in wild radish (*Raphanus raphanistrum*). I. Selection through lifetime female fitness. *Evolution* 50:1127–36.
- Dacks AM, Christensen TA, Agricola H-J, Wollweber L, Hildebrand JG. 2005. Octopamine-immunoreactive neurons in the brain and subesophageal ganglion of the hawkmoth *Manduca sexta*. *J Comp Neurol* 488:255–68.
- Daly KC, Chandra S, Durtschi ML, Smith BH. 2001. The generalization of an olfactory-based conditioned response reveals unique but overlapping odour representations in the moth *Manduca sexta*. *J Exp Biol* 204:3085–95.
- Daly KC, Christensen TA, Lei H, Smith BH, Hildebrand JG. 2004. Learning modulates the ensemble representations for odors in primary olfactory networks. *Proc Natl Acad Sci USA* 101:10476–81.
- de Belle J, Heisenberg M. 1994. Associative odor learning in *Drosophila* abolished by chemical ablation of mushroom bodies. *Science* 263:692–5.
- Deisig N, Giurfa M, Lachnit H, Sandoz J-C. 2006. Neural representation of olfactory mixtures in the honeybee antennal lobe. *E J Neurosci* 24:1161–74.
- Dekker T, Steib B, Cardé RT, Geier M. 2002. L-lactic acid: a human-signifying host cue for the anthropophilic mosquito *Anopheles gambiae*. *Med Vet Entomol* 16:91–8.
- Denker M, Finke R, Schaupp F, Grün S, Menzel R. 2010. Neural correlates of odor learning in the honeybee antennal lobe. *E J Neurosci* 31:119–33.
- Dobson HEM. 2006. Relationship between floral fragrance composition and type of pollinator. In: Dudareva N, Pickersky E, editors. *Biology of floral scent*. Boca Raton: CRC Press. p. 147–98.
- Dornhaus A, Chittka L. 2005. Bumble bees (*Bombus terrestris*) store both food and information in honeypots. *Behav Ecol* 16:661–6.
- Dubnau J, Grady L, Kitamoto T, Tully T. 2001. Disruption of neurotransmission in *Drosophila* mushroom body blocks retrieval but not acquisition of memory. *Nature* 411:476–80.
- Dudareva N, Pickersky E. 2006. Floral scent metabolic pathways: their regulation and evolution. In: Dudareva N, Pickersky E, editors. *Biology of floral scent*. Boca Raton: CRC Taylor and Francis. p. 55–78.
- Dukas R, Visscher PK. 1994. Lifetime learning by foraging honey bees. *Anim Behav* 48:1007–12.
- Durst C, Eichmüller S, Menzel R. 1994. Development and experience lead to increased volume of subcompartments of the honeybee mushroom body. *Behav Neural Biol* 62:259–63.
- Dyer AG, Neumeyer C, Chittka L. 2005. Honeybee (*Apis mellifera*) vision can discriminate between and recognise images of human faces. *J Exp Biol* 208:4709–14.

- Eltz T, Whitten WM, Roubik DW, Linsenmair KE. 1999. Fragrance collection, storage, and accumulation by individual male orchid bees. *J. Chem. Ecol.* 25:157–76.
- Faber T, Joerges J, Menzel R. 1999. Associative learning modifies neural representations of odors in the insect brain. *Nat Neurosci* 2:74–8.
- Farina WM, Grüter C, Díaz PC. 2005. Social learning of floral odours inside the honeybee hive. *Proc Roy Soc B* 272:1923–8.
- Farooqui T, Vaessin H, Smith BH. 2004. Octopamine receptors in the honeybee (*Apis mellifera*) brain and their disruption by RNA-mediated interference. *J Insect Physiol* 50:701–13.
- Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization. *Annu Rev Ecol Evol Syst* 35:375–403.
- Fernandez PC, Locatelli FF, Person-Rennell N, Deleo G, Smith BH. 2009. Associative conditioning tunes transient dynamics of early olfactory processing. *J Neurosci* 29:10191–202.
- Free JB. 1987. Pheromones of social bees. London: Chapman and Hall.
- Friedrich A, Thomas U, Müller U. 2004. Learning at different satiation levels reveals parallel functions for the cAMP protein kinase A cascade in formation of long-term memory. *J Neurosci* 24:4460–8.
- Gabel B, Thiery D, Suchy V, Marion-Poll F, Hradsky P, Farkas P. 1992. Floral volatiles of *Tanacetum vulgare* L. attractive to *Lobelia botrana* den. et. schiff. females. *J Chem Ecol* 18:693–701.
- Gallai N, Salles J-M, Settele J, Vaissière BE. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol Econ* 68:810–21.
- Gómez JM, Abdelaziz M, Camacho JPM, Muñoz-Pajares AJ, Perfectti F. 2009. Local adaptation and maladaptation to pollinators in a generalist geographic mosaic. *Ecol Lett* 12:672–82.
- Granero A, Sanz J, Gonzalez F, Vidal J, Dornhaus A, Ghani J, Serrano A, Chittka L. 2005. Chemical compounds of the foraging recruitment pheromone in bumblebees. *Naturwissenschaften* 92:371–4.
- Grisson-Pigé L, Bessièrre J-M, Hossaert-McKey M. 2002. Specific attraction of fig-pollinating wasps: role of volatile compounds released by tropical figs. *J Chem Ecol* 28:283–95.
- Hammer M. 1993. An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. *Nature* 366:59–63.
- Hammer M, Menzel R. 1998. Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. *Learning Memory* 5:146–56.
- Heisenberg M, Borst A, Wagner S, Byers D. 1985. *Drosophila* Mushroom body mutants are deficient in olfactory learning. *J Neurogenet* 2:1–30.
- Hildebrand JG, Shepherd GM. 1997. Mechanisms of olfactory discrimination: evidence for common principles across Phyla. *Annu. Rev Neurosci* 20:595–631.
- Hill CA, Fox AN, Pitts RJ, Kent LB, Tan PL, Chrystal MA, Cravchik A, Collins FH, Robertson HM, Zweibel LJ. 2002. G protein coupled receptors in *Anopheles gambiae*. *Science* 298:176–8.
- Hopkins TL, Young H. 1990. Attraction of the grasshopper, *Melanoplus sanguinipes*, to host plant odors and volatile components. *Entomol Experiment Appl* 56:249–58.
- Ibba I, Angioy A, Hansson B, Dekker T. 2010. Macrogglomeruli for fruit odors change blend preference in *Drosophila*. *Naturwissenschaften* 97:1059–66.
- Ito I, Ong RC-y, Raman B, Stopfer M. 2008. Sparse odor representation and olfactory learning. *Nat Neurosci* 11:1177–84.
- Jordano P, Bascompte J, Olesen JM. 2003. Invariant properties in coevolutionary networks of plant–animal interactions. *Ecol Lett* 6:69–81.
- Jørgensen K, Strandén M, Sandoz J-C, Menzel R, Mustaparta H. 2007. Effects of two bitter substances on olfactory conditioning in the moth *Heliothis virescens*. *J Exp Biol* 210:2563–73.
- Kaiser-Bunbury CN, Muff S, Memmott J, Müller CB, Calfisch A. 2010. The robustness of pollination networks to the loss of species and interactions: a quantitative approach incorporating pollinator behaviour. *Ecol Lett* 13:442–52.
- Kearns CA, Inouye DW, Waser NM. 1998. Endangered mutualisms: the conservation of plant–pollinator interactions. *Annu Rev Ecol Syst* 29:83–112.
- Kelber A. 2002. Pattern discrimination in a hawkmoth: innate preferences, learning performance and ecology. *Proc Roy Soc B* 269:2573–7.
- Knudsen JT, Gershenzon J. 2006. The chemical diversity of floral scent. In: Dudareva N, Pickersky E, editors. *Biology of floral scent*. Boca Rotan: CRC Taylor Francis. p. 27–54.
- Knudsen JT, Klitgaard BB. 1998. Floral scent and pollination in *Brownneopsis disepala* (Leguminosae: Caesalpinioideae) in western Ecuador. *Brittonia* 50:174–82.
- Knudsen JT, Tollsten L, Bergstrom LG. 1993. Floral scents: a checklist of volatile compounds isolated by head-space techniques. *Phytochemistry* 33:253–80.
- Kreissl S, Eichmüller S, Bicker G, Rapus J, Eckert M. 1994. Octopamine-like immunoreactivity in the brain and subesophageal ganglion of the honeybee. *J Comp Neurol* 348:583–95.
- Kulahci IG, Dornhaus A, Papaj DR. 2008. Multimodal signals enhance decision making in foraging bumble-bees. *Proc Roy Soc B* 275:797–802.
- Lei H, Christensen TA, Hildebrand JG. 2002. Local inhibition modulates odor-evoked synchronization of glomerulus-specific output neurons. *Nat Neurosci* 5:557–65.
- Lewis WH, Zenger VE. 1983. Breeding systems and fecundity in the American ginseng, *Panax quinquefolium* (Araliaceae). *Am J Bot* 70:466–8.
- Lindauer M. 1967. Recent advances in bee communication and orientation. *Annu Rev Entomol* 12:439–70.
- Mares S, Ash L, Gronenberg W. 2005. Brain allometry in bumblebee and honey bee workers. *Brain Behav Evol* 66:50–61.
- McGuire SE, Le PT, Davis RL. 2001. The role of *Drosophila* Mushroom body signaling in olfactory memory. *Science* 293:1330–3.
- Menzel R. 1967. Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (*Apis mellifera*). *Zeitschrift für vergleichende Physiologie* 56:22–62.

- Menzel R. 1985. Learning in honeybees in an ecological and behavioral context. In: Holldobler B, Lindauer M, editors. *Experimental behavioral ecology*. Stuttgart: Gustav Fischer. p. 55–74.
- Menzel R, Muller U. 1996. Learning and memory in honeybees: from behavior to neural substrates. *Annu Rev Neurosci* 19:379–404.
- NRC. 2007. *Status of Pollinators in North America*. Washington DC: National Academies Press.
- Ollerton J, Alarcón R, Waser NM, Price MV, Watts S, Cranmer L, Hingston A, Peter CI, Rotenberry J. 2009. A global test of the pollination syndrome hypothesis. *Ann Bot* 103:1471–80.
- Olesen JM, Bascompte J, Elberling H, Jordano P. 2008. Temporal dynamics in a pollination network. *Ecology* 89:1573–82.
- Olsen SR, Wilson RI. 2008. Lateral presynaptic inhibition mediates gain control in an olfactory circuit. *Nature* 452:956–60.
- Ômura H, Honda K. 2005. Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. *Oecologia* 142:588–96.
- Pasquet RS, Peltier A, Hufford MB, Oudin E, Saulnier J, Paul L, Knudsen JT, Herren HR, Gepts P. 2008. Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. *Proc Natl Acad Sci USA* 105:13456–61.
- Piñero JC, Giovanni Galizia C, Dorn S. 2008. Synergistic behavioral responses of female oriental fruit moths (Lepidoptera: Tortricidae) to synthetic host plant-derived mixtures are mirrored by odor-evoked calcium activity in their antennal lobes. *J Insect Physiol* 54:333–43.
- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. 2010. Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* 25:345–53.
- Raguso RA. 2008. Wake up and smell the roses: the ecology and evolution of floral scent. *Annu Rev Ecol Evol Syst* 39:549–69.
- Raguso RA, Henzel C, Buchman SL, Nabhan GP. 2003. Trumpet flowers of the Sonoran Desert: floral biology of *Peniocereus* cacti and Sacred Datura. *Int J Plant Sci* 164:877–92.
- Raguso RA, Roy BA. 1998. ‘Floral’ scent production by *Puccinia* rust fungi that mimic flowers. *Mol Ecol* 7:1127–36.
- Reisenman CE, Heinbockel T, Hildebrand JG. 2008. Inhibitory interactions among olfactory glomeruli do not necessarily reflect spatial proximity. *J Neurophysiol* 100:554–64.
- Reisenman CE, Riffell JA, Bernays EA, Hildebrand JG. 2010. Antagonistic effects of floral scent in an insect–plant interaction. *Proc Roy Soc B* 277:2371–9.
- Riffell JA, Abrell L, Hildebrand J. 2008b. Physical processes and real-time chemical measurement of the insect olfactory environment. *J Chem Ecol* 34:837–53.
- Riffell JA, Alarcón R, Abrell L, Davidowitz G, Bronstein JL, Hildebrand JG. 2008a. Behavioral consequences of innate preferences and olfactory learning in hawkmoth-flower interactions. *Proc Natl Acad Sci USA* 105:3404–9.
- Riffell JA, Lei H, Christensen TA, Hildebrand JG. 2009a. Characterization and coding of behaviorally significant odor mixtures. *Curr Biol* 19:335–40.
- Riffell JA, Lei H, Hildebrand JG. 2009b. Neural correlates of behavior in the moth *Manduca sexta* in response to complex odors. *Proc Natl Acad Sci USA* 106:19219–26.
- Riley JR, Greggers U, Smith AD, Reynolds DR, Menzel R. 2005. The flight paths of honeybees recruited by the waggle dance. *Nature* 435:205–7.
- Robertson HM, Wanner KW. 2006. The chemoreceptor superfamily in the honey bee, *Apis mellifera*: expansion of the odorant, but not gustatory, receptor family. *Genome Res* 16:1395–403.
- Robertson HM, Warr CG, Carlson JR. 2003. Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 100:14537–42.
- Rodríguez I, Gumbert A, Hempel de Ibarra N, Kunze J, Giurfa M. 2004. Symmetry is in the eye of the ‘beeholder’: innate preference for bilateral symmetry in flower-naïve bumblebees. *Naturwissenschaften* 91:374–7.
- Roy BA, Raguso RA. 1997. Olfactory versus visual cues in a floral mimicry system. *Oecologia* 109:414–26.
- Sachse S, Galizia CG. 2002. Role of inhibition for temporal and spatial odor representation in olfactory output neurons: a calcium imaging study. *J Neurophysiol* 87:1106–17.
- Schemske DW, Bradshaw HD. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proc Natl Acad Sci USA* 96:11910–5.
- Schiestl FP. 2010. The evolution of floral scent and insect chemical communication. *Ecol Lett* 13:643–56.
- Schiestl FP, Peakall R, Mant JG, Ibarra F, Schulz C, Franke S, Francke W. 2003. The chemistry of sexual deception in an orchid-wasp pollination system. *Science* 302:437–8.
- Schippers M-P, Dukas R, Smith RW, Wang J, Smolen K, McClelland GB. 2006. Lifetime performance in foraging honeybees: behaviour and physiology. *J Exp Biol* 209:3828–36.
- Schlieff ML, Wilson RI. 2007. Olfactory processing and behavior downstream from highly selective receptor neurons. *Nat Neurosci* 10:623–30.
- Schroll C, Riemensperger T, Bucher D, Ehmer J, Völler T, Erbguth K, Gerber B, Hendel T, Nagel G, Buchner E, Fiala A. 2006. Light-induced activation of distinct modulatory neurons triggers appetitive or aversive learning in *Drosophila* larvae. *Curr Biol* 16:1741–7.
- Schwaerzel M, Monastirioti M, Scholz H, Friggi-Grelin F, Birman S, Heisenberg M. 2003. Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*. *J Neurosci* 23:10495–502.
- Seeley TD. 1994. Honey bee foragers as sensory units of their colonies. *Behav Ecol Sociobiol* 34:51–62.
- Silbering AF, Galizia CG. 2007. Processing of odor mixtures in the *Drosophila* antennal lobe reveals both global inhibition and glomerulus-specific interactions. *J Neurosci* 27:11966–77.
- Sinakevitch I, Strausfeld NJ. 2006. Comparison of octopamine-like immunoreactivity in the brains of the fruit fly and blow fly. *J Comp Neurol* 494:460–75.
- Stökl J, Strutz A, Dafni A, Svatos A, Doubsky J, Knaden M, Sachse S, Hansson BS, Stensmyr MC. 2010. A deceptive pollination system targeting *Drosophilids* through olfactory mimicry of yeast. *Curr Biol* 20:1846–52.

- Straw AD, Branson K, Neumann TR, Dickinson MH. 2011. Multi-camera real-time three-dimensional tracking of multiple flying animals. *J Roy Soc Interface* 8:395–409.
- Tholl D, R ose UR. 2006. Detection and identification of floral scent compounds. In: Dudareva N, Pickersky E, editors. *Biology of floral scent*. Boca Rotan: CRC Taylor Francis. p. 3–26.
- Touhara K, Vosshall LB. 2009. Sensing odorants and pheromones with chemosensory receptors. *Annu Rev Physiol* 71:307–22.
- Tully T, Preat T, Boynton SC, Del Vecchio M. 1994. Genetic dissection of consolidated memory in *Drosophila*. *Cell* 79:35–47.
- Tylianakis JM, Didham RK, Bascompte J, Wardle DA. 2008. Global change and species interactions in terrestrial ecosystems. *Ecol. Lett* 11:1351–63.
- Vainstein A, Lewisohn E, Weiss D. 2006. An integrated genomics approach to identifying floral scent genes in rose. In: Dudareva N, Pickersky E, editors. *Biology of floral scent*. Boca Rotan: CRC Taylor Francis. p. 91–104.
- Vereecken NJ, Schiestl FP. 2008. The evolution of imperfect floral mimicry. *Proc Natl Acad Sci USA* 105:7484–8.
- von Helversen O, Winkler L, Bestmann HJ. 2000. Sulphur-containing "perfumes" attract flower-visiting bats. *J Comp Physiol A* 186:143–53.
- Waser NM, Chittka L, Price MV, Williams NM, Ollerton J. 1996. Generalization in pollination systems, and why it matters. *Ecology* 77:1043–60.
- Waser NM, Ollerton J. 2006. Specialization and generalization in plant-pollinator interactions: a historical perspective. In: Waser NM, Ollerton J, editors. *Plant-pollinator interactions: from specialization to generalization*. Chicago: University of Chicago Press. p. 3–17.
- Weiss MR. 1997. Innate colour preferences and flexible colour learning in the pipevine swallowtail. *Anim Behav* 53:1043–52.
- Weiss MR. 2001. Vision and learning in some neglected pollinators: beetles, flies, moths, and butterflies. In: Chittka L, Thomson JD, editors. *Cognitive ecology of pollination; animal behavior and floral evolution*. Cambridge: Cambridge University Press. p. 171–90.
- Wilson RI. 2008. Neural and behavioral mechanisms of olfactory perception. *Curr Opin Neurobiol* 18:408–12.
- Wright GA, Smith BH. 2004. Variation in complex olfactory stimuli and its influence on odour recognition. *Proc Roy Soc B* 271:147–52.
- Yu D, Ponomarev A, Davis RL. 2004. Altered representation of the spatial code for odors after olfactory classical conditioning: memory trace formation by synaptic recruitment. *Neuron* 42:437–49.