Olfaction in context – sources of nuance in plant-pollinator communication

Claire Rusch^{*,a}, Geoffrey T. Broadhead^{*,b}, Robert A. Raguso^{*,b,†}, Jeffrey A. Riffell^{a,†}

^aDepartment of Biology, University of Washington, Seattle, WA 98195

^bDepartment of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

^{*}contributed equally to this paper

[†]correspondence: jriffell@uw.edu and rar229@cornell.edu

Abstract

Floral scents act as long-distance signals to attract pollinators, but volatiles emitted from the vegetation and neighboring plant community may modify this mutualistic communication system. What impact does the olfactory background have on pollination systems and their evolution? We consider recent behavioral studies that address the context of when and where volatile backgrounds influence a pollinator's perception of floral blends. In parallel, we review neurophysiological studies that show the importance of blend composition and background in modifying the representation of floral blends in the pollinator brain, as well as experience-dependent plasticity in increasing the representation of a rewarding odor. Here, we suggest that the efficacy of the floral blend in different environments may be an important selective force shaping differences in pollinator olfactory receptor expression and underlying neural mechanisms that mediate flower visitation and plant reproductive isolation.

Introduction

A challenge inherent to communication is how senders convey reliable information to receivers within a noisy and unpredictable environment. Effective communication is

fundamental to mutualistic as well as deceptive relationships between flowering plants and their animal pollinators, as it mediates gene flow and reproductive isolation for plants while driving flower choice and foraging efficiency for pollinators. There have been several recent reviews on multimodal floral signals and pollinator choice [1, 2]; thus in this review we focus on recent progress in understanding the behavioral and neural underpinnings of the olfactory channel in plant-pollinator communication, with an emphasis on how context and background influence the detection and perception of floral volatile signals. We begin with an overview of the insect olfactory system relevant to distinguishing complex volatile blends from a noisy background. Here we discuss how environmental factors influence how insect brains process floral scent bouquets. Next, we explore how experience modifies olfactory processing. Finally, we conclude with an exciting topic for future research by examining how signal efficacy – the interplay between signal composition and environmental background – may feedback on the speciation process in plants, through reproductive isolation and reinforcement.

I. Pollinator behavior to complex odor sources and environments:

A. Efficiency and accuracy of behavioral decisions in response to behaviorally effective odors and environmental background odors

Insect pollinators forage for nectar and pollen in habitats rife with potentially distracting sources of volatile organic compounds (VOCs). These “background odorants” include the volatile signatures of organic decay and of living vegetation in a community assemblage of flowering and fruiting plants [3], including those of competing floral resources. Floral scent blends have long been characterized as species-specific [4], and there is growing experimental evidence that when bouquets of neighboring plants are too similar, they suffer reduced pollinator constancy or breakdowns in reproductive isolation [5].

The challenge of distinguishing a target VOC blend - whether innately attractive or learned [6,7] – within a complex olfactory scene recalls the problem of signal acceptance threshold in social evolution, in which uncertainty increases the risk of an incorrect decision [8]. From an information theory standpoint, Wilson et al. [9] suggest that redundant VOC bouquets reduce uncertainty, which may explain why many resource-indicating odors are blends [10]. Thus, distinguishing an olfactory target from “background” might require it to be chemically, spatially or temporally distinct from other features in the same habitat [11].

Beyond the distinctiveness of a floral bouquet is its compatibility with background VOCs. This might include whether neighboring plants emit masking compounds or repellents [3,12], reducing floral visitation or constancy. By swapping floral and vegetative VOCs from *Datura wrightii* and *Nicotiana attenuata*, Karpati et al. [13] discovered unexpected coaction between floral and vegetative volatiles, such that floral visitation by naïve *Manduca sexta* moths (which use these plants as larval hosts) was reduced in cross-species combinations. These findings reflect conflicting selective pressures driving plants to enhance pollinator services while mitigating herbivory [14]. In a more extreme example of signal integration, Dufaÿ et al. [15] showed that the flowers of the dwarf palm (*Chamaerops humilis*) are pollinated by a

specialized weevil attracted by the scent of leaves subtending the inflorescence, which emit volatiles only when flowers are receptive. It remains unclear whether certain community contexts synergize floral attraction through signal contrast between neighboring species. This idea differs from the “magnet species” effect, in which food deceptive plants are more frequently visited when they co-bloom with other, rewarding species, often with contrasting floral signals [16,17]. However, their similarity resides in the notion that some neighbors enhance pollination by altering the information landscape [7], an intriguing extension of associational resistance and susceptibility [18].

The concept of synergy between olfactory inputs is well established in the case of insect sex pheromone signals emitted in the presence of host-plant volatile cues [19]. More recent work demonstrates that the enhancement or inhibition of olfactory signals is dependent on the source of background odorants as well as physical odor plume characteristics [20,21]. Within the context of plant-pollinator interactions, Riffell et al. [11] evaluated the neural and behavioral responses of *M. sexta* moths tracking floral VOCs against various chemical backdrops using wind tunnel assays. These results highlight the potential for co-occurring plants to alter the olfactory perception and behavior of pollinators. Larue et al. [22] further explored the influence of chemical context in a manipulative field experiment. Here, plant-pollinator network links were significantly altered after the reciprocal application of floral scent extracts, in some cases due to attractants, in other cases due to repellents. Nevertheless, few network interactions were lost entirely, suggesting that in the face of confusing olfactory information pollinator experience or multimodal integration (e.g. with visual display) may reinforce plant-pollinator interactions. Collectively, these experiments support the importance of olfactory background and context and indicate that models of floral constancy should account for multimodal floral displays rather than focusing on a single, isolated modality [23].

B. How the olfactory system detects and discriminates between focal odorants and environmental background

The ability of pollinators to locate floral resources amidst a complex olfactory environment requires an olfactory system that can detect and discriminate target VOC blends at short (<500 ms) timescales. Building on recent reviews [24-26], we focus on neural mechanisms in the peripheral (olfactory receptor neurons [ORNs] located on antennae and maxillary palps) and higher-level olfactory centers (antennal lobe [AL] and mushroom body [MB]) by which insect pollinators distinguish a target VOC blend from a complex chemical background (Fig. 1; Table 1). The magnitude and temporal dynamics of ORN responses allow rapid detection of salient VOCs from a floral source. For instance, ORNs are extremely sensitive to the onset of odorant delivery; latency between ORN responses thus allows the system to respond to spatially separated odor sources whose plumes are not entirely mixed. Szyszka and coworkers [27,28] found that bees and moths can resolve odorant fluctuations greater than 100 Hz, with response latencies as short as 2 ms. Thus, even when odor plumes begin to blend together, the VOC filaments from the different plumes are distinct enough to enable a pollinator to resolve the differences between the two plumes. An additional feature for processing stimuli from background is that ORNs which respond to different constituents in a blend are often housed in the same sensillum [29,30], enabling coincident activation of ORN types for detection of

behaviorally effective blends. Finally, sensory adaptation may be another process by which pollinators detect floral VOCs from background, but few field studies have linked adaptation with characterization of the VOC environment. In wind tunnel bioassays, moths adapted to natural backgrounds were rarely able to locate the odor sources; this effect was due to the combination of sensory adaptation and the background ‘masking’ the floral VOCs because of its overlapping composition [11].

The balance of excitatory input by ORNs and inhibition by LNs is critical both for processing floral VOCs and for suppressing activation of PNs in response to background (Fig. 2). Recent studies of *Spodoptera littoralis* moths exemplify the importance of physiological state and AL modulation in olfactory processing. In virgin females, the AL glomerular representation of lilac scent is enhanced relative to vegetative VOC blends, but after mating, the representation of host (cotton leaf) VOCs is enhanced and other scents are suppressed [31]. Similarly, Stierle et al. [32] showed that separability in AL glomerular representations of two complex VOC blends improves with increased time between stimulation of the two blends in honey bees. Longer time between stimulations enhances the representation of blend constituents in the AL glomerular response patterns [32], such that levels of inhibition and blend separability are correlated with temporal offset between the competing blends. In a similar manner, *M. sexta* moths require an intact AL inhibitory network for effective navigation to a blend of a few key odorants. When floral VOCs are presented in a background that shares similar constituents – including those from anthropogenic sources – both the spatial AL glomerular representations and the temporal dynamics of PN coding are lost (Fig. 2) [11].

These examples highlight how a complex background can alter the neural representation of floral VOCs, but what occurs when the background synergizes responses to floral scent, as discussed above for the flowers and vegetation of *D. wrightii* and the dwarf palm (*C. humilis*)? One explanation is that the background shares the same VOC ratio as the floral blend, thereby maintaining patterns of glomerular representation, excitation and inhibition in the AL. Alternately, the background may include a key volatile that, when combined with the scent, elicits a new neural representation and increase in behavioral response [33].

II. How previous experience impacts locating a focal odor and amidst the environmental background

A. Pollinator behavioral ecology and the role of learning

Recent studies have advanced our understanding of how volatile blends are perceived, coded and tracked by insects seeking floral rewards and hostplants [34,35]. For example, inhibitory interactions among antennal lobe glomeruli enhance the distinctiveness of specific volatile blends as perceived by honey bees ([36]; see Fig. 2). A major question complementing such studies is how experience modifies innate olfactory and behavioral discrimination by foraging insects. Do the volatile templates used by naïve insects to find floral resources change when those insects learn to associate some aspect of profitability (quality, abundance) with volatile stimuli [37,38]? Naïve bumblebees (*Bombus terrestris*) are innately attracted to the floral scent of *Brassica rapa*, but show no behavioral preferences to electrophysiologically active

compounds (e.g. phenylacetaldehyde). However, experienced bees prefer phenylacetaldehyde, which is strongly correlated with nectar and pollen content in *B. rapa* flowers [39]. The salience of learned volatiles may also reflect statistical aspects of conditioned volatile stimuli, including their concentration and (in)variance [37]. Finally, innate preferences may be overcome through the learning process, as was shown for *M. sexta* moths learn to associate a chemically novel scent with the copious nectar of *Agave palmeri* in the absence of *D. wrightii* flowers, whose scent they innately prefer ([6]; see Fig. 2).

Few natural environments resemble the conditions found in laboratory olfactory associative learning experiments. For naïve *M. sexta*, background odorants significantly affect odor-tracking ability [11], however wild, experienced flower-visitors have clearly learned to navigate in complex natural landscapes of competing olfactory stimuli. Despite the olfactory milieu characteristic of these natural scenes, few experiments have translated key concepts of olfactory learning into more biologically relevant contexts. For example, odor generalization, or the ability to transfer learned associations despite variability in the olfactory stimulus, is generally described as a potential mechanism for organisms to cope with the intrinsic variation characteristic of naturally occurring odor sources [40]. Yet, in odor discrimination tasks in which an incorrect decision risks a negative outcome, pollinators may preferentially respond to more extreme versions of the original stimulus that are more detectably different from stimuli associated with a negative outcome. Thus, the potential for negative outcomes can modify or narrow signal acceptance thresholds resulting in an olfactory ‘peak-shift’ [8,41,42]. From controlled laboratory-based associative learning experiments (e.g. [41,42]), it is clear that both generalization and peak-shifted biases are strongly influenced by the conditioning paradigm, stimulus variability, and relative reward quality – suggesting the potential for extensive plasticity in olfactory learning and recognition in more nuanced, natural environments. Further complicating matters, chemical backgrounds and foraging contexts are not static and many species of flower-visiting insect are highly mobile such that a given pollinator may experience a number of chemical landscapes within a single foraging event. Consequently, in addition to learning phenomena (e.g. peak-shifts and generalization) we must also consider the temporal dynamics of stimulus acquisition and extinction in combination with species’ inherent, individual learning abilities [43] to fully understand the learning and recognition of rewarding floral resources in a dynamic environment.

B. Experience related plasticity in olfactory processing and focal blend-background separation

Prior experience can influence pollinator olfactory processing through different mechanisms, including non-associative and associative processes. For example, Locatelli et al.[44] showed in honeybees that repeated stimulation of one floral odorant causes a shift in its AL representation such that when it is paired with another odorant, the resulting blend-evoked representation is more similar to the second odorant. This non-associative change in neural representation is thought to be mediated by increased inhibition from the LNs.

As mentioned above in section IIA, a current gap lies in linking environmental processes in the field with controlled laboratory experiments examining the neural and behavioral effects of learning on plant-pollinator interactions. Nonetheless, controlled laboratory studies using various pollinator species have shown glimpses of how olfactory learning may influence these interactions. For example, after olfactory conditioning, a honeybee's glomerular responses are increased compared with those of untrained bees; the increased responses serve to improve the discrimination of trained stimuli [45]. Recently Chen et al.[46] examined how learning-mediated plasticity in the AL modifies the representation of constituents in a blend. They found that training the association between a sugar reward and an odorant, and stimulating with a blend containing that odorant, the AL neural representation is shifted toward the representation of the trained odorant alone. Beyond single odorants, pollinators also readily learn natural scents and complex floral blends. As mentioned above, *M. sexta* moths learn to associate nectar reward with the floral scent of the century plant (*Agave palmeri*). During training, the AL neurons increase their blend-evoked responses, thereby causing the AL representation to change. Similar to what was shown with honeybees, plasticity in the moth AL serves to increase the separability between complex flower scents: during a discrimination task between the scents of *A. palmeri* (rewarded) and the Saguaro cactus (*Carnegiea gigantea*; unrewarded), the separability between AL representations of the two scents increased ([47]; Fig. 2).

Learning-evoked changes in the pollinator olfactory system – often mediated by neuromodulators like octopamine and dopamine, or GABA – can influence the representation of floral VOCs in the pollinator AL and higher-order centers, including the MB[48]. For instance, in honeybees, activation of an octopaminergic neuron, VUMmx1, and stimulation with an odorant was sufficient to trigger formation of an appetitive memory [48,49]. In the AL octopamine increases responses in both PN and LNs and increases the coordinated activity of glomeruli[47]. The coordinated glomerular responses “bind” together the representation of key VOCs from a blend – thereby creating a synergistic representation – and increase responses of MB neurons (Kenyon cells) that receive input from the AL (Table 1). The coordinated input from the AL and octopamine release serves to form a “memory trace” of the learned flower scent in the Kenyon cells. The MB also provides inhibitory feedback (*via* GABA release) to other brain regions, including the AL-PNs [50] and the Kenyon cells. This inhibitory feedback may be critical for focal blend discrimination from a background; a tantalizing glimpse into this mechanism comes from an elegant learning study in honeybees, where bees were trained to a rewarded blend but not its constituents, or the converse situation, in which the constituents were rewarded, but not the blend (termed positive and negative patterning, respectively). The authors showed that inhibitory feedback in the MBs is required for accurate discrimination of the trained stimulus [51]. This feedback is not necessary for simple olfactory conditioning, where the pollinator learns the association between one odorant and the reward, but is critical for blends or distinguishing an individual constituent within a blend. Thus, inhibition and plasticity in the AL and MBs may provide the mechanisms by which experienced pollinators rapidly and accurately separate focal floral blends and key constituents from background.

III. Conclusions and prospects for future work: Olfactory/environmental complexity as a selective force

We have provided an overview of recent studies on how the olfactory environment can modify plant-pollinator interactions. Increasing numbers of studies have highlighted the impact of background VOCs – emitted from the flowers and/or vegetation of neighboring plants – on pollinator behavior. Background VOCs may improve or mask the floral signal, demonstrating the context-dependency of these effects for pollinators in local environments. However, missing from many ecological studies are the mechanisms by which background VOCs and focal floral blends are processed in the pollinator olfactory system. Such an understanding could provide predictions on the efficacy of plant-pollinator interactions in different environments, and the role of pollinator experience in shaping these interactions[35]. Finally, given the strong behavioral and neurophysiological effects of background VOCs on the pollinators, and distinct population-level differences in pollinator preferences, flower phenotypes and plant communities in which they exist, we suggest that the VOC background may operate as a selective force in reproductive isolation and reinforcement. Future studies examining the mechanisms by which these rapid population-level differences develop – possibly through epigenetic modifications of olfactory receptor expression [52,53] or memory-related effects [54,55] – should shed light on these transgenerational processes. Although here in this review we focus on the role of floral scent and pollinator behavior, we recognize that flowers are sensory ‘billboards’, with multiple signals or cues [56,57]. Interestingly, some of these cues (e.g., water vapor and temperature) may be processed in the same region of the insect brain, the AL. Here we provide impetus for identifying the role of background VOCs in impacting plant-pollinator interactions and reproductive isolation, as well as studying the neural substrates involved in sensory integration and processing floral VOCs from background.

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Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

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Fig. 1. Peripheral and central regions of the olfactory system for the detection and processing of floral and background scents. (A) Honey bee approaching a flower. (B) Depiction of a sensillum on the antennae. ORNs response (latencies, coincidence detectors, etc. [27-30]) may play a key role in processing floral scents from background. Inset: location of antennal sensillum on honeybee head. (C) Processing of floral scents and associated background may rely on lateral inhibition. Inset: location of the AL in the honeybee brain [11,32]. (D) Multisensory integration and retrieval of previously experienced sensory information in the MB may increase the accuracy of a pollinator locating a flower [1, 49]. Inset: location of the MB in the honeybee brain. ORN: olfactory receptor neurons, AL: antennal lobe, MB: mushroom body. *Photo credit: Emile Pitre.*

Fig. 2. Impact of olfactory environment on AL processing of floral scents. (Left, top): moth navigating to, and feeding from a *Datura* flower that emits a distinct scent from the local plant community. **(Left, bottom):** in the AL, the floral scent activates strong lateral inhibition between neighboring glomeruli that serves to increase the neural representation of the flower scent and separate it from the background scents [11,31,32]. **(Middle, top):** moth navigating to, and feeding from an *Agave* flower as a result of a learned behavior. **(Middle, bottom):** in the AL, the learned floral scent impact the ratio of lateral inhibition, modifying olfactory preference [6, 36, 47-50]. **(Right, top):** moth navigating in a low contrast olfactory environment. **(Right, bottom):** the plant community may emit a key volatile present also in the floral scent, thereby altering the excitation and inhibition and thus reducing distinctiveness of its representation [11]. *Photo credit:* Charles Hedgcock.

Table 1. The ABCs of the beginning stages of the pollinator olfactory system implicated in processing focal floral blends and volatile backgrounds

Level	Cell types	Effect	Reference
Antenna and maxillary palps	ORNs	Activated by odorants; tuning and temporal specificity	24, 27
		Spatial coincidence detectors	29
Antennal Lobe	PNs	Receive input from ORNs; show tuning and temporal specificity to floral odorants	32, 34, 36, 44, 47
	LNs	Receive input from ORNs; GABA-mediated inhibition of ORNs and PNs	11, 24, 44
	Circuit level activity of PNs, LNs	Synchronous firing to floral blend constituents	34, 47
	Circuit level activity of PNs, LNs	Changing the volatile blend or background can change the balance of excitation and inhibition in the AL	11, 33, 34, 35
	Octopaminergic neurons; circuit level activity of PNs, LNs	Octopamine release increases gain of AL responses; increases PN synchrony	46, 47
Mushroom Body - Calyces	Kenyon cells	Kenyon cells receive input from many PNs; forms a memory trace	24, 26
	Octopaminergic neurons; Kenyon cells	Coincident activation of PN input and octopamine increases synaptic strength of Kenyon cells	26, 47-50