REVIEW

Mixture and odorant processing in the olfactory systems of insects: a comparative perspective

Marie R. Clifford · Jeffrey A. Riffell

Abstract Natural olfactory stimuli are often complex mixtures of volatiles, of which the identities and ratios of constituents are important for odor-mediated behaviors. Despite this importance, the mechanism by which the olfactory system processes this complex information remains an area of active study. In this review, we describe recent progress in how odorants and mixtures are processed in the brain of insects. We use a comparative approach toward contrasting olfactory coding and the behavioral efficacy of mixtures in different insect species, and organize these topics around four sections: (1) Examples of the behavioral efficacy of odor mixtures and the olfactory environment; (2) mixture processing in the periphery; (3) mixture coding in the antennal lobe; and (4) evolutionary implications and adaptations for olfactory processing. We also include pertinent background information about the processing of individual odorants and comparative differences in wiring and anatomy, as these topics have been richly investigated and inform the processing of mixtures in the insect olfactory system. Finally, we describe exciting studies that have begun to elucidate the role of the processing of complex olfactory information in evolution and speciation.

Keywords Mixtures · Olfaction · Antennal lobe · Olfactory coding

M. R. Clifford (✉) · J. A. Riffell (✉)
Department of Biology, University of Washington,
Seattle, WA 98195, USA
e-mail: cliffmar@u.washington.edu

J. A. Riffell
e-mail: jriffell@u.washington.edu

Abbreviations

AL	Antennal lobe
AN	Antennal nerve
GABA	γ-Aminobutyric acid
LH	Lateral horn of the protocerebrum
LN	Local interneuron
eLN	Excitatory local interneuron
iLN	Inhibitory local interneuron
MB	Mushroom bodies
MGC	Macroglomerular complex
OB	Olfactory bulb
OR	Olfactory receptor
OBP	Odorant binding protein
ORC	Olfactory receptor cell
PN	Projection neuron

Introduction

For an insect attempting to locate an odor source, the olfactory environment is dynamic in space and time. The olfactory environment is also complex—a single point source can emit more than 300 volatiles that, in turn, are mixed with the odor "background." Insects use this complexity to identify and discriminate odor sources by processing a few volatiles in the bouquet (Riffell et al. 2009a, b). The olfactory processing of the "key" constituents in the bouquet can depend on their presence and proportion in the bouquet. For instance, the apple maggot, *Rhagoletis pomonella*, can discriminate between the fruit from different host plants based on a few compounds in the fruit scent (Linn et al. 2012), and the honeybee, *Apis mellifera*, can generalize between mixtures based on the constituent proportions (Wright et al. 2005). The behavioral efficacy of

mixtures compared to single odorants in a variety of insects further demonstrates that the olfactory system transforms multiple input streams into a coherent percept (reviewed Lei and Vickers 2008; Riffell et al. 2009a).

Olfactory systems have evolved to distinguish behaviorally relevant odors from irrelevant ones efficiently and in a variety of contexts (Baker 2008; Ibba et al. 2010; Riffell 2012). As reviewed in Lei and Vickers (2008), the different ways that the olfactory system processes an odor mixture relative to its component parts aid in this discrimination: One type of processing is termed elemental, analytical, additive, or dissociative. In this type of processing, the response to a mixture is the same as a linear sum of the responses to its components. Elemental processing retains information about the individual components of a mixture when representing the mixture in the olfactory system. Another type of processing is termed configural, synthetic, non-linear, non-additive, or associative (Fig. 1). In this case, the response to a mixture is not the same as a linear sum of the responses to its components. Configural processing allows for the perception of an odor mixture as something novel—distinct from, and even potentially unrelated to its chemical components. The olfactory system may code a given mixture using both types of processing, potentially retaining information about components of a mixture while still representing it as a novel percept (reviewed by Lei and Vickers 2008).

Representation of this complex olfactory information can occur in the insect peripheral sensory organs (antennae, maxillary palps), the brain, or both. In recent years, there has been considerable interest into how the antennal lobe (AL), the first synaptic connection in the insect brain, modifies the olfactory input from the periphery, in particular when the stimulus is a complex mixture of odorants (Laurent 2002; Wilson 2008; Kuebler et al. 2012). The AL receives afferent input from both the maxillary palps and antennae (Pitts et al. 2004; Guerenstein and Hildebrand 2008; reviewed by Stocker 1994; Ignell et al. 2005; Hansson and Stensmyr 2011), further demonstrating that this region is an important site for combinatorial processing of odor.

The study of processing of olfactory information in a variety of insect species has recently expanded in the last decade (reviewed by Martin et al. 2011). This new information provides a comparative approach toward understanding how olfactory information is processed in the insect brain. Furthermore, it allows the generation of hypotheses for the evolution of olfactory circuits and how ecological processes may select for different coding strategies in the olfactory system.

In this review, we describe recent progress in how mixtures are processed in the brain of insects. More recent insect olfaction reviews, as well as reviews in this special issue, have described some of the mechanisms associated with the processing of odorant (Wilson 2008; Martin et al. 2011) and mixture input (Lei and Vickers 2008; Riffell 2012; Smith 2013, submitted). In this review, we use a comparative approach toward comparing and contrasting olfactory coding and the behavioral efficacy of mixtures in different insect species. Specifically, this review is organized around four foci:

- Examples of the behavioral efficacy of odor mixtures and the olfactory environment.
- How this complex information is initially processed in the periphery.
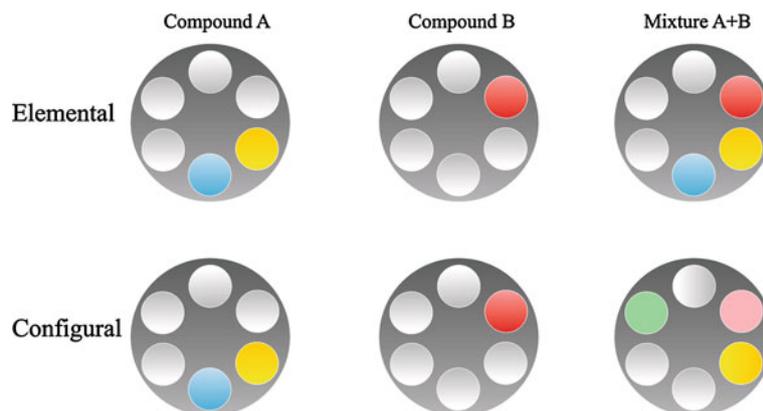- Mixture coding in the AL.

Fig. 1 Configural and elemental processing in the AL. In this illustration of processing in the antennal lobe, we show the response of glomeruli to imaginary compounds A and B alone, as well as to a binary mixture of the two. When processed elementally, the response to the mixture is a linear summation of the responses to compound A and compound B. By contrast, when processed configurally non-additive representation occurs. Additional glomeruli may be recruited by lateral excitation (*green*) and the responses of some glomeruli may be inhibited by higher activity in others (*red* in compound B, *pink* in mixture), potentially resulting in mixture suppression, or lower activity in response to a mixture than what would be expected by summing the responses to its components

- Evolutionary implications and adaptations for olfactory processing.

Finally, we describe exciting future topics for olfactory research, and highlight studies that illuminate how the processing of complex olfactory information drives behavior, and how olfaction-driven behaviors interact with ecological and evolutionary processes.

The olfactory environment

Volatile olfactory stimuli are restricted by molecular weight (<300 Da) and polarity. Thus, there are a limited number of natural volatiles, on the order of a few thousand, in the environment (El-Sayed 2012). The number of insect species that use these volatiles for reproduction, food resource identification, and host site location far exceeds this number (reviewed by Hansson and Stensmyr 2011). While one class of volatiles may be more important for one insect species over another—e.g., mosquitoes that are highly responsive to aliphatic compounds while fruit flies are responsive to esters—it has repeatedly been shown that mixtures of volatiles at specific ratios are an important classifier for stimulus identity.

The mixture of volatiles emitted from a source can vary in its complexity. Certain odor sources may emit a scent that is dominated by a few volatiles of a particular chemical class, but these volatiles may be particularly important in attracting a given insect (Peakall 1990; Schiestl et al. 1999, 2003; Ayasse et al. 2000). For example, the night-blooming Cereus flower, *Peniocereus gregii*, emits a fragrance dominated by benzenoid compounds that are attractive to crepuscular hawkmoths (Raguso et al. 2003; Riffell et al. 2013). By contrast, other odor sources can be complex in either volatile number and/or chemical class; a rose emits more than 400 volatiles in its bouquet and body odor from a human can contain more than 300 volatiles. In many cases, the insect olfactory system has been shown to "filter" this complexity by processing only a few volatiles in the scent for odor identity (Wright et al. 2005; Riffell et al. 2009a; Reinhard et al. 2010).

Beyond odor identity and complexity, olfactory stimuli are temporally and spatially dynamic. Once emitted by a source, volatiles become subject to the physical forces of moving air. The advection and turbulent mixing by the air flow cause the odor to be broken up to discrete packets, or filaments, that are spatially and temporally complex (reviewed by Riffell et al. 2008). Mixing of the odor-laden eddies with clean air causes odor intensities to drop. Furthermore, turbulent and molecular diffusivity cause volatiles from the odor source to become embedded with the ambient air—which contains its own unique "signature" of volatiles. Thus, the odor "background" may modify the "signal" of the odor source. The mechanisms by which the insect olfactory system filters the signal from the background remain unclear.

Behavioral responses to mixtures

Determination of how the olfactory system processes natural stimuli has benefitted from behavioral experiments, in particular in response to innately attractive cues. Behavioral responses to sex pheromones have been well studied, and the recent work on the neurobiological mechanisms controlling motor output and behavioral response has continued to expand our understanding of how these cues are processed. The male-specific olfactory system in moths, which is specialized to detect the features of conspecific females' sex-pheromone volatiles, has been a model system for the last 40 years. Male moth attraction to the female's pheromone has been shown to be driven by the blend of two or three volatiles in the female's bouquet (Baker 2008). By contrast, 11-*cis*-vaccenyl acetate, an apparently multifunctional pheromone in *Drosophila melanogaster*, is one component sufficient for mediating aggregation in the fruit flies (Bartelt et al. 1985; Wertheim et al. 2002) as well as indicating the mating state of a female to responsive males (Ha and Smith 2006; Ejima et al. 2007).

Beyond volatile pheromone signals in Lepidoptera and Diptera, Hymenoptera employ a wide suite of pheromone signals that are diverse in the number, class, and intensities of volatiles in the odor, as well as the diversity of effects the pheromones have on behavior (Slessor et al. 2005; Le Conte and Hefetz 2008). For instance, bee pheromones mediate hive defense (Hunt 2007), foraging (Thom et al. 2007), and queen primacy (Vergoz et al. 2007; Strauss et al. 2008). Taken together, pheromone signals often are complex mixtures of volatiles with multiple physical and behavioral effects (Ferveur 2005; Siwicki et al. 2005).

Finally, diverse insects also can learn associations between volatile stimuli and rewards in the natural environment. For example, honey bees can learn the scent of flowers that are profitable to visit (i.e., are yielding nectar and/or pollen) (Chittka and Raine 2006), and locusts—voracious, generalist feeders—have the ability to associate olfactory stimuli with the quantity and nutritional quality of food sources (Behmer et al. 2005). In two related species of parasitic wasps, the ability to associate volatiles with the presence of host caterpillars correlates with the behavioral plasticity demanded by the distribution of their respective host species (Bleeker et al. 2006; Smid et al. 2007). The Carolina sphinx moth *Manduca sexta* exhibits an innate attraction to certain host flowers, but can learn to feed from

others when the preferred flowers are scarce (Riffell et al. 2008, 2013).

Peripheral processing

The first level of the neural coding of odors occurs in the periphery. The binding of an odorant to an odorant receptor (OR) on the surface of a sensory neuron (located on the antenna or maxillary palp) is the first step in the transformation of environmental chemical signal to olfaction-mediated behavior in an insect. There have been several recent reviews of insect olfactory processing at the periphery (Vosshall and Stocker 2007; de Bruyne and Baker 2008; Su et al. 2009), but here we discuss past and more recent findings of mixture processing at this level.

Olfactory receptors, olfactory receptor cell (ORC) populations, and mixture coding in the periphery

ORs are capable of binding diverse sets of odorants; variation in their genetic sequences provides different binding affinities for volatiles present in the environment (Robertson et al. 2003, 2010; Robertson and Wanner 2006). This greater or lesser affinity results in a broader or narrower "tuning," or specificity for a given chemical (cf. Qiu et al. 2006). It is thought that narrowly tuned receptors are meant to transmit information about biologically important odorants (Wilson and Mainen 2006). This tuning may in turn cause competitive interactions between odorants to take place, resulting in non-linear responses by the sensory cell, including additive or suppressive responses to a stimulus (Derby 2000; Münch et al. 2013). Some of these interactions may occur when ligands for an OR compete for its binding site; known as "syntopic interaction," this phenomenon can cause mixture suppression at high concentrations and may contribute to concentration invariance, or the ability of an organism to classify a mixture as the same percept at different concentrations (Münch et al. 2013).

Beyond the receptor and corresponding ORC that expresses the receptor protein, the ORC itself is housed in sensilla on the antenna or maxillary palp. Sensilla contain multiple ORCs with distinct neuronal identities, thus providing a mechanism by which mixtures can be processed at the periphery (de Bruyne et al. 2001). Although the structure and function of insect ORs appears superficially similar to those of vertebrates, there are some crucial differences. Unlike vertebrate ORs, insect ORs require the assistance of OR 83b ("Orco") to migrate to the ORC membrane (Benton et al. 2006; Vosshall and Hansson 2011), and OR 83b is highly conserved across the insect phylogeny. Additional differences exist in the structure of

the ORs and their roles in negative feedback, complicating the use of insects as models for vertebrate olfaction (reviewed in Benton 2006) and highlighting the diversity of olfactory systems that have evolved across the animal phylogeny.

For invertebrates, some of the most thorough work on mixture processing at the periphery has come from work with lobsters. Using electrophysiological recordings of ORCs on lobster antennule, Derby and collaborators demonstrated that mixtures elicited responses significantly different from the responses expected based on single odorant responses (Derby et al. 1984, 1985, 1991a, b; Carr and Derby 1986; Gentilcore and Derby 1998). This work demonstrated four key points. First, mixtures often elicit non-additive responses in ORCs, with mixture suppression, where the response to the mixture is less than the response predicted by its components, tending to dominate (Derby et al. 1991a). Second, suppression in the population of ORCs to binary mixtures results in a decrease in sensitivity that is further reflected in suppressive responses in olfactory interneurons in the brain (Derby et al. 1985, 1991a). Third, the change in response by the ORC population provides a coding mechanism to represent the odor stimulus (Derby et al. 1991b). Finally, the unique mixture-evoked responses could be due to competitive binding interactions at the receptor (Gentilcore and Derby 1998), as well as non-competitive mechanisms like ephaptic coupling (Derby et al. 1991a) (Fig. 2a). Importantly, electrophysiological responses could also be correlated with behavior (Carr and Derby 1986; Daniel and Derby 1991; Finelevy and Derby 1992; Livermore et al. 1997).

In parallel with work with lobsters, electrophysiological recordings of ORCs in the insect sensilla also showed non-additive effects when the insect was presented with odor mixtures. For example, vegetative odorants were demonstrated to modulate pheromone receptors when presented in isolation or simultaneously (van der Pers et al. 1980), and host plant odorants could synergize with, or inhibit, pheromone-evoked responses (synergize: Ochieng et al. 2002; inhibit: Party et al. 2009; Andersson et al. 2010; Rouyar et al. 2011; Deisig et al. 2012 and references therein). More recent work in D. melanogaster showed similar non-additive effects that can operate on a single OR, presumably through competitive binding (Su et al. 2011) and through interactions between ORCs housed in the same sensilla (Su et al. 2012), known as ephaptic coupling. When an ORC—expressing a well-characterized OR—was stimulated with a binary mixture of suppression- and excitatation-evoking odorants, the temporal response characteristics were modified such that duration of the excitatory response was increased or decreased depending upon the ratio of the two odorants (Su et al. 2011). In a second study, the response of one ORC was non-synaptically inhibited by transient
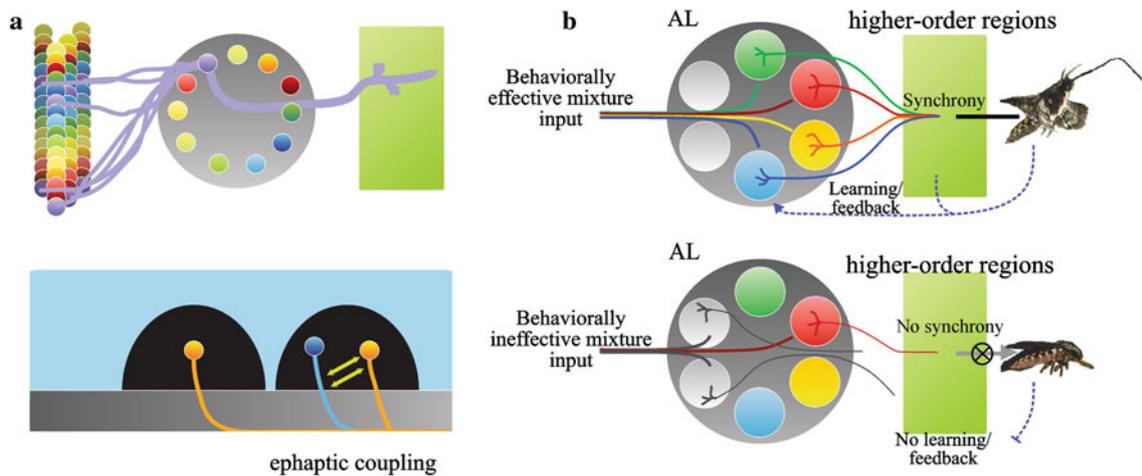
Fig. 2 Mixture processing in the periphery, antennal lobe, and higher-order regions. **a** Peripheral processing: *top* in most insects, all olfactory receptor cells (*lines of colored circles*) that express a given olfactory receptor protein in the periphery converge to the same antennal lobe glomerulus (*large gray circle*) before information is transmitted to higher-order brain regions (*green square*). *Bottom* ephaptic coupling occurs between two olfactory receptor cells that share the same sensillum on the insect antenna. This can be a mechanism for configural mixture processing in the periphery.

b Mixture processing and behavior: synchronized activity in the antennal lobe can result in changes in higher-order processing that lead to behavior and/or learning. The *top panel* shows the moth *Manduca sexta* responding to a behaviorally effective floral scent mixture with proboscis extension. A reward for this behavior can cause positive feedback, resulting in changes in the brain. The *bottom panel* shows the same moth reacting to a behaviorally ineffective odor mixture

activation of the neighboring ORC (Su et al. 2012). Together, these results suggest that mixture interactions at both the receptor and cellular scales may contribute to the unique coding of the mixture and the binding of the odorant "features" into a unified percept.

Evolutionary changes in the periphery: olfactory specialization and speciation

Because olfaction is thought to be the most indispensable sensory system to most insects (Strausfeld 2012), changes to the olfactory system may profoundly change how a given insect perceives the world and how it behaves. Such olfactory changes can mediate significant ecological and social changes in an insect species, such as niche shifting to a new host plant or altering mate choice; more broadly, these changes can lead to reproductive isolation and speciation. Alteration in the olfactory periphery may take the form of transformation of the tuning of ORs, the abundance of a given ORC type, or the ratio of ORC types present.

Some of the well-studied examples of sympatric speciation (speciation without geographic separation) result from shifts in insect–host plant relationships due to changes in the insect olfactory system. The relationship between species of *Drosophila* and *Morinda* fruits and the presently occurring host shifts of the apple maggot fly (*R. pomonella*) are particularly good models (Coyne and Orr 2004). *Drosophila sechellia* is closely related to the model organism, *D. melanogaster*, which is an ecological generalist. By contrast, *D. sechellia* is extremely specialized, ovipositing

exclusively on the fruit of *Morinda citrifolia* (Farine et al. 1996; Dekker et al. 2006). *Morinda* is toxic to other *Drosophila* larvae, but *D. sechellia* has evolved resistance to these toxins (Jones 1998). The olfactory system of *D. sechellia* is specialized to detect the mixture of volatiles released by *Morinda*. Relative to *D. melanogaster*, *D. sechellia* has increased expression of sensillum ab3 by 150–200 % (Dekker et al. 2006). Sensillum ab3 houses the ab3A neuron, which is activated by hexanoate esters, and the ab3B neuron, which is activated by 2-heptanone (Ibba et al. 2010); both of these compounds are important for mediating attraction to *Morinda* fruit (Dekker et al. 2006; Ibba et al. 2010). This specialization comes at the expense of other sensilla; compared to the closely related *D. melanogaster*, *D. sechellia* has reduced expression of ab1 by 60–80 % and of ab2 by 93–100 %, and has altered the tuning of ab1 (Dekker et al. 2006). The ab3A neurons of *D. sechellia* are also ten times more sensitive to methyl hexanoate than those of *D. melanogaster*, which could be due to a narrowing in tuning of OR22a mediated by a change of only nine amino acids, and also potentially by an increase in the expression of OR22a within that ORC (Dekker et al. 2006). It is thought that this *Morinda* specialization in *D. sechellia* may also have been mediated by a loss of innate repulsion to *Morinda*'s toxic acids due to a change in expression of two types of odorant binding protein (OBP); these OBPs are found in the leg gustatory sensilla and are responsible mediating repulsion to hexanoic and octanoic acids in other drosophilids (Matsuo et al. 2007; reviewed by Hansson and Stensmyr 2011). Previous

research has shown that these peripheral changes lead to changes in the antennal lobe and to behavioral differences in response to mixtures of *Morinda* fruit headspace volatiles, which are later discussed in detail in this review. In light of the recent findings by Su et al. (2012), future work might investigate if there are any interactions between ab3A and ab3B neurons through ephaptic coupling (Fig. 2a), since they share the ab3 sensillum and both mediate attraction to *Morinda* fruit.

The three races of *R. pomonella*, commonly known as the apple maggot fly, also show specialization in the olfactory periphery. Each race of this species is specialized to mate and oviposit on the fruits of apple, dogwood, or hawthorn trees, and shows behavioral preference for the scent of their host (Zhang et al. 1999; Nojima et al. 2003a, b; Linn et al. 2003, 2005). It has been proposed that these preferences are mediated by differences in the tuning of ORs, specifically their response thresholds and temporal firing patterns (Olsson et al. 2006c), rather than differences in the types of ORs present or in the ratio of OR types present on the antenna (Olsson et al. 2006b). This may have led to reproductive isolation between the races of *R. pomonella*, even though they live in sympatry. Remarkably, the host shift from the native downy hawthorn to the introduced domesticated apple, *Malus domestica*, in the eastern United States has occurred in the last 150 years (McPheron et al. 1988; Linn et al. 2003). The evolution of additional shifts between host plants in the western United States may have occurred even more recently, perhaps within the last 60 years (Linn et al. 2012)—an incredible pace for the process of speciation. This rapid change underscores the critical importance of olfaction in insect behavior, ecological niche selection, and evolution. It also underscores the importance of mixture processing in host shifts, since dogwood, apple, and hawthorn fruits share physiologically effective volatiles (Olsson et al. 2006a). More work needs to be done to identify modifications in the central olfactory system, such as differences in glomerular size or organization in the AL between races, since these alterations likely occur in parallel to modifications in the periphery (see the review by Hansson and Stensmyr (2011) for additional information).

Future work in the periphery

Valuable future work in blood-feeding insects (e.g., mosquitoes, kissing bugs) could lead to important advances in our understanding of mixture processing and the evolutionary processes regulating shifts between zoophily (animal-preferring) and anthropophily (human-preferring) insects. Because the volatiles emitted from human hosts are blends of many compounds, understanding olfaction-mediated host-seeking and mixture processing in the olfactory systems of disease vectors may help us find ways to repel, trap, or confound them, and thus help us control the spread of diseases like malaria, dengue, leishmaniasis, Chagas disease, and more (reviewed by Carey and Carlson 2011). In addition, future work could investigate peripheral changes in other classic sympatric speciation models as listed in Coyne and Orr (2004), such as the soapberry bug. Finally, the contribution of processes like ephaptic coupling, interaction between chemicals prior to reaching receptor sites, competition between chemicals for access to receptor sites, and feedback from the antennal lobe on natural mixture processing in the periphery could be studied in greater depth to determine if these processes might play a role in downstream spatial and temporal coding.

Antennal lobe processing

After the airborne chemical mixture is transduced into an electrical signal by the ORCs, it is processed in the antennal lobe, the primary processing center in the insect brain (Hildebrand and Shepherd 1997), which is partitioned into functional subunits called "glomeruli." The olfactory system can recognize and distinguish odor mixtures as patterns of spatial and temporal activity in AL glomeruli. A common feature for many, but not all, insects is that ORCs that express a given OR project to the same glomerulus within the antennal lobe (Bargmann 2006) (Fig. 2a). When a complex mixture encounters the insect antennae, many types of ORCs can respond, thereby driving activity in multiple glomeruli. Local interneurons (LNs), whose processes are fully contained within the AL, may connect glomeruli such that activity in one glomerulus can modify activity in another when both are stimulated. These interactions—as well as characteristics of the synapses that connect ORCs and the neurons that project from the AL to higher-order regions of the brain (projection neurons, or PNs)—may modify the signal for a given mixture substantially from what was input into the ALs by the ORCs (Olsen and Wilson 2008). This can result in the coding of the mixture as a percept unique from its components even before the signal is processed by higher-order brain regions, termed configural processing (Kuebler et al. 2012). For example, work by Deisig et al. (2006, 2010) in the honey bee found that mixture responses from ORCs were largely elemental, or linearly predictable from responses to mixture components, while output from the AL to higher-order brain regions by PNs was more configural, which allowed for greater separation of similar mixtures in olfactory space.

This section of our review will explore how odorants and mixtures are coded by the population of AL neurons,

the mechanisms of such coding, comparative differences in AL organization and coding, and how changes in AL processing can play a role in larger evolutionary shifts.

Population coding

The identity, concentration, and temporal dynamics of a mixture are coded by the spatial and temporal activity of the population of AL neurons (Galizia and Szyszka 2008).

Spatial and temporal coding

Olfaction is unlike other senses, such as audition and vision, where the spatial pattern of receptors at the periphery is mirrored by a similar spatial organization in the brain. Instead, ORCs express a specific OR, but are distributed semi-randomly on the antennal surface. All of the ORCs that express a given OR type project to a specific glomerulus in the antennal lobe, thereby forming a chemotopic map, the temporal and spatial activity of which encodes odor properties such as identity and concentration. Again unlike in vision and audition, the spatial representation of the odor in the brain reflects odor properties like identity rather than spatial characteristics of the stimulus in the environment (reviewed by Martin et al. 2011; Strausfeld 2012). The spatial patterns of glomerular activity that encode a given odorant or odor mixture are often consistent within a species (Galizia et al. 1999), indicating that conspecifics process and perceive odorants similarly within the AL. Temporal features of AL activity, such as spike synchrony and latency (Meyer and Galizia 2012; reviewed by Laurent 2002), are also critical for coding chemical as well as temporal features of an odor plume.

However, processing within the AL can modify the afferent input. Insect AL glomeruli exist at the interface of ORCs, PNs, and LNs (Silbering and Galizia 2007), so mixture information transmitted to the AL by the ORCs is modified—in both spatial and temporal aspects—by interaction with the population of LNs. LNs and PNs also have been shown to have a much larger array of neurotransmitter and neuropeptide profiles than historically thought (Utz et al. 2007; Neupert et al. 2012; reviewed by Nässel and Winther 2010), in addition to morphological and physiological differences (Chou et al. 2010), adding complexity to the AL network responsible for mixture processing. Mixture information is also modulated by centrifugal neurons within the AL before being conveyed to higher-order brain regions by the PNs. PN and LN activity is modified with respect to firing frequency, latency, spatial location, and temporal pattern in ways that cannot be predicted by any general rule (Kuebler et al. 2011). Temporal patterns of activity by neurons, such as synchrony in

the firing of PNs, can selectively drive higher-order neurons to mediate behavior (Perez-Orive et al. 2002; Riffell et al. 2009a, b; reviewed by Martin et al. 2011) (Fig. 2b).

These modifications of coding by the AL network, in addition to properties of the AL neurons themselves, allow the AL neural population to encode mixtures as percepts distinct from their individual components, as well as allow the animal to perceive mixtures differently in different ambient odor environments, as different contexts for a given odor might require different behavioral responses. The pheromone systems of Lepidoptera present fantastic examples of both of these. Studies in some moths have shown that there are different types of neurons in the macroglomerular complex (MGC) of male moths, a set of AL glomeruli specialized to process sexual odors. Some of these of neurons (both projection neurons and interneurons, which are discussed later in this section) are broadly responsive to both pheromone blends and to single components of blends; others are more selective, responding to a smaller range of these stimuli, or only to specific blends of certain components in certain ratios. Thus, during processing in the AL, some information about individual pheromone components is preserved, but there is also mixture-specific AL activity (Christensen et al. 1989; Wu et al. 1996). There are also effects of environmental context on pheromone detection for male moths. In some moth species, the response to the plant odor + pheromone blend is suppressed, or decreased in strength relative to that of the pheromone blend alone, but this suppression may be paired with an increase in temporal resolution (Chaffiol et al. 2012; Deisig et al. 2012). However, in other species, pheromone blend and plant odor responses synergize and result in a response of greater strength than the pheromone blend alone in MGC neurons (Namiki et al. 2008; Trona et al. 2010). These interactions underscore the importance of mixture processing not just for coding blends that originate from a given source (eg., a given flower, a female moth, etc.), but also for modulating that coding based on odorants emitted by other environmental sources.

Comparison between species in spatial and temporal coding

Laurent (2002) proposed that a large "coding space" is necessary to process olfactory stimuli, given the complexity and noisiness of environmental odor mixtures. The large size of the "coding space" created by the AL is made possible by the large number of possible permutations of spatial and temporal activity. However, the ways in which different insect species create an olfactory "coding space" large enough to meet their computational needs differ dramatically. Neural wiring and glomerular organization play a large role in this process, as each additional

glomerulus can add an additional dimension to "coding space" (Galizia and Szyszka 2008). These differences are particularly stark in the locusts, which exhibit many more intra-AL connections than other insects, resulting in a dramatic expansion of "coding space" (reviewed by Laurent 2002; Martin et al. 2011). As a probable result of these differences in organization, locusts appear to show markedly greater reliance than other insects on temporal coding strategies to encode odor identity and concentration. However, it is also possible that the anatomical connectivity of microglomeruli could simply reflect "expanded" glomeruli; in other words, that groups of microglomeruli form functional subunits that are analogous to unique glomeruli in other taxa. If this is the case, coding space in locusts would not be as expanded relative to other insects as proposed by Laurent (2002).

In locusts, the population of neurons involved in the response to an odorant evolves over the course of the response, synchronized with local field potential oscillations of about 20 Hz. While other insects show temporal signatures in their neural responses that may be especially important for mixture coding (Riffell et al. 2009a, b), as well as some oscillatory synchronization (bees: Galizia and Szyszka 2008; moths: Heinbockel et al. 1998; Christensen et al. 2003; flies: Wilson and Laurent 2005), the locust's comparatively extreme change of the spatial representation over time provides a mechanism for odorant input that produce very small differences in ORC response that can be coded uniquely in the AL over the course of the oscillations (Laurent 2002). The locust's "coding space" may be enlarged by the inclusion of dynamics of the response patterns, as well as increased unique spatial possibilities as a result of their microglomerular organization (Laurent 2002); this may result in greater resolution between similar odors, and perhaps greater resolution between similar odorant mixtures. However, the greatly expanded "coding space" of the locust may come at the price of response speed: locusts can respond to olfactory stimuli with PN spikes up to 20 Hz, the frequency of their local field potential oscillations, while moths can respond to stimuli at up to 100 Hz, and flies and bees up to 200 Hz (moths: Vickers et al. 2001; flies: Bhandawat et al. 2007). In locusts, moths, flies, and bees, the important temporal coding feature of PN spike synchronization is the result of the activity of inhibitory LNs in the AL (MacLeod and Laurent 1996; Lei et al. 2002; Galizia and Szyszka 2008; Ito et al. 2009; Tanaka et al. 2009). The locust's atypical AL coding may also require additional downstream decoding strategies like decorrelation (Laurent 2002) not observed in other insects. These phylogenetic differences in the architecture of the "coding space" affect how both mixtures and single odorants are processed and discriminated.

Role of ORCs and projection neurons in gain control

Control of gain (the change in signal amplitude between input and output) is critical for the maintenance of sensitivity to a given stimulus, the encoding of stimulus intensity, and the capability to reliably encode the identity of a given stimulus despite a wide range of possible concentrations in the environment (Bhandawat et al. 2007; Kazama and Wilson 2008). In the AL, gain control is the result of properties of the synapses between ORCs and AL projection neurons, the network of LNs connecting glomeruli, and the aminergic neurons innervating the ALs from higher-order brain regions.

Work in *Drosophila* suggests that synapses between ORCs and PNs are strong, likely containing a large number of release sites and a high probability of neurotransmitter release (Kazama and Wilson 2008). As such, stimulation of an ORC results in a reliable spiking in a PN even when the stimulus is weak (Kazama and Wilson 2008). However, another consequence of this high probability is that the synapse quickly depresses in response to the high-frequency spiking induced by a strong stimulus. This results in a non-linear relationship between ORC input and PN output, in which PN responses to weak ORC input are amplified and those to strong ORC input are subdued (Bhandawat et al. 2007; Kazama and Wilson 2008). This may allow for high sensitivity to odors that are at a low concentration in the environment, while still preventing saturation and allowing for discrimination when odors are at a high concentration in the environment. This echoes the effect of concentration on configural processing that occurs at the periphery due to syntopic interactions between mixture components, where suppression effects are most pronounced at high concentrations. Perhaps high concentrations of mixtures composed of similar ligands experience increased suppression because of the potential for suppression in both the periphery and the AL.

Role of local interneurons in gain control, sharpening, and broadening

Local interneurons in the AL, first described by Hildebrand and Matsumoto in the moth *M. sexta* (Matsumoto and Hildebrand 1981; Strausfeld 2012), synapse onto PNs (Yaksi and Wilson 2010) and can modulate the activity of a given PN based on activity in other parts of the AL. LNs can be excitatory (Olsen et al. 2007; Yaksi and Wilson 2010) or inhibitory (Olsen and Wilson 2008) and, though the overall effect of interglomerular inhibition dominates that of interglomerular excitation (Olsen and Wilson 2008), simulations have shown that these competing forces must be finely balanced to discriminate similar odors (Assisi et al. 2012). LN activity can influence a variety of AL

coding mechanisms: sharpening and broadening, which alter the signal-to-noise ratio; gain, which alters the relative strength of output to input; and synchrony, which helps to bind neural representations of mixture components into unitary percepts. LN-mediated inhibition—via release of γ-aminobutyric acid (GABA)—also helps enhance the accuracy of the time of onset and offset of an odor (Sachse and Galizia 2002), likely aiding the insect in negotiating the physical structure of an odor plume.

Importantly, modifying LNs, and thus the olfactory coding in which they participate, has been shown to modify perception and odor-evoked behavior (Acebes et al. 2011), notably altering configural processing in response to odor mixtures (Choudhary et al. 2012). Both experiments and simulations have shown how configural processing in the AL can aid in odor discrimination, particularly via interglomerular inhibition, where the most activated glomerulus inhibits activity in co-activated ones with similar response profiles (Linster et al. 2005; Schmuker and Schneider 2007). Behavioral response to mixtures in honey bees supports the idea that configural processing is more prevalent when mixtures are similar: when trained to associate single odorants with a sugar reward, honey bees are better able to discriminate rewarded from unrewarded mixtures when they had fewer components in common; the corollary was true as well (Deisig et al. 2002).

Recent interest in LN properties has increased our understanding of their role in odor processing; but many of the studies investigating LNs have used single odorants as stimuli, so the contribution of LNs to mixture coding is still less well known. Finally, LNs can differ in their morphology and tuning. LNs within the ALs can project globally, innervating all glomeruli more or less equally, or locally, innervating a subset of glomeruli (Chou et al. 2010; Strausfeld 2012). For the rest of this review these will be called global LNs and local LNs, respectively. They also can be tuned to single odorants, or be blend specific, and may have differences in temporal characteristics such as latency (Meyer and Galizia 2012)—all of which are important for mixture processing.

Role of global LNs

Global LNs play an important role in gain control in flies, as the global lateral inhibition and excitation they mediate scales with input to the ALs (Root et al. 2007). It has been suggested, but not yet demonstrated, that this may allow for a consistent representation of an odor mixture in the AL across a range of concentrations. This representation may be coded for by a consistent pattern of relative activation across PNs if global LNs increase gain when stimulation is weak and decrease it when stimulation is strong. Further affecting gain, global inhibition dominates global excitation, facilitating sharpening, the reduction in overlap for different components of the mixture in the population code (Sachse and Galizia 2002; reviewed by Martin et al. 2011), in addition to the global reduction of PN responses. Differences between insect taxa with respect to the role of excitation versus inhibition may be a result of taxon-specific olfactory system architecture. LNs play an important role in other facets of AL mixture coding as well, including mixture suppression, the decrease in response to a mixture relative to the expected response given its components. Mediated by an increase in LN activity, interglomerular interaction increases when the number of odors that make up a mixture increases (Silbering and Galizia 2007). Although inhibitory global LNs play a role in mixture suppression, local LNs are also important for mediating this process. As the complexity of the mixture (and thus the total input to the AL) increases, so too does the magnitude of mixture suppression; this, along with the effects of experimental pharmaceutical manipulation, implicates the participation of the network of global LNs in mixture suppression. However, the total input to the ALs does not predict global PN interaction, so local LNs are involved in mediating mixture suppression as well (Silbering and Galizia 2007). Some inhibitory LNs may also play a role in computation that occurs within, not just between, glomeruli (Sachse and Galizia 2006; Silbering and Galizia 2007).

Role of local LNs

Local LNs also play an important role in coding mixture responses in the antennal lobe, particularly in the selective sharpening and broadening that contribute to configural processing (reviewed by Martin et al. 2011). Configural processing occurs in the olfactory systems of many organisms across the animal phylogeny (fish, mammals, and humans: reviewed Lei and Vickers 2008), including insects (moths: Christensen et al. 1991; Hartlieb et al. 1997; honey bees: Sun et al. 1993). Excitatory and inhibitory LNs may play a role in configural mixture processing through sharpening and broadening. Local LNs that have different patterns of innervation connecting different glomeruli show physiological differences in spike rate and timing; thus, morphologically distinct LNs may have fundamentally different functions in modulating PN activity (Chou et al. 2010), allowing for more control over how mixtures are coded based on these patterns of innervation and the physiological properties that accompany them. As mentioned previously, local LNs also help to mediate mixture suppression (Silbering and Galizia 2007). Excitatory local LNs can sharpen or broaden the representation of a mixture because they may synapse onto a PN or onto an inhibitory LN (Yaksi and Wilson 2010); excitatory LNs tend to be local in general, so their effects should not

propagate throughout the AL. If an excitatory LN synapses onto and drives a PN, activity will be broadened by direct excitation. If an excitatory LN synapses onto an inhibitory LN, it may stimulate additional inhibition that results in the sharpening of the signal.

Inhibitory local LNs can sharpen the representation of a mixture, preventing weakly stimulated neurons from contributing to the PN output and allowing the most strongly stimulated ones to dominate: Strong stimulation of a given glomerulus may, depending on the pattern of innervation by local LNs, inhibit a more weakly stimulated glomerulus; this is especially true for chemically similar mixture components, whose glomeruli often share more inhibitory connections than those of dissimilar components (Wiltrout et al. 2003; Wilson and Laurent 2005; Linster et al. 2005; reviewed by Sachse and Galizia 2006). Thus, configural processing tends to dominate when components of a mixture are more similar, while elemental processing tends to dominate when components of a mixture are more different. Although many optophysiological studies postulated that elemental processing dominates in the AL (reviewed by Lei and Vickers 2008), such studies largely reflect the output of the ORCs rather than any downstream processing occurring within the antennal lobe (Heil et al. 2007). Because ORCs largely process odors elementally, studies using optophysiological methods report elemental processing in the AL more frequently than it actually occurs; this occurs largely as an artifact of the calcium bath method used—ORCs activate glial cells, which subsequently show a calcium signal (Heil et al. 2007). Other studies have shown that PN responses are substantially different from ORC input (Kuebler et al. 2012).

Centrifugal input and modulation of the AL

Gain can also be adjusted by aminergic and peptidergic neurons that project into the AL from higher-order brain regions, providing what is known as centrifugal input (Anton and Homberg 1999). These neurons release neuromodulators such as serotonin, dopamine, octopamine, or histamine into the antennal lobe. These compounds may alter the K+ conductances of AL neurons (dopamine and serotonin; reviewed by Ellen and Mercer 2012), and thus modulate neuronal activity. Neuromodulators released in the AL can signal the behavioral context of the olfactory response and modulate AL activity accordingly: for example, it is thought that serotonin modulates olfactory response based on circadian rhythm (Kloppenburg and Mercer 2008), dopamine based on association of the odor with an aversive stimulus (Dacks et al. 2012), and octopamine based on association of the odor with an appetitive stimulus (Schwaerzel et al. 2003). Although the release of these neuromodulators signals dramatically different

behavioral contexts, their effect on AL responses can elicit similar responses in AL neurons (octopamine: Barrozo et al. 2010; Riffell et al. 2013; serotonin: Dacks et al. 2008; Kloppenburg et al. 1999; *Drosophila*: Yu et al. 2004; *Manduca*: Daly et al. 2004; dopamine: Dacks et al. 2012).

Comparison of neural wiring and its effects on coding

Previous sections in this review have discussed on how ORC-PN connections and interactions between glomeruli can affect mixture processing, but this is further complicated by comparative differences in the insects. A diversity of AL morphologies and olfactory wiring schemes have evolved across the insect phylogeny, with pronounced differences in glomerular size and organization (Ignell et al. 2001), the way that ORCs innervate the AL, the wiring of PNs to higher-order brain regions, and other factors (reviewed by Martin et al. 2011). These differences have resulted in novel coding strategies in certain taxa and in differential emphasis on coding strategies that are shared between taxa. Below, we detail these differences in the insect taxa that have been most extensively used for olfactory studies.

Comparison of wiring: ORCs to glomeruli

The wiring of neurons that project to the AL differs dramatically between taxa. In addition, ORCs can be organized in different ways as they project to the AL. The connection of ORCs to glomeruli in the AL is particularly important because ORCs seem to play a key role in the formation of glomeruli, as glomeruli do not form when ORC axons are removed and groups of ORCs spontaneously form glomerulus-like structures with their axons (Oland and Tolbert 1996). In the dipteran *D. melanogaster*, all ORCs that express a given olfactory receptor project to a single glomerulus in the ipsilateral AL, which in turn generally sends the signal along to the corresponding glomerulus in the contralateral AL via an antennal commissure, a feature that has not been found outside the dipterans (reviewed by Martin et al. 2011). In *Drosophila*, five glomeruli receive information from only the ipsilateral antenna, but the rest receive information from both (Stocker et al. 1990). In Lepidoptera, the case is similar, except with no antennal commissure: ORCs control the functional identity of moth glomeruli (Oland and Tolbert 1987), as demonstrated by antennal transplant experiments between male and female conspecifics (Schneiderman et al. 1982; Rössler et al. 1999; Kalberer et al. 2010) and between individuals from closely related species (Vickers et al. 2005). It was thought that Hymenoptera exhibited a higher level organization of the AL : that their ALs were innervated by ORCs forming separate tracts that led to sets

of neighboring glomeruli grouped by odor specificity (Galizia et al. 1998); recent imaging studies, however, have shown that this is not the case (Carcaud et al. 2012; Galizia et al. 2012). Any given glomerulus receives input from a single ORC type in most insects that have been studied (Hildebrand and Shepherd 1997, reviewed by Bargmann 2006). In contrast, locusts, with their microglomerular AL organization, operate differently: locust ORCs each project to one to three microglomeruli in the AL. (Hansson et al. 1996). In the locust, a given microglomerulus thus likely samples information from multiple ORC types and therefore samples a wider chemical range of mixture components than a given glomerulus might in another insect.

Comparative glomerular architecture and organization

There is great variety in the size, quantity, and wiring of glomeruli in the ALs of insects. Honey bees and cockroaches have many small glomeruli (163 and 125, respectively) that each project multiple PNs into the higher-order brain (Anton and Homberg 1999; Galizia and Rössler 2010; Brill et al. 2013). However, it should be noted that the large number of glomeruli in higher Hymenoptera appears to be derived, as the basal sawflies have only 44 (Dacks and Nighorn 2011). In bees, these glomeruli are grouped by odor specificity within the AL (Galizia et al. 1998), and the AL as a whole is divided into two hemi-lobes whose outputs follow two different tracts to higher brain regions (reviewed by Martin et al. 2011). The ALs of flies and moths have a smaller number of glomeruli (40 and 63, respectively; reviewed by Rospars and Hildebrand 1992; Anton and Homberg 1999), and each glomerulus also gives rise to multiple PNs. In addition to having fewer, larger glomeruli, the ALs of many moths also have a MGC, a series of large glomeruli specialized to process odorants important for reproduction such as pheromones; these glomeruli have correspondingly larger numbers of PNs (reviewed by Martin et al. 2011). For all the above insects studied, researchers have found that any given glomerulus can be uniquely identified by its position in the AL relative to that of other glomeruli. In hemimetabolous insects this means that as the insect and its antennae grow, the glomeruli grow larger while the number of glomeruli remains fixed, with relatively slight rearrangement in glomerular position (reviewed by Rospars and Chambille 1985; Strausfeld 2012). This is important for coding because it may mean that a relatively consistent odortypic map can be maintained as the insect moves through life stages, and that such a map may be consistent within a species regardless of individual size.

Beyond the specific glomerular organization of certain hemimetabolous insects, other insects show remarkably different AL organization. Some hemipterans, such as plant lice and some aphids, appear to have no glomeruli at all, or have very ill-defined ones (Kollmann et al. 2011). By contrast, the ALs of locusts, *Schistocerca gregaria* (Orthoptera: Acrididae), are composed of over 1,000 microglomeruli, as well as the glomerular lobe, a separate area of the AL reserved for processing olfactory information from the maxillary palps (Frambach and Schurmann 2004). Although microglomerular structure of some kind is thought to have evolved twice within the Orthoptera, once in the grasshoppers (Orthoptera: Caelifera: Acrididae), with locusts possessing fully microglomerular ALs, and once in the crickets (Orthoptera: Ensifera: Gryllidae), with microglomerular substructures within each unique glomerulus found in field cricket *Gryllus bimaculatus* (Ignell et al. 2001; reviewed by Hansson and Stensmyr 2011), this type of organization is quite rare in the insects. Microglomeruli have only been tentatively observed in one other insect taxon, and ancestral orthopterans likely displayed a more typical glomerular organization similar to that of the cockroach (reviewed by Ignell et al. 2001); as a result, microglomeruli appear to be evolutionarily derived in the Orthoptera. The rarity of this microglomerular organization and its stark differences with that of most other insect taxa call into question the generality of observations made on locusts. Because of their microglomerular ALs, the olfactory codes on which locusts rely are quite different from those of other insects; however, it should be noted that the heavily connected AL arrangement observed in locusts is also seen in all studied decapods (reviewed by Strausfeld 2012). Unlike all other insects studied, locusts add more microglomeruli to their ALs each time they molt and add new ORCs (Ochieng et al. 1998), implying relatively unique demands for olfactory coding in this species; the number of PNs projecting from these microglomeruli, however, remain constant as the locust ages, expanding their dendritic arbors (Anton et al. 2002). As a result, a perfectly consistent odortypic map cannot be maintained as the locust ages. This raises questions about how coding changes over the life of the insect, what new local and higher-order connections are forged with each molt, and how consistency of odor-evoked behaviors is maintained. However, this may account for the elevated role in temporal coding—versus spatial coding—in locust olfaction.

Additional information on the projection of PNs to higher-order regions can be found in Galizia and Rössler (2010), and Rössler and Brill (2013).

Evolutionary changes in the AL: olfactory specialization and speciation

Changes to the olfactory system may profoundly alter insect behavior and can mediate changes in selection of host plants, prey, or mates. Because all ORCs of a given

type often converge in the same glomerulus in insects, evolutionary changes to the AL—such as an increase in the volume of a glomerulus or the change in the tuning of its PNs—often follow from changes in the periphery.

Host plant switches and sympatric speciation, or speciation without geographic separation, are mediated by changes in the olfactory system in many different species of *Drosophila* (Fig. 3a). As mentioned earlier in this review, *D. sechellia* is specialized to oviposit on the toxic fruit of *M. citrifolia*, in contrast with its generalist sister species, *D. melanogaster* (Farine et al. 1996; Dekker et al. 2006; Ibba et al. 2010). Compared to *D. melanogaster*, *D. sechellia* overexpresses sensillum ab3, and its neurons ab3A and ab3B (Dekker et al. 2006). Accordingly the glomeruli that receive afferent input from the ab3A and ab3B ORCs, the DM2 and VM5d, respectively, are enlarged in *D. sechellia* compared to *D. melanogaster* (Dekker et al. 2006; Ibba et al. 2010). These anatomical changes translate into behavioral ones. *D. melanogaster* and *D. sechellia* are both attracted to methyl hexanoate and 2-heptanone, but *D. sechellia* has a higher attraction than *D. melanogaster*. Furthermore, *D. sechellia* is more strongly attracted to the mixture of methyl hexanoate and 2-heptanone than it is to each of the odorants separately, while *D. melanogaster* is repulsed by the mixture, suggesting that changes in glomerular size can mediate changes in the coding and preference of the olfactory information (Ibba et al. 2010).

Research on pheromone-mediated speciation in moths has shown similar trends. Major shifts in pheromone blends that females moths produce can occur in sister species, such as the European corn borer and the Asian corn borer *Ostrinia nubilalis* and *O. furnacalis*, respectively (Roelofs et al. 2002). Since tracking of female pheromones directly influences mating success, changes in the pheromone blends may spur evolutionary change in the olfactory systems of male moths (Roelofs et al. 2002). Two different strains of the European corn borer that produce and prefer almost reversed ratios of a two component pheromone mixture also show that changes in the periphery and corresponding changes in the relative sizes of glomeruli within the MGC mediate their differing pheromone preferences (Karpati et al. 2010). Rather than modifying glomerular size within the MGC, the males of the closely related *Heliothis virescens* and *H. subflexa* use PN tuning to mediate their differing pheromone preferences (Fig. 3b). The two species have morphologically and volumetrically identical MGCs, but the tuning of one of the MGC glomeruli (the DM glomerulus) is modified to process information about a secondary compound unique to each conspecific pheromone mixture (Vickers and Christensen 2003). In these sister species, tracking conspecific pheromone—a critical process in maintaining reproductive isolation and avoiding costly hybrids—is mediated by the

activation of specific subsets of glomeruli to trigger behavior (reviewed by Lei and Vickers 2008).

Future work in mixture coding in the AL

Despite the incredible volume of work already done on coding in the AL, there remains a great deal to investigate, especially with regards to mixture processing. Most studies that have parsed out the mechanisms of AL coding have done so using single odorants (Christensen et al. 1991; Olsen et al. 2007; Root et al. 2007; Olsen and Wilson 2008; Yaksi and Wilson 2010; but see Deisig et al. 2002, 2012; Silbering and Galizia 2007; Silbering et al. 2008; Eschbach et al. 2011; Choudhary et al. 2012; Meyer and Galizia 2012), and many studies have found evidence of configural mixture coding, but did not elucidate its neural mechanisms (Dekker et al. 2002; Zhang and Schlyter 2003; Tasin et al. 2006; Riffell et al. 2009a). Such work might be approached in insect models for olfaction such as *Drosophila*, *Manduca*, *Schistocerca*, and/or *Apis*, to elucidate the neural substrates for configural mixture processing as well as the comparative differences in mechanisms of AL mixture processing across the insect phylogeny.

More comparative work between close relatives also needs to be done to see how AL morphology and coding shifts accompany changes in ecology. One promising avenue would be to investigate AL differences in the races of *R. pomonella*, a model of sympatric speciation where shifts between fruit tree hosts is linked with changes in the olfactory periphery (Olsson et al. 2006a, b) (Fig. 3c). Similarly, work on AL processing in disease vector mosquitoes, such as *Anopheles gambiae*, should be done to follow up on work done in the periphery (reviewed by Carey and Carlson 2011). It would be especially interesting to compare mixture processing in the periphery and ALs of anthropophilic and zoophilic mosquitoes, since configural processing seems very likely to aid these animals in discriminating host from non-host animals. Understanding these differences in host-odor processing may help aid in the fight to preferentially target these disease vectors with traps, repellents, and other methods, without harming beneficial insects. Broader comparisons between AL coding in locusts and other orthopterans could also be done, given the extreme morphological and organizational differences in the AL that have been found within the order Orthoptera (Ignell et al. 2001); this will serve as a model for disentangling phylogenetic effects from ecological ones in determining glomerular features of the AL. In addition, comparisons could also be very interesting in the Hymenoptera: contrasting generalist and specialist pollinating bees against parasitic relatives and investigating processing and AL differences in the development of sociality. By investigating multiple models of olfactory processing and
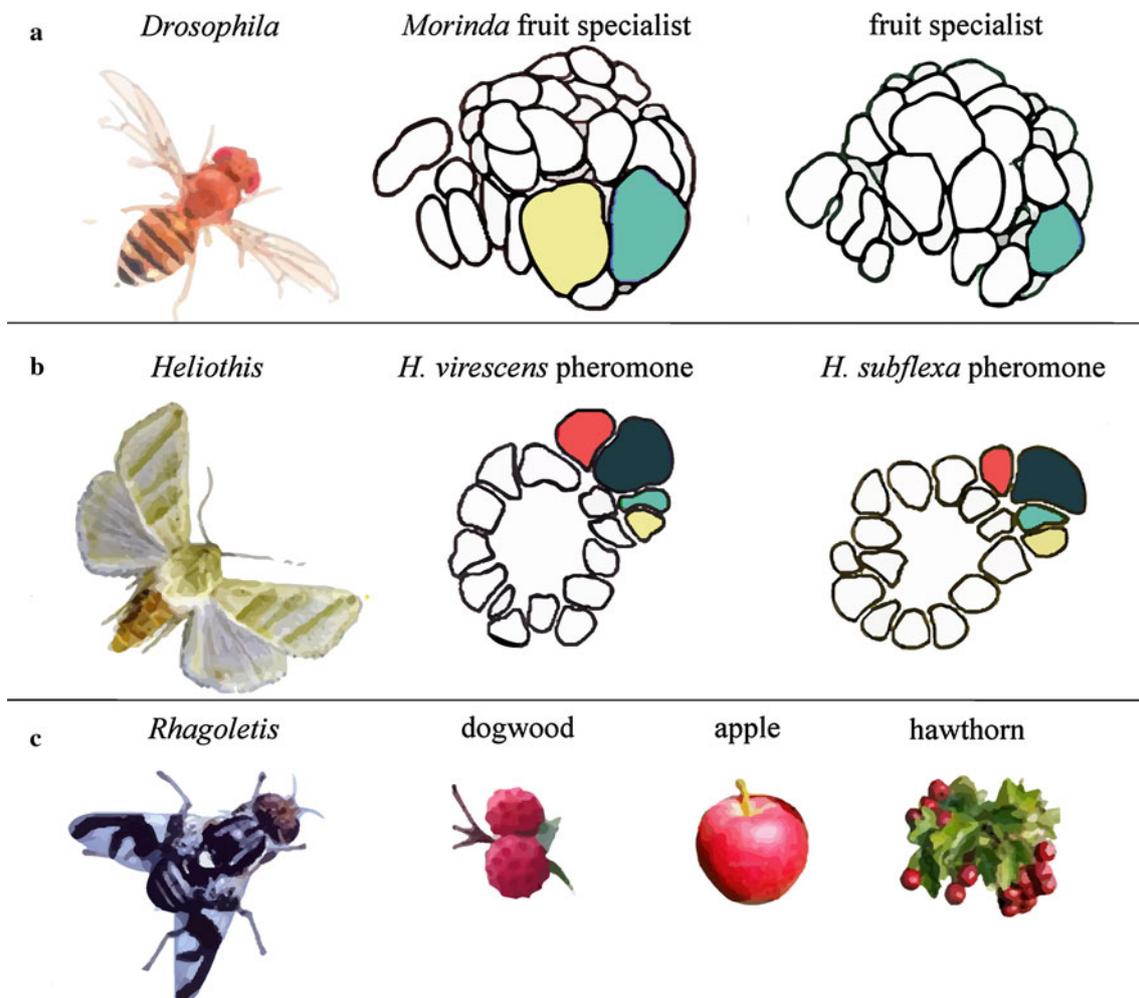
Fig. 3 Speciation is mediated by olfactory changes that affect mixture processing. **a** AL anatomical and tuning changes: *Drosophila sechellia* and *Drosophila melanogaster* show differences in volumes of AL glomeruli that reflect their oviposition preferences as specialists on *Morinda* (*left*) fruit and as generalists (*right*), respectively. *D. sechellia* exhibits two glomeruli that are enlarged compared to those in *D. melanogaster* (*light blue* and *light yellow*), as well as different tuning for hexanoates. Images adapted from Ibba et al. (2010), and Flybase. **b** AL tuning changes only: *Heliothis virescens* (*left*) and *Heliothis subflexa* (*right*) do not exhibit differently sized or arranged glomeruli in their macroglomerular complexes (*blue, pink, light blue, yellow*) although they are attracted to different pheromone blends that share components, but differ in ratio. Rather, the glomeruli in their MGCs are tuned for components that are more important in the conspecific pheromone blend. **c** Future work: different races of *Rhagoletis pomonella* can prefer dogwood, apple, or hawthorn fruits, which emit volatile mixtures composed of many of the same odorant components. While the mechanisms of this behavioral difference, and its rapid evolution have been investigated in the olfactory periphery, follow-up work in the AL must still be done for this species. Images adapted from Vickers and Christensen (2003) and with permission from the Michigan State University Diagnostic Services Lab and Jim Vargo, North American Moth Photographers Group at Mississippi State

coding in the AL, we can gain broader insights into the evolution of insect olfactory processing in the brain and its role in the interaction between insects and their environment. Finally, downstream coding of mixture input to the LH and MB, and how those centers decode that information, are exciting future topics of research.

Summary

Insects interact with a complex olfactory environment—most stimuli are not single odorants but are instead mixtures of odorants. These odorant mixtures may serve as cues for ecologically important objects such as host plants, food sources, and conspecifics—so it is critical to their survival to be able to process these efficiently. Mixtures of odorants are more behaviorally effective than single odorants so ratios and key odorants in a given mixture can be critical to perceptual success. Many stimuli contain secondary volatiles (e.g., plant defense compounds), so being able to pick out the ones that are most important (e.g., floral volatiles) is critical. Olfactory processing of mixtures is often non-additive—the mixture is coded differently than the sum of its parts, and mixtures are treated

differently from individual components. This processing happens through multiple steps—the chemical cues are first processed in the periphery, then in the glomeruli of the antennal lobe, and then in higher-order regions like the mushroom bodies (MB) and lateral horn (LH). Although this general model of insect olfaction holds true for all insects, details of the processing and AL structure differ between taxa. Differences in olfactory processing are known to underlie sympatric speciation (e.g., the apple maggot) and reproductive isolation between closely related insects (e.g., moth pheromones), and also play a role in the evolution of associated organisms such as flowering plants, whose insect pollinators may process chemical cues from related species in different fashions, leading to reproductive isolation in them as well. Beyond these areas, understanding how insects process chemosensory information in multiple levels of the olfactory system may be able to give us insights into how humans and other animals translate olfactory input into associations and actions.

References

Acebes A, Martin-Pena A, Chevalier V, Ferrus A (2011) Synapse loss in olfactory local interneurons modifies perception. J Neurosci 31:2734–2745

Andersson MN, Larsson MC, Blazenec M, Jakus R, Zhang Q-H, Schlyter F (2010) Peripheral modulation of pheromone response by inhibitory host compound in a beetle. J Exp Biol 213:3332–3339

Anton S, Homberg U (1999) Antennal lobe structure. In: Hansson B (ed) Insect olfaction. Springer, Berlin, pp 97–124

Anton S, Ignell R, Hansson BS (2002) Developmental changes in the structure and function of the central olfactory system in gregarious and solitary desert locusts. Microsc Res Tech 56:281–291

Assisi C, Stopfer M, Bazhenov M (2012) Excitatory local interneurons enhance tuning of sensory information. PLoS Comp Biol 8:e1002563

Ayasse M, Schiestl FP, Paulus HF, Löfstedt C, Hansson B, Ibarra F, Francke W (2000) Evolution of reproductive strategies in the sexually deceptive orchid Ophrys sphegodes: how does flower-specific variation of odor signals influence reproductive success? Evolution 54:1995–2006

Baker TC (2008) Balanced olfactory antagonism as a concept for understanding evolutionary shifts in moth sex pheromone blends. J Chem Ecol 34:971–981

Bargmann CI (2006) Comparative chemosensation from receptors to ecology. Nature 444:295–301

Barrozo RB, Jarriault D, Simeone X, Gaertner C, Gadenne C, Anton S (2010) Mating-induced transient inhibition of responses to sex pheromone in a male moth is not mediated by octopamine or serotonin. J Exp Biol 213:1100–1106

Bartelt RJ, Schaner AM, Jackson LL (1985) Cis-vaccenyl acetate as an aggregation pheromone in Drosophila melanogaster. J Chem Ecol 11:1747–1756

Behmer ST, Belt CE, Shapiro MS (2005) Variable rewards and discrimination ability in an insect herbivore: what and how does a hungry locust learn? J Exp Biol 208:3463–3473

Benton R (2006) On the origin of smell: odorant receptors in insects. Cell Mol Life Sci 63:1579–1585

Benton R, Sachse S, Michnick SW, Vosshall LB (2006) Atypical membrane topology and heteromeric function of Drosophila odorant receptors in vivo. PLoS Biol 4:240–257

Bhandawat V, Olsen SR, Gouwens NW, Schlief ML, Wilson RI (2007) Sensory processing in the Drosophila antennal lobe increases the reliability and separability of ensemble odor representations. Nat Neurosci 10:1474–1482

Bleeker MAK, Smid HM, Steidle JLM, Kruidhof HM, Van Loon JJA (2006) Differences in memory dynamics between two closely related parasitoid wasp species. Anim Behav 71:1343–1350

Brill MF, Rosenbaum T, Reus I, Kleineidam CJ, Nawrot MP, Rössler W (2013) Parallel processing via a dual olfactory pathway in the honeybee. J Neurosci 33:2443–2456

Carcaud J, Hill T, Giurfa M, Sandoz J-C (2012) Differential coding by two olfactory subsystems in the honeybee brain. J Neurophysiol 108:1106–1121

Carey AF, Carlson JR (2011) Insect olfaction from model systems to disease control. Proc Nat Acad Sci USA 108:12987–12995

Carr W, Derby CD (1986) Chemically stimulated feeding-behavior in marine animals—importance of chemical-mixtures and involvement of mixture interactions. J Chem Ecol 12:989–1011

Chaffiol A, Kropf J, Barroxo RB, Gadenne C, Rospars J-P, Anton S (2012) Plant odour stimuli reshape pheromonal representation in neurons of the antennal lobe macroglomerular complex of a male moth. J Exp Biol 215:1670–1680

Chittka L, Raine NE (2006) Recognition of flowers by pollinators. Curr Opin Plant Biol 9:428–435

Chou YH, Spletter ML, Yaksi E, Leong JC, Wilson RI, Luo L (2010) Diversity and wiring variability of olfactory local interneurons in the Drosophila antennal lobe. Nat Neurosci 13:439–449

Choudhary AF, Laycock I, Wright GA (2012) Gamma-aminobutyric acid receptor A-mediated inhibition in the honeybee's antennal lobe is necessary for the formation of configural olfactory percepts. Eur J Neurosci 35:1718–1724

Christensen TA, Mustaparta H, Hildebrand JG (1989) Discrimination of sex pheromone blends in the olfactory system of the moth. Chem Senses 14(3):463–477

Christensen TA, Mustaparta H, Hildebrand JG (1991) Chemical communication in heliothine moths 2. Central processing of intraspecific and interspecific olfactory message in the male corn-earworm Helicoverpa zea. J Comp Physiol A 169:259–274

Christensen TA, Lei H, Hildebrand JG (2003) Coordination of central odor representations through transient non-oscillatory synchronization of glomerular output neurons. Proc Nat Acad Sci USA 100:11076–11081

Coyne JA, Orr HA (2004) Speciation. Sinauer Associates, Sunderland

Dacks AM, Nighorn AJ (2011) The organization of the antennal lobe correlates not only with phylogenetic relationship, but also life history: a basal hymenopteran as exemplar. Chem Senses 36:209–220

Dacks AM, Christensen TA, Hildebrand JG (2008) Modulation of olfactory information processing in the antennal lobe of Manduca sexta. J Neurophysiol 99:2077–2085

Dacks AM, Riffell JA, Martin JP, Gage SL, Nighorn AJ (2012) Olfactory modulation by dopamine in the context of aversive learning. J Neurophysiol 108:539–550

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: 10476–10481

Daniel PC, Derby CD (1991) Mixture suppression in behavior—the antennular flick response in the spiny lobster towards binary odorant mixtures. Physiol Behav 49:591–601

de Bruyne M, Baker TC (2008) Odor detection in insects: volatile codes. J Chem Ecol 34:882–897

de Bruyne M, Foster K, Carlson JF (2001) Odor coding in the Drosophila antenna. Neuron 30:537–552

Deisig N, Lachnit H, Giurfa M (2002) The effect of similarity between elemental stimuli and compounds in olfactory patterning discriminations. Learn Memory 9:112–121

Deisig N, Giurfa M, Lachnit H, Sandoz JC (2006) Neural representation of olfactory mixtures in the honeybee antennal lobe. Eur J Neurosci 24(4):1161–1174

Deisig N, Giurfa M, Sandoz JC (2010) Antennal lobe processing increases separability of odor mixture representations in the honeybee. J Neurophysiol 103(4):2185–2194

Deisig N, Kropf J, Vitecek S, Pevergne D, Rouyar A, Sandoz J-C, Lucas P, Gadenne C, Anton S, Barrozo R (2012) Differential interactions of sex pheromone and plant odour in the olfactory pathway of a male moth. PLoS ONE 7:e33159

Dekker T, Steib B, Carde RT, Geier M (2002) L-lactic acid: a human-signifying host cue for the anthropophilic mosquito Anopheles gambiae. Med Vet Entomol 16:91–98

Dekker T, Ibba I, Siju KP, Stensymyr MC, Hansson BS (2006) Olfactory shifts parallel superspecialism for toxic fruit in Drosophila melanogaster sibling. D. sechellia. Curr Biol 16:101–109

Derby CD (2000) Learning from spiny lobsters about chemosensory coding of mixtures. Physiol Behav 69:203–209

Derby CD, Hamilton KA, Ache BW (1984) Processing of olfactory information at 3 neuronal levels in the spiny lobster. Brain Res 300:311–319

Derby CD, Ache BW, Kennel EW (1985) Mixture suppression in olfaction—electrophysiological evaluation of the contribution of peripheral and central neural components. Chem Senses 10:301–316

Derby CD, Girardot MN, Daniel PC (1991a) Responses of olfactory receptor cells of spiny lobsters to binary mixtures. 1. Intensity mixture interactions. J Neurophysiol 66:112–130

Derby CD, Girardot MN, Daniel PC (1991b) Responses of olfactory receptor cells of spiny lobsters to binary mixtures. 2. Pattern mixture interactions. J Neurophysiol 66:131–139

Ejima A, Smith BPC, Lucas C, Van Naters WV, Miller CJ, Carlson JR, Levine JD, Griffith LC (2007) Generalization of courtship learning in Drosophila is mediated by cis-vaccenyl acetate. Curr Biol 17:599–605

Ellen CW, Mercer AR (2012) Modulatory actions of dopamine and serotonin on insect antennal lobe neurons: insights from studies in vitro. J Mol Histol 43:401–404

El-Sayed AM (2012) The pherobase: database of pheromones and semiochemicals. http://www.pherobase.com

Eschbach C, Vogt K, Schmuker M, Gerber B (2011) The similarity between odors and their binary mixtures in Drosophila. Chem Senses 36:613–621

Farine JP, Legal L, Moreteau B, LeQuere JL (1996) Volatile components of ripe fruits of Morinda citrifolia and their effects on Drosophila. Phytochem 41:433–438

Ferveur JF (2005) Cuticular hydrocarbons: their evolution and roles in Drosophila pheromonal communication. Behav Genet 35:279–295

Finelevy JB, Derby CD (1992) Behavioral discrimination of binary mixtures and their components—effects of mixture interactions

on coding of stimulus-intensity and quality. Chem Senses 17:307–323

Frambach I, Schurmann FW (2004) Separate distribution of deutocerebral projection neurons in the mushroom bodies of the cricket brain. Acta Biol Hung 55:21–29

Galizia CG, Rossler W (2010) Parallel olfactory systems in insects: anatomy and function. Annu Rev Entomol 55:399–420

Galizia CG, Szyszka P (2008) Olfactory coding in the insect brain: molecular receptive ranges, spatial and temporal coding. Entomol Exp Appl 128:81–92

Galizia CG, Nagler K, Hölldobler B, Menzel R (1998) Odour coding is bilaterally symmetrical in the antennal lobes of honeybees (Apis mellifera). Eur J Neurosci 10:2964–2974

Galizia CG, McIlwrath SL, Menzel R (1999) A digital three-dimensional atlas of the honeybee antennal lobe based on optical sections acquired by confocal microscopy. Cell Tissue Res 295:383–394

Galizia CG, Franke T, Menzel R, Sandoz JC (2012) Optical imaging of concealed brain activity using a gold mirror in honeybees. J Insect Physiol 58:743–749

Gentilcore LR, Derby CD (1998) Complex binding interactions between multicomponent mixtures and odorant receptors in the olfactory organ of the Caribbean spiny lobster Panulirus argus. Chem Senses 23:269–281

Guerenstein PG, Hildebrand JG (2008) Roles and effects of environmental carbon dioxide in insect life. Annu Rev Entomol 53:161–178

Ha TS, Smith DP (2006) A pheromone receptor mediates 11-cis-vaccenyl acetate-induced responses in Drosophila. J Neurosci 26:8728–8733

Hansson BS, Stensmyr MC (2011) Evolution of insect olfaction. Neuron 72:698–711

Hansson BS, Ochieng' SA, Grosmaitre X, Anton S, Njagi PGN (1996) Physiological responses and central nervous projections of antennal olfactory receptor neurons in the adult desert locust, Schistocerca gregaria (Orthoptera: Acrididae). J Comp Physiol A 179:157–167

Hartlieb E, Anton S, Hansson BS (1997) Dose-dependent response characteristics of antennal lobe neurons in the male moth Agrotis segetum (Lepidoptera: Noctuidae). J Comp Physiol A 181:469–476

Heil JE, Oland LA, Lohr C (2007) Acetylcholine-mediated axon-glia signaling in the developing insect olfactory system. E J Neurosci 26:1227–1241

Heinbockel T, Kloppenburg P, Hildebrand JG (1998) Pheromone-evoked potentials and oscillations in the antennal lobes of the sphinx moth Manduca sexta. J Comp Physiol A 182:703–714

Hildebrand JG, Shepherd GM (1997) Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. Annu Rev Neurosci 20:595–631

Hunt GJ (2007) Flight and fight: a comparative view of the neurophysiology and genetics of honey bee defensive behavior. J Insect Physiol 53:399–410

Ibba I, Angioy AM, Hansson BS, Dekker T (2010) Macroglomeruli for fruit odors change blend preference in Drosophila. Naturwissenschaften 97:1059–1066

Ignell R, Anton S, Hansson BS (2001) The antennal lobe of Orthoptera—anatomy and evolution. Brain Behav Evol 57:1–17

Ignell R, Dekker T, Ghaninia M, Hansson BS (2005) Neuronal architecture of the mosquito deutocerebrum. J Comp Neurol 493:207–240

Ito I, Bazhenov M, Ong RCY, Raman B, Stopfer M (2009) Frequency transitions in odor-evoked neural oscillations. Neuron 64:692–706

Jones CD (1998) The genetic basis of Drosophila sechellia's resistance to a host plant toxin. Genetics 149(4):1899–1908

Kalberer NM, Reisenman CE, Hildebrand JG (2010) Male moths bearing transplanted female antennae express characteristically female behaviour and central neural activity. J Exp Biol 213:1272–1280

Karpati Z, Olsson S, Hansson B, Dekker T (2010) Inheritance of central neuroanatomy and physiology related to pheromone preference in the male European corn borer. BMC Evol Biol 10:286

Kazama H, Wilson RI (2008) Homeostatic matching and nonlinear amplification at identified central synapses. Neuron 58:401–418

Kloppenburg P, Mercer AR (2008) Serotonin modulation of moth central olfactory neurons. Annu Rev Entomol 53:179–190

Kloppenburg P, Ferns D, Mercer AR (1999) Serotonin enhances central olfactory neuron responses to female sex pheromone in the male sphinx moth Manduca sexta. J Neurosci 19:8172–8181

Kollmann M, Ninolis S, Bonhomme J, Homberg U, Schactner J, Taju D, Anton S (2011) Revisiting the anatomy of the central nervous system of a hemimetabolous model insect: the pea aphid Acyrthosiphon pisum. Cell Tissue Res 343:343–355

Kuebler LS, Olsson SB, Weniger R, Hansson BS (2011) Neuronal processing of complex mixtures establishes a unique odor representation in the moth antennal lobe. Front Neural Circuits 5:16

Kuebler LS, Schubert M, Karpati Z, Hansson BS, Olsson SB (2012) Antennal lobe processing correlates to moth olfactory behavior. J Neurosci 32:5772–5782

Laurent G (2002) Olfactory network dynamics and the coding of multidimensional signals. Nat Rev Neurosci 3:884–895

Le Conte Y, Hefetz A (2008) Primer pheromones in social hymenoptera. Ann Rev Entomol 53:523–542

Lei H, Vickers N (2008) Central processing of natural odor mixtures in insects. J Chem Ecol 34:915–927

Lei H, Christensen TA, Hildebrand JG (2002) Local inhibition modulates odor-evoked synchronization of glomerulus-specific output neurons. Nat Neurosci 5:557–565

Linn C, Feder JL, Nojima S, Dambroski HR, Berlocher SH, Roelofs W (2003) Fruit odor discrimination and sympatric host race formation in Rhagoletis. Proc Natl Acad Sci USA 100:11490–11493

Linn C, Nojima S, Roelofs W (2005) Antagonist effects of non-host fruit volatiles on discrimination of host fruit by Rhagoletis flies infesting apple (Malus pumila), hawthorn (Crataegus spp.), and flowering dogwood (Cornus florida). Entomol Exp App 114:97–105

Linn CE, Yee WL, Sim SB, Cha DH, Powell THQ, Goughnour RB, Feder JL (2012) Behavioral evidence for fruit odor discrimination and sympatric host races of Rhagoletis pomonella flies in the western United States. Evolution 66:3632–3641

Linster C, Sachse S, Galizia CG (2005) Computational modeling suggests that response properties rather than spatial position determine connectivity between olfactory glomeruli. J Neurophysiol 93:3410–3417

Livermore A, Hutson M, Ngo V, Hadjisimos R, Derby CD (1997) Elemental and configural learning and the perception of odorant mixtures by the spiny lobster Panulirus argus. Physiol Behav 62:169–174

MacLeod K, Laurent G (1996) Distinct mechanisms for synchronization and temporal patterning of odor-encoding neural assemblies. Science 274:976–979

Martin JP, Beyerlein A, Dacks AM, Reisenman CE, Riffell JA, Lei H, Hildebrand JG (2011) The neurobiology of insect olfaction: sensory processing in a comparative context. Prog Neurobiol 95:427–447

Matsumoto SG, Hildebrand JG (1981) Olfactory mechanisms in the moth Manduca sexta—response characteristics and morphology of central neurons in the antennal lobe. Proc R Soc B 213:249–277

Matsuo T, Sugaya S, Yasukawa J, Aigaki T, Fuyama Y (2007) Odorant-binding proteins OBP57d and OBP57e affect taste perception and host-plant preference in Drosophila sechellia. PLoS Biol 5:e118

McPheron BA, Smith DC, Berlocher SH (1988) Genetic differences between host races of Rhagoletis pomonella. Nature 336:64–66

Meyer A, Galizia CG (2012) Elemental and configural olfactory coding by antennal lobe neurons of the honeybee (Apis mellifera). J Comp Physiol A 198:159–171

Münch D, Schmeichel B, Silbering AF, Galizia CG (2013) Weaker ligands can dominate an odor blend due to syntopic interactions. Chem Senses doi:10.1093/chemse/bjs138

Namiki S, Iwabuchi S, Kanzaki R (2008) Representation of a mixture of pheromone and host plant odor by antennal lobe projection neurons of the silkmoth Bombyx mori. J Comp Physiol A 194:501–515

Nässel DR, Winther AM (2010) Drosophila neuropeptides in regulation of physiology and behavior. Prog Neurobiol 92:42–104

Neupert S, Fusca D, Schachtner J, Kloppenburg P, Predel R (2012) Toward a single-cell-based analysis of neuropeptide expression in Periplaneta americana antennal lobe neurons. J Comp Neurol 520:694–716

Nojima S, Linn CE, Zhang A, Morris B, Roelofs WL (2003a) Identification of host fruit volatiles from hawthorn (Crataegus spp.) attractive to hawthorn-origin Rhagoletis pomonella flies. J Chem Ecol 29:319–334

Nojima S, Linn CE, Roelofs WL (2003b) Identification of host fruit volatiles from flowering dogwood (Cornus florida) attractive to dogwood-origin Rhagoletis pomonella flies. J Chem Ecol 29:2347–2357

Ochieng SA, Hallberg E, Hansson BS (1998) Fine structure and distribution of antennal sensilla of the desert locust, Schistocerca gregaria (Orthoptera: Acrididae). Cell Tissue Res 291:525–536

Ochieng SA, Park K, Baker T (2002) Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male Helicoverpa zea. J Comp Physiol A 188:325–333

Oland LA, Tolbert LP (1987) Glial patterns during early development of antennal lobe of Manduca sexta—a comparison between normal lobes and lobes deprived of antennal axon. J Comp Neurol 255:196–207

Oland LA, Tolbert LP (1996) Multiple factors shape development of olfactory glomeruli: insights from an insect model system. J Neurobiol 30:92–109

Olsen SR, Wilson RI (2008) Lateral presynaptic inhibition mediates gain control in an olfactory circuit. Nature 452:956–960

Olsen SR, Bhandawat V, Wilson RI (2007) Excitatory interactions between olfactory processing channels in the Drosophila antennal lobe. Neuron 54:89–103

Olsson SB, Linn CE, Michel A, Dambroski HR, Berlocher SH, Feder JL, Roelofs WL (2006a) Receptor expression and sympatric speciation: unique olfactory receptor neuron responses in F-1 hybrid Rhagoletis populations. J Exp Biol 209:3729–3741

Olsson S, Linn CE, Roelofs WI (2006b) The chemosensory basis for behavioral divergence involved in sympatric host shifts I: characterizing olfactory receptor neuron classes responding to key host volatiles. J Comp Physiol A 192:289–300

Olsson S, Linn CE, Roelofs WI (2006c) The chemosensory basis for behavioral divergence involved in sympatric host shifts II: olfactory receptor neuron sensitivity and temporal firing pattern to key host volatiles. J Comp Physiol A 192:289–300

Party V, Hanot C, Said I, Rochat D, Renou M (2009) Plant terpenes affect intensity and temporal parameters of pheromone detection in a moth. Chem Senses 34:763–774

Peakall R (1990) Responses of male *Zaspilothynnus trilobatus* Turner wasps to females and the sexually deceptive orchid it pollinates. Funct Ecol 4:159–167

Perez-Orive J, Mazor O, Turner GC, Cassenaer S, Wilson RI, Laurent G (2002) Oscillations and sparsening of odor representations in the mushroom body. Science 297:359–365

Pitts RJ, Fox AN, Zwiebel LJ (2004) A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector *Anopheles gambiae*. Proc Natl Acad Sci USA 101:5058–5063

Qiu YT, van Loon JJA, Takken W, Meijerink J, Smid HM (2006) Olfactory coding in antennal neurons of the malaria mosquito, *Anopheles gambiae*. Chem Senses 31:845–863

Raguso RA, Henzel C, Buchmann SL (2003) Trumpet flowers of the Sonoran Desert: floral biology of *Peniocereus* cacti and sacred *Datura*. Int J Plant Sci 164:877–892

Reinhard J, Sinclair M, Srinivasan MV, Claudianos C (2010) Honeybees learn odour mixtures via a selection of key odorants. PLoS ONE 5:e9110

Riffell JA (2012) Olfactory ecology and the processing of complex mixtures. Curr Opin Neurobiol 22:236–242

Riffell JA, Abrell L, Hildebrand JG (2008) Physical processes and real-time chemical measurement of the insect olfactory environment. J Chem Ecol 34:837–853

Riffell JA, Lei H, Christensen TA, Hildebrand JG (2009a) Characterization and coding of behaviorally significant odor mixtures. Curr Biol 19:335–340

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 106:19219–19226

Riffell JA, Hong L, Abrell L, Hildebrand JG (2013) Neural basis of a pollinator's buffet: olfactory specialization and learning in *Manduca sexta*. Science 339:200–204

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 16:1395–1403

Robertson HM, Warr CG, Carlson JR (2003) Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. Proc Natl Acad Sci USA 100:14537–14542

Robertson HM, Gadau J, Wanner KW (2010) The insect chemoreceptor superfamily of the parasitoid jewel wasp *Nasonia vitripennis*. Insect Mol Biol 19:121–136

Roelofs WL, Liu WT, Hao GX, Jiao HM, Rooney AP, Linn CE (2002) Evolution of moth sex pheromones via ancestral genes. Proc Natl Acad Sci USA 99:13621–13626

Root CM, Semmelhack JL, Wong AM, Flores J, Wang JW (2007) Propagation of olfactory information in *Drosophila*. Proc Natl Acad Sci USA 104:11826–11831

Rospars JP, Chambille I (1985) Neurons and identified glomeruli of antennal lobes during postembryonic development in the cockroach *Blaberus caniifer* Burn (Dictyoptera: Blaberidae). Int J Insect Morphol Embryol 14:203–226

Rospars JP, Hildebrand JG (1992) Anatomical identification of glomeruli in the antennal lobes of the male sphinx moth *Manduca sexta*. Cell Tissue Res 270:205–227

Rössler W, Brill M (2013) Parallel processing in the honeybee olfactory pathway: structure, function and evolution. J Comp Physiol A (this issue). doi:10.1007/s00359-013-0821-y

Rössler W, Randolph PW, Tolbert LP, Hildebrand JG (1999) Axons of olfactory receptor cells of transsexually grafted antennae induce development of sexually dimorphic glomeruli in *Manduca sexta*. J Neurobiol 38:521–541

Rouyar A, Party V, Prešern J, Blejec A, Renou M (2011) A general odorant background affects the coding of pheromone stimulus intermittency in specialist olfactory receptor neurones. PLoS ONE 6:e26443

Sachse S, Galizia CG (2002) Role of inhibition for temporal and spatial odor representation in olfactory output neurons: a calcium imaging study. J Neurophysiol 87:1106–1117

Sachse S, Galizia CG (2006) Topography and dynamics of the olfactory system. In: Grillner S, Graybiel AM (eds) Microcircuits: the interface between neurons and global brain function. Dahlem UP–MIT, Cambridge, pp 251–274

Schiestl FP, Ayasse M, Paulus HF, Löfstedt C, Hansson BS, Ibarra F, Francke W (1999) Orchid pollination by sexual swindle. Nature 399:421

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 302:437–438

Schmuker M, Schneider G (2007) Processing and classification of chemical data inspired by insect olfaction. Proc Natl Acad Sci USA 104:20285–20289

Schneiderman AM, Matsumoto SG, Hildebrand JG (1982) Trans-sexually granted antennae influence development of sexually dimorphic neurons in moth brain. Nature 298:844–846

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 23:10495–10502

Silbering AF, Galizia CG (2007) Processing of odor mixtures in the *Drosophila* antennal lobe reveals both global inhibition and glomerulus-specific interactions. J Neurosci 27:11966–11977

Silbering AF, Okada R, Ito K, Galizia CG (2008) Olfactory information processing in the *Drosophila* antennal lobe: anything goes? J Neurosci 28:13075–13087

Siwicki KK, Riccio P, Ladewski L, Marcillac F, Dartevelle L, Cross SA, Ferveur JF (2005) The role of cuticular pheromones in courtship conditioning of *Drosophila* males. Learn Memory 12:636–645

Slessor KN, Winston ML, Le Conte Y (2005) Pheromone communication in the honeybee (*Apis mellifera* L.). J Chem Ecol 31:2731–2745

Smid HM, Wang GH, Bukovinszky T, Steidle JLM, Bleeker MAK, Van Loon JJA, Vet LEM (2007) Species-specific acquisition and consolidation of long-term memory in parasitic wasps. Proc R Soc B 274:1539–1546

Stocker RF (1994) The organization of the chemosensory system in *Drosophila melanogaster*—a review. Cell Tissue Res 275:3–26

Stocker RF, Lienhard MC, Borst A, Fischbach KF (1990) Neuronal architecture of the antennal lobe in *Drosophila melanogaster*. Cell Tissue Res 262:9–34

Strausfeld NJ (2012) Arthropod brains: evolution, functional elegance, and historical significance. Harvard University Press, Cambridge

Strauss K, Scharpenberg H, Crewe RM, Glahn F, Foth H, Moritz RFA (2008) The role of the queen mandibular gland pheromone in honeybees (*Apis mellifera*): honest signal or suppressive agent? Behav Ecol Sociobiol 62:1523–1531

Su CY, Menuz K, Carlson JR (2009) Olfactory perception: receptors, cells, and circuits. Cell 139:45–59

Su CY, Martelli C, Emonet T, Carlson JR (2011) Temporal coding of odor mixtures in an olfactory receptor neuron. Proc Natl Acad Sci USA 108:5075–5080

Su CY, Menuz K, Reisert J, Carlson JR (2012) Non-synaptic inhibition between grouped neurons in an olfactory circuit. Nature 492:66

Sun XJ, Fonta C, Masson C (1993) Odor quality processing by bee antennal lobe interneurons. Chem Senses 18:355–377

Tanaka NK, Ito K, Stopfer M (2009) Odor-evoked neural oscillations in *Drosophila* are mediated by widely branching interneurons. J Neurosci 29:8595–8603

Tasin M, Backman AC, Bengtsson M, Varela N, Ioriatti C, Witzgall P (2006) Wind tunnel attraction of grapevine moth females,

Lobesia botrana, to natural and artificial grape odour. Chemoecol 16:87–92

Thom C, Gilley DC, Hooper J, Esch HE (2007) The scent of the waggle dance. PLoS Biol 5(9):1862–1867

Trona F, Anfora G, Bengtsson M, Witzgall P, Ignell R (2010) Coding and interaction of sex pheromone and plant volatile signals in the antennal lobe of the codling moth *Cydia pomonella*. J Exp Biol 213:4291–4303

Utz S, Huetteroth W, Wegener C, Kahnt J, Predel R, Schachtner J (2007) Direct peptide profiling of lateral cell groups of the antennal lobes of Manduca sexta reveals specific composition and changes in neuropeptide expression during development. Dev Neurobiol 67:764–777

van der Pers JNC, Thomas G, Denotter CJ (1980) Interactions between plant odors and pheromone reception in small ermine moths (Lepidoptera: Yponomeutidae). Chem Senses 5:367–371

Vergoz V, Schreurs HA, Mercer AR (2007) Queen pheromone blocks aversive learning in young worker bees. Science 317:384–386

Vickers NJ, Christensen TA (2003) Functional divergence of spatially conserved olfactory glomeruli in two related moth species. Chem Senses 28:325–338

Vickers NJ, Christensen TA, Baker TC, Hildebrand JG (2001) Odor-plume dynamics influence the brain's olfactory code. Nature 410:466–470

Vickers NJ, Poole K, Linn CE (2005) Plasticity in central olfactory processing and pheromone blend discrimination following interspecies antennal imaginal disc transplantation. J Comp Neurol 491:141–156

Vosshall LB, Hansson BS (2011) A united nomenclature system for the insect olfactory coreceptor. Chem Senses 36:497–498

Vosshall LB, Stocker RE (2007) Molecular architecture of smell and taste in *Drosophila*. Ann Rev Neurosci 30:505–533

Wertheim B, Dicke M, Vet LEM (2002) Behavioural plasticity in support of a benefit for aggregation pheromone use in *Drosophila melanogaster*. Entomol Exp App 103:61–71

Wilson RI (2008) Neural and behavioral mechanisms of olfactory perception. Curr Opin Neurobiol 18:408–412

Wilson RI, Laurent G (2005) Role of GABAergic inhibition in shaping odor-evoked spatiotemporal patterns in the *Drosophila* antennal lobe. J Neurosci 25:9069–9079

Wilson RI, Mainen ZF (2006) Early events in olfactory processing. Ann Rev Neurosci 29:163–201

Wiltrout C, Dogra S, Linster C (2003) Configurational and nonconfigurational interactions between odorants in binary mixtures. Behav Neurosci 117:236–245

Wright GA, Lutmerding A, Dudareva N, Smith BH (2005) Intensity and the ratios of compounds in the scent of snapdragon flowers affect scent discrimination by honeybees (*Apis mellifera*). J Comp Physiol A 191:105–114

Wu W, Anton S, Löfstedt C, Hansson BS (1996) Discrimination among pheromone component blends by interneurons in male antennal lobes of two populations of the turnip moth, *Agrotis segetum*. Proc Natl Acad Sci USA 93(15):8022–8027

Yaksi E, Wilson RI (2010) Electrical coupling between olfactory glomeruli. Neuron 67:1034–1047

Yu DH, Ponomarey A, Davis RL (2004) Altered representation of the spatial code for Odors after olfactory classical conditioning: memory trace formation by synaptic recruitment. Neuron 42:437–449

Zhang QH, Schlyter F (2003) Redundancy, synergism, and active inhibitory range of non-host volatiles in reducing pheromone attraction in European spruce bark beetle *Ips typographus*. Oikos 101:299–310

Zhang AJ, Linn C, Wright S, Prokopv R, Reissig W, Roelofs W (1999) Identification of a new blend of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella*. J Chem Ecol 25:1221–1232