Chemosensation and Evolution of *Drosophila* Host Plant Selection

Robert R.H. Anholt

The ability to respond to chemosensory cues is critical for survival of most organisms. Among insects, *Drosophila melanogaster* has the best characterized olfactory system, and the availability of genome sequences of 30 *Drosophila* species provides an ideal scenario for studies on evolution of chemosensation. Gene duplications of chemoreceptor genes allow for functional diversification of the rapidly evolving chemoreceptor repertoire. Although some species of the genus *Drosophila* are generalists for host plant selection, rapid evolution of olfactory receptors, gustatory receptors, odorant-binding proteins, and cytochrome P450s has enabled diverse host specializations of different members of the genus. Here, I review diversification of the chemoreceptor repertoire among members of the genus *Drosophila* along with co-evolution of detoxification mechanisms that may have enabled occupation of diverse host plant ecological niches.

**BEHAVIORS AS A PLATFORM FOR EVOLUTION**

Behaviors, i.e., interactions of an organism with its environment, are the ultimate expression of the nervous system. Behaviors mediate interactions between the environment and conspecific and heterospecific individuals, which are essential for survival and reproductive success. Thus, behaviors provide a substrate for natural selection and “survival of the fittest.” From a genetics perspective, behaviors are quantitative traits because their manifestation is determined by multiple segregating genes and influenced by the environment. Genetic variation is mediated through mutation-selection balance, as well as genetic drift within a population and gene flow between populations. Selective forces, often driven by environmental pressures, act upon genetic variation within a population, leading to fixation or shifts in frequencies of alleles (Slatkin, 1987; Orr and Betancourt, 2001; Yeaman and Otto, 2011; Huang et al., 2016; Alexander et al., 2017). Behavioral traits are prime targets for natural selection, because these traits are often highly variable and plastic, and behaviors associated with survival and reproduction are determinants of fitness. *Drosophila* provides an exceptional model system for understanding the evolution of behavior (Glossary).

The well-studied species *Drosophila melanogaster* offers powerful tools that can be used to genetically dissect complex behaviors. Complete, well-annotated genome sequences for 30 species in the genus *Drosophila* (Drosophila 12 Genomes Consortium, 2007; Song et al., 2011; Miller et al., 2018; Yang et al., 2018; Wiegmann and Richards, 2018) (Figure 1) enable comparative evolutionary studies on complex behaviors. Olfactory behaviors toward food sources, mating partners (Ahmed et al., 2019), oviposition site selection (reviewed in Anholt et al., 2020), and avoidance of predators, parasites (Ebrahim et al., 2015), and harmful microbes (Stensmyr et al., 2012) are especially important drivers of behavioral evolution.

In this review I will focus on chemoreceptors as evolutionary substrates for host plant selection. Members of the genus *Drosophila* can be categorized as generalists and specialists. The evolutionary advantage of generalism is the ability to exploit diverse resources for feeding and oviposition providing behavioral flexibility under conditions of competition or limited availability of any one resource. Specialists have an advantage in that they can occupy an ecological niche that provides a reliable food source and oviposition site but is toxic to related species.

**DROSOPHILA CHEMOSENSATION**

The recognition of chemical signals is essential for the survival and reproductive success of insects, and large and diverse gene families of chemoreceptors have evolved (Box 1). Among insects, *D. melanogaster* has the best-characterized chemoreceptor repertoire (Joseph and Carlson, 2015). The evolutionarily most ancient family of chemoreceptors is represented by the ionotropic receptors (IRs) (Benton et al., 2009), which are found in early diverging insects (Archaeognatha and Zygentoma; Missbach et al., 2014) and as far back as Protista (Croset et al., 2010). IRs recognize, among others, water-soluble molecules,
Glossary

Accessory gland: Male glands that provide products to sustain the sperm and include seminal fluid proteins that affect female physiology and behavior after mating

Arista: A feather-like appendage that emanates from the base of the antenna

Circadian: Biological rhythms that occur in approximately 24-hour periods

cis-regulatory elements: Regions of non-coding DNA that regulate the transcription of neighboring genes

Copy number variants: Insertions, deletions, and duplications of segments of DNA that vary among individuals within a population

Directional selection: A type of natural selection in which a single phenotype is favored, causing the allele frequency to continuously shift in one direction thereby favoring extreme values over intermediate values

Drift: Random variation in allele frequency caused by sampling in finite populations

Evolvability: The capacity to undergo adaptive evolution

Fitness: The genetic contribution of an individual to the next generation

Functional redundancy: The situation in which multiple genes contribute in equivalent ways and can substitute for one another in generating the phenotype

Gene duplication: Duplication of a region of DNA that contains a gene, resulting in an extra copy of that gene

Gene flow: The introduction of genetic material by interbreeding from one population of a species to another, thereby changing the composition of the gene pool of the receiving population

Gene ontology enrichment analyses: A bioinformatics technique for interpreting whether sets of genes with similar functional classifications are over-represented in the dataset.

Ionotropic receptors: Membrane-bound receptor proteins that respond to ligand binding by opening an ion channel

Knockout: An animal from whose genome a gene has been removed

Loss-of-function allele: An allele in which a mutation has occurred so that the altered gene product lacks the molecular function of the wild-type gene

Molecular response profiles: The spectrum of molecules that can elicit a biological response from a receptor

Mutation-selection balance: The equilibrium between the rate at which mutations arise and their elimination by natural selection

Neofunctionalization: Acquisition of a novel function

Odorant: A molecule that carries an odor

Oviposition: Egg laying

Paralogs: Genes that derive from the same ancestral gene

Phylogeny: The relationship among different groups of organisms based on their evolutionary history

Plasticity: The ability of one genotype to produce more than one phenotype when exposed to different environments

Polymorphisms: Naturally occurring DNA variants among individuals in a population as a result of a spontaneous mutations

Positive selection: The process by which new advantageous genetic variants sweep through a population favoring advantageous alleles toward fixation

Proboscis: The elongated mouthpart of an insect used for food intake

Pseudogenization: An evolutionary process whereby mutations cause a gene to become dysfunctional by disruption of its regulatory or coding sequence

RNAi-mediated inhibition: A process by which expression of a double-stranded RNA activates ribonucleases that degrade homologous mRNA into short fragments.

RNA-seq: Large-scale sequencing of all the RNA, or at least messenger RNA, in a cell, tissue, or animal

Subfunctionalization: The acquisition of complementary functions of two genes after gene duplication

Sympatric: Occurring in the same geographic area overlapping in distribution

Transcript: The RNA product of a gene

Transposon: A DNA sequence that can move and change its position within a genome

Xenobiotics: Substances that are foreign to the body or to an ecological system
including alcohols, amines, and acids (Ai et al., 2010; Min et al., 2013). With the advent of a terrestrial lifestyle, a large and diverse family of gustatory receptors (GRs) that evaluates food and regulates nutrient intake evolved (Scott et al., 2001; Scott, 2018; Robertson, 2019). With the appearance of flying insects, the family of canonical odorant receptors (ORs), which recognize airborne odorants, (Robertson et al., 2003; Missbach et al., 2014; Brand et al., 2018) emerged along with the common co-receptor, ORCO. The latter may be ancestral to the ORs and may have been derived from the GR family (Robertson, 2019). ORCO is an obligatory partner for all individual OR receptors and is necessary for their localization to the chemosensory dendritic membrane (Larsson et al., 2004) and for activation by their cognate ligands (Wicher et al., 2008; Sato et al., 2008).

Odorant-binding proteins (OBPs) may have evolved to serve as carriers for hydrophobic ligands in the aqueous perilymph that surrounds olfactory sensory neurons (Pelosi et al., 2014; Larter et al., 2016). However, their precise role in modulating olfactory responses remains unclear. RNAi-mediated inhibition of OBPs modulates behavioral responses in a sexually dimorphic manner toward a variety of odorants (Swarup et al., 2011), and deletion of Obp83a and Obp83b affects deactivation kinetics of olfactory responses (Scheuermann and Smith, 2019). Simultaneous deletion of four highly expressed OBPs retained robust responses against a wide variety of odorants measured electrophysiologically (Xiao et al., 2019). Furthermore, despite their nomenclature, it has become increasingly clear that members of the OBP family also participate in still poorly understood non-chemosensory functions (Findlay et al., 2008; Arya et al., 2010).

Figure 1. A Phylogenetic Tree Based on Combined Phylogenetic Relationships of 13 Enzymes
Numbers above each branch indicate the percentage agreement across all enzymes, and numbers below each branch indicate the number of phylogenetic trees for each individual enzyme that supports the branch. From Seetharam and Stuart, 2013, reproduced under the Creative Commons Attribution License.

Two members of the D. melanogaster Gr family, Gr21a and Gr63a, are co-expressed in antennal chemosensory neurons and mediate chemosensation of carbon dioxide (Jones et al., 2007), an odorant that can serve both as an alarm signal to trigger avoidance behavior (Suh et al., 2004) and as an attractant because CO2 is a by-product of yeast fermentation, a food source for flies. Attractive responses require IR25a, which is a co-receptor for members of the IR family, indicating that different chemosensory modalities may mediate repulsion and attraction to this ecologically relevant odorant (van Breugel et al., 2018).
Finally, members of the *D. melanogaster* Pickpocket (PPK) ion channel family mediate recognition of water via water-sensing taste sensilla on the proboscis that express ppk28 (Chen et al., 2010; Cameron et al., 2010) and courtship pheromones via a channel complex that includes ppk23 expressed on male forelegs (Lu et al., 2012; Toda et al., 2012; Thistle et al., 2012; Mast et al., 2014; Seeholzer et al., 2018). Although ecologically relevant ligands have not been identified for the majority of chemoreceptors, molecular response profiles and ligand specificities of ORs, IRs, and GRs have been characterized using commercially available odorants and tastants (Joseph and Carlson, 2015). Interactions between several OBPs and odorants have also been documented (Kim et al., 1998; Matsuo et al., 2007; Swarup et al., 2011). Sensory neurons that express the same ORs converge on the same output neurons in the antennal lobe of the brain, forming complex synaptic processing units, glomeruli (Vosshall et al., 2000; Grabe et al., 2016). Activation of chemosensory neurons is translated in a pattern of glomerular activity, which is decoded in higher brain regions (Vosshall et al., 2000; Marin et al., 2002; Wong et al., 2002; Masse et al., 2009; Caron et al., 2013; Joseph and Carlson, 2015).

### RAPID EVOLUTION AND DIVERSIFICATION OF CHEMORECEPTOR GENES

Insect chemoreceptor genes evolve rapidly, leading to extensive diversification and birth and death of gene family members (McBride, 2007; McBride et al., 2007; Sánchez-Gracia et al., 2009; Vieira and Rozas, 2011; Cande et al., 2013). One striking example of ecological adaptations of chemosensory responsiveness comes from a direct comparison between molecular response profiles of *D. melanogaster* and *Anopheles gambiae*, which showed that odorant recognition has been adapted to the distinct ecological needs of each of these species. Female mosquitoes require a blood meal to produce eggs, whereas *D. melanogaster* feeds on fruit. Odorant perception in *A. gambiae* is tuned predominantly to aromatics found in human sweat, whereas olfactory perceptions in *D. melanogaster* are shaped primarily by esters, which are prevalent in fruit (Carey et al., 2010).

Although the earliest evolutionary origins of the insect chemoreceptor families remain unknown, their evolution likely involved gene duplication and diversification events along with chromosomal rearrangements (Hekmat-Scafe et al., 2002; Nozawa and Nei, 2007). The functions of many members of the *D. melanogaster* chemoreceptor families remain unknown, and the functional consequences of receptor gene duplication and subsequent neo- or subfunctionalization have remained largely unexplored. Gene duplication could relax evolutionary constraint on the daughter genes enabling rapid adaptive evolution. Expansion of chemoreceptor subfamilies can lead to expansion of molecular response profiles within the chemosensory recognition repertoire, which might buffer chemosensory ability by generating functional redundancy within an expanded family of chemoreceptors. Alternatively, chemoreceptor genes might undergo neo-functionalization and functionally diversify to adopt functions not directly related to the recognition of external odorants or tastants.

---

**Box 1. Organization of Olfactory Sensory Neurons in *Drosophila melanogaster***

Olfaction in *Drosophila* is mediated through olfactory receptor neurons housed in three types of morphologically distinct sensilla on the third antennal segments and the maxillary palps. Neurons that express members of the Or family are housed in basiconic sensilla. There are 10 distinct types of basiconic sensilla on the third antennal segment, designated ab1 through ab10 (de Bruyne et al., 2001; Couto et al., 2005). The ab1 sensilla contains four chemosensory neurons (A–D), and the others each contain two chemosensory neurons, designated A and B, which can be distinguished by their spiking pattern. Neurons housed in the same sensilla types follow a strict pairing rule, so that a neuron with a defined molecular response profile always occurs together with the same partner (de Bruyne et al., 2001). Similarly, each maxillary palp contains 60 basiconic sensilla (pb1–pb3), divided into three sensillar classes, each of which contains an A and a B neuron, again following a strict pairing rule (de Bruyne et al., 1999). The third antennal segment also contains four functional types of coeloconic sensilla, which house neurons that express Ir receptors (Yao et al., 2005), and four functional types of trichoid sensilla (at1–at4), which house neurons that respond to pheromones (Miller and Carlson, 2010) (e.g., the Or67d receptor expressed in T1 sensilla responds to the pheromone 11-cis-vaccenyl acetate; Ha and Smith, 2006). The nomenclature of chemoreceptors designates the family (e.g., Or for olfactory receptors), the cytological location on the chromosome, e.g., location 47 is on the right arm of the second chromosome, and the sequence of related family members, e.g., a, b, etc. (*Drosophila Odorant Receptor Nomenclature Committee*, 2000).
One intriguing question is how expression of daughter genes after a duplication event becomes segregated in different olfactory sensory neurons. One can speculate that expression of Or genes is silenced and that activation of individual receptors is accomplished through higher-order chromatin conformational modifications, which bring enhancers for specific transcriptional regulators in close proximity to single Or genes, as has been shown for regulation of singular olfactory receptor expression in the mouse (Monahan et al., 2017 and 2019). In Drosophila, combinatorial usage of transcription factors during development appears to play a prominent role in receptor choice determination (Jafari et al., 2012; Barish and Volkan, 2015). However, the mechanisms that regulate expression of singular Or genes in insects remain to be further clarified.

One example of evolutionary diversification is evident in the family of IRs, where IR40a and IR93A along with the common IR25a co-receptor have become specialized for humidity sensation (Enjin et al., 2016; Knecht et al., 2017). In addition to members of the IR family, an OBP, OBPS9a, has also been implicated in humidity sensing (Sun et al., 2018). IR93a and IR25a, along with IR21a, also mediate temperature sensation and are expressed in thermosensory neurons in the arista (Enjin et al., 2016; Budelli et al., 2019). In addition, a member of the GR family, GR28b, has been identified as a peripheral thermosensor that responds to rapid warming (Ni et al., 2013). It is of interest that IR25a has been implicated in temperature-dependent regulation of the circadian clock (Chen et al., 2015).

Another example of evolutionary diversification of chemoreceptors comes from the large IR20a clade of IR genes. Members of this clade are expressed in diverse gustatory neurons in the labellum, the pharynx, the wing margin, and the front legs. This clade includes 35 genes with on average 16% sequence identity and 7 genes with premature stop codons, which bears testimony to their rapid evolution (Koh et al., 2014). Comparisons of sequence variation of members of this clade among the sequenced inbred wild-derived lines of the Drosophila melanogaster Genetic Reference Panel (Huang et al., 2014; Mackay et al., 2012) with divergence between D. melanogaster and Drosophila simulans showed evidence for positive selection among the related paralogs Ir52c and Ir52d (Koh et al., 2014). These genes are expressed in neurons on the forelegs of D. melanogaster males and are associated with mating behavior, possibly through recognition of pheromones (Koh et al., 2014).

Other examples of likely sub- and neofunctionalization are evident among paralogs of a cluster of Obp genes on the D. melanogaster X chromosome. Association analyses in wild-derived inbred D. melanogaster lines showed polymorphisms in Obp19a and Obp19b associated with variation in behavioral responses to benzaldehyde, whereas Obp19c harbored a SNP associated with variation in behavioral response to hexanal (Arya et al., 2010). In addition, two polymorphic markers in Obp19d were associated with variation in lifespan. In the antenna, Obp19a is expressed in a subset of basiconic sensilla, whereas Obp19d is associated with extrasensillar uninnervated spinules (Larter et al., 2016). Gene ontology enrichment analyses of ensembles of coregulated genes with each of the focal genes implicated Obp19c in oviposition and postmating behavior (Arya et al., 2010). In this light, it is of interest that Obp19c is also expressed in ovaries. Furthermore, Obp8a on the D. melanogaster X chromosome shows high expression in the male accessory gland, suggesting that Obp8a and Obp19c, and potentially other OBPs found in seminal fluid (Findlay et al., 2008), may bind thus far unidentified hydrophobic molecules associated with the transfer of sperm during mating and stimulation of oviposition.

**CO-EVOLUTION OF CHEMOSENSATION AND HOST PLANT SELECTION**

Adaptation to host plants on which flies oviposit and on which larvae can develop depends to a large extent on chemosensation. Such adaptation has been extensively studied in Drosophila sechellia, which prefers to feed on Morinda citrifolia fruit, which is avoided by its sister species D. simulans (Jones, 2005). The ability to feed on a food source that is toxic to competing species ensures survival. M. citrifolia produces hexanoic and octanoic acids, fatty acids to which D. sechellia are attracted and that are toxic and repellent to other Drosophila species (Amlou et al., 1998). This specialization is accompanied by rapid evolutionary changes in the chemoreceptor repertoire of D. sechellia with accumulation of loss-of-function alleles, especially among the Gr family (McBride, 2007; McBride et al., 2007). One well-characterized change in the chemoreceptor repertoire involves Obp57d and Obp57e, which are expressed in cells in the tarsi (Matsuo et al., 2007). Expression of these OBPs is controlled by conserved cis regulatory elements (Tomoka et al., 2012). A 4-bp CCAT insertion upstream of the D. sechellia Obp57e gene prevents its expression, even though its open reading frame is intact (Matsuo et al., 2007). In addition, a premature
Several other genes that showed extensive upregulation in *D. simulans* or *D. sechellia* showed significant upregulation of several OR genes compared with flies from Catalina Island, notably *D. mojavensis*. Differences between the different genome-wide transcript abundances showed differential expression of members of the *Or* gene family between the different *D. mojavensis* populations (Crowley-Gall et al., 2016). Flies from the Mojave desert showed significant upregulation of several OR genes compared with flies from Catalina Island, notably Or67b and Or71a, which are expressed in ab3A neurons, respectively, and are excited by aromatics. Differential expression of receptors was correlated with differential activity, measured electrophysiologically, and reflected in differences in the proportions of specific olfactory sensory neurons, in which they are expressed (Crowley-Gall et al., 2016).

Different habitats and different host plant specializations among the *D. mojavensis* populations could ultimately lead to reproductive barriers (Pfeiler et al., 2009). Analyses of genome sequences between *D. mojavensis* and its close relatives *D. arizonae* and *D. navojoa* revealed chromosomal inversion differences that form a barrier to interbreeding (Sanchez-Flores et al., 2016), although host plant specialization may not necessarily have been the driving force for this reproductive isolation.

Olfactory adaptations can have economic consequences. *Drosophila suzukii* has emerged as a major agricultural pest during the last decade as it has spread from Southeast Asia to Europe and North America (Walsh et al., 2011). Most *Drosophila* species are attracted to decaying fruit, whereas *D. suzukii* females oviposit on ripening fruit. *D. suzukii* females have evolved an enlarged serrated ovipositor, which enables ovipositing on resistant substrates, which produce terpenes that are detected via the OR19a receptor (Dweck et al., 2013). Studies on wild populations of *D. melanogaster* in Zimbabwe show that these African flies feed and oviposit almost exclusively on marula fruit (*Sclerocarya birrea*), a citrus-like endemic fruit. This specialization is not observed in sympatric *D. simulans* (Mansourian et al., 2018). Ethyl isovalerate produced by the marula fruit acts as an olfactory cue for oviposition site preference by activating ab3A neurons, which project to the DM2 glomerulus and express a distinct Or22a/Or22b variant in this fly population. Even laboratory-reared flies of the Canton-S strain still favor marula fruit in preference assays (Mansourian et al., 2018). Thus, specialization on marula of African *D. melanogaster* may be ancestral to the generalist host plant relationships of cosmopolitan *D. melanogaster*, illustrating plasticity and evolvability of insect-host plant relationships.

A similar example of host specialization comes from *Drosophila erecta*, another close relative of *D. melanogaster*, endemic in forests of west central Africa. *D. erecta* has evolved a specialized relationship with screw pine fruits (*Pandanus sp.*). These fruits produce 3-methyl-2-butenyl acetate. The proportion of olfactory sensory neurons that respond to this odorant (ab3A neurons) has increased by ~40% in *D. erecta* with a concomitant ~2.5-fold increase in volumes of its corresponding glomeruli in the antennal lobes. Exposure to 3-methyl-2-butenyl acetate induces egg laying in *D. erecta*, but not in *D. melanogaster* (Linz et al., 2013).

One of the best studied examples of host plant adaptations in the genus *Drosophila* comes from *Drosophila mojavensis*, which feeds on decomposing cactus in Arizona, the Mojave desert and Baja California, the Sonoran Desert, and Catalina Island. Different races of *D. mojavensis* have developed specialized host plant relationships with different cacti that are endemic at each location (Newby and Etges, 1998). Olfactory adaptations to distinct odorants emanating from each cactus species have been characterized both electrophysiologically and through behavioral studies (Date et al., 2013). In addition, analyses of genome-wide transcript abundances showed differential expression of members of the *Or* gene family between the different *D. mojavensis* populations (Crowley-Gall et al., 2016). Flies from the Mojave desert showed significant upregulation of several OR genes compared with flies from Catalina Island, notably Or67b and Or71a, which are expressed in ab9B and pb1B neurons, respectively, and are excited by aromatics. Differential expression of receptors was correlated with differential activity, measured electrophysiologically, and reflected in differences in the proportions of specific olfactory sensory neurons, in which they are expressed (Crowley-Gall et al., 2016).

Although cosmopolitan *D. melanogaster* are considered generalist feeders, they prefer to lay eggs on citrus substrates, which produce terpenes that are detected via the OR19a receptor (Dweck et al., 2013). Studies on wild populations of *D. melanogaster* in Zimbabwe show that these African flies feed and oviposit almost exclusively on marula fruit (*Sclerocarya birrea*), a citrus-like endemic fruit. This specialization is not observed in sympatric *D. simulans* (Mansourian et al., 2018). Ethyl isovalerate produced by the marula fruit acts as an olfactory cue for oviposition site preference by activating ab3A neurons, which project to the DM2 glomerulus and express a distinct Or22a/Or22b variant in this fly population. Even laboratory-reared flies of the Canton-S strain still favor marula fruit in preference assays (Mansourian et al., 2018). Thus, specialization on marula of African *D. melanogaster* may be ancestral to the generalist host plant relationships of cosmopolitan *D. melanogaster*, illustrating plasticity and evolvability of insect-host plant relationships.

A similar example of host specialization comes from *Drosophila erecta*, another close relative of *D. melanogaster*, endemic in forests of west central Africa. *D. erecta* has evolved a specialized relationship with screw pine fruits (*Pandanus sp.*). These fruits produce 3-methyl-2-butenyl acetate. The proportion of olfactory sensory neurons that respond to this odorant (ab3A neurons) has increased by ~40% in *D. erecta* with a concomitant ~2.5-fold increase in volumes of its corresponding glomeruli in the antennal lobes. Exposure to 3-methyl-2-butenyl acetate induces egg laying in *D. erecta*, but not in *D. melanogaster* (Linz et al., 2013).

One of the best studied examples of host plant adaptations in the genus *Drosophila* comes from *Drosophila mojavensis*, which feeds on decomposing cactus in Arizona, the Mojave desert and Baja California, the Sonoran Desert, and Catalina Island. Different races of *D. mojavensis* have developed specialized host plant relationships with different cacti that are endemic at each location (Newby and Etges, 1998). Olfactory adaptations to distinct odorants emanating from each cactus species have been characterized both electrophysiologically and through behavioral studies (Date et al., 2013). In addition, analyses of genome-wide transcript abundances showed differential expression of members of the *Or* gene family between the different *D. mojavensis* populations (Crowley-Gall et al., 2016). Flies from the Mojave desert showed significant upregulation of several OR genes compared with flies from Catalina Island, notably Or67b and Or71a, which are expressed in ab9B and pb1B neurons, respectively, and are excited by aromatics. Differential expression of receptors was correlated with differential activity, measured electrophysiologically, and reflected in differences in the proportions of specific olfactory sensory neurons, in which they are expressed (Crowley-Gall et al., 2016).

Different habitats and different host plant specializations among the *D. mojavensis* populations could ultimately lead to reproductive barriers (Pfeiler et al., 2009). Analyses of genome sequences between *D. mojavensis* and its close relatives *D. arizonae* and *D. navojoa* revealed chromosomal inversion differences that form a barrier to interbreeding (Sanchez-Flores et al., 2016), although host plant specialization may not necessarily have been the driving force for this reproductive isolation.

Olfactory adaptations can have economic consequences. *Drosophila suzukii* has emerged as a major agricultural pest during the last decade as it has spread from Southeast Asia to Europe and North America (Walsh et al., 2011). Most *Drosophila* species are attracted to decaying fruit, whereas *D. suzukii* females oviposit on ripening fruit. *D. suzukii* females have evolved an enlarged serrated ovipositor, which enables
them to penetrate the soft skin of ripe fruit, for example, a variety of berries (Atallah et al., 2014). Behavioral studies show that D. suzukii is attracted to the odor of ripe strawberry and that oviposition behavior on ripe fruit is reduced when the common odorant co-receptor gene, Orco, is knocked down by RNAi or eliminated through CRISPR deletion (Karageorgi et al., 2017).

In contrast to its closely related species Drosophila biarmipes, the Or gene repertoire of D. suzukii has undergone duplications at the Or23a and Or67a loci and there is evidence for positive selection at the Or67a locus (Hickner et al., 2016). Elegant experiments in D. melanogaster in which different ORs were ectopically expressed in sensilla that lack expression of the endogenous Or22a receptor showed that the Or67a receptor could be activated strongly by methyl benzoate and ethyl benzoate, both of which have fruity odor qualities (Hallem and Carlson, 2006). However, volatiles arising from berries are complex; e.g., strawberries exude as many as 147 volatiles (Kim et al., 2013), making assessment of causal attractants challenging. D. suzukii also responds to the leaf odor β-cyclocitrinal, which does not elicit responses from its close relatives D. biarmipes and Drosophila takahashii. The responses to β-cyclocitrinal are mediated via the A neurons of ab3 sensilla (Keesey et al., 2015). Thus, both fruit volatiles and leaf volatiles may play a role in feeding and oviposition site selection by D. suzukii. Further examination of the Or gene repertoire in D. suzukii revealed pseudogenization of Or74a, Or85a, and Or98b. B neurons of the ab2 sensilla, which express Or85a, in D. melanogaster and D. biarmipes respond strongly to the fruity odorant ethyl 3-hydroxybutyrate, these neurons in D. suzukii are not responsive to this odorant (Keesey et al., 2015). Thus the functional significance of pseudogenization of this gene and other genes in D. suzukii remains to be established (Hickner et al., 2016).

Another adaptation driven by chemosensation is exemplified by the leaf-mining drosophilid Scaptomyza flava, which oviposits, and its larvae feed on leaves of the family Brassicaceae, which includes Arabidopsis thaliana (Whiteman et al., 2011; Goldman-Huertas et al., 2015). Evolution of S. flava herbivory has been accompanied by extensive changes in its OR repertoire with pseudogenization of multiple Or genes that respond to short-chain aliphatic esters, commonly found in yeast. The most striking change in the Or repertoire in this species is duplication at the Or67b locus, which has given rise to three paralogs with evidence for positive selection (Goldman-Huertas et al., 2015). It is possible that positive selection at this locus is intimately associated with feeding behavior on plant leaves, because its counterpart, the D. melanogaster OR67b receptor, responds to the green-leaf volatile (2)-3-hexenol (Galizia et al., 2010). The diversity of specializations within the genus Drosophila is illustrated in Figure 2. Finally, it should be noted that the absence of toxicity and lack of preference for a potential food source in the wild does not necessarily imply inability per se to feed and develop on that food source. For example, in the absence of screw pine fruits, D. erecta can feed on fungi, Ficus capensis fruits (Lachaise and Tsacas, 1974), and even on bananas (Rio et al., 1983).

**CO-EVOLUTION OF NEURAL PROJECTIONS WITH DEVELOPMENT OF PREFERENCE FOR CHEMOSENSORY CUES**

Development of preference for chemical cues from host plants is accompanied by alterations in the functional organization of the fly’s olfactory system. The D. sechellia antennae respond to femtogram quantities of methyl hexanoate, produced by its host plant, and this extraordinary sensitivity is reflected by an approximately 3-fold overrepresentation of neurons responding to this odorant compared with D. melanogaster (Dekker et al., 2006). Overrepresentation of this neuronal population is accompanied by a corresponding increase in volume of the glomerulus to which they project (Dekker et al., 2006). The A neuron of the ab3 sensillum responds to hexanoate esters and projects to its corresponding enlarged DM2 glomerulus, whereas B neurons in the ab3 sensilla respond to 2-heptanone, which is also produced by the Morinda fruit, and the glomerulus to which these neurons project is also enlarged (Ibba et al., 2010). Furthermore, a single amino acid change in the D. sechellia IR75b receptor, which is expressed in the ac3 sensilla, confers sensitivity and attraction to hexanoic acid (Prieto-Godino et al., 2017). This amino acid substitution and the resulting change in odorant response profile is accompanied by expansion of the DL2d glomerulus, which receives projections from IR75b-expressing neurons. However, no neuroanatomical changes were observed in higher-order circuits (Prieto-Godino et al., 2017). Thus, adaptations to specific olfactory cues that mediate host plant specialization can be accompanied by overrepresentation of olfactory receptor neuron populations and their projections to the antennal lobes.
CO-EVOLUTION OF CYTOCHROME P450S DURING HOST PLANT ADAPTATION

Specialized adaptations of insects to host plants depend not only on chemosensation but also require mechanisms that can neutralize toxic substances that plants produce to defend against herbivory. Members of the cytochrome P450 family play a major role in detoxification of xenobiotics. Cytochrome P450s are a diverse class of enzymes that perform a variety of functions from synthesis and degradation of ecdysteroids and juvenile hormone to the processing of various toxic chemicals insects may encounter in their environments (Feyereisen, 1999). Rapid evolution of the large family of cytochrome P450s, driven by gene duplications and diversification, accompanies olfactory adaptations to host plant specializations (Wu et al., 2011; McDonnell et al., 2012; Good et al., 2014, Harrop et al., 2014). Analysis of the cytochrome P450 gene family along the evolutionary trajectory of 12 Drosophila species has detected 114 gene gains and 74 gene losses (Figure 3) (Good et al., 2014). The cytochrome P450 gene family in D. melanogaster encompasses 90 genes, of which 83 encode functional transcripts, most of which belong to the CYP4 and CYP6 families (Tijet et al., 2001). Duplication of the Cyp6g1 gene has occurred at least four times in the Drosophila lineage, and Cyp6g1 paralogs are associated with insecticide resistance both in D. melanogaster and its sister species D. simulans (Harrop et al., 2014). Furthermore, copy number variants and transposon insertions at the 5’ regulatory region of the D. melanogaster Cyp6g1 locus have been associated with increased transcription of Cyp6g1 (Daborn et al., 2002) and resistance of Cyp6g1 alleles to dichlorodiphenyltrichloroethane in field populations (Schmidt et al., 2010). Strong directional selection in a California population of D. simulans has resulted in fixation of a Doc transposable element in the 5’ flanking region of Cyp6g1, which is also associated with increased transcription (Schlenke and Begun, 2004).

Molecular modeling of the effects of allelic variants of cytochrome P450s on protein folding, based on structural information from mammalian cytochrome P450s, shows that changes in protein conformation can have diverse effects on catalytic activity across different insect species. Phylogenetically closely related CYP450s can display distinct substrate specificities, whereas distantly related CYP450s may act on similar substrates. These studies also provided insight into the range of specificities of different members of the CYP450 family, ranging from narrow to broad (Schuler and Berenbaum, 2013).
Alkaloid-metabolizing P450 enzymes have been implicated in host adaptations of the cosmopolitan species *D. hydei* (Danielson et al., 1997) and in cactophilic *Drosophila* (Danielson et al., 1995). Two cytochrome P450 genes, *Cyp28a1* and *Cyp4d10*, have evolved to detoxify alkaloids from cactus host plants to enable host plant utilization by the cactophilic species *Drosophila mettleri* (Bono et al., 2008). Thus, evolutionary specialization on host plants may have been facilitated by adaptive tuning of the chemosensory gene repertoire along with evolution of a spectrum of cytochrome P450s targeted toward detoxification of potentially harmful or aversive plant-derived xenobiotics.

The adaptive mechanisms that enable co-evolution of different members of the chemoreceptor repertoire (e.g., OBPs and ORs) and detoxification enzymes remain poorly understood. One can hypothesize that selection of alleles of members of the cytochrome P450 family might occur first to enable a generalist species to access a previously unavailable food source, which would result in a selective advantage, followed by adaptation of chemoreceptors. However, in the absence of clear evidence this hypothesis remains speculative.

**Box 2. Outstanding Questions**

A central question related to evolution of the chemoreceptor repertoire is how evolution of the odorant receptor repertoire is accommodated in the neural projection to the antennal lobe. Does the total number of olfactory sensory neurons increase or does expansion of one neuronal specificity and enlargement of a single glomerulus occur at the expense of others? Do the same OR-expressing sensory neurons target the same glomeruli in all Drosophilids? Is it possible that corresponding olfactory sensory neurons in some species express different ORs tuned to their relevant host plant?

Finally, occupation of a specialized niche for oviposition and feeding establishes a reproductive barrier. Thus, host plant adaptation is intimately associated with speciation. How host plant adaptation functions in the transition from prezygotic to postzygotic isolation remains an area of current interest.

The genus *Drosophila* provides an ideal system to address these questions.
CONCLUDING REMARKS
Evolution of specialization requires co-evolution of multigene families of chemoreceptors and detoxification enzymes concomitant with modifications of neural circuitry in the brain. This complex process raises questions about the evolutionary mechanisms and adaptive forces that drive the acquisition of behavioral specializations (Box 2). The vast amount of information and resources available for Drosophilids make this genus an excellent model system to explore the evolutionary ecology of behavioral diversification.

ACKNOWLEDGMENTS
The author wishes to thank the peer review process for improving this manuscript. Work in the author’s laboratory is supported by grants from the National Institutes of Health (DA041613 and GM128974).

REFERENCES
Ahmed, O. M., Avila-Herrera, A., Tun, K. M., Serpa, P. H., Peng, J., Parthasarathy, S., Knapp, J. M., Stern, D. L., Davis, G. W., Pollard, K. S., and Shah, N. M. (2019). Evolution of mechanisms that control xenobiotic-inducible drosophilid cytochrome P450 enzymes in Drosophila melanogaster. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 214, 10797–10802.

Anholt, R. R. H., Wolfner, M. F., O’Grady, P., and Harbison, S. T. (2020). Evolution of reproductive behavior. Genetics 214 In press.

Arya, G. H., Weber, A. L., Wang, P., Magwire, M. M., Negron, Y. L., Mackay, T. F., and Anholt, R. R. (2011). Natural variation, functional pleiotropy and transcriptional networks of Odonart binding protein genes in Drosophila melanogaster. Genetics 186, 1475–1485.

Atallah, J., Teixeira, L., Salazar, R., Zaragoza, G., and Kopp, A. (2014). The making of a pest: the evolution of fruit-penetrating ovipositor in Drosophila suzukii and related species. Proc. Biol. Sci. 281, 20132840.

Barrish, S., and Volkman, P. C. (2015). Mechanisms of olfactory receptor neuron specification in Drosophila. Wiley Interdiscip. Rev. Dev. Biol. 4, 609–621.

Benton, R., Vannice, K. S., Gomez-Diaz, C., and Vosshall, L. B. (2009). Variant ionotropic glutamate receptors as chemosensory receptors in Drosophila. Cell 136, 149–162.

Bono, J. M., Matzkin, L. M., Castreanza, S., and Markow, T. A. (2008). Molecular evolution and population genetics of two Drosophila mettleri cytochrome P450 genes involved in host plant utilization. Mol. Ecol. 17, 3211–3221.

Brand, P., Robertson, H. M., Lin, W., Pothula, R., Klingeman, W. E., Jurat-Fuentes, J. L., and Johnson, B. R. (2018). The origin of the odorant receptor gene family in insects. Elife 7, e38340.

Budelli, G., Ni, L., Berciu, C., van Giesen, L., Knecht, Z. A., Chang, E. C., Kaminsky, B., Silbering, A. F., Samuel, A., Klein, M., et al. (2019). Ionotropic receptors specify the morphogenetic phasic sensory neurons controlling rapid thermal preference in Drosophila. Neuron 101, 738–747.

Cameron, P., Hriri, M., Ngai, J., and Scott, K. (2010). The molecular basis for water taste in Drosophila. Nature 465, 91–95.

Cande, J., Prud’homme, B., and Gompel, N. (2013). Smells like evolution: the role of chemoreceptor evolution in behavioral change. Curr. Opin. Neurobiol. 23, 152–158.

Carey, A. F., Wang, G., Su, C. Y., Zwiebel, L. J., and Carlson, J. R. (2010). Odorant receptor in the malaria mosquito Anopheles gambiae. Nature 464, 66–71.

Caron, S. J., Ruta, V., Abbott, L. F., and Axel, R. (2013). Random convergence of olfactory inputs in the Drosophila mushroom body. Nature 497, 113–117.

Chen, Z., Wang, Q., and Wang, Z. (2010). The amiloride-sensitive epithelial Na+ channel PPK28 is essential for drosophila gustatory water perception. J. Neurosci. 30, 6427–6432.

Chen, C., Buhl, E., Xu, M., Croset, V., Rees, J. S., Lilley, K. S., Benton, R., Hodge, J. J., and Stanewsky, R. (2015). Drosophila Ionotropic Receptor 25a mediates circadian clock resetting by temperature. Nature 527, 516–520.

Couto, A., Alenius, M., and Dickson, B. J. (2005). Molecular, anatomical, and functional organization of the Drosophila olfactory system. Curr. Biol. 15, 1535–1547.

Croset, V., Rytz, R., Cummings, S. F., Budd, A., Bravard, D., Kaessmann, H., Gibson, T. J., and Benton, R. (2010). Ancient protostome origin of chemosensory ionotropic glutamate receptor and the evolution of insect taste and olfaction. PLoS Genet. 6, e1000164.

Crowley-Gall, A., Date, P., Han, C., Rhodes, N., Andolfatto, P., Layne, J. E., and Rollmann, S. M. (2016). Population differences in olfactory experience accompany host shift in Drosophila mohojensis. Proc. Biol. Sci. 283, 20161562.

Daborn, P. J., Yen, J. L., Bogwitz, M. R., Le Goff, G., Feil, E., Jeffers, S., Tijet, N., Perry, T., Heckel, D., Batterham, P., et al. (2002). A single p450 allele associated with insecticide resistance in Drosophila. Science 297, 2253–2256.

Danielson, P. B., Letman, J. A., and Fogleman, J. C. (1999). Alkaloid metabolism by cytochrome P-450 enzymes in Drosophila melanogaster. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 110, 683–688.

Danielson, P. B., Macintyre, R. J., and Fogleman, J. C. (1997). Molecular cloning of a family of xenobioto-inducible drosophilid cytochrome p450s: evidence for involvement in host-plant allelochemical resistance. Proc. Natl. Acad. Sci. U. S. A 94, 10797–10802.

Date, P., Dweck, H. K., Stensmyr, M. C., Shann, J., Hansson, B. S., and Rollmann, S. M. (2013). Divergence in olfactory host plant preference in D. mojavensis in response to cactus host use. PLoS One 8, e70027.

de Bruyne, M., Clyne, P. J., and Carlson, J. R. (1999). Olfact coding in a model olfactory organ: the Drosophila maxillary palp. J. Neurosci. 19, 4520–4532.

de Bruyne, M., Foster, K., and Carlson, J. R. (2001). Olfact coding in the Drosophila antenna. Neuron 30, 537–552.

Dekker, T., Ibba, I., Siju, K. P., Stensmyr, M. C., and Hansson, B. S. (2006). Olfactory shifts parallel superspecializm for toxic fruit in Drosophila melanogaster sibling, D. sechellia. Curr. Biol. 16, 101–109.

Drosophila 12 Genomes Consortium (2007). Evolution of genes and genomes on the Drosophila phylogeny. Nature 450, 203–218.

Drosophila Odorant Receptor Nomenclature Committee (2000). A unified nomenclature system for the Drosophila odorant receptors. Cell 102, 145–146.

Dweck, H. K., Ebrahim, S. A., Kromann, S., Bown, D., Hillbur, Y., Sachse, S., Hansson, B. S., and Stensmyr, M. C. (2013). Olfactory preference for egg laying on citrus substrates in Drosophila. Curr. Biol. 23, 2472–2480.

Dwarkin, I., and Jones, C. D. (2009). Genetic changes accompanying the evolution of host specialization in Drosophila sechellia. Genetics 181, 721–736.
Ebrishm, S.A., Dweck, H.K., Stokl, J., Hofkerberth, J.E., Trona, F., Weniger, K., Rybak, J., Seki, Y., Stensmyr, M.C., Sachse, S., et al. (2015). Drosophila avoids parasitoids by sensing their semiochemicals via a dedicated olfactory circuit. PLoS Biol. 13, e1002318.

Enjin, A., Zaharieva, E.E., Frank, D.D., Mansournia, S., Suh, G.S., Gallio, M., and Stensmyr, M.C. (2016). Humidity sensing in Drosophila. Curr. Biol. 26, 1352–1358.

Feyerisen, R. (1999). Insect P450 enzymes. Annu. Rev. Entomol. 44, 507–533.

Findlay, G.D., Yi, X., Maccoss, M.J., and Swanson, W.J. (2008). Proteomics reveals novel Drosophila secretion of proteins transferred at mating. PLoS Biol. 6, e178.

Galizia, C.G., Münch, D., Strauch, M., Nissler, A., and Ma, S. (2010). Integrating heterogeneous odor response data into a common response model: a DoOR to the complete olfactome. Chem. Senses 35, 551–563.

Goldman-Huertas, B., Mitchell, R.F., Lapoint, R.T., Faucher, C.P., Hildebrand, J.G., and Whiteman, W.J. (2008). Proteomics reveals novel Drosophila seminal fluid proteins transferred at mating. PLoS One 3, e118.

Ha, T.S., and Smith, D.P. (2006). A pheromone receptor mediates 11-cis-vaccenyl acetate-induced responses in Drosophila. J. Neurosci. 26, 8727–8733.

Hallet, E.A., and Carlson, J.R. (2006). Coding of odors by a receptor repertoire. Cell 125, 143–160.

Harrap, T.W., Sztal, T., Lumb, C., Good, R.T., Daborn, P.J., Batterham, P., and Chung, H. (2014). Evolutionary changes in gene expression, coding sequence and copy-number at the Cyp12g1 locus contribute to resistance to multiple insecticides in Drosophila. PLoS One 9, e84879. Erratum in PLoS One (2014) 9, e92490.

Hekmat-Scafe, D.S., Scafe, C.R., McKinney, A.J., and Tanouye, M.A. (2002). Genome-wide analysis of the odorant-binding protein gene family in Drosophila melanogaster. Genom. Res. 12, 1357–1369.

Hickner, P.V., Rivaldi, C.L., Johnson, C.M., Siddappaji, M., Raster, G.J., and Syed, Z. (2016). The making of a pest: insights from the evolution of chemosensory receptor families in pestiferous and invasive fly, Drosophila suzukii. BMC Genomics 17, 648.

Huang, W., Lyman, R.F., Lyman, R.A., Carbone, M.A., Harbison, S.T., Magwire, M.M., and Mackay, T.F. (2016). Spontaneous mutations and the origin and maintenance of quantitative genetic variation. Elife 5, e14625.

Huang, W., Massouras, A., Inoue, Y., Peiffer, J., Ramia, M., Taron, A.M., Turlapati, T., Zichner, T., Zhu, D., Lyman, R.F., et al. (2014). Natural variation in genome architecture among 205 Drosophila melanogaster Genetic Reference Panel lines. Genome Res. 24, 1193–1208.

Ibáñez, I., Angiò, A.M., Hansson, B.S., and Dekker, T. (2010). Macroglomeruli for fruit odors change blend preference in Drosophila. Naturwissenschaften 97, 1059–1066.

Jafari, S., Alkhori, L., Schleffer, A., Brochtrup, A., Hummel, T., and Alenius, M. (2012). Combinatorial activation and repression by seven transcription factors specify Drosophila odorant receptor expression. PLoS Biol. 10, e1001280.

Jones, C.D. (2005). The genetics of adaptation in Drosophila sechellia. Genetica 123, 137–145.

Jones, W.D., Cayirlioglu, P., Kadow, I.G., and Vosshall, L.B. (2007). Two chemosensory receptors together mediate carbon dioxide detection in Drosophila. Nature 445, 86–90.

Joseph, R.M., and Carlson, J.R. (2015). Drosophila chemoreceptors: a molecular interface between the chemical world and the brain. Trends Genet. 31, 683–695.

Karageorgi, M., Bräcker, L.B., Lebretton, S., Minervino, C., Cavey, M., Siju, K.P., Grunwald Kadow, I.C., Gompel, N., and Prud’homme, B. (2017). Evolution of multiple sensory systems drives novel egglaying behavior in the fruit pest Drosophila suzukii. Curr. Biol. 27, 847–853.

Kim, M.S., Repp, A., and Smith, D.P. (1998). LUSH odorant-binding protein mediates chemosensory responses to alcohols in Drosophila melanogaster. Genetics 150, 711–721.

Keesey, I.W., Knaden, M., and Hansson, B.S. (2015). Olfactory specialization in Drosophila suzukii supports an ecological shift in host preference from rotten to fresh fruit. J. Chem. Ecol. 41, 121–128.

Kim, Y.H., Kim, K.H., Szulejko, J.E., Gorur-Shandilya, S., Menuz, K., Kopp, A., Barmina, O., Hamilton, A.M., Rehermann, G., Becher, P.G., Pool, J.E., and Stensmyr, M.C. (2018). Wild African Drosophila melanogaster are seasonal specialists on marula fruit. Curr. Biol. 28, 3960–3968.

Marin, E.C., Jefferis, G.S., Komiyama, T., Zhu, H., and Luo, L. (2002). Representation of the glomerular olfactory map in the Drosophila brain. Cell 109, 243–255.

Masse, N.Y., Turner, G.C., and Jefferis, G.S. (2009). Olfactory information processing in Drosophila. Curr. Biol. 19, R700–R713.

Mast, J.D., De Moraes, C.M., Alborn, H.T., Lavis, L.D., and Stern, D.L. (2014). Evolved differences in larval social behavior mediated by novel pheromones. Elife 3, e04205.

Matsuou, T., Sugaya, S., Yasukawa, J., Aigaki, T., and Fuyama, Y. (2007). Odonator-binding proteins OBPs7 and OBPs7-e affect taste perception and host-plant preference in Drosophila sechellia. PLoS Biol. 5, e118.

McBride, C.S. (2007). Rapid evolution of smell and taste receptor genes during host specialization in Drosophila sechellia. Proc. Natl. Acad. Sci. U S A 104, 4996–5001.

McBride, C.S., Arguello, J.R., and O’Meara, B.C. (2007). Five Drosophila genomes reveal nonneutral evolution and the signature of host specialization in the chemoreceptor superfamily. Genetics 177, 1395–1416.

McDonnell, C.M., King, D., Comeron, J.M., Li, H., Sun, W., Berenbaum, M.R., Schuler, M.A., and Pittendrigh, R.B. (2012). Evolutionary toxicogenomics: diversification of the Cyp12d1 and Cyp12d3 genes in Drosophila species. J. Mol. Evol. 74, 281–296.
Miller, C.J., and Carlson, J.R. (2010). Regulation of odor receptor gene in trichoid sensilla of the Drosophila antenna. Genetics 186, 79–95.

Miller, D.E., Staber, C., Zeitlinger, J., and Hawley, R.S. (2018). Highly contiguous genome assemblies of 15 Drosophila species generated using nanopore sequencing. G3 (Bethesda) 8, 3131–3141.

Min, S., Ai, M., Shin, S.A., and Suh, G.S. (2013). Dedicated olfactory neurons mediating attraction behavior to ammonia and amines in Drosophila. Proc. Natl. Acad. Sci. U. S. A 110, E1321–E1329.

Missbach, C., Dweck, H.K., Vogel, H., Vilcinskas, A., Stensmyr, M.C., Hansson, B.S., and Grosse-Wilde, E. (2014). Evolution of insect olfactory receptors. Elife 3, e02115.

Monahan, K., Schieren, I., Cheung, J., Mumbey-Wafala, A., Monuki, E.S., and Lomvardas, S. (2017). Cooperative interactions enable singular olfactory receptor expression in mouse olfactory neurons. Elife 6, e28620.

Monahan, K., Horta, A., and Lomvardas, S. (2019). LRQ2- and LDB1-mediated trans interactions regulate olfactory receptor choice. Nature 565, 448–453.

Nexby, B.D., and Etges, W.J. (1998). Host preference among populations of Drosophila mojavensis (Diptera: Drosophilidae) that use different host cacti. J. Insect Behav. 11, 691–712.

Ni, L., Bronk, P., Chang, E.C., Lowell, A.M., Flam, J.O., Panzano, V.C., Theobald, D.L., Griffith, L.C., and Garrity, P.A. (2013). A gustatory receptor paralogue controls rapid warmth avoidance in Drosophila. Nature 500, 580–584.

Nozawa, M., and Nei, M. (2007). Evolutionary dynamics of olfactory receptor genes in Drosophila species. Proc. Natl. Acad. Sci. U. S. A 104, 7122–7127.

Orr, H.A., and Betancourt, A.J. (2001). Haldane’s sieve and adaptation from the standing genetic variation. Genetics 157, 875–884.

Pelosi, P., Iovinella, I., Felicioli, A., and Dani, F.R. (2010). Copy number variation and transposable elements feature in recent, ongoing adaptation at the Cyp4g1 locus. PLoS Genet. 6, e1000998.

Schuler, M.A., and Berenbaum, M.R. (2013). Structure and function of cytochrome P450s in insect adaptation to natural and synthetic toxins: insights gained from molecular modeling. J. Chem. Ecol. 39, 1232–1245.

Scott, K., Brady, R., Jr, Cravchik, A., Morozov, P., Razhetsky, A., Zuker, C., and Axel, R. (2001). A chemosensory gene family encoding candidate gustatory and olfactory receptors in Drosophila. Cell 104, 661–673.

Scott, K. (2018). Gustatory processing in Drosophila melanogaster. Annu. Rev. Entomol. 63, 15–30.

Seelholzer, L.F., Seppo, M., Stern, D.L., and Ruta, V. (2018). Evolution of central neural circuit underlies Drosophila mate preference. Nature 559, 564–569.

Seetharam, A.S., and Stuart, G.W. (2013). Whole genome phylogeny for 21 Drosophila species using predicted 2b-RAD fragments. PeerJ 1, e226.

Shiao, M.S., Chang, J.M., Fan, W.L., Lu, M.Y., Notredame, C., Fang, S., Kondo, R., and Li, W.H. (2015). Expression divergence of chemosensory genes between Drosophila sechellia and its sibling species and its implications for host shift. Genome Biol. Evol. 7, 2843–2858.

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. Science 236, 787–792.

Song, X., Goicoechea, J.L., Ammiraju, J.S., Luo, M., He, R., Lin, J., Lee, S.J., Sisneros, N., Watts, T., Kudrna, D.A., et al. (2011). The 19 genomes of Drosophila: a BAC library resource for genome-wide and scale comparative evolutionary research. Genetics 187, 1023–1030.

Stensmyr, M.C., Dweck, H.K., Farhan, A., Ibba, I., Strutz, A., Mukunda, L., Linz, J., Grabe, V., Steck, K., Lavista-Llano, S., et al. (2012). A conserved dedicated olfactory circuit for detecting harmful microbes in Drosophila. Cell 151, 1345–1357.

Suh, G.S., Wang, A.M., Hergarden, A.C., Wang, J.W., Simon, A.F., Benzer, S., Axel, R., and Anacker, D.J. (2004). A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila. Nature 431, 854–859.

Sun, J.S., Larter, N.K., Chahda, J.S., Rioux, D., Gumaste, A., and Carlson, J.R. (2018). Humidity response depends on the small soluble protein Obp59a in Drosophila. Elife 7, e39249.

Swarup, S., Williams, T.I., and Anholt, R.R. (2011). Functional dissection of Odorant binding protein genes in Drosophila melanogaster. Genes Brain Behav. 10, 648–657.

Thistle, R., Cameron, P., Ghorayshi, A., Dennison, L., and Scott, K. (2012). Contact chemoreceptors mediate male-male repulsion and male-female attraction during Drosophila courtship. Cell 149, 1140–1151.

Tijet, N., Helvig, C., and Feyereisen, R. (2001). The cytochrome P450 gene superfamily in Drosophila melanogaster: annotation, intron-exon organization and phylogeny. Gene 262, 189–198.

Toda, H., Zhao, X., and Dickson, B.J. (2012). The Drosophila female aphrodisiac pheromone activates ppk23(+) sensory neurons to elicit male courtship behavior. Cell Rep. 1, 599–607.

Tomiska, S., Aigaki, T., and Matsuo, T. (2012). Conserved cis-regulatory elements of two odorant-binding protein genes, Obp57d and Obp57e, in Drosophila. Genes Genet. Syst. 87, 323–329.

van Breugel, F., Huda, A., and Dickinson, M.H. (2010). Distinct activity-gated pathways mediate attraction and aversion to CO2 in Drosophila. Nature 564, 420–424.

Vieira, F.G., and Rozas, J. (2011). Comparative genomics of the odorant-binding and chemosensory protein gene families across the arthropods: origin and evolutionary history of the chemosensory system. Genome Biol. Evol. 3, 476–490.

Vosshall, L.B., Wong, A.M., and Axel, R. (2000). An olfactory sensory map in the fly brain. Cell 102, 147–159.

Walsh, D.B., Bolda, M.P., Goodhue, R.E., Drees, A.J., Lee, J., Bruck, D.J., Walton, V.M.,
O’Neal, S.D., and Zalom, F.G. (2011). Drosophila suzukii (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. J. Integr. Pest Manag. 2, G1–G7.

Whiteman, N.K., Groen, S.C., Chevasco, D., Bear, A., Beckwith, N., Gregory, T.R., Denoux, C., Mammarella, N., Ausubel, F.M., and Pierce, N.E. (2011). Mining the plant-herbivore interface with a leaf mining Drosophila of Arabidopsis. Mol. Ecol. 20, 995–1014.

Wicher, D., Schäfer, R., Bauernfeind, R., Stensmyr, M.C., Heller, R., Heinemann, S.H., and Hansson, B.S. (2008). Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 452, 1007–1011.

Wiegmann, B.M., and Richards, S. (2018). Genomes of Diptera. Curr. Opin. Insect Sci. 25, 116–124.

Wong, A.M., Wang, J.W., and Axel, R. (2002). Spatial representation of the glomerular map in the Drosophila protocerebrum. Cell 109, 229–241.

Wu, D.D., Irwin, D.M., and Zhang, Y.P. (2011). Correlated evolution among six gene families in Drosophila revealed by parallel change of gene numbers. Genome Biol. Evol. 3, 986–999.

Xiao, S., Sun, J.S., and Carlson, J.R. (2019). Robust olfactory responses in the absence of odorant binding proteins. Elife 8, e51040.

Yang, H., Jaime, M., Polihronakis, M., Kanegawa, K., Markow, T., Kaneshiro, K., and Oliver, B. (2018). Re-annotation of eight Drosophila genomes. Life Sci. Alliance 1, e201800156.

Yao, C.A., Ignell, R., and Carlson, J.R. (2005). Chemosensory coding by neurons in the coeloconic sensilla of the Drosophila antenna. J. Neurosci. 25, 8359–8367.

Yassin, A., Debat, V., Bastide, H., Gidaszewski, N., David, J.R., and Pool, J.E. (2016). Recurrent specialization on a toxic fruit in an island Drosophila population. Proc. Natl. Acad. Sci. U S A 113, 4771–4776.

Yeaman, S., and Otto, S.P. (2011). Establishment and maintenance of adaptive genetic divergence under migration, selection, and drift. Evolution 65, 2123–2129.