**Entomologia
Experimentalis et Applicata**

MINI REVIEW

Special Issue: Insect Chemical Communication

Evolutionary neuroecology of olfactory-mediated sexual communication and host specialization in *Drosophila* – a review

Mohammed A. Khallaf[1,2] | **Markus Knaden[1]**

[1]Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, Jena, D-07745, Germany

[2]Department of Zoology and Entomology, Faculty of Science, Assiut University, Assiut, Egypt

Correspondence
Markus Knaden, Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, D-07745, Jena, Germany.
Email: mknaden@ice.mpg.de

Mohammed A. Khallaf, Department of Neuroscience, Max Delbrück Center for Molecular Medicine, Berlin, D-13122, Germany.
Email: mohammed.khallaf@mdc-berlin.de

Present address
Mohammed A. Khallaf, Department of Neuroscience, Max Delbrück Center for Molecular Medicine, Berlin, D-13122, Germany

Abstract

In humans, finding a partner is quite a difficult task because there are many criteria that one needs to consider. However, in comparison to many animals, when choosing a partner, we easily discriminate between ourselves and members of other species through various communication systems. On the contrary, many fly species (Diptera) are morphologically similar and overlap in their geographical distributions and ecological habitats. Sexual interactions of most drosophilid flies occur on their hosts. Therefore, flies rely on olfactory sex pheromones, as well as on non-pheromonal chemicals such as host volatiles – which guide and restrict the search for conspecifics within limited locations – as honest signals for pre-mating reproductive isolation. A subtle divergence in the perception of these signals can lead to accumulated changes among populations of the same species, and ultimately to a reduction in gene flow and reproductive isolation. In recent years, we have seen an increased interest in how olfactory systems diverge to drive host adaptation and speciation. In this review, we discuss the evolutionary changes of the neural circuits that underlie mate recognition. We shed light onto sex pheromone communication systems, the construction of olfactory nervous systems, and the role of host specialization in reproductive isolation. Finally, leveraging the incipient speciation of *Drosophila mojavensis* Patterson populations, we highlight the underlying sensory mechanisms of the reproductive isolation barriers. In the end, we propose future research topics of the evolutionary neuroecology field of sexual communication.

KEYWORDS
sex pheromones, drosophilid flies, mating systems, olfaction, Diptera, Drosophilidae, honest signal, pre-mating reproductive isolation, partner choice, sexual communication, mate recognition, sensory mechanism

INTRODUCTION

We live in a world full of odors – at least a trillion that our noses can distinguish (Bushdid et al., 2014) and that have significant impact on our life and emotions. Amazingly, our noses are also developed to smell feeling-related chemicals (fear, joy, sexual arousal) of other people, and therefore, we, like other creatures, 'talk' to each other through chemical signals (de Groot et al., 2012).

In the 1870s, 90 years before the olfactory intra-specific communication signals were directly explored and named, Jean-Henri Fabre wondered how a male moth found a

hidden female behind wire-gauze while ignoring a visible female. Fabre (1916) suspected the presence of scents that attract conspecific partners and are not detected by the human nose. In 1959, Karlson & Lüscher (1959) proposed the new term 'pheromone' – derived from the Greek *pherein*, to transfer, and *hormōn*, to excite – for the chemicals that mediate communication between individuals of the same species, in which both the sender and the receiver of the signal gain benefit (Wyatt, 2017). Two months later, the German scientist Adolf Butenandt and his team succeeded in isolating and identifying the first sex pheromone – bombykol – which the female silk moth *Bombyx mori* L. releases to attract a male (Butenandt et al., 1959, 1961a,b). In these early days, half a million female moths were needed to extract and identify the sex pheromone (Butenandt et al., 1959). Currently, the development of analytical techniques, particularly gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectroscopy (LC-MS), has enabled us to identify numerous odorants emitted from a single insect as small as a vinegar fly, *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Khallaf et al., 2021).

Moreover, in the light of modern technological innovations such as CRISPR-Cas9 genome editing, we have seen an increased interest in how olfactory systems diverge to drive speciation and adaptation to new ecological niches (Zhao & McBride, 2020). Therefore, the study of evolutionary changes of neural circuits that underlie species-specific behaviors in an animal's ecological niche – a field referred to as evolutionary neuroecology – has become an attractive topic in the last 2 decades (de Bono & Bargmann, 1998; Lim et al., 2004; Newcomb & Katz, 2009; Bendesky et al., 2017; Prieto-Godino et al., 2017; Seeholzer et al., 2018; Ding et al., 2019; Hong et al., 2019; Zhao & McBride, 2020). By tracing the accumulated changes in the olfactory communication systems between closely related *Drosophila* spp., this review advances our understanding of how peripheral and central neural circuits are modulated over relatively short evolutionary time scales. The following sections provide a comprehensive overview on intra-specific olfactory communication via sex pheromones to attract a mating partner, the architecture of the insect olfactory system, and the transformation of the sex pheromone signal into an electrical signal to elicit sexual behaviors. Furthermore, we discuss how host shift, and the usually accompanying changes in olfactory preferences in flies, can contribute to sexual isolation and, hence, speciation.

THE INS AND OUTS OF OLFACTORY COMMUNICATION

Since the first olfactory receptors have been described (Buck & Axel, 1991), more and more scientists started to investigate the evolution of chemical communication among species with respect to the chemical signal and the molecular and neural basis of the signal's perception. However, olfaction is a challenging sense for several reasons. First, unlike with optical, thermosensory, hygrosensory, and mechanosensory stimuli that can be measured (such as wavelength, temperature, humidity, and pressure), olfactory stimuli do not follow a physical scale but present a nearly infinite number of possible combinations of molecules. Second, in contrast to vision and audition, where the stimulus travels with the huge velocity of light or sound, olfactory signals rely on the complex physical movement of molecules that take time to travel in air (or water). These molecules convey information not only about the sender, but also about the distance to the emitting source and the time elapsed since their release. Third, a relatively large portion of the genome is devoted to encoding the olfactory receptor genes. It is, therefore, hardly surprising that, aside from the ability to discriminate among millions of chemicals, the olfactory system can detect odorants that have never before naturally occurred on earth (i.e., that are synthesized for the first time by chemists) (Meunier & Rampin, 2017). The olfactory system is well suited for a prompt adaptation to ecological changes. Fourth, olfaction relies on large numbers of receptors, larger than for any of the other senses. For example, humans could differ significantly in how they perceive the same odor due to potential variation in ca. 400 functional olfactory genes, compared to limited differences in vision due to changes in the four opsin genes (Menashe et al., 2003; Wyatt, 2014). Fifth, the rapid evolution of olfactory sensory genes that have emerged independently in insects and vertebrates makes them a rich and challenging evolutionary subject to be investigated. Finally, in comparison to taste neural pathways that elicit attraction or aversion through labeled-line coding (Zhang et al., 2019), olfaction is to a large extent organized through high-level combinatorial processes (Malnic et al., 1999). This postulates that the olfactory system uses combinations of receptors to encode odor identities, which permit an enormous flexibility of signaling to evolve (Haverkamp et al., 2018).

Sex pheromone communication: a 'curriculum vitae for mating'

Sex pheromones have a large impact on the life of an animal and represent a very detailed curriculum vitae to engage in copulation. They are the first and arguably the best-studied olfactory communication signals, from their production to detection and elicited behaviors (Dickson, 2008; Yu et al., 2010; Dahanukar & Ray, 2011). Sex pheromones are produced by members of almost all orders of animals, from worms (Jeong et al., 2005) and insects (Wyatt, 2017) to fish (Clarke et al., 1991), reptiles (Schoralkova et al., 2018), birds (Caro et al., 2015), and mammals (Goodwin et al., 1979). Animals use various mechanisms for releasing sex pheromones into the environment, e.g., they have been reported in the saliva of pigs, in urine and tears of mice, in vaginal

fluids of hamsters, and in anal glands of insects (Brennan, 2010; Wyatt, 2017).

Although closely related species tend to produce similar sex pheromones, as a result of shared biosynthetic pathways that were present in common ancestors (Figure 1), they gain specificity by blending compounds in a species-specific concentration ratio. Aphids provide a fascinating example, as most species utilize the same four chemicals (nepetalactone and three isomers of nepetalactol), but each has its unique sex pheromonal blend due to a specific concentration ratio (Pickett et al., 1992; Hardie et al., 1994). Besides the various ratios, an enormous diversity of sex pheromones could be achieved by utilizing simple modifications (e.g., reduction and oxidation) to an existing sex pheromone (Lebreton et al., 2017; Borrero-Echeverry et al., 2021), which can explain the presence of different, but biosynthetically related (Figure 1), components in closely related species (Symonds & Elgar, 2008).

Contrary to the concept of species specificity, the same molecules could be used as a sex pheromone in different species, if: (1) they live in non-overlapping geographical regions, i.e., allopatric species (Wyatt, 2017), (2) the release time of sex pheromones is segregated to avoid heterospecific attraction (Ishikawa et al., 1999; Cardé & Haynes, 2004), or (3) the animals are unlikely to mate, as is the case for elephants and several moth species that utilize the same sex pheromone (Wyatt, 2014).

Animals often synthesize sex pheromones in a single form of two stereoisomers, i.e., enantiomers. Enantiomers are two compounds with the same molecular formula and order of atoms, but with different spatial orientations of the atoms, similar to the right and left human hands. For example, the mammalian pheromone

3,4-dehydro-exo-brevicomin has two chiral centers (RR and SS enantiomers); only the RR enantiomer is produced and detected by male mice (Novotny et al., 1995). Moreover, the biologically 'wrong' enantiomer sometimes acts as an inhibitor of the physiological activity of the 'right' component. It is, therefore, not surprising that racemic mixtures of pheromones (1:1 mixture of both enantiomers) often result in poor behavioral responses (Leal et al., 1998). An interesting example is provided by two Japanese beetles species, *Popillia japonica* Newman and *Anomala osakana* Sawada, that produce different enantiomers of γ-lactone. The two species can accurately distinguish between the two signals, because one of the enantiomers is an attractant whereas the other acts as an inhibitor, and vice versa for the other species (Leal et al., 1998).

Sex pheromones induce hard-wired and innate behaviors with immediate (i.e., releaser effects, such as courtship rituals) and long-lasting (i.e., primer effects, such as phase changes in locusts) consequences. However, the interpretation of the meaning of sex pheromones could vary among individuals due to context, age, sex, or other factors including internal or mating state (Wyatt, 2014). For example, the urinal male-specific pheromone of mice induces aggression in males, attracts females, induces estrus in mature females, and accelerates maturation of young females (Novotny, 2003). Similarly, in the vinegar fly, 11-*cis*-vaccenyl acetate (*c*VA), a male-specific sex pheromone that transfers to females during copulation, triggers many behavioral outputs (Figure 2). The compound induces sexual receptivity in virgin females (Bartelt et al., 1985; Ha & Smith, 2006; Kurtovic et al., 2007), but not in mated females (Lebreton et al., 2014; Das et al., 2017). On the other hand, it suppresses courtship and elicits aggression in males (Jallon et al., 1981;
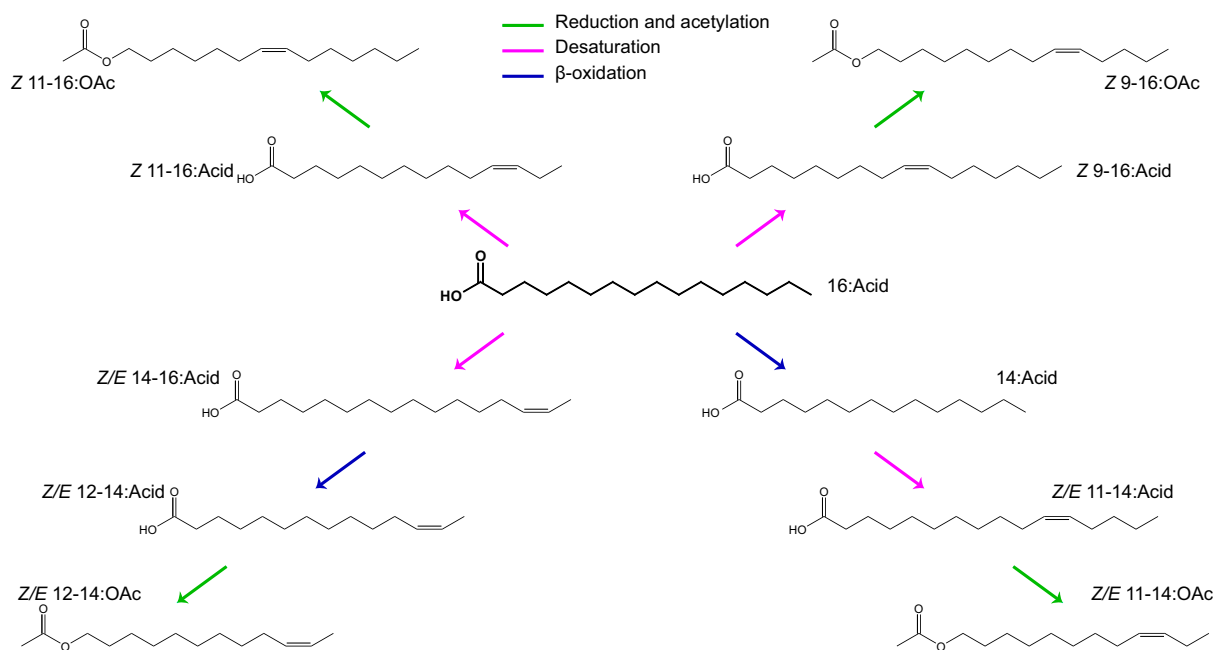

FIGURE 1 Proposed pheromone biosynthetic pathways from a single compound (palmitic acid, in the center) in drosophilid flies. Adapted from Lassance & Löfstedt (2009)
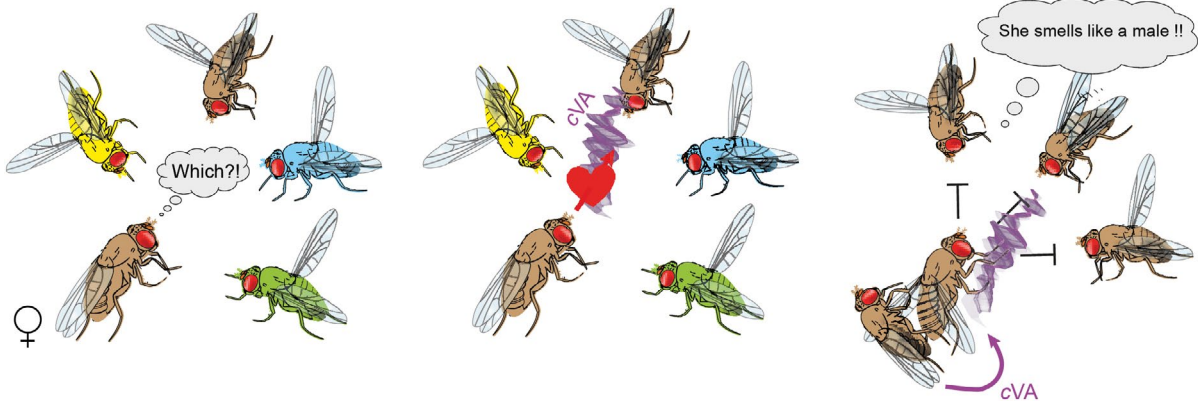
FIGURE 2 Sexual roles of 11-*cis*-vaccenyl acetate (*c*VA) in *Drosophila melanogaster*. (A) Female flies are often courted by conspecific as well as heterospecific males. (B) Among other cues, *c*VA emitted by *D. melanogaster* males helps the female choose the conspecific male. (C) During mating, the male transfers *c*VA to the female, which reduces the female's attractiveness to other *D. melanogaster* males. Drawings by Mohammed Khallaf

Ejima et al., 2007; Wang & Anderson, 2010). Furthermore, *c*VA works as an oviposition cue (Dumenil et al., 2016) and aggregation pheromone for both sexes (Bartelt et al., 1985), and induces long-range attraction behavior that could be modulated by starvation (Lebreton et al., 2015).

Sex pheromone processing: turning the chemical codes into electrical signals

Insects detect odors with their antennae – specifically the third antennal segment (the funiculus) – and maxillary palps (Figure 3). Olfaction starts when odor molecules diffuse into the pores of hair-like structures called sensilla, which cover the surfaces of these olfactory organs (Hansson, 1999; Menini, 2010). Each sensillum houses a different number of olfactory sensory neurons (OSNs). Once an odor binds to the cognate chemoreceptor, which is located on the surface of the ciliary membranes of OSNs, the responsive OSN transforms this chemical information into electric signals that are conveyed further to the primary olfactory neuropil (Figure 3). In insects, this neuropil is located in a region called the antennal lobe (AL), which is analogous to the olfactory lobe in crustaceans or the olfactory bulb in vertebrates (Ache & Young, 2005; Homberg et al., 1989). The OSNs send their axons to the AL (Figure 3) to innervate discrete spherical subunits of this neuropil – the olfactory glomeruli.

The number of glomeruli varies widely across animal species and correlates to the number of odorant receptors (ORs), e.g., 62 ORs and 52 glomeruli in *D. melanogaster*, 170 ORS and 165 glomeruli in honeybees, around 380 ORs and 400 glomeruli in ants, and ca. 1000 ORs and 1800 glomeruli in the olfactory bulb of mice (Galizia et al., 1999a,b; Schachtner et al., 2005; Maresh et al., 2008; Zube et al., 2008; Krieger et al., 2010; Braubach et al., 2012; Grabe et al., 2016). In both invertebrates and vertebrates, each glomerulus is innervated by a group of OSNs that express the same chemoreceptor(s) (i.e., OSNs with identical odor response

profiles) (Mombaerts et al., 1996; Gao et al., 2000; Vosshall et al., 2000). The glomeruli are connected by a network of local interneurons (LNs), which represent an early processing step for shaping the efferent olfactory information into the higher brain centers [reviewed in Grabe & Sachse (2018) and Yang et al. (2019)]. The LNs' arborization patterns diversify their anatomical structure, with some LNs innervating only a few glomeruli (so-called patchy LNs) and others targeting many, if not all, glomeruli (so-called panglomerular LNs) (Chou et al., 2010; Seki et al., 2010; Liou et al., 2018; Mohamed et al., 2019). Two types of LNs are distinguished by the impact of the signal and use of neurotransmitters: inhibitory and excitatory neurons (Shang et al., 2007; Silbering et al., 2008).

After processing by LNs, afferent olfactory information is conveyed from the AL to higher brain centers via second-order neurons, so-called olfactory projection neurons (PNs, analogous to mitral/tufted cells in vertebrates). The PNs send their dendrites to the AL glomeruli and their axons to higher-order brain centers. The PNs reveal a clear spatial segregation with respect to their response, to either pheromones or food odors (Jefferis et al., 2007), attractive amines or aversive acids (Min et al., 2013), and opposing hedonic valences and odor intensity (Strutz et al., 2014). The PNs convey information through the inner, middle, and outer antennocerebral tract (iAct, mAct, and oAct) (Stocker et al., 1990) to the mushroom body (MB) and the lateral horn (LH) (Figure 3) (Vosshall & Stocker, 2007). The mushroom body is analogous to the piriform cortex in mammals (Su et al., 2009) and is thought to be a center for olfactory learning and memory (Davis, 1993; Heisenberg, 2003; Fiala, 2007; Waddell, 2013), whereas the lateral horn shares many similarities with the mammalian amygdala (Miyamichi et al., 2011; Sosulski et al., 2011) and is presumed to mediate innate behavior (Heimbeck et al., 2001). However, recent studies have documented many roles of the MB in innate behaviors (Bracker et al., 2013; Lin et al., 2014; Lewis et al., 2015) and of the LH in modulation of the learning processing of the MB (Dolan et al., 2019). The LH output neurons
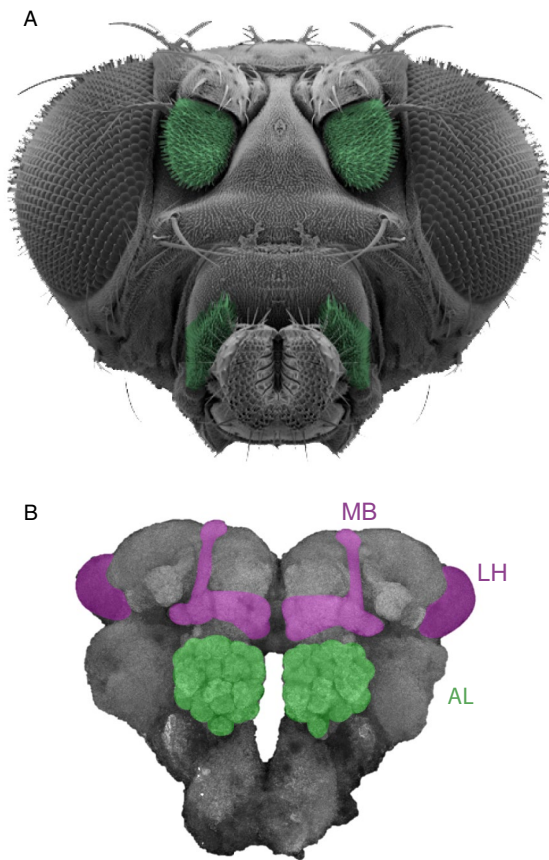
FIGURE 3 The olfactory system in *Drosophila* spp. (A) Detection: odors are detected by olfactory sensory neurons (OSNs) housed in sensilla on the olfactory organs (antennae and maxillary palps, green) of the fly. (B) Processing: odor information is sent to the antennal lobe (AL, green) and from there to higher brain centers such as the lateral horn (LH, dark purple) and mushroom bodies (MB, light purple) Photo credit: (A) Jürgen Rybak, (B) Veit Grabe

project their axonal terminals to many other brain regions (Tanaka et al., 2004; Frechter et al., 2019) and then to descending neurons that will ultimately activate the motor system, to enable the animal to perform odor-guided behaviors.

Drosophila as a model to study the evolution of sex pheromone communication

Since 1903, when William Ernest Castle started to use *D. melanogaster* as a research organism in the laboratory, five Nobel prizes in physiology or medicine have been awarded to nine scientists for their vinegar fly-based discoveries: (1) In 1933, to Thomas Hunt Morgan for his pioneering discovery that the 'chromosome plays a key role in heredity' (Morgan, 1910). (2) In 1946, to Hermann Joseph Muller for the discovery that the 'mutation rate of genes increases by x-ray radiation' (Muller, 1930). (3) In 1995, to Edward B Lewis, Christiane Nüsslein-Volhard, and Eric F Wieschaus for understanding the genetic control of embryonic

development (Lewis, 1978; Nüsslein-Volhard & Wieschaus, 1980). (4) In 2011, to Jules A Hoffmann for discoveries concerning the activation of innate immunity (Lemaitre et al., 1996). And (5) in 2017, to Jeffrey C Hall, Michael Rosbash, and Michael W Young for uncovering the molecular mechanisms that control the circadian rhythm (Bargiello et al., 1984; Zehring et al., 1984; Siwicki et al., 1988; Hardin et al., 1990; Liu et al., 1992; Vosshall et al., 1994; Price et al., 1998).

All of these discoveries were possible due to the ease of manipulating fly genomes by unique and sophisticated genetic tools, which are capable of activating or silencing the gene(s) of interest (Senturk & Bellen, 2018). Besides our ever-increasing bounty of knowledge and tools, and the ability to extrapolate to related species, their wealth of ecological diversity has made *Drosophila* spp. an excellent model to elucidate the genetic and neural correlates of their evolution. The genus *Drosophila* includes more than 1500 described species that live in quite diverse environments (Markow & O'Grady, 2005; Jezovit et al., 2017). Drosophilids' various ecological niches and species-specific behaviors and neural circuits, as well as the available genome sequences and experimental tools, have made insect sexual communication a valuable model to study evolutionary neuroecology.

Sex pheromone signaling in *D. melanogaster* is well understood, from sensory signals and their cognate receptors to neurons and their connectivity (Auer & Benton, 2016). Therefore, sex pheromone signaling in drosophilid flies represents an ideal system to address the evolutionary basis of various sexual traits. Moreover, the small number of pheromone-sensing ORs compared to other ORs tuned to general odors (Couto et al., 2005) makes a comprehensive evolutionary study feasible. Out of the 52 OSN classes in *D. melanogaster* (Grabe et al., 2016), only four express OSNs tuned to pheromones, which are localized in a particular sensillum type (trichoid sensillum) (Couto et al., 2005). The OSNs housed in trichoid sensilla are activated exclusively by olfactory sex pheromones and consist of two classes (at1 and at4). Whereas at1 sensilla are located on the ventro-distal region of the funiculus and house one OSN, at4 sensilla are present on the dorso-distal region and house three OSNs (Clyne et al., 1997; van der Goes van Naters & Carlson, 2007; Datta et al., 2008; Dweck et al., 2015; Lin & Potter, 2015). The pheromone-tuned OSNs and their corresponding pheromones are Or67d and Or65a/b/c, which are both tuned to *c*VA, Or47b, tuned to methyl laurate, and Or88a, tuned to methyl laurate, methyl myristate, and methyl palmitate (Kurtovic et al., 2007; van der Goes van Naters & Carlson, 2007; Dweck et al., 2015).

ROLE OF HOST VOLATILES AND SEX PHEROMONES IN SPECIATION

Like other animals, drosophilids rely on chemical cues to locate and choose an appropriate mating partner (Johansson & Jones, 2007; Smadja & Butlin, 2009; Wyatt,

2010; Thomas & Simmons, 2011). These cues might vary among individuals of the same species to facilitate choosing an appropriate conspecific, i.e., at the right age and reproductive status and the most genetically compatible (Andersson & Iwasa, 1996; Andersson & Simmons, 2006). Olfactory sex pheromones, as well as non-pheromonal chemicals such as host volatiles – which guide and restrict the search for conspecifics within limited locations – are pre-mating isolation and speciation signals (Smadja & Butlin, 2009). Any change in these pre-mating signals and their associated preferences (Ritchie, 2007) may, hence, lead to reproductive isolation and ecological speciation (Mackay et al., 2005; Arbuthnott, 2009). It is still not well understood how sender and receiver of sexual communication signals coevolve, as novel signals may not be preferred and/or recognized by conspecifics, and would thus fall out of the population. The same would be expected for novel preferences.

Ecological speciation is the process by which adaptation to various ecological environments leads to the formation of reproductive barriers between populations of the same species that will later split to form different species (Schluter, 2000; Coyne & Orr, 2004; Gross & Rieseberg, 2005; Rundle & Nosil, 2005; Funk et al., 2006). For example, populations that adapt to a specific environment (e.g., desert vs. forest habitats) may evolve genetic differences affecting the way that individuals communicate or behave. Therefore, speciation occurs as a by-product of accumulated ecologically related variation that results in individuals from different populations that avoid mating with each other (Mayr, 1947, 1963; Rundle & Nosil, 2005; Vines & Schluter, 2006). Ecological speciation can occur under either geographic arrangement, allopatry (where populations are geographically separated), or sympatry (where populations speciate in the absence of geographical barriers) (Rundle & Nosil, 2005). However, sympatric speciation requires a pronounced divergent selection to overcome the homogenizing effects of gene flow. On the other hand, the unimpeded divergence by gene flow due to geographic barriers may lead to more distinct pre- and post-zygotic mechanisms between allopatric populations (Turelli et al., 2001).

An important line of inquiry to help understand isolation forces is to investigate the genetic and neural changes that underlie preferences among closely related species, especially those that diverged across short evolutionary time scales. The following sub-sections provide an overview of the impact of host volatiles and sex pheromones on reproductive isolation among drosophilids.

Reproductive isolation through host specialization

Colonization of a new host could directly lead to divergence in the olfactory communication systems through many ways (Figure 4). First, host shift has severe impact on the biology of animals, e.g., body size, shape, and color, as well as population density (Nosil et al., 2003; Messina, 2004). These changes, therefore, may impact communication signals, mate-searching strategies, and sexual preferences (Etges, 1998). For example, searching for mates in less dense environments could lead to the adoption of long-range sexual signals. Second, new hosts could provide new ways of signal transmission and perception, resulting in differential sexual signaling (Funk et al., 2002), a process called sensory drive (Endler & Basolo, 1998). Sensory drive concerns how sexual traits and preferences coevolve in response to new environments. Lastly, host volatiles may be perceived and preferred as background or as components of the sex pheromone blend. For example, exposure to a particular host plant while mating has a strong impact on the subsequent reproductive preferences of male moths (Proffit et al., 2015; Zakir et al., 2017).

Similarly, in *D. melanogaster*, food odors (vinegar) boost the perception of the male sex pheromone – *c*VA – in virgin females, but not in males nor in mated females (Das et al., 2017). Although vinegar does not directly activate glomerulus DA1 – a specific glomerulus that responds to *c*VA (Kurtovic et al., 2007) – vinegar odors potentiated the *c*VA response on the PNs projecting to this glomerulus through electrical synapses between excitatory LNs and PNs in the antennal lobe. In line with these findings, other food odors (phenyl acetic acid and phenyl acetaldehyde) induce courtship in male flies through the ionotropic receptor (IR84a) (Grosjean et al., 2011). Vinegar is a complex blend that consists of many odorants, of which acetic acid is the most abundant compound (Becher et al., 2010). Although acetic acid alone is sufficient to elicit attraction in flies, it failed to potentiate the *c*VA response in combination with *c*VA, indicating the vinegar blend is necessary to mediate this synergism. Notably, the synergistic pattern, which was observed on the electrophysiological level, has been translated into increased female receptivity (Das et al., 2017). Interestingly, males showed neither synergistic effects nor behavioral responses in the presence of vinegar and *c*VA (Das et al., 2017). In behavioral assays, fed virgin *Drosophila* females were more attracted to the blend of *c*VA and vinegar than to vinegar alone, whereas males were not (Lebreton et al., 2014). Obviously, female flies benefit from not only focusing on male odors when looking for a mating partner, but also keeping the odors of potential food sources in mind.

Drosophila spp. live in a wide range of habitats across all climatic conditions, from deserts and caves to mountains and forests (Markow & O'Grady, 2005; Jezovit et al., 2017). In these environments, they feed and breed on diverse hosts such as decaying fruits, slime fluxes, mushrooms, and flowers, as well as frog spawn. On the one hand, many drosophilids share the same habitat (Markow & O'Grady, 2005; Jezovit et al., 2017); to reduce competition, they might allocate more energy to a distinct
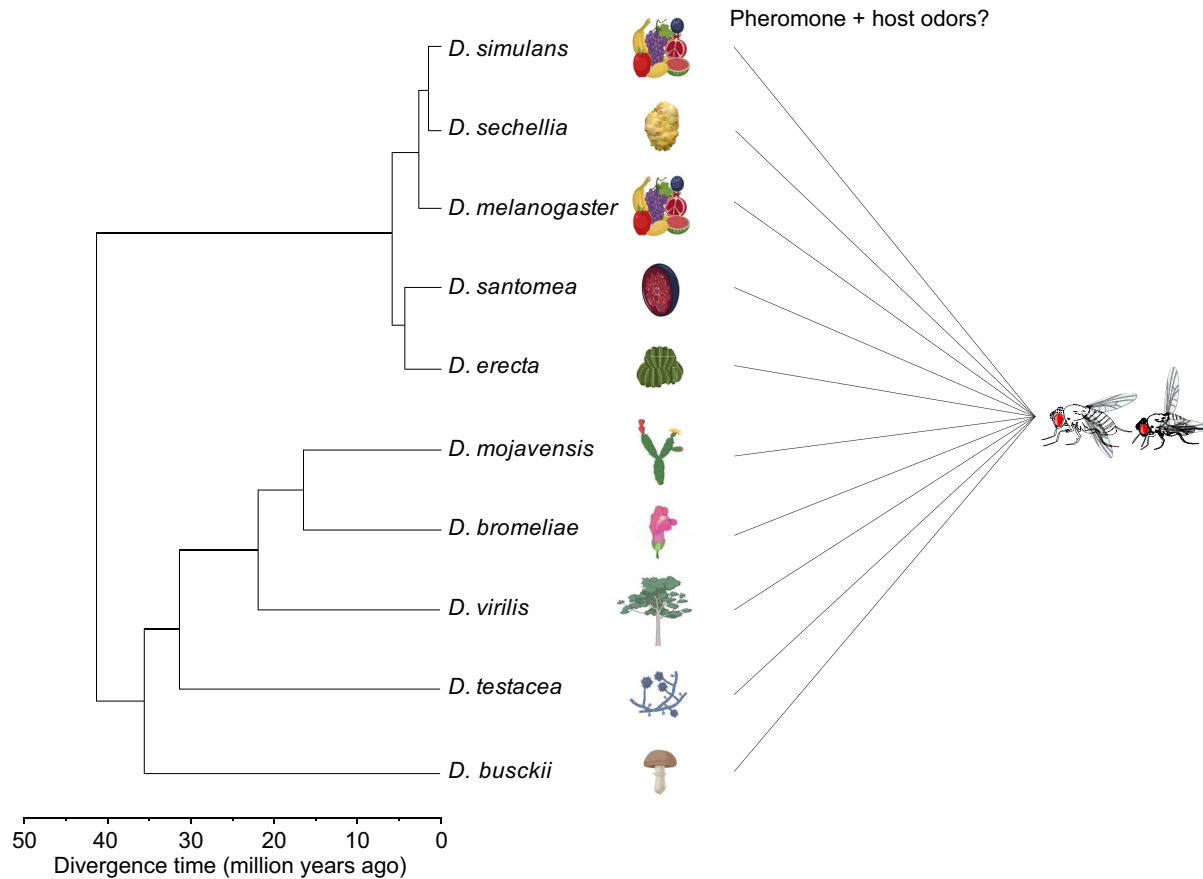
FIGURE 4 Host odors potentially affect courtship of various species of drosophilid flies through influencing their perception of sex pheromones. Examples of host specialization: *Drosophila melanogaster* and *D. simulans* on fermented fruits, *D. sechellia* on noni (*Morinda citrifolia*), *Drosophila santomea* Lachaise & Harry on fig [*Ficus chlamydocarpa* ssp. *fernandesiana* (Hutch.) C.C. Berg], *D. erecta* on *Pandanus* spp., *D. mojavensis* on cacti, *Drosophila bromeliae* Sturtevant on flowers, *Drosophila virilis* Sturtevant on tree slime flux, *Drosophila testacea* Roser on fungi, and *Drosophila busckii* Coquillett on mushrooms. Phylogenetic tree adapted from Khallaf et al. (2021)

sensory modality (Keesey et al., 2019). On the other hand, many drosophilids specialize on a single host resource, such as *Drosophila mojavensis* Patterson on cacti, and *Drosophila erecta* Tsacas & Lachaise on *Pandanus* fruit. Another notable drosophilid that has received particular attention with respect to its chemoreception is *Drosophila sechellia* Tsacas & Bachli, which is endemic to the Seychelles archipelago and has evolved extreme specialism for noni fruits (Morinda citrifolia L.), which are toxic for most (if not all) other drosophilids, including its sibling species *Drosophila simulans* Sturtevant and *D. melanogaster* (Legal et al., 1992).

The extreme specialism that *D. sechellia* has evolved compared to its close cousins did not only result in an increased metabolic tolerance of noni fruit toxins (R'Kha et al., 1991; Jones, 1998), but also in changes in courtship and mate choice (Cobb et al., 1989; Coyne, 1992), oviposition (Moreteau et al., 1994; Amlou et al., 1998), and pupariation site selection (Erezyilmaz & Stern, 2013). Recently, these shifts were shown to be correlated with changes at the periphery of the fly's olfactory system, i.e., whereas the odorant receptor OR22b is lost, the flies exhibit an increased number of neurons expressing OR22a (Auer et al.,

2020). However, the many behavioral shifts raise the possibility that the peripheral changes of olfactory coding are accompanied by novel central projection patterns. Indeed, circuit tracing experiments revealed the presence of increased sensory pooling onto interneurons and novel branches of PNs (Auer et al., 2020). Consistent with these findings, *Or22a* displays substantial intra- and interspecific nucleotide and copy number variation in many drosophilids (Guo & Kim, 2007; Nozawa & Nei, 2007; Aguade, 2009; Goldman-Huertas et al., 2015). In addition, the number of Or22a neurons has also expanded in *D. erecta*, a specialist on *Pandanus* fruit (Linz et al., 2013). Obviously, OR22a is an important receptor when it comes to host specificity. However, despite the importance of the Or22a pathway, several other olfactory channels play a role in noni attraction. These include Or85c/b neurons, which have conserved physiological properties among drosophilids but increased in number in *D. sechellia*, and Ir75b neurons, which have changed in both function and number while preserving central projections (Prieto-Godino et al., 2017). Such observations indicate that neural pathways may adapt in distinct ways, possibly reflecting diverse selection pressures and roles that enable animals to specialize to a

unique host, and thus limit encountering heterospecific flies.

Reproductive isolation through mate selection

Sexual traits involved in communication between the sexes often provide an instant contribution to reproductive isolation (Blair, 1955; Bridle & Ritchie, 2001). Upon search for a mate, females rely on honest signals – including visual, acoustic, and chemical signals – to find high-quality males (Bateman, 1948; Grafen, 1990). Therefore, sexual selection has led to the evolution of sexual signals in males and the cognate sensory systems in females for many animal species. Compared to other sensory information, olfactory sex pheromones provide the first cue to recognize a conspecific individual and can include much valuable information, such as its sex, age, body size, reproductive status, and health condition (Martin et al., 2007; Nielsen, 2017). These olfactory cues not only provide information on the sender, but may also have negative or positive effects on the receiver. For example, in mice, male scent induces estrus in females, whereas female scent suppresses ovarian activity in other females (Whitten, 1959, 1966; Nielsen, 2017). Once they are attracted by sex pheromones, males and females engage in species-specific courtship rituals, by which both partners ensure their reproductive success.

Sex pheromones and courtship behaviors of drosophilid flies differ quantitatively and qualitatively (Spieth, 1952; Symonds & Wertheim, 2005) and therefore represent an attractive model to identify the genetic basis of phenotypic evolution. These species-specific behaviors represent a pre-mating isolation barrier, especially between sympatric species (Spieth, 1952). When meeting at the host, flies identify conspecific mating partners through integration of various sensory modalities including vision, audition, olfaction, and gustation (Markow & O'Grady, 2005), which leads to either increased or inhibited courtship behaviors. Fruitful insights have been gained regarding the evolution of sensory modalities that underlie the interspecific variations in drosophilid courtship behavior (Manoli et al., 2005; Ding et al., 2016; Tanaka et al., 2017; Seeholzer et al., 2018; Ahmed et al., 2019). Sexual behaviors in drosophilids consist of a series of actions and reactions that vary qualitatively and quantitively between species (Spieth, 1952) and result in species and sexual discrimination. At first, males orient to and follow the females. Subsequently, males tap the females' bodies with their forelegs, followed by wing spreading and fanning for vibrational song production. Males follow the females by extending their proboscis to lick the females' genitalia and then attempt copulation. Obviously, the male has to invest to convince the rather choosy female. Interestingly, female choosiness varies even within species. When population size is small, i.e., when potential mates are rare, females that are too choosy may not find any suitable males, whereas females that are less choosy might still reproduce, although with less satisfying males (de Jong & Sabelis, 1991; Martínez-Ruiz & Knell, 2017).

One remarkable drosophilid that splits into four geographically isolated and ecologically distinct populations, and represents a model of incipient speciation and host adaptation, is *D. mojavensis* (Etges, 2019). The northern subspecies *D. m. wrigleyi* and *D. m. mojavensis* utilize the prickly pear cacti in Santa Catalina Island (CA, USA) and the red barrel cactus in the Mojave Desert (CA, NV, AZ, and UT, USA), respectively. The southern subspecies *D. m. sonorensis* and *D. m. baja* breed and feed on the organ pipe cactus in the mainland Sonoran Desert (Mexico and AZ and CA, USA) and the agria cactus in Baja California (Mexico), respectively (Ruiz et al., 1990). Phylogenetic analyses revealed that the two northern and the two southern subspecies cluster with each other (Allan & Matzkin, 2019). These closely-related subspecies exhibit many differences, including in their morphological features (Pfeiler et al., 2009), neurophysiological responses (Crowley-Gall et al., 2016; Date et al., 2013; Nemeth et al., 2018), genomic (Matzkin, 2014) and transcriptomic (Matzkin & Markow, 2013) characteristics, and behavioral traits (Newby & Etges, 1998). Experimental reciprocal crosses between these allopatric subspecies revealed various pre- and post-zygotic isolation barriers (Zouros & Dentremont, 1980; Krebs & Markow, 1989; Knowles & Markow, 2001).

Leveraging the short evolutionary time scale between the closely related *D. mojavensis* populations, we elucidated the evolution of the reproductive isolation barriers among these populations and the underlying sensory mechanisms (Khallaf et al., 2020). We identified four male-specific acetates of which three were exclusively produced by the northern subspecies. This dramatic change in chemical profile was surprising given the short divergence time, ca. 0.25 million years, between *D. mojavensis* subspecies (Matzkin, 2014; Etges, 2019). The four populations were feeding on similar artificial media, suggesting that the differential male-specific compounds are not a consequence of nutrition but are rather genetically determined. When checking for the function of these novel compounds, we realized that R-(*Z*)-10-heptadecen-2-yl acetate (R-HDEA) induces female receptivity, whereas (*Z*,*Z*)-19,22-octacosadien-1-yl acetate (OCDA) reduces male courtship. Together, these two substances fulfil the same roles that are described for *c*VA in *D. melanogaster*. Our results support the findings of Chin et al. (2014), who showed that *D. m. wrigleyi* males avoid courting females perfumed with ejaculatory bulb extracts of mature males (which, according to our analyses, contain OCDA). By contrast, similar extracts from immature males (which do not produce OCDA) did not elicit this behavior (Chin et al., 2014). We provided direct evidence that, in the northern subspecies, the dedicated R-HDEA-sensing neurons and auditory cues collaborate to mediate mate recognition. However, the southern subspecies rely on auditory cues only (Etges et al., 2006) and rather ignore R-HDEA for mate recognition. As all *D. mojavensis* flies can detect R-HDEA (which is only present in the northern subspecies),

the striking change of R-HDEA-induced behaviors among *D. mojavensis* subspecies implies the existence of differences in the central processing pathways (Khallaf et al., 2020). These findings are reminiscent of sexually dimorphic behavioral responses to *c*VA in *D. melanogaster* (Ruta et al., 2010; Kohl et al., 2015), where both males and females detect *c*VA, but interpret this in different ways.

Most drosophilids are gregarious in mating, except the Hawaiian species, whose males display lekking behavior, i.e., they select and defend a unique territory on their host (Spieth, 1952; Carson et al., 1970). Similar to Hawaiian species, we demonstrated that *D. m. wrigleyi* males advertise their presence by pulsating a droplet of anal secretions, which contains the volatile sex pheromone, R-HDEA, in close proximity to females to enhance their receptivity (Khallaf et al., 2020). Such a trait is supportive to the observed sexual behaviors of *D. mojavensis* in nature, where males conquer undamaged areas next to feeding sites on cactus and attract females to this spot (O'Grady & Markow, 2012). Similarly, other members of the *Drosophila* genus show species-specific courtship behaviors (Spieth, 1952), which might include nuptial gift donation (Steele, 1986), partners' song duet (O'Grady & Markow, 2012; LaRue et al., 2015), or territorial dating (O'Grady & Markow, 2012).

Divergence of the chemosensory genes could be accompanied by a rewiring of the central circuitry (Ding et al., 2016; Seeholzer et al., 2018). Indeed, neurons of *D. mojavensis* expressing the pheromone receptor *Or65a* do not innervate to the same glomerulus (DL3) as in *D. melanogaster*. Furthermore, despite expressing homologues of *Or65a*, the corresponding pheromone circuits of *D. mojavensis* and *D. melanogaster* govern different behaviors: the *Dmoj*-Or65a circuit detects R-HDEA and mediates female receptivity and conspecific recognition, whereas the homologous circuitry in *D. melanogaster* detects *c*VA and suppresses courtship and aggression behaviors in males (Ejima et al., 2007; Liu et al., 2011), as well as attraction towards *c*VA in mated females (Lebreton et al., 2014). These differences also suggest that the target domains in the higher brain centers of both circuits have changed. Notably, the processing pathway of a comparable pheromonal circuitry, between *D. melanogaster* and *D. simulans* (Seeholzer et al., 2018), has changed during less than one-tenth of the divergence time between *D. melanogaster* and *D. mojavensis* (ca. 40 million years ago). Thus, divergent functions of a homologous neural circuitry during a longer divergence time could be presumed.

FUTURE PERSPECTIVES

This review sheds light on many aspects in the evolutionary neuroecology field of sexual communication. However, there remains much to be accomplished. With special focus on how hosts guide and restrict the search for conspecifics, several aspects and open questions remain to be explored to get a more complete picture on how food volatiles and olfactory sex pheromones interact:

1. What is the interplay between sexual and natural selection? Do these forces act in concert to result in speciation events?
2. How do ecological conditions influence the evolution of sex pheromones and their perceptions?
3. Do host volatiles influence the perception of sex pheromones in a species-specific manner? In other words, are sex pheromones of conspecifics perceived differently in a background of host volatiles?
4. Does host specialization relieve flies of investment in sexual selection barriers (i.e., sex pheromones) as they may encounter only conspecific flies?
5. Do genetic and neural variations act together to reinforce sexual isolation barriers, especially in sympatric species that require prominent divergences?
6. How many and what types of genes need to mutate for flies to specialize on a new host?

With more than 1500 species, the genus *Drosophila* offers a fantastic resource to target these questions. Many of these species are nowadays available from *Drosophila* stock centers, and the establishment of molecular tools, e.g., CRISPR Cas9, allow us to investigate various aspects, e.g., the neuronal circuits of non-*D. melanogaster* drosophilids. However, what is lacking for many species is detailed knowledge about their ecology and distribution areas. Future drosophilists should, therefore, consider to visit more often their study flies in the field.

AUTHOR CONTRIBUTIONS
Mohammed A. Khallaf: Conceptualization (lead); Visualization (lead); Writing – original draft (lead); Writing – review & editing (equal). Markus Knaden: Conceptualization (supporting); Supervision (lead); Writing – review & editing (equal).

ORCID
Mohammed A. Khallaf https://orcid.org/0000-0002-1402-3858
Markus Knaden https://orcid.org/0000-0002-6710-1071

REFERENCES
Ache BW & Young JM (2005) Olfaction: diverse species, conserved principles. *Neuron* 48: 417–430.
Aguade M (2009) Nucleotide and copy-number polymorphism at the odorant receptor genes *Or22a* and *Or22b* in *Drosophila melanogaster*. *Molecular Biology and Evolution* 26: 61–70.

Ahmed OM, Shah NM, Stern DL, Davis GW, Pollard KS et al. (2019) Evolution of mechanisms that control mating in *Drosophila* males. *Cell Reports* 27: 2527–2536.

Allan CW & Matzkin LM (2019) Genomic analysis of the four ecologically distinct cactus host populations of *Drosophila mojavensis*. *BMC Genomics* 20: 732.

Amlou M, Moreteau B & David JR (1998) Genetic analysis of *Drosophila sechellia* specialization: oviposition behaviour toward the major aliphatic acids of its host plant. *Behavior Genetics* 28: 455–464.

Andersson M & Iwasa Y (1996) Sexual selection. *Trends in Ecology & Evolution* 11: 53–58.

Andersson M & Simmons LW (2006) Sexual selection and mate choice. *Trends in Ecology & Evolution* 21: 296–302.

Arbuthnott D (2009) The genetic architecture of insect courtship behavior and premating isolation. *Heredity* 103: 15–22.

Auer TO & Benton R (2016) Sexual circuitry in *Drosophila*. *Current Opinion in Neurobiology* 38: 18–26.

Auer TO, Khallaf MA, Silbering AF, Zappia G, Ellis K et al. (2020) Olfactory receptor and circuit evolution promote host specialization. *Nature* 579: 402–408.

Bargiello TA, Jackson FR & Young MW (1984) Restoration of circadian behavioral rhythms by gene-transfer in *Drosophila*. *Nature* 312: 752–754.

Bartelt RJ, Schaner AM & Jackson LL (1985) *Cis*-vaccenyl acetate as an aggregation pheromone in *Drosophila melanogaster*. *Journal of Chemical Ecology* 11: 1747–1756.

Bateman AJ (1948) Intra-sexual selection in *Drosophila melanogaster*. *Heredity* 2: 277.

Becher PG, Bengtsson M, Hansson BS & Witzgall P (2010) Flying the fly: long-range flight behavior of *Drosophila melanogaster* to attractive odors. *Journal of Chemical Ecology* 36: 599–607.

Bendesky A, Kwon YM, Lassance JM, Lewarch CL, Yao S et al. (2017) The genetic basis of parental care evolution in monogamous mice. *Nature* 544: 434–439.

Blair WF (1955) Mating call and stage of speciation in the *Microhyla-Olivacea-M-Carolinensis* complex. *Evolution* 9: 469–480.

de Bono M & Bargmann CI (1998) Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. *Cell* 94: 679–689.

Borrero-Echeverry F, Solum M, Trona F, Wallin EA, Bengtsson M et al. (2021) The female pheromone (*Z*)-4-undecenal mediates flight attraction and courtship in *Drosophila melanogaster*. *bioRxiv*: 2021.2001.2006.425638.

Bracker LB, Siju KP, Varela N, Aso Y, Zhang M et al. (2013) Essential role of the mushroom body in context-dependent CO_2 avoidance in *Drosophila*. *Current Biology* 23: 1228–1234.

Braubach OR, Fine A & Croll RP (2012) Distribution and functional organization of glomeruli in the olfactory bulbs of zebrafish (*Danio rerio*). *Journal of Comparative Neurology* 520: 2317–2339.

Brennan PA (2010) Pheromones and mammalian behavior. *The Neurobiology of Olfaction* (ed. by A Menini), pp. 157–180. CRC Press, Boca Raton, FL, USA.

Bridle JR & Ritchie MG (2001) Assortative mating and the genic view of speciation - Commentary. *Journal of Evolutionary Biology* 14: 878–879.

Buck L & Axel R (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65: 175–187.

Bushdid C, Magnasco MO, Vosshall LB & Keller A (2014) Humans can discriminate more than 1 trillion olfactory stimuli. *Science* 343: 1370–1372.

Butenandt A, Beckmann R & Hecker E (1961a) Über den Sexuallockstoff des Seidenspinners. I. Der biologische Test und die Isolierung des reinen Sexuallockstoffes Bombykol. *Biological Chemistry* 324: 71–83.

Butenandt A, Beckmann R & Hecker E (1961b) Über den Sexuallockstoff des Seidenspinners. II. Konstitution und Konfiguration des Bombykols. *Biological Chemistry* 324: 84–87.

Butenandt A, Beckmann R, Stamm D & Hecker E (1959) Über den Sexual-Lockstoff des Seidenspinners *Bombyx mori*. Reindarstellung und Konstitution. *Zeitschrift für Naturforschung* 14b: 283–284.

Cardé RT & Haynes KF (2004) Structure of the pheromone communication channel in moths. *Advances in Insect Chemical Ecology* (ed. by RT Cardé & JG Millar), pp. 283–332. Cambridge University Press, Cambridge, UK.

Caro SP, Balthazart J & Bonadonna F (2015) The perfume of reproduction in birds: chemosignaling in avian social life. *Hormones and Behavior* 68: 25–42.

Carson HL, Hardy DE, Spieth HT & Stone WS (1970) The evolutionary biology of the Hawaiian Drosophilidae. *Essays in Evolution and Genetics in Honor of Theodosius Dobzhansky – a Supplement to Evolutionary Biology* (ed. by MK Hecht & WC Steere), pp. 437–543. North-Holland Publishing, Amsterdam, The Netherlands.

Chin JSR, Ellis SR, Pham HT, Blanksby SJ, Mori K et al. (2014) Sex-specific triacylglycerides are widely conserved in Drosophila and mediate mating behavior. *eLife* 3: e01751.

Chou YH, Spletter ML, Yaksi E, Leong JCS, Wilson RI & Luo LQ (2010) Diversity and wiring variability of olfactory local interneurons in the *Drosophila* antennal lobe. *Nature Neuroscience* 13: 439–449.

Clarke DJ, George SG & Burchell B (1991) Glucuronidation in fish. *Aquatic Toxicology* 20: 35–56.

Clyne P, Grant A, O'Connell R & Carlson JR (1997) Odorant response of individual sensilla on the *Drosophila* antenna. *Invertebrate Neuroscience* 3: 127–135.

Cobb M, Burnet B, Blizard R & Jallon JM (1989) Courtship in *Drosophila sechellia* - its structure, functional aspects, and relationship to those of other members of the *Drosophila melanogaster* species subgroup. *Journal of Insect Behavior* 2: 63–89.

Couto A, Alenius M & Dickson BJ (2005) Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. *Current Biology* 15: 1535–1547.

Coyne JA (1992) Genetics of sexual isolation in females of the *Drosophila simulans* species complex. *Genetical Research* 60: 25–31.

Coyne JA & Orr HA (2004) *Speciation*. Sinauer Associates, Sunderland, MA, USA.

Crowley-Gall A, Date P, Han C, Rhodes N, Andolfatto P et al. (2016) Population differences in olfaction accompany host shift in *Drosophila mojavensis*. *Proceedings of the Royal Society B* 283: 20161562.

Dahanukar A & Ray A (2011) Courtship, aggression and avoidance: pheromones, receptors and neurons for social behaviors in *Drosophila*. *Fly* 5: 58–63.

Das S, Trona F, Khallaf MA, Schuh E, Knaden M et al. (2017) Electrical synapses mediate synergism between pheromone and food odors in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA* 114: E9962–E9971.

Date P, Dweck HKM, Stensmyr MC, Shann J, Hansson BS & Rollmann SM (2013) Divergence in olfactory host plant preference in *D. mojavensis* in response to cactus host use. *PLoS ONE* 8: e70027.

Datta SR, Vasconcelos ML, Ruta V, Luo S, Wong A et al. (2008) The *Drosophila* pheromone cVA activates a sexually dimorphic neural circuit. *Nature* 452: 473–477.

Davis RL (1993) Mushroom bodies and *Drosophila* learning. *Neuron* 11: 1–14.

Dickson BJ (2008) Wired for sex: the neurobiology of *Drosophila* mating decisions. *Science* 322: 904–909.

Ding Y, Berrocal A, Morita T, Longden KD & Stern DL (2016) Natural courtship song variation caused by an intronic retroelement in an ion channel gene. *Nature* 536: 329–332.

Ding Y, Lillvis JL, Cande J, Berman GJ, Arthur BJ et al. (2019) Neural evolution of context-dependent fly song. *Current Biology* 29: 1089–1099.

Dolan M-J, Frechter S, Bates AS, Dan C, Huoviala P et al. (2019) Neurogenetic dissection of the *Drosophila* lateral horn reveals major outputs, diverse behavioural functions, and interactions with the mushroom body. *eLife* 8: e43079.

Dumenil C, Woud D, Pinto F, Alkema JT, Jansen I et al. (2016) Pheromonal cues deposited by mated females convey social information about egg-laying sites in *Drosophila melanogaster*. *Journal of Chemical Ecology* 42: 259–269.

Dweck HKM, Ebrahim SAM, Thoma M, Mohamed AAM, Keesey IW et al. (2015) Pheromones mediating copulation and attraction in *Drosophila*. *Proceedings of the National Academy of Sciences of the USA* 112: E2829–E2835.

Ejima A, Smith BPC, Lucas C, van der Goes van Naters W, Miller CJ et al. (2007) Generalization of courtship learning in *Drosophila* is mediated by *cis*-vaccenyl acetate. *Current Biology* 17: 599–605.

Endler JA & Basolo AL (1998) Sensory ecology, receiver biases and sexual selection. *Trends in Ecology & Evolution* 13: 415–420.

Erezyilmaz DF & Stern DL (2013) Pupariation site preference within and between *Drosophila* sibling species. *Evolution* 67: 2714–2727.

Etges WJ (1998) Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. IV. Correlated responses in behavioral isolation to artificial selection on a life-history trait. *American Naturalist* 152: 129–144.

Etges WJ (2019) Evolutionary genomics of host plant adaptation: insights from *Drosophila*. *Current Opinion in Insect Science* 36: 96–102.

Etges WJ, Over KF, de Oliveira CC & Ritchie MG (2006) Inheritance of courtship song variation among geographically isolated populations of *Drosophila mojavensis*. *Animal Behaviour* 71: 1205–1214.

Fabre JH (1916) *The Life of the Caterpillar*. Dodd, Mead and Company, New York, NY, USA.

Fiala A (2007) Olfaction and olfactory learning in *Drosophila*: recent progress. *Current Opinion in Neurobiology* 17: 720–726.

Frechter S, Bates AS, Tootoonian S, Dolan M-J, Manton JD et al. (2019) Functional and anatomical specificity in a higher olfactory centre. *eLife* 8:e44590.

Funk DJ, Filchak KE & Feder JL (2002) Herbivorous insects: model systems for the comparative study of speciation ecology. *Genetica* 116: 251–267.

Funk DJ, Nosil P & Etges WJ (2006) Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences of the USA* 103: 3209–3213.

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

Galizia CG, Sachse S, Rappert A & Menzel R (1999b) The glomerular code for odor representation is species specific in the honeybee *Apis mellifera*. *Nature Neuroscience* 2: 473–478.

Gao Q, Yuan BB & Chess A (2000) Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. *Nature Neuroscience* 3: 780–785.

van der Goes van Naters W & Carlson JR (2007) Receptors and neurons for fly odors in *Drosophila*. *Current Biology* 17: 606–612.

Goldman-Huertas B, Mitchell RF, Lapoint RT, Faucher CP, Hildebrand JG & Whiteman NK (2015) Evolution of herbivory in *Drosophilidae* linked to loss of behaviors, antennal responses, odorant receptors, and ancestral diet. *Proceedings of the National Academy of Sciences of the USA* 112: 3026–3031.

Goodwin M, Gooding KM & Regnier F (1979) Sex pheromone in the dog. *Science* 203: 559–561.

Grabe V, Baschwitz A, Dweck HKM, Lavista-Llanos S, Hansson BS & Sachse S (2016) Elucidating the neuronal architecture of olfactory glomeruli in the *Drosophila* antennal lobe. *Cell Reports* 16: 3401–3413.

Grabe V & Sachse S (2018) Fundamental principles of the olfactory code. *Biosystems* 164: 94–101.

Grafen A (1990) Biological signals as handicaps. *Journal of Theoretical Biology* 144: 517–546.

de Groot JH, Smeets MA, Kaldewaij A, Duijndam MJ & Semin GR (2012) Chemosignals communicate human emotions. *Psychological Science* 23: 1417–1424.

Grosjean Y, Rytz R, Farine JP, Abuin L, Cortot J et al. (2011) An olfactory receptor for food-derived odours promotes male courtship in *Drosophila*. *Nature* 478: 236–240.

Gross BL & Rieseberg LH (2005) The ecological genetics of homoploid hybrid speciation. *Journal of Heredity* 96: 241–252.

Guo S & Kim J (2007) Molecular evolution of *Drosophila* odorant receptor genes. *Molecular Biology and Evolution* 24: 1198–1207.

Ha TS & Smith DP (2006) A pheromone receptor mediates 11-*cis*-vaccenyl acetate-induced responses in *Drosophila*. *Journal of Neuroscience* 26: 8727–8733.

Hansson BS (1999) *Insect Olfaction*. Springer, Berlin, Germany.

Hardie J, Storer JR, Nottingham SF, Peace L, Harrington R et al. (1994) The interaction of sex pheromone and plant volatiles for field attraction of male bird-cherry aphid, *Rhopalosiphum padi*. *Brighton Crop Protection Conference - Pests and Diseases 1994*: 1223–1230.

Hardin PE, Hall JC & Rosbash M (1990) Feedback of the *Drosophila* period gene-product on circadian cycling of its messenger-RNA levels. *Nature* 343: 536–540.

Haverkamp A, Hansson BS & Knaden M (2018) Combinatorial codes and labeled lines: how insects use olfactory cues to find and judge food, mates, and oviposition sites in complex environments. *Frontiers in Physiology* 9: 49.

Heimbeck G, Bugnon V, Gendre N, Keller A & Stocker RF (2001) A central neural circuit for experience-independent olfactory and courtship behavior in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA* 98: 15336–15341.

Heisenberg M (2003) Mushroom body memoir: from maps to models. *Nature Reviews Neuroscience* 4: 266–275.

Homberg U, Christensen TA & Hildebrand JG (1989) Structure and function of the deutocerebrum in insects. *Annual Review of Entomology* 34: 477–501.

Hong RL, Riebesell M, Bumbarger DJ, Cook SJ, Carstensen HR et al. (2019) Evolution of neuronal anatomy and circuitry in two highly divergent nematode species. *eLife* 8: e47155.

Ishikawa Y, Takanashi T, Kim C, Hoshizaki S, Tatsuki S & Huang Y (1999) *Ostrinia* spp. in Japan: their host plants and sex pheromones. *Entomologia Experimentalis et Applicata* 91: 237–244.

Jallon JM, Antony C & Benamar O (1981) Un anti-aphrodisiaque produit par les males de *Drosophila melanogaster*. *Comptes Rendus de l'Académie des Sciences* 292: 1147–1149.

Jefferis GSXE, Potter CJ, Chan AI, Marin EC, Rohlfing T et al. (2007) Comprehensive maps of *Drosophila* higher olfactory centers: spatially segregated fruit and pheromone representation. *Cell* 128: 1187–1203.

Jeong PY, Jung M, Yim YH, Kim H, Park M et al. (2005) Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-inducing pheromone. *Nature* 433: 541–545.

Jezovit JA, Levine JD & Schneider J (2017) Phylogeny, environment and sexual communication across the *Drosophila* genus. *Journal of Experimental Biology* 220: 42–52.

Johansson BG & Jones TM (2007) The role of chemical communication in mate choice. *Biological Reviews* 82: 265–289.

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

de Jong MCM & Sabelis MW (1991) Limits to runaway sexual selection: the wallflower paradox. *Journal of Evolutionary Biology* 4: 637–655.

Karlson P & Luscher M (1959) Pheromones: a new term for a class of biologically active substances. *Nature* 183: 55–56.

Keesey IW, Grabe V, Gruber L, Koerte S, Obiero GF et al. (2019) Inverse resource allocation between vision and olfaction across the genus *Drosophila*. *Nature Communications* 10: 1162.

Khallaf MA, Auer TO, Grabe V, Depetris-Chauvin A, Ammagarahalli B et al. (2020) Mate discrimination among subspecies through a conserved olfactory pathway. *Science Advances* 6: eaba5279.

Khallaf MA, Cui R, Weissflog J, Erdogmus M, Svatos A et al. (2021) Large-scale characterization of sex pheromone communication systems in *Drosophila*. *Nature Communications* 12: 4165.

Knowles LL & Markow TA (2001) Sexually antagonistic coevolution of a postmating-prezygotic reproductive character in desert *Drosophila*. *Proceedings of the National Academy of Sciences of the USA* 98: 8692–8696.

Kohl J, Huoviala P & Jefferis GSXE (2015) Pheromone processing in *Drosophila*. *Current Opinion in Neurobiology* 34: 149–157.

Krebs RA & Markow TA (1989) Courtship behavior and control of reproductive isolation in *Drosophila mojavensis*. *Evolution* 43: 908–913.

Krieger J, Sandeman RE, Sandeman DC, Hansson BS & Harzsch S (2010) Brain architecture of the largest living land arthropod, the giant robber crab *Birgus latro* (Crustacea, Anomura, Coenobitidae): evidence for a prominent central olfactory pathway? *Frontiers in Zoology* 7: 25.

Kurtovic A, Widmer A & Dickson BJ (2007) A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone. *Nature* 446: 542–546.

LaRue KM, Clemens J, Berman GJ & Murthy M (2015) Acoustic duetting in *Drosophila virilis* relies on the integration of auditory and tactile signals. *eLife* 4: e07277.

Lassance JM & Löfstedt C (2009) Concerted evolution of male and female display traits in the European corn borer, *Ostrinia nubilalis*. *BMC Biology* 7: 10.

Leal WS, Wojtasek H, Picimbon JF, Kuwahara S, Saito H & Hasegawa M (1998) Perireceptor events in pheromone perception in scarab beetles. *Journal of Asia-Pacific Entomology* 1: 1–8.

Lebreton S, Borrero-Echeverry F, Gonzalez F, Solum M, Wallin EA et al. (2017) A *Drosophila* female pheromone elicits species-specific long-range attraction via an olfactory channel with dual specificity for sex and food. *BMC Biology* 15: 88.

Lebreton S, Grabe V, Omondi AB, Ignell R, Becher PG et al. (2014) Love makes smell blind: mating suppresses pheromone attraction in *Drosophila* females via Or65a olfactory neurons. *Scientific Reports* 4: 7119.

Lebreton S, Trona F, Borrero-Echeverry F, Bilz F, Grabe V et al. (2015) Feeding regulates sex pheromone attraction and courtship in *Drosophila* females. *Scientific Reports* 5: 13132.

Legal L, David JR & Jallon JM (1992) Toxicity and attraction effects produced by *Morinda citrifolia* fruits on the *Drosophila melanogaster* complex of species. *Chemoecology* 3: 125–129.

Lemaitre B, Nicolas E, Michaut L, Reichhart JM & Hoffmann JA (1996) The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. *Cell* 86: 973–983.

Lewis EB (1978) Gene complex controlling segmentation in *Drosophila*. *Nature* 276: 565–570.

Lewis LP, Siju KP, Aso Y, Friedrich AB, Bulteel AJ et al. (2015) A higher brain circuit for immediate integration of conflicting sensory information in *Drosophila*. *Current Biology* 25: 2203–2214.

Lim MM, Wang Z, Olazabal DE, Ren X, Terwilliger EF & Young LJ (2004) Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene. *Nature* 429: 754–757.

Lin CC & Potter CJ (2015) Re-classification of *Drosophila melanogaster* trichoid and intermediate sensilla using fluorescence-guided single sensillum recording. *PLoS ONE* 10: e0139675.

Lin S, Owald D, Chandra V, Talbot C, Huetteroth W & Waddell S (2014) Neural correlates of water reward in thirsty *Drosophila*. *Nature Neuroscience* 17: 1536.

Linz J, Baschwitz A, Strutz A, Dweck HK, Sachse S et al. (2013) Host plant-driven sensory specialization in *Drosophila erecta*. *Proceedings of the Royal Society B* 280: 20130626.

Liou NF, Lin SH, Chen YJ, Tsai KT, Yang CJ et al. (2018) Diverse populations of local interneurons integrate into the *Drosophila* adult olfactory circuit. *Nature Communications* 9: 2232.

Liu WW, Liang XH, Gong JX, Yang Z, Zhang YH et al. (2011) Social regulation of aggression by pheromonal activation of Or65a olfactory neurons in *Drosophila*. *Nature Neuroscience* 14: 896–902.

Liu X, Zwiebel LJ, Hinton D, Benzer S, Hall JC & Rosbash M (1992) The period gene encodes a predominantly nuclear-protein in adult *Drosophila*. *Journal of Neuroscience* 12: 2735–2744.

Mackay TFC, Heinsohn SL, Lyman RF, Moehring AJ, Morgan TJ & Rollmann SM (2005) Genetics and genomics of *Drosophila* mating behavior. *Proceedings of the National Academy of Sciences of the USA* 102: 6622–6629.

Malnic B, Hirono J, Sato T & Buck LB (1999) Combinatorial receptor codes for odors. *Cell* 96: 713–723.

Manoli DS, Foss M, Villella A, Taylor BJ, Hall JC & Baker BS (2005) Male-specific fruitless specifies the neural substrates of *Drosophila* courtship behaviour. *Nature* 436: 395–400.

Maresh A, Gil DR, Whitman MC & Greer CA (2008) Principles of glomerular organization in the human olfactory bulb - implications for odor processing. *PLoS ONE* 3: e2640.

Markow TA & O'Grady PM (2005) Evolutionary genetics of reproductive behavior in *Drosophila*: connecting the dots. *Annual Review of Genetics* 39: 263–291.

Martin J, Moreira PL & Lopez P (2007) Status-signalling chemical badges in male Iberian rock lizards. *Functional Ecology* 21: 568–576.

Martínez-Ruiz C & Knell RJ (2017) Sexual selection can both increase and decrease extinction probability: reconciling demographic and evolutionary factors. *Journal of Animal Ecology* 86: 117–127.

Matzkin LM (2014) Ecological genomics of host shifts in *Drosophila mojavensis*. *Ecological Genomics: Ecology and the Evolution of Genes and Genomes* (ed. by CR Landry & N Aubin-Horth), pp. 233–247. Springer, Dordrecht, The Netherlands.

Matzkin LM & Markow TA (2013) Transcriptional differentiation across the four cactus host races of *Drosophila mojavensis*. *Speciation: Natural Processes, Genetics and Biodiversity* (ed. by P Michalak), pp. 119–136. Nova Science Publishers, Hauppauge, NY, USA.

Mayr E (1947) Ecological factors in speciation. *Evolution* 1: 263–288.

Mayr E (1963) *Animal Species and Evolution*. Harvard University Press, Cambridge, MA, USA.

Menashe I, Man O, Lancet D & Gilad Y (2003) Different noses for different people. *Nature Genetics* 34: 143–144.

Menini A (2010) *The Neurobiology of Olfaction*. CRC Press, Boca Raton, FL, USA.

Messina FJ (2004) Predictable modification of body size and competitive ability following a host shift by a seed beetle. *Evolution* 58: 2788–2797.

Meunier N & Rampin O (2017) The nuts and bolts of olfaction. *Olfaction in Animal Behaviour and Welfare* (ed. by BL Nielsen), pp. 1–15. CABI, Wallingford, UK.

Min SH, Ai MR, Shin SA & Suh GSB (2013) Dedicated olfactory neurons mediating attraction behavior to ammonia and amines in *Drosophila*. *Proceedings of the National Academy of Sciences of the USA* 110: E1321–E1329.

Miyamichi K, Amat F, Moussavi F, Wang C, Wickersham I et al. (2011) Cortical representations of olfactory input by trans-synaptic tracing. *Nature* 472: 191–196.

Mohamed AAM, Retzke T, Das Chakraborty S, Fabian B, Hansson BS et al. (2019) Odor mixtures of opposing valence unveil inter-glomerular crosstalk in the *Drosophila* antennal lobe. *Nature Communications* 10: 1201.

Mombaerts P, Wang F, Dulac C, Vassar R, Chao SK et al. (1996) The molecular biology of olfactory perception. *Cold Spring Harbor Symposia on Quantitative Biology* 61: 135–145.

Moreteau B, Rkha S & David JR (1994) Genetics of a nonoptimal behaviour – oviposition preference of *Drosophila mauritiana* for a toxic resource. *Behavior Genetics* 24: 433–441.

Morgan TH (1910) Sex limited inheritance in *Drosophila*. *Science* 32: 120–122.

Muller H j & Altenburg E (1930) The frequency of translocations produced by x-rays in *Drosophila*. *Genetics* 15: 283–311.

Nemeth DC, Ammagarahalli B, Layne JE & Rollmann SM (2018) Evolution of coeloconic sensilla in the peripheral olfactory system of *Drosophila mojavensis*. *Journal of Insect Physiology* 110: 13–22.

Newby BD & Etges WJ (1998) Host preference among populations of *Drosophila mojavensis* (Diptera: Drosophilidae) that use different host cacti. *Journal of Insect Behavior* 11: 691–712.

Newcomb JM & Katz PS (2009) Different functions for homologous serotonergic interneurons and serotonin in species-specific rhythmic behaviours. *Proceedings of the Royal Society B* 276: 99–108.

Nielsen BL (2017) *Olfaction in Animal Behaviour and Welfare*. CABI, Wallingford, UK.

Nosil P, Crespi BJ & Sandoval CP (2003) Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. *Proceedings of the Royal Society B 270*: 1911–1918.

Novotny MV (2003) Pheromones, binding proteins and receptor responses in rodents. *Biochemical Society Transactions 31*: 117–122.

Novotny MV, Xie TM, Harvey S, Wiesler D, Jemiolo B & Carmack M (1995) Stereoselectivity in mammalian chemical communication: male mouse pheromones. *Experientia 51*: 738–743.

Nozawa M & Nei M (2007) Evolutionary dynamics of olfactory receptor genes in *Drosophila* species. *Proceedings of the National Academy of Sciences of the USA 104*: 7122–7127.

Nüsslein-Volhard C & Wieschaus E (1980) Mutations affecting segment number and polarity in *Drosophila*. *Nature 287*: 795–801.

O'Grady PM & Markow TA (2012) Rapid morphological, behavioral, and ecological evolution in *Drosophila*: comparisons between the endemic Hawaiian *Drosophila* and the cactophilic repleta species group. *Rapidly Evolving Genes and Genetic Systems* (ed. by RS Singh, JP Xu & RJ Kulathinal), pp. 176–186. Oxford University Press, Oxford, UK.

Pfeiler E, Castrezana S, Reed LK & Markow TA (2009) Genetic, ecological and morphological differences among populations of the cactophilic *Drosophila mojavensis* from southwestern USA and northwestern Mexico, with descriptions of two new subspecies. *Journal of Natural History 43*: 923–938.

Pickett JA, Wadhams LJ, Woodcock CM & Hardie J (1992) The chemical ecology of aphids. *Annual Review of Entomology 37*: 67–90.

Price JL, Blau J, Rothenfluh A, Abodeely M, Kloss B & Young MW (1998) Double-time is a novel *Drosophila* clock gene that regulates PERIOD protein accumulation. *Cell 94*: 83–95.

Prieto-Godino LL, Rytz R, Cruchet S, Bargeton B, Abuin L et al. (2017) Evolution of acid-sensing olfactory circuits in drosophilids. *Neuron 93*: 661–667.

Proffit M, Khallaf MA, Carrasco D, Larsson MC & Anderson P (2015) 'Do you remember the first time?' Host plant preference in a moth is modulated by experiences during larval feeding and adult mating. *Ecology Letters 18*: 365–374.

R'Kha S, Capy P & David JR (1991) Host-plant specialization in the *Drosophila melanogaster* species complex: a physiological, behavioural, and genetical analysis. *Proceedings of the National Academy of Sciences of the USA 88*: 1835–1839.

Ritchie MG (2007) Sexual selection and speciation. *Annual Review of Ecology, Evolution, and Systematics 38*: 79–102.

Ruiz A, Heed WB & Wasserman M (1990) Evolution of the *mojavensis* cluster of cactophilic *Drosophila* with descriptions of two new species. *Journal of Heredity 81*: 30–42.

Rundle HD & Nosil P (2005) Ecological speciation. *Ecology Letters 8*: 336–352.

Ruta V, Datta SR, Vasconcelos ML, Freeland J, Looger LL & Axel R (2010) A dimorphic pheromone circuit in *Drosophila* from sensory input to descending output. *Nature 468*: 686–690.

Schachtner J, Schmidt M & Homberg U (2005) Organization and evolutionary trends of primary olfactory brain centers in Tetraconata (Crustacea plus Hexapoda). *Arthropod Structure & Development 34*: 257–299.

Schluter D (2000) Ecological character displacement in adaptive radiation. *American Naturalist 156*: S4–S16.

Schoralkova T, Kratochvil L & Kubicka L (2018) Female sexual attractiveness and sex recognition in leopard gecko: males are indiscriminate courters. *Hormones and Behavior 99*: 57–61.

Seeholzer LF, Seppo M, Stern DL & Ruta V (2018) Evolution of a central neural circuit underlies *Drosophila* mate preferences. *Nature 559*: 564–569.

Seki Y, Rybak J, Wicher D, Sachse S & Hansson BS (2010) Physiological and morphological characterization of local interneurons in the *Drosophila* antennal lobe. *Journal of Neurophysiology 104*: 1007–1019.

Senturk M & Bellen HJ (2018) Genetic strategies to tackle neurological diseases in fruit flies. *Current Opinion in Neurobiology 50*: 24–32.

Shang Y, Wu Y, Yao SL, Wang XJ, Feng D & Yang WQ (2007) Protective effect of erythropoietin against ketamine-induced apoptosis in cultured rat cortical neurons: involvement of PI3K/Akt and GSK-3 beta pathway. *Apoptosis 12*: 2187–2195.

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

Siwicki KK, Eastman C, Petersen G, Rosbash M & Hall JC (1988) Antibodies to the period gene product of *Drosophila* reveal diverse tissue distribution and rhythmic changes in the visual system. *Neuron 1*: 141–150.

Smadja C & Butlin RK (2009) On the scent of speciation: the chemosensory system and its role in premating isolation. *Heredity 102*: 77–97.

Sosulski DL, Bloom ML, Cutforth T, Axel R & Datta SR (2011) Distinct representations of olfactory information in different cortical centres. *Nature 472*: 213–216.

Spieth HT (1952) Mating behavior within the genus *Drosophila* (Diptera). *Bulletin of the American Museum of Natural History 99*: 401–474.

Steele RH (1986) Courtship feeding in *Drosophila subobscura*. 1. The nutritional significance of courtship feeding. *Animal Behaviour 34*: 1087–1098.

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

Strutz A, Soelter J, Baschwitz A, Farhan A, Grabe V et al. (2014) Decoding odor quality and intensity in the Drosophila brain. *eLife 3*: e04147.

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

Symonds MR & Elgar MA (2008) The evolution of pheromone diversity. *Trends in Ecology & Evolution 23*: 220–228.

Symonds MRE & Wertheim B (2005) The mode of evolution of aggregation pheromones in *Drosophila* species. *Journal of Evolutionary Biology 18*: 1253–1263.

Tanaka NK, Awasaki T, Shimada T & Ito K (2004) Integration of chemosensory pathways in the *Drosophila* second-order olfactory centers. *Current Biology 14*: 449–457.

Tanaka R, Higuchi T, Kohatsu S, Sato K & Yamamoto D (2017) Optogenetic activation of the fruitless-labeled circuitry in *Drosophila subobscura* males induces mating motor acts. *Journal of Neuroscience 37*: 11662–11674.

Thomas ML & Simmons LW (2011) Crickets detect the genetic similarity of mating partners via cuticular hydrocarbons. *Journal of Evolutionary Biology 24*: 1793–1800.

Turelli M, Barton NH & Coyne JA (2001) Theory and speciation. *Trends in Ecology & Evolution 16*: 330–343.

Vines TH & Schluter D (2006) Strong assortative mating between allopatric sticklebacks as a by-product of adaptation to different environments. *Proceedings of the Royal Society B 273*: 911–916.

Vosshall LB, Price JL, Sehgal A, Saez L & Young MW (1994) Block in nuclear-localization of *period* protein by a second clock mutation, *timeless*. *Science 263*: 1606–1609.

Vosshall LB & Stocker RE (2007) Molecular architecture of smell and taste in *Drosophila*. *Annual Review of Neuroscience 30*: 505–533.

Vosshall LB, Wong AM & Axel R (2000) An olfactory sensory map in the fly brain. *Cell 102*: 147–159.

Waddell S (2013) Reinforcement signalling in *Drosophila*; dopamine does it all after all. *Current Opinion in Neurobiology 23*: 324–329.

Wang L & Anderson DJ (2010) Identification of an aggression-promoting pheromone and its receptor neurons in *Drosophila*. *Nature 463*: 227–231.

Whitten WK (1959) Occurrence of anoestrus in mice caged in groups. *Journal of Endocrinology 18*: 102–107.

Whitten WK (1966) Pheromones and mammalian reproduction. *Advances in Reproductive Physiology 1*: 155–177.

Wyatt TD (2010) Pheromones and signature mixtures: defining species-wide signals and variable cues for identity in both invertebrates and vertebrates. *Journal of Comparative Physiology A 196*: 685–700.

Wyatt TD (2014) Introduction to chemical signaling in vertebrates and invertebrates. *Neurobiology of Chemical Communication* (ed. by C Mucignat-Caretta), pp. 1–22. CRC Press, Boca Raton, FL, USA.

Wyatt TD (2017) Pheromones. *Current Biology 27*: R739–R743.

Yang CJ, Tsai KT, Liou NF & Chou YH (2019) Interneuron diversity: toward a better understanding of interneuron development in the olfactory system. *Journal of Experimental Neuroscience 13*: 1–5.

Yu JY, Kanai MI, Demir E, Jefferis GS & Dickson BJ (2010) Cellular organization of the neural circuit that drives *Drosophila* courtship behavior. *Current Biology 20*: 1602–1614.

Zakir A, Khallaf MA, Hansson BS, Witzgall P & Anderson P (2017) Herbivore-induced changes in cotton modulates reproductive behavior in the moth *Spodoptera littoralis*. *Frontiers in Ecology and Evolution 5*: 49.

Zehring WA, Wheeler DA, Reddy P, Konopka RJ, Kyriacou CP et al. (1984) P-element transformation with *period* locus DNA restores rhythmicity to mutant, arrhythmic *Drosophila melanogaster. Cell 39*: 369–376.

Zhang J, Jin H, Zhang WY, Ding C, O'Keeffe S et al. (2019) Sour sensing from the tongue to the brain. *Cell 179*: 392–402.

Zhao Z & McBride CS (2020) Evolution of olfactory circuits in insects. *Journal of Comparative Physiology A 206*: 353–367.

Zouros E & Dentremont CJ (1980) Sexual isolation among populations of *Drosophila mojavensis* – response to pressure from a related species. *Evolution 34*: 421–430.

Zube C, Kleineidam CJ, Kirschner S, Neef J & Rossler W (2008) Organization of the olfactory pathway and odor processing in the antennal lobe of the ant *Camponotus floridanus*. *Journal of Comparative Neurology 506*: 425–441.