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Drosophila as a holistic model for insect pheromone signaling and processing

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In recent years, research into the chemical ecology of the vinegar fly, *Drosophila melanogaster*, has yielded a wealth of information on the neural substrates that detect and process pheromones and control behavior. The studies reveal at the cellular and molecular level how behavioral responses to pheromones are initiated and modulated by social, environmental, and physiological factors. By taking into account both the complexity of the chemical world and the intricacies of the animal's physiological state, the emerging holistic perspective provides insight not only into chemical communication but more generally, how organisms balance internal and external signals when making behavioral decisions.

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Introduction

The identification of pheromones and their influence on behavior have been studied in *Drosophila* since 1969 [1,2]. Early studies largely focused on the characterization of pheromone molecules and the genetic control of pheromone production [3]. The advent of next generation sequencing technologies and improved methods for chemical detection have accelerated the rate of both receptor and pheromone discovery. Together with the development of automated systems for behavioral analysis [4] and extensive libraries of genetic tools for neural circuit mapping [5], the cellular and molecular pathways underlying chemical communication can now be examined with exquisite spatial and temporal resolution. In this review, we summarize recent discoveries of pheromones and receptors in *Drosophila* and propose how research using *Drosophila* provides a holistic model for chemical communication that integrates pheromone perception, other channels of sensory information, and the physiological state of the animal.

Behavior, pheromones, and receptors

Until recently, the known pheromones of *Drosophila melanogaster* consisted of cuticular hydrocarbons (CHCs) produced in the oenocytes, and a male specific lipid, (*Z*)-11-octadecenyl acetate (cVA), produced in the ejaculatory bulb (EB) [6,7]. The use of alternative analytical methods has revealed other chemical classes of pheromones, including medium-length fatty acids (FAs) and high-molecular weight lipids [8,9•,10••,11]. Similarly, the receptors that detect these pheromones were thought to be mainly heteromeric ligand-gated cation channels and were divided into olfactory receptors (Ors) and gustatory receptors (Grs) [12–14]. Recent bioinformatics and genetic screens have identified two new classes of receptors that respond to pheromones [15•]: ionotropic glutamate receptors (Irs), which are expressed in the antenna as well as gustatory organs in *Drosophila* [16,17], and members of the degenerin/epithelial sodium channel subunits (*ppk* genes) [18]. These new discoveries in *Drosophila* highlight the complexity of pheromone signaling and response within a single species.

Aphrodisiacs and attractants

Early studies using thin layer chromatography and gas chromatography identified the aphrodisiacs cVA and (*Z*)-7-tricosene (7-T) in males, as well as (*Z*,*Z*)-7,11-heptacosadiene (7,11-HD) and (*Z*,*Z*)-7,11-nonacosadiene (7,11-ND) in females. cVA acts through the receptor Or67d in females to promote mating behavior but also inhibits mating behavior in males [19] and promotes aggression (see below). Recently identified members of the ppk family, ppk23, ppk25 and ppk29, are involved in the detection of male and female pheromones, including 7,11-HD [20–23]. The olfactory receptors Or88a and Or47b have been known since 2007 to respond electrophysiologically to cuticular signals [24] though it was only recently that the ligands were identified as medium length fatty acids and their methyl esters, including palmitoleic acid, palmitoleic acid, myristic acid, myristoleic acid, methyl laurate, methyl myristate and methyl palmitate [9•,10••]. Despite these recent discoveries, our picture of the pheromonal component in *Drosophila* courtship is still incomplete as there are several receptors that affect courtship but the ligands are unknown, including Gr32a [25], Gr33a [26] and Gr39a [27]. Gr33a may function as a co-receptor for other Grs since it is broadly expressed and responds to a number of bitter tastants. In addition, an ionotropic receptor, Ir52c, is shown to promote male courtship behavior but its ligand(s) is also unknown [17].

In addition to mate attraction, chemosensory signals play a significant role in species identification, as shown by both manipulations of oenocyte and receptor function. Removing most of the cuticular pheromones by genetic ablation of the oenocytes results in cross-species courtship [28]. Strikingly, inactivation of a gustatory receptor, Gr32a, leads to heterospecific courtship between *D. melanogaster* and distant species such as *D. virilis* [25]. Though the ligand(s) have not been definitively identified by electrophysiology, behavioral experiments indicate that 7-T, (*Z*)-9-tricosene (9-T), and (*Z*)-11-pentacosene function as aversive molecules that function through Gr32a to inhibit inter-species mating [25]. Some classes of CHCs, such as methyl-branched CHCs (mbCHCs) can play a role in both environmental adaptation as well as mating success in a single species of *Drosophila*, suggesting that ecological adaptation can drive speciation via pheromonal diversification [29].

Anti-aphrodisiacs

In many insect taxa, males mark females with an anti-aphrodisiac pheromone during mating in order to deter other potential mates [7]. cVA has been known to serve this function in *Drosophila* since it was identified in 1970 [30]. With the application of new analytical methods, a second male-specific pheromone, (*R,Z,Z*)-3-acetoxy-11,19-octacosadien-1-ol (CH503), was discovered in 2009 [11]. The anti-aphrodisiac is conserved in other drosophilids and remarkably, functions as a broad courtship inhibitor across numerous species, even in species that do not produce the molecule [31]. The gustatory receptor Gr68a was shown recently to respond to CH503 [32•]. A new chemical class of pheromones, triacylglycerides (TAGs), was also identified across numerous *Drosophila* subgroups and shown to function as anti-aphrodisiac [8]. The receptors for TAGs remain unknown.

Aggression

In *D. melanogaster*, the first pheromone shown to promote aggression is cVA [33]. cVA is detected by both Or67d [34] and Or65a [35,36], depending on social context. The former mediates aggression during acute encounters between males whereas the latter mediates prolonged cVA exposure, which occurs during prolonged social grouping. While cVA may enhance aggression, other components of the male pheromone bouquet are needed to trigger an attack [37]. Both 7-T and cVA regulate aggression in a hierarchical manner with sensitivity to 7-T dominant to cVA in eliciting aggressive responses [38]. Consistent with these findings, the putative receptor for 7-T, Gr32a, is important for the perception of pheromonal cues that induce male–male aggression [39].

Aggregation

Aggregation provides benefits for larval and adult flies, allowing food to be processed more efficiently [40] and offering protection in numbers from predators. The first pheromone identified in *Drosophila*, cVA, has long been known to induce aggregation in both males and females [41]. Recently, other aggregation pheromones have been found for larval and adult animals. The medium length fatty acids (*Z*)-5-tetradecenoic acid and (*Z*)-7-tetradecenoic acid are released by *D. melanogaster* larvae, induce aggregation of other larvae. ppk23 and ppk29 respond to one of these molecules, (*Z*)-7-tetradecenoic acid, though the ppks appear to have broader specificity for classes of molecules rather than specific ligands [42]. In adults, 9-T is deposited by males upon stimulation with food odor and induces aggregation of males and females as well as increasing female oviposition. 9-T is detected by Or7a [43•].

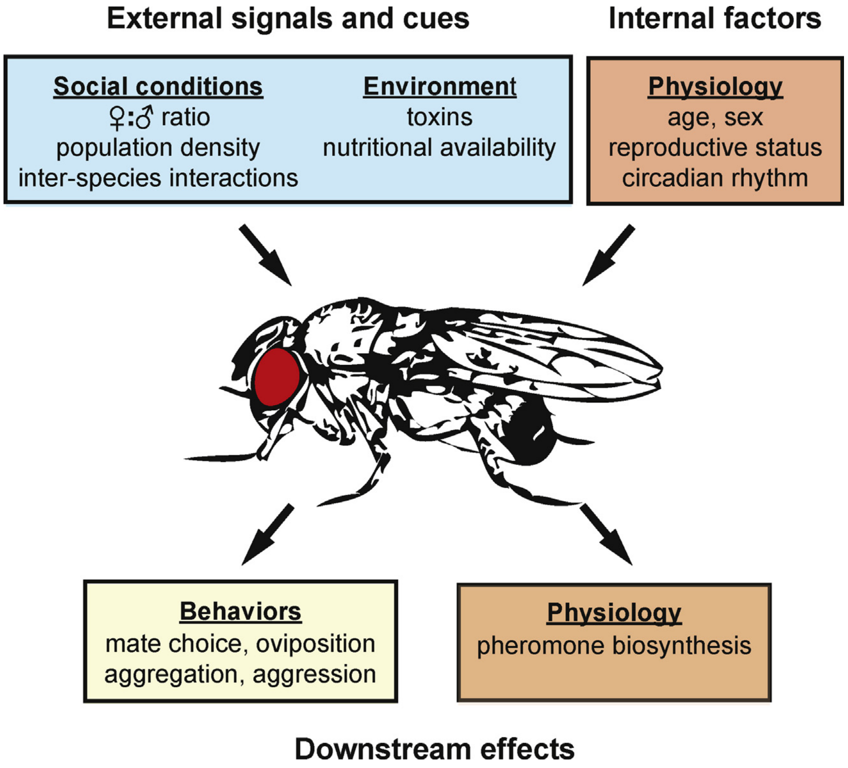
Toward a holistic understanding of chemical communication

The long tradition of behavioral studies in *Drosophila* provides a rich ethological context to connect chemical communication to other behaviors and systemic processes. The identification of ligands and peripheral sensory neurons for pheromone detection lays the foundation for a map of central brain regions that underlie chemical communication. This framework has allowed us to begin uncovering the mechanisms by which multiple types of sensory information are integrated and how physiological state modulates chemical communication (Figure 1).

The reverse engineering of behavior to identify circuits

Several mechanisms can account for the observation that the same pheromone elicits different types of behaviors depending on the sex of the receiver. In some cases, distinct male or female responses to the same compounds are encoded by a difference in receptor expression at the periphery. For example, Or47b, Gr68a, Ir52c, Ir52d, and ppk23 are enriched in forelegs of males but not females [17,20–22,32•,44,45]. In contrast, Gr32a [25], Gr33a [26] and Gr39a [27] each play a role in male courtship, have no discernible function in female courtship, but are found expressed in both males and females. In these cases, sexually dimorphic responses are likely encoded in higher order brain regions. The best described example of this mechanism pertains to the detection of cVA. The pheromone induces opposite behavioral responses from males and females yet sensory and second order neurons respond similarly in both sexes [46]. The sexual dimorphism in response patterns is attributed to two cell clusters in the lateral horn region of the protocerebrum and their pattern of innervation. Projection neurons

Figure 1



Pheromones and chemical cues from the environment play potent roles in guiding the behavior of *Drosophila*. Pheromones dictate an animal's choice to mate or fight by revealing the presence of potential mates or rivals. In addition, food odors and toxins influence mating and egg-laying decisions. Behavioral decisions can be enhanced or inhibited by a number of physiological factors which modulate sensory perception and pheromone production. With the availability of detailed neural circuits underlying each of these complex processes, the next challenge will be to understand how behavioral and physiological modules are integrated at the cellular and molecular level.

conveying pheromone input differentially innervate the clusters in males and females, functioning as a courtship circuit 'switch' that underlies the sexually dimorphic response to cVA [46,47]. Identifying other examples of pheromone circuits beyond primary and secondary processing centers will allow us to determine if neural switchboards are a common mechanism for specifying sexually dimorphic behavioral responses.

Behavioral output is not necessarily elicited solely by the presence of a pheromone — the context in which pheromone detection occurs can also modulate the response elicited by pheromone detection. For instance, courtship for males and females is known to be enhanced by food-derived odors [48,49]. One neural circuit underlying this response is mediated by Ir84a sensory neurons, which detect food-derived odors phenylacetic acid and phenlacetaldehyde and send information to the VL2a glomerulus in the antennal lobe [50]. Critically, projection neurons from VL2a co-project to the lateral horn with other pheromone pathways, namely projection neurons from glomeruli associated with Or67d and Or47b neurons. By coupling food odor perception to the activation of the courtship circuit, Ir84a facilitates male courtship behavior even though it does not convey pheromone information. The perception of a chemosensory bouquet rather than a single molecule may also explain how cuticular FAs attract males to females even though the compounds are found in equal quantities on the cuticles of both sexes [10••]. In each of these examples, information integrated from multiple receptors for multiple signals can enhance or dampen pheromone input. An alternative mechanism by which context modulates behavior involves the use of multiple receptors for the same molecule, each with distinct upstream projection patterns and different activation patterns depending on the social context (see cVA and aggression section).

Information from multiple sensory modalities can also influence the behavioral response to a pheromone. Both female movement and chemical stimuli were proposed to stimulate male courtship since Sturtevant's first observations in 1915 [51]. Intriguingly, two recent studies reveal a neural map connecting gustatory and mechanical input. Input from sweet sensing neurons extend to the subesophageal zone (SEZ) [52], a site of convergence for gustatory information, and the anterior motor and mechanosensory center (AMMC), a region associated with auditory and mechanical input. In addition, pheromone-sensing Gr68a neurons were shown to provide parallel input to both the SEZ and AMMC [32•,53], consistent with observations that the receptor is needed by males to detect female movement [53] and the gustatory pheromone CH503. It may be the case that the AMMC is a processing center for gustation; alternatively, mechanical stimulation may accompany taste perception and facilitate Gr68a activation. Information from each sensory domain may eventually converge in the P1 region within the protocerebrum, a group of cells designated as a courtship command center. This possibility is supported by recent findings showing that pathways originating from ppk25 gustatory sensory neurons and Or67d olfactory neurons converge onto sexually dimorphic P1 interneurons and that the combination of excitatory and inhibitory activity in males is integrated to regulate courtship initiation [54••].

Physiological state and its influence on pheromone production and reception

CHC profiles can change both over the course of a day [55] and over the lifespan of the animal [10••,56]. CHC production in the oenocytes follows a circadian rhythm that is partly modulated by the neuropeptide pigment-dispersing factor (PDF). Sensitivity of pheromone reception is also modulated by age-related hormonal release — changes in juvenile hormone (JH) levels during the first week of adulthood regulate transcription of Or47b, which confers sensitivity to medium-length chain FA pheromones [10••]. Long-term changes in CHC profiles, which vary throughout an animal's life, are mediated by three hormones whose levels fluctuate throughout development and adulthood: insulin-like signals [57], JH [58], and 20-hydroxyecdysone (20E) [59]. The close association between CHCs and age allow the CHC profile to serve as an honest indicator of age for males, who prefer to mate with younger females [56]. Interestingly, since 20E levels are associated with ovary development [60,61], changes in fertility may be reflected in CHC levels due to fluctuations in 20-HE. Beyond intrinsic processes of aging and circadian rhythm, changes in physiological state also reflect social, environmental, and dietary conditions, all of which are known to impact the CHC profile [62]. In many of these examples, the identification of key neuroendocrine hormones and peptides has provided a mechanistic tie across multiple organ systems that coordinates physiological changes with CHC perception and production.

Conclusion

Pheromones are commonly categorized in one of two types: fast-acting releasers with an immediate behavioral response and slow-acting primers which have a gradual or delayed response on behavior and physiology. Increasingly, we are recognizing that even the response to releaser pheromones are nuanced and represent the integration of numerous internal and external factors. Having identified the ligands, receptors, central pathways and pheromone biosynthesis effectors, we now have the tools to elucidate at the cellular level how sensory information is weighed against physiological signals when directing a behavioral response.

As more neural modules for information transduction, motor patterns, and physiological drive are delineated, the challenge will be to elucidate the organization of each assembly to each other, whether they function as a hierarchy or in parallel, and to determine how modulation occurs, whether at the level of neural switches, hormonal control, or epigenetic mechanisms.

Conflicts of interest

The authors declare no conflicts of interest.

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