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Smells like evolution: the role of chemoreceptor evolution in behavioral change

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In contrast to physiology and morphology, our understanding of how behaviors evolve is limited.This is a challenging task, as it involves the identification of both the underlying genetic basis and the resultant physiological changes that lead to behavioral divergence. In this review, we focus on chemosensory systems, mostly in *Drosophila*, as they are one of the best-characterized components of the nervous system in model organisms, and evolve rapidly between species. We examine the hypothesis that changes at the level of chemosensory systems contribute to the diversification of behaviors. In particular, we review recent progress in understanding how genetic changes between species affect chemosensory systems and translate into divergent behaviors. A major evolutionary trend is the rapid diversification of the chemoreceptor repertoire among species. We focus mostly on functional comparative studies involving model species, highlighting examples where changes in chemoreceptor identity and expression are sufficient to provoke changes in neural circuit activity and thus behavior. We conclude that while we are beginning to understand the role that the peripheral nervous system (PNS) plays in behavioral evolution, how the central nervous system (CNS) evolves to produce behavioral changes is largely unknown, and we advocate the need to expand functional comparative studies to address these questions.

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Introduction

Finding food or mates, avoiding predation, or choosing a site to lay eggs all depend on the perception of and response to diverse environmental cues. Adapting behaviors is therefore a critical component of evolutionary success. How an organism interacts with its environment can be divided into three parts: first, the sensory perception of diverse auditory, visual, tactile, chemosensory or other cues; second, the processing of this information by the central nervous system (CNS), leading to a representation of the sensory signal; and third, a behavioral response. Thus, behaviors could evolve either through changes in the peripheral nervous system (PNS) (e.g. [1•]), or through changes in higher-order neural circuitry (Figure 1). While the latter remain elusive, recent work on chemosensation in insects illustrates how the PNS shapes behavioral evolution.

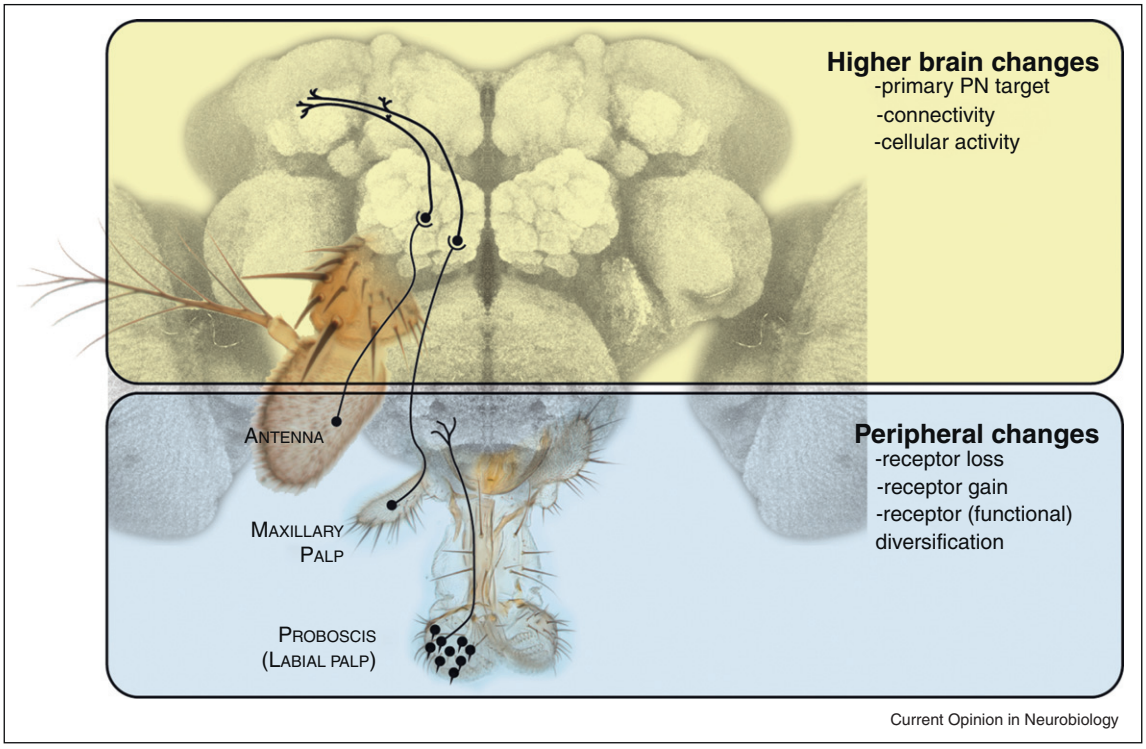
Chemosensation in insects depends on three classes of receptors expressed in peripheral neurons housed in specialized sensilla [2–4]. Olfaction depends on the insect-specific odorant receptors (ORs) on the antennae and palpae [2], as well as the more ancient ionotropic receptors (IRs) expressed in the antennae [4]. ORs vary rapidly between species, while IRs tend to be conserved across insect orders, possibly representing an ancestral insect chemodetection module for environmental cues of general interest [5,6•]. Gustation is mediated by gustatory receptors (GRs) housed in taste sensilla concentrated around the mouthparts, but also scattered throughout the body [7].

These receptors for chemosensation are evolving quickly, both in number and identity. For example, the number of IRs in protostome species ranges from 3 in *Caenorhabditis elegans*, to 85 in *Daphnia pulex* [5]. While this number is more stable across *Drosophila* species (58–66), phylogenetic analysis indicates tens of gains and losses in this genus [5]. The same pattern holds for ORs [8] and GRs [9•]. In all cases, there is extensive lineage-specificity of protein family members; for instance, only a handful of GRs are conserved even across flies [9•]. Clearly, the chemoreceptor families are gaining and losing genes all the time. Is this just neutral drift under a 'birth and death' model of gene family evolution [9•,10], or are there examples of gain, loss or diversification of chemoreceptors associated with changes in behavior?

Gain of chemoreceptor-encoding genes

There are several potential mechanisms by which a novel chemoreceptor could be added to an organism's pre-existing sensory repertoire to change behavior. Neural circuits are defined not only by the actual wiring of interconnected cells, but also by the genes controlling the development of those cells, their activity, and for the

Figure 1



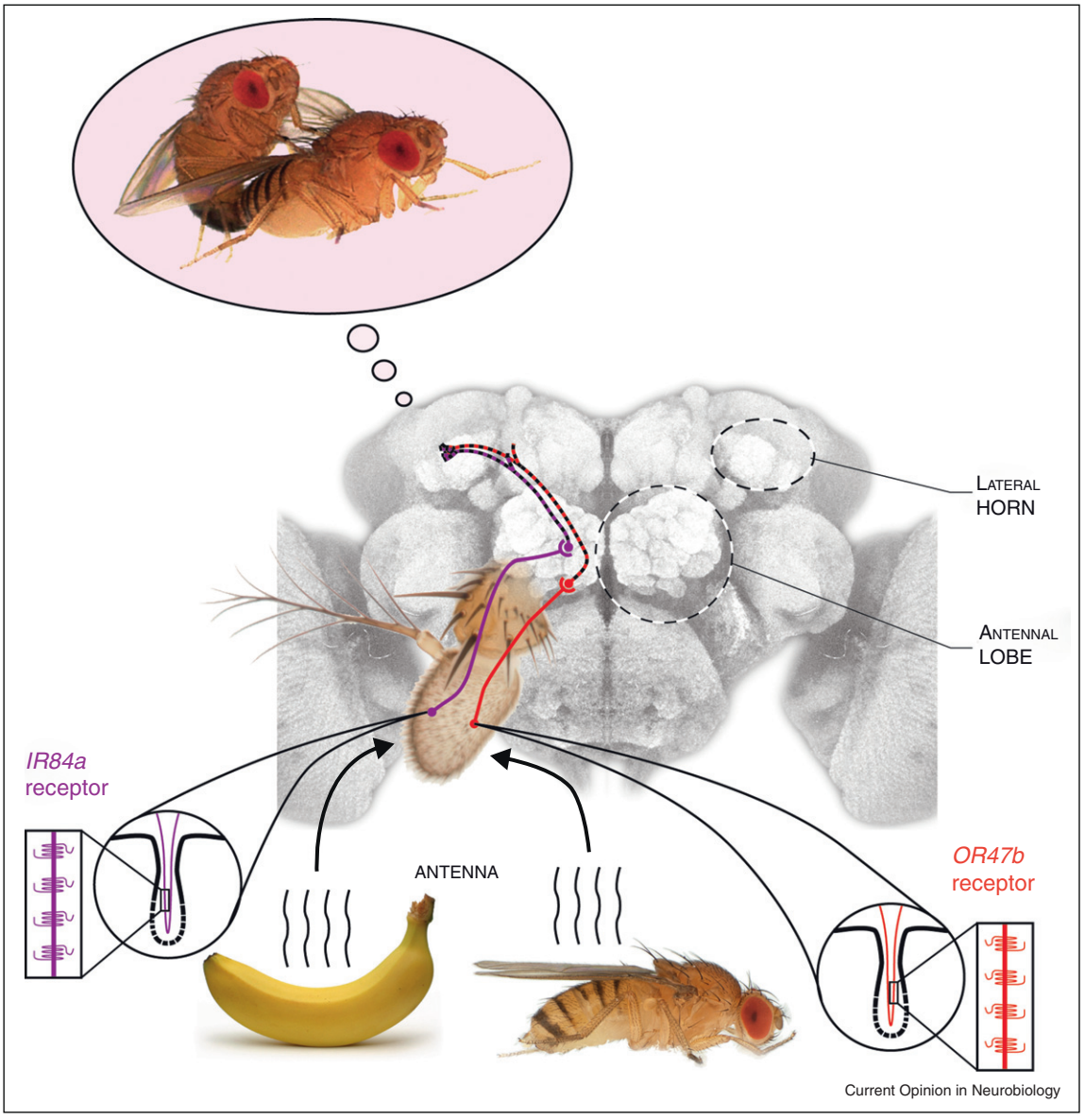
Possible levels of neuronal changes leading to divergent behaviors. In theory, changes in the nervous system leading to a divergent output response between closely related species can occur at the level of perception (blue box) or at the level of signal processing (higher brain centers, yellow box). In this review, we examine evolutionary examples of such changes in the context of chemosensation.

PNS, what chemoreceptors are expressed. While each OR is expressed in a single neuron [11], the neurons housing GRs and IRs typically express multiple receptor classes [4,12], allowing the expression of new chemoreceptors in a pre-existing cellular framework. Alternatively, new chemoreceptors might accompany additional changes at the cellular and genetic level.

The *Drosophila* IR84a receptor illustrates well how a novel chemoreceptor may modulate mating behavior through the integration of pre-existing neural circuits (Figure 2). IR84a is conserved in drosophilid flies, which court on food substrates, but is absent in other Dipterans, which do not [13••]. IR84a-expressing OSNs are activated by the fruit odors phenylacetic acid and phenylacetaldehyde. These sensory neurons express the male-specific courtship transcription factor Fru^M, and their activity is required for proper male courtship behavior. Importantly, wild type males, but not *IR84a* mutants, court more vigorously in the presence of IR84a ligands. Projection neurons downstream of this type of IR OSN typically innervate food odor-processing areas of the brain [13••,14]. Surprisingly though, the projection neurons downstream of IR84a-expressing OSNs innervate a pheromone-processing center, where they intermingle with other Fru^M-positive interneurons [13••,15]. Therefore, IR84a-mediated integration of food sensing circuits with the pre-existing Fru^M courtship circuitry may be responsible of the shift toward mating on food substrates in *Drosophila* species [13••].

A novel receptor may alter behavior by being expressed in pre-existing sensory neurons, and thus activate a downstream neural circuit in response to a new ligand. For instance, when the *D. melanogaster* receptor for the cVA pheromone, OR67d, was experimentally swapped for the *Bombyx mori* BmOR1 bombykol receptor, a normal cVA-mediated courtship suppression response was elicited with bombykol in these transgenic flies [16]. Similarly, ectopic expression of the GR64e glycerol receptor in CO_2 sensing neurons in the antenna was sufficient to confer glycerol sensitivity in these cells [17••]. One potential natural example of this process is the GR pxutGR1, which is expressed in the female legs of the swallowtail butterfly *Papilio xuthus* [18••]. Swallowtail butterflies are specialists, feeding and laying their eggs on specific host plants whose identity they check by drumming on the leaves with their forelegs [19]. PxutGR1 is the receptor for synephrine, one of 10 oviposition stimulants known for *P. xuthus*, and *pxutGR1* RNAi butterflies drum but fail to lay eggs on artificial leaves soaked in synephrine [18••]. While this example awaits comparative work, it is easy to envisage how changing the suite of GRs expressed on the

Figure 2



Fruit flies meet and mate at the restaurant. *Drosophila* males are aroused by the simultaneous exposure to two kinds of chemosensory inputs, pheromones produced by females and odors emanating from food. The former is mediated by a canonical OR pathway, whereby projection neurons (dashed black and red) targeted by the OR-expressing peripheral neurons (red) project to a pheromone-processing center in the mushroom body and the lateral horn. The latter is perceived by IR84a peripheral neurons of the antenna (purple). While IR84a sensory neurons perceive food cues, their target projection neurons project to the pheromone-processing center, thus achieving a first level in the sensory integration.

T1 leg in swallowtails could readily change host plant affinity among these species while leaving oviposition behaviors unchanged.

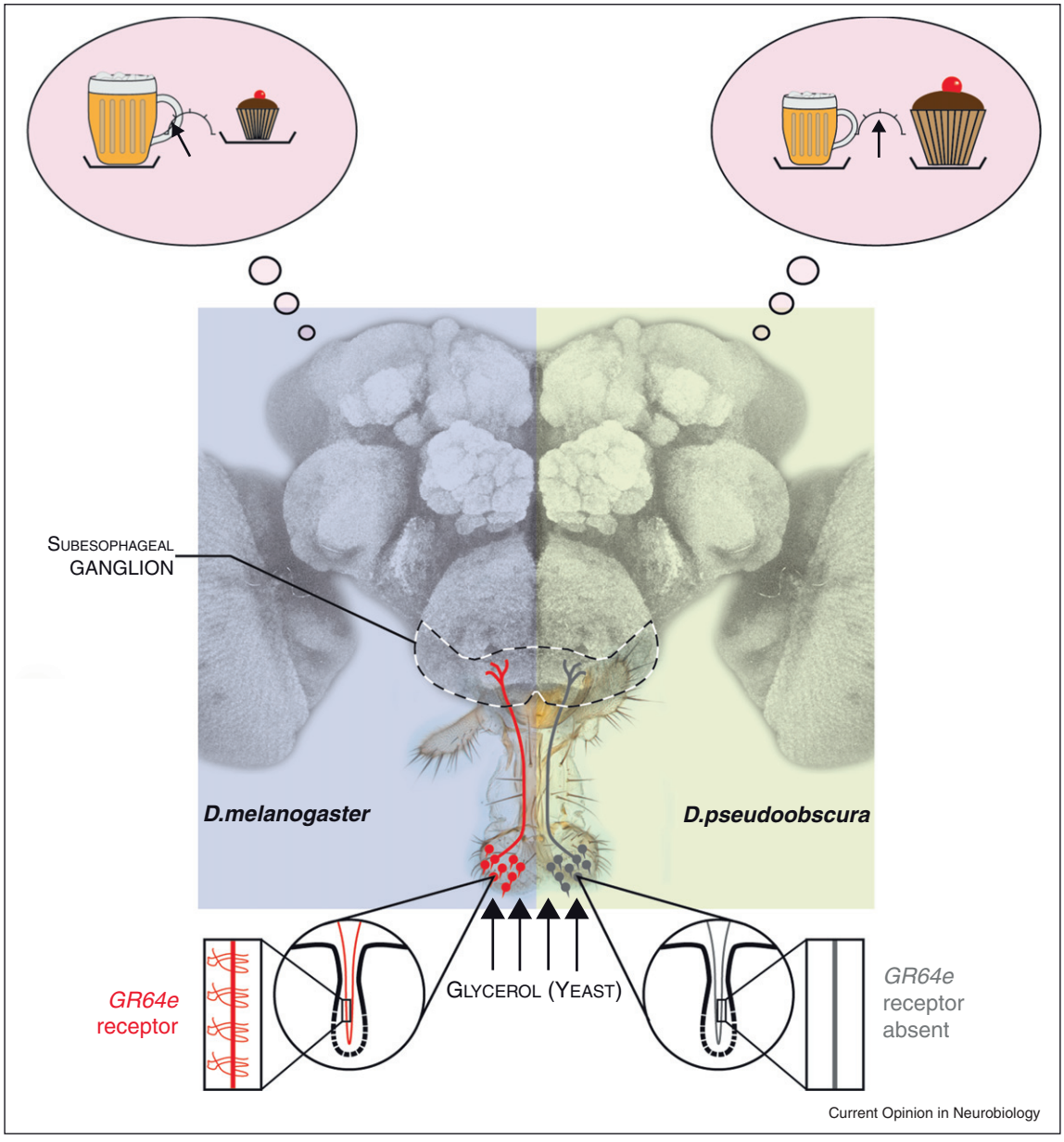
Alternatively, new chemoreceptors may accompany significant rewiring in the central and PNS. How, then, is a new chemosensory circuit assembled? Analysis of the projection patterns of OSNs onto the *Drosophila* antennal lobe hints at this process. Axon projections from OSNs expressing the same OR or IR converge onto the same glomerulus [4,11]. Comparing the projection patterns of OR or IR to their phylogenetic relationships has shown that similar, closely related ORs and IRs tend to project to adjacent glomeruli [6•,20], indicating that glomerular number has increased with chemoreceptor number, possibly through fission [21]. Recent work by Ray *et al.* [22••] has shown conserved *cis*-regulatory elements regulating OR expression are also found upstream of genes regulating OSN axon targeting and guidance, providing a potential genetic mechanism for the concerted evolution

of chemoreceptor expression and neural circuitry. Nevertheless, the mechanisms by which new circuits are assembled in the CNS are almost completely unknown.

Loss of chemoreceptor-encoding genes

When particular chemoreceptor genes are lost, organisms are now no longer able to muster a behavioral response to a previously important environmental cue. This may explain the pattern of accelerated chemoreceptor loss in species such as *Drosophila sechellia*, a host-plant specialist that presumably retains only receptors that recognize ligands from a narrow range of hosts [5,23,24]. This pattern of loss may, however, be due to endemism that might drive accelerated pseudogenization rather than host plant specialization [25], reflecting the tailoring of chemoreceptor repertoires to suit a local environment. Greater genome sampling of species from various niches and ranges will be needed to sort out the tangled influences of environment and evolutionary history on chemoreceptor gene evolution.

Figure 3



Modulation of preference for glycerol via the pseudogenization of its gustatory receptor. The difference in preference for beer/glycerol versus sugar between *Drosophila melanogaster* and *D. pseudoobscura* is due, at least partially, to the differential expression of the glycerol receptor GR64e in the gustatory neurons of the proboscis. *D. melanogaster* neurons express this receptor and *D. melanogaster* shows a preference for beer over sugar, while the ortholog of GR64e is a pseudogene in *D. pseudoobscura*, which is less attracted to beer.

Similarly, Wisotsky *et al.* [17••] have shown that pseudogenization of a single GR may be sufficient to alter food preference in some *Drosophila* species. Given the choice, *D. melanogaster* prefers beer to sugar, while *D. pseudoobscura* is relatively indifferent to beer (Figure 3). In *D. melanogaster*, this response is mediated by the glycerol receptor GR64e, and *GR64e* mutants lose their beer preference [17••]. *D. melanogaster* GR64e expression overlaps substantially with sugar sensing neurons, and GR64e OSNs send projections to a known sugar recognition center in the subesophageal ganglion [12,17••]. Glycerol sensing is thus integrated with the neural circuitry for other attractive food cues by expressing multiple GRs in the same sensory neurons. In species such as *D. pseudoobscura*, in which *GR64e* has become a pseudogene, loss of the receptor modulates the attractive signals coming in from the PNS. The response of the food reward circuitry to this different input is sufficient to alter food preference [17••].

Gene diversification

Apart from gene gain or loss, evolution can stem from the diversification of existing genes. Genes are redeployed in new developmental contexts, or their protein domains evolve to confer new functions. Hence, *D. sechellia*, again, preferentially feeds only on *Morinda citrofolia* fruits, attracted by its odorants hexanoic acid (HA) and octanoic acid (OA). Conversely *D. simulans*, *D. mauritiana* and *D. melanogaster* find *M. citrofolia* toxic, and avoid its odors. This difference in odor preference between *D. sechellia* and its sibling species maps to the OBP57d/e locus [26••]. Small odorant binding proteins (OBPs) are likely involved in the solubilization and transport of odor molecules in the sensillum [2]. While knocking out *OBP57d* and *OBP57e* in *D. melanogaster* failed to convert *D. melanogaster* responsiveness into that of *D. sechellia*, exogenous transfer of the *D. sechellia* locus to *D. melanogaster* was able to partially transform the *D. melanogaster* response to HA/OA. The changed expression of *OBP57e* between *D. sechellia* and *D. melanogaster* is due to regulatory changes, and maps to a 4-bp insertion in the promoter of the *D. sechellia* allele [26••].

The evolution of CO_2 sensing provides another well-characterized example of how the chemosensory system can be reshaped through changes in gene regulation. Among Dipterans, blood-sucking insects such as mosquitoes target their prey by localizing plumes of CO_2, while CO_2 deters *Drosophila*. The difference in CO_2 perception between the mosquito and *Drosophila* involves, among other changes, the relocation of the CO_2 sensilla, which are on the maxillary palps of the mosquito, but are confined to the antennae in *Drosophila*. The absence of CO_2 receptors on the *Drosophila* maxillary palp is due to the repression of their developmental program by a micro-RNA, *miR-279* [27••], and mutants for this micro-RNA look like a possible evolutionary intermediate between mosquitoes and flies.

Conclusion

The past several decades of research into evolution and development highlighted the general principles of morphological evolution. In particular, we now understand better how diversity can stem from a relatively stable number of genes across taxa, through the diversification of existing genes [28], although examples of important gene gains [29] and losses [30] also exist. This diversification appears to be fueled by regulatory changes [31] affecting individual effector genes at the bottom of a developmental genetic hierarchy, or upstream regulators such as transcription factors [32], where a single change has the potential to shift the expression of hundreds of target genes.

To what extent does this also hold true for the evolution of neural circuitry and behavior? Gene family level turnover in chemoreceptors and their associated support proteins is emerging as a major theme in sensory evolution. This is in stark contrast to morphological evolution, where the genetic players are largely invariant but the connections between them are reshuffled to produce diversity [33]. The rapid turnover of chemoreceptor genes may reflect a biological reality: chemoreceptors may be interchangeable modules, the most readily evolvable part of a neural circuit, as gaining, losing or changing a receptor alters the circuit's function but leaves it structural properties largely intact. In contrast, the developmental programs controlling circuit patterning may be too constrained to respond readily to selective pressure. Alternatively, this emerging trend might reflect a sampling bias: we see chemoreceptor evolution because this where we have looked. Beyond the perception level, there might also be numerous changes in the representation of chemosensory signals, and therefore in cell identity determinants acting during brain development and wiring.

The emerging picture of the evolution of chemosensation in insects is reminiscent of the evolution of visual neurons in primates, where the expression of various opsins tune the neurons to different wavelengths, resulting in a modified perception [34]. What is still a black box is the extent to which changes in the connectivity or the activity of chemosensory neurons play a role, or more radically, entirely novel neural circuits evolve, and what sorts of changes affect neural circuits in the higher brain centers, downstream of the periphery. We know that the cellular architecture of the CNS and PNS is not static [2,35,36], and that these changes must have a genetic and developmental basis. Answering these questions will require additional fine-scale comparative studies between closely related (e.g. *Drosophila*) species with divergent behaviors. Fortunately, the last decade has seen an explosion of genome sequencing [24], and the adaption of transgenic tools and technology to flies beyond the model species [37]

which should allow us to tackle this problem by carrying out studies previously only possible in *D. melanogaster*.

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