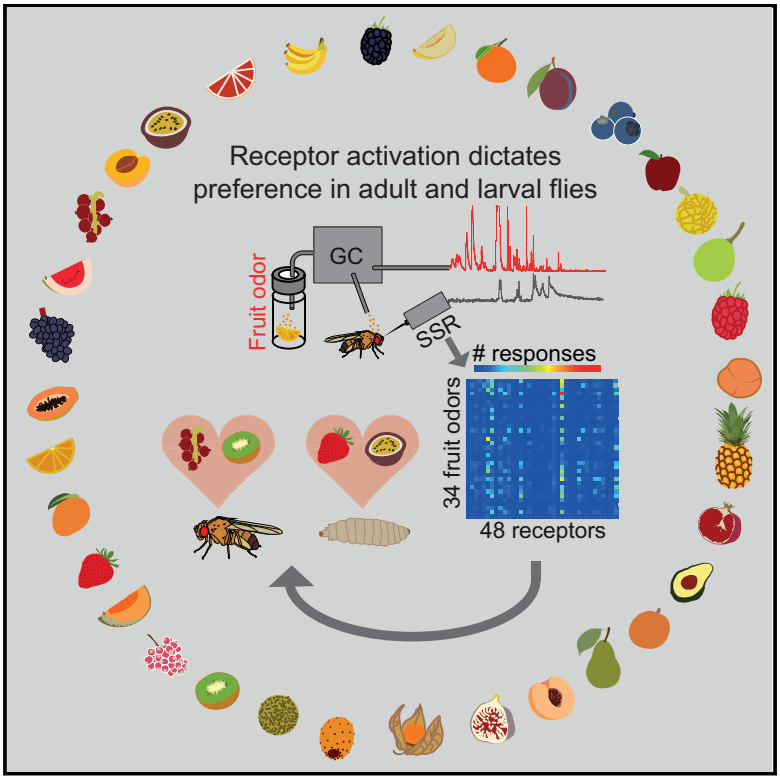
Resource

Cell Reports

# The Olfactory Logic behind Fruit Odor Preferences in Larval and Adult *Drosophila*

## Graphical Abstract




## Highlights

- The preference for fruit odors differs in larval and adult flies
- 90% of larval and 53% of adult olfactory receptors detect fruit compounds
- Olfactory neuronal activity correlates positively with attraction in larvae
- Olfactory neuronal activity correlates negatively with attraction in adult flies

## Authors

Hany K.M. Dweck, Shimaa A.M. Ebrahim, Tom Retzke, ..., Ales Svatoš, Bill S. Hansson, Markus Knaden

## Correspondence

hany.dweck@yale.edu (H.K.M.D.), mknaden@ice.mpg.de (M.K.)

## In Brief

Dweck et al. investigate the attraction of larval and adult flies to 34 different fruit odors. They show that both developmental stages differ in their preferences. The authors furthermore identify those olfactory receptors that are responsible for the detection and evaluation of the 116 chemical compounds in the fruit odors.

Dweck et al., 2018, Cell Reports *23*, 2524–2531
May 22, 2018 
https://doi.org/10.1016/j.celrep.2018.04.085

CellPress

OPEN ACCESS
CellPress

Cell Reports
Resource

# The Olfactory Logic behind Fruit Odor Preferences in Larval and Adult *Drosophila*

Hany K.M. Dweck,[1,3,4,*] Shimaa A.M. Ebrahim,[1,3,4] Tom Retzke,[1] Veit Grabe,[1] Jerrit Weißflog,[2] Ales Svatoš,[2] Bill S. Hansson,[1,5] and Markus Knaden[1,5,6,*]

[1]Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Hans-Knoell-Strasse 8, 07745 Jena, Germany
[2]Mass Spectrometry Group, Max Planck Institute for Chemical Ecology, Hans-Knoell-Strasse 8, 07745 Jena, Germany
[3]Present address: Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520, USA
[4]These authors contributed equally
[5]Senior author
[6]Lead Contact
*Correspondence: hany.dweck@yale.edu (H.K.M.D.), mknaden@ice.mpg.de (M.K.)
https://doi.org/10.1016/j.celrep.2018.04.085

## SUMMARY

**Despite the comprehensive knowledge on odor coding, our understanding of the relationship between sensory input and behavioral output in *Drosophila* remains weak. Here, we measure the behavioral responses generated by larval and adult flies in response to 34 fruit odors and find that larval preference for fruit odors differs from that of adult flies. Next, we provide a functional analysis of the full repertoire of the peripheral olfactory system using the same comprehensive stimulus spectrum. We find that 90% and 53% of larval and adult olfactory receptors tested here, respectively, are involved in evaluating these fruit odors. Finally, we find that the total amount of olfactory neuronal activity correlates strongly positively with behavioral output in larvae and correlates weakly negatively in adult flies. Our results suggest that larval and adult flies have evolved different mechanisms for detection and computation of fruit odors, mechanisms likely mirroring the different lifestyles of both developmental stages.**

## INTRODUCTION

Larval and adult flies, *Drosophila melanogaster*, evaluate olfactory information emitted from their ecological niche, decaying fruit (Hansson et al., 2010), via the olfactory system. The larval olfactory system consists of 21 olfactory sensory neurons (OSNs) housed in a single morphological structure, the dorsal organ, located at the tip of the head (Ramaekers et al., 2005). These 21 OSNs express, in addition to the coreceptor Orco, 25 members of the odorant receptor (Or) family. Out of these, 13 are also expressed in the adult (Couto et al., 2005; Fishilevich et al., 2005; Kreher et al., 2005, 2008). In contrast, the 48 OSN types of the adult olfactory system are housed in three large antennal basiconic (ab1–ab3), seven small antennal basiconic (ab4–ab10), two antennal intermediate (ai1 and ai2, previously known as at2 and at3, respectively), two antennal trichoid (at1 and at4), four antennal coeloconic (ac1-ac4), and three palp basiconic (pb1–pb3) sensilla (Couto et al., 2005; Shanbhag et al., 1999). OSNs housed in basiconic, intermediate, and trichoid sensilla express 44 Or genes along with the Orco coreceptor (Couto et al., 2005). However, with the exception of Or35a/Orco, OSNs housed in coeloconic sensilla express 12 ionotropic receptor (Ir) genes, including three co-receptors, Ir8a, Ir25a and Ir76b (Benton et al., 2009). Finally, the ab1C neuron expresses Gr21a and Gr63a and detects $CO_2$ (Kwon et al., 2007).

There are two ways to address the question of how sensory input is converted into behavioral output. The first is to dissect the circuit of each sensory input from the peripheral OSN to the primary processing center in the brain, the antennal lobe (AL), and from the AL to higher brain centers like mushroom body and lateral horn, finally leading to a behavioral output. However, this approach is currently unattainable. The second is to describe sensory input and behavioral output quantitatively, considering the processing of information in the CNS as a black box. In the vinegar fly, *Drosophila melanogaster*, several studies have used the second way (Bell and Wilson, 2016; Hernandez-Nunez et al., 2015; Knaden et al., 2012; Kreher et al., 2008; Thoma et al., 2014). However, none of these studies analyzed the sensory input of the entire olfactory system, particularly in adult flies, and none of them dealt with ecologically relevant complex stimuli.

In this study, we measured the behavioral responses of larval and adult flies to the headspaces of 34 different fruits (Figure 1A). Subsequently, we dissected how these fruit odors are detected by the entire olfactory systems of larval and adult flies. Finally, we correlated the physiological responses with the behavioral output to decipher the logic behind food preference in larval and adult flies.

## RESULTS AND DISCUSSION

### Behavioral Responses of Larval and Adult *Drosophila* to the Headspaces of 34 Fruits

We first investigated olfactory behavioral responses by quantifying the chemotaxis of larvae in a binary-choice assay (Figure 1B), and of adult *Drosophila* in the Flywalk assay (Steck et al., 2012; Thoma et al., 2015) (Figure 1C) to headspace extracts

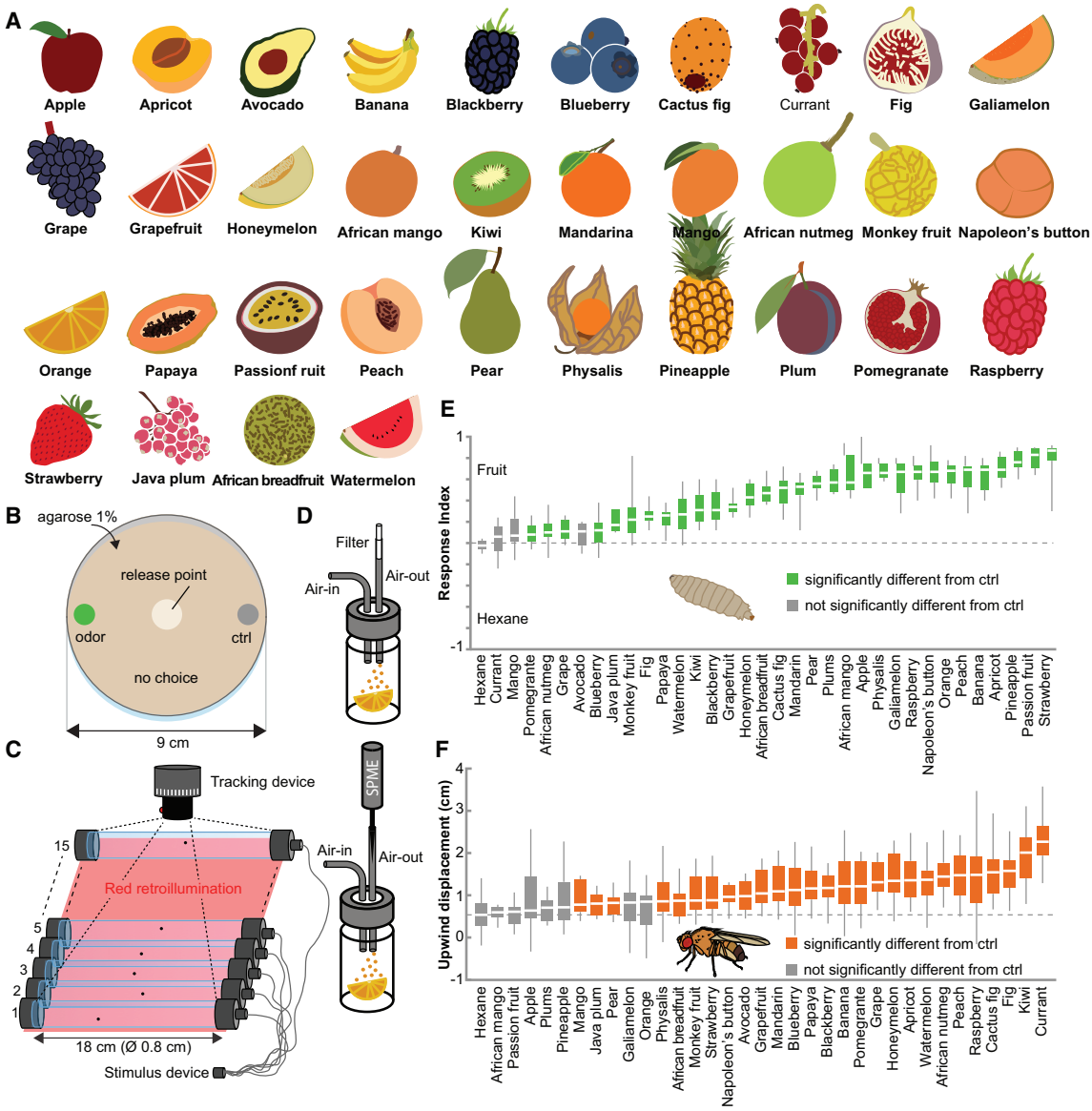



**Figure 1. Behavioral Responses of Larval and Adult *Drosophila* to Fruit-Headspace Extracts**

(A) Clip art of 34 different fruits.

(B) Schematic drawing of the two-choice larval assay used in (E).

(C) Schematic drawing of the Flywalk assay used in (F).

(D) Schematic drawing of headspace odor collection equipment for behavioral assays (top) and GC-SSR experiments via solid-phase micro-extraction (SPME, bottom).

(E) Larval response indices from the two-choice assay. Green boxes indicate significant differences from the solvent control ($p < 0.05$, Wilcoxon signed-rank test; $n = 10$). White line indicates median; boxes indicate upper and lower quartiles; whiskers indicate minimum and maximum values.

(F) Net upwind displacement of mated female flies within 4 s after encountering 500-ms pulses of different fruit headspaces. Orange boxes indicate significantly higher upwind displacement compared with the negative control hexane ($p < 0.05$, Wilcoxon signed-rank test; $n = 15$). White line indicates median; boxes indicate upper and lower quartiles; whiskers indicate minimum and maximum values.

from 34 different fruits. These extracts were collected from either ripe fruit or fruit in the early fermentation stage according to standard procedures (Figure 1D; see also Experimental Procedures). One should mention that the ripening state of a fruit strongly influences its odor emission with increasing amounts of alcohols and acids at later stages and, hence, influences the attractiveness of a given fruit to *D. melanogaster*. Our behavioral screen, hence, does not claim to identify the most attractive fruit at whatever stage but gives a comprehensive overview on the attractiveness of a varying set of fruit headspaces.

We found that larval and adult *Drosophila* displayed significant and differential positive chemotaxis to 31 and 27 of the tested

fruit extracts, respectively (Figures 1E and 1F). Neither larvae nor adult flies showed negative chemotaxis to any of the extracts. The most attractive fruit-headspace extracts for larvae—such as strawberry, passion fruit, and pineapple—elicited either no or mild positive chemotaxis in adult flies. The most attractive headspace extracts for adult flies were red currant and kiwi. In larvae, currant triggered no significant behavioral responses, while kiwi triggered only mild responses. These results clearly indicate that larvae show fruit-specific preferences that differ from those of adult flies.

## Coding of Fruit Volatiles by Ors Expressed in Larval and Adult *Drosophila*

Having established the innate behavioral responses of larval and adult *Drosophila* to the fruit-headspace extracts, we next examined how these fruit extracts are detected by the complete olfactory systems of larval and adult *Drosophila*. To do this, we performed a system-wide electrophysiological screen from these olfactory systems using the same 34 fruit odors.

We carried out our screen from the 48 OSN types of the adult olfactory system and identified them by using a diagnostic set of odorants (Ebrahim et al., 2015). By doing so, we also recorded from OSNs expressing several Irs and two coexpressed gustatory receptors (Grs). Furthermore, we screened the responses of 21 larval Ors that were previously found to be functional in the adult empty neuron system (Kreher et al., 2005; Mathew et al., 2013). The identification of individual OSN responses in larvae is extremely demanding, as all OSNs are co-localized in a single morphological structure, the dorsal organ (Hoare et al., 2008), which reported recordings from up to 11 OSNs in a single preparation. Therefore, we mis-expressed the 11 larval-specific receptors in mutant ab3A OSNs (lacking a functional Or22a) on the adult antenna using the Gal4-UAS system (Dobritsa et al., 2003). This so-called empty neuron system does not necessarily reveal the exact activation pattern of each tested Or, as larval-specific odorant-binding proteins or other xenoproteins that play a significant role in the activity of larval OSNs might not be expressed. Furthermore ephaptic interactions in adult OSNs have been reported (Su et al., 2012) that might influence the results. However, to our knowledge, it is, so far, the most accurate way to analyze receptor-ligand interactions of Ors that cannot be measured directly. For the ensuing analysis, recordings from adult neurons expressing the 10 receptors that are expressed in both larval and adult flies were used for the analysis of both developmental stages (for an overview of all neuronal responses, see Table S1).

This system-wide screen was performed using linked gas chromatography-single sensillum recording (GC-SSR) measurements (Wadhams, 1982) (Figures 2A and 2B). The combination of GC with SSR allowed us to pinpoint the physiologically active flame ionization detection (FID) peaks in each extract and their retention times. Because, in some cases, the active FID peaks were within the solvent peak, we used the linked solid-phase microextraction (SPME)-GC-SSR technique to overcome this limitation.

Our screen revealed that the tested fruit elicited 1,085 and 1,668 excitatory responses from the screened Ors in larvae and adult flies, respectively (Figures 2C and 2D). Surprisingly, but consistent with the findings of another study (Stensmyr et al., 2003), none of the fruit volatiles elicited inhibitory responses, neither in larvae nor in adult flies (data not shown). The number of peaks eliciting responses in single extracts ranged in larvae from 65, as in the case of African breadfruit, down to seven, as in case of blueberry (Figure S1A). In adult flies, extracts elicited responses ranging from 101 (passionfruit) down to nine (avocado) (Figure S1B). Monkey fruit activated the highest number of OSN types, while avocado activated the lowest number (Figures S1C and S1D) (for a list of all active compounds present in the different fruit headspaces, see Table S2).

Furthermore, our screen revealed that fruit volatiles activated 90% of the screened larval Ors and 53% of the adult Ors. 89% of the activated Ors were activated more than once (Figures S1E and S1F). In larvae, Or45a, Or74a, and Or85c responded to 247 (22.8%), 147 (13.5%), and 88 (8%), respectively, out of 1,085 FID peaks. In adult flies, Or67a, Or22a/Or22b, and Or98a responded to 235 (14%), 143 (8.6%), and 124 (7.4%), respectively, out of 1,668 FID peaks. Contrary to these obviously rather widely tuned receptors, either Or33b or Or46a was activated only once. In a former study, Mathew et al. (2013) screened all *Drosophila* larval Ors with a set of almost 500 synthetic odorants and identified cognate odorants for most of the receptors (i.e., odorants to which the individual receptors are most sensitive). Interestingly, only four of the cognate odorants identified in that comprehensive screen turned out to be present at physiologically active concentrations in our fruit headspaces (2-nonanone, pentyl acetate, geranyl acetate, and 3-octanol). These odorants, however, activated the same Ors in both studies, confirming the explanatory power of our GC-coupled SSR analysis.

Our screen also revealed that none of the fruit volatiles triggered responses from receptors that belong to ecologically labeled lines (Figures 2C and 2D; Table S1). Neither Gr21a and Gr63a (which detect $CO_2$; Kwon et al., 2007) nor Or49a and Or85f (which govern the fly's avoidance to parasitoid wasps of the genus *Leptopilina*; Ebrahim et al., 2015), nor Or56a (which signify the presence of harmful microbes by detecting the key odor geosmin; Stensmyr et al., 2012) were activated by any of the fruit odors. Also, none of the Ors expressed in neurons of trichoid sensilla, which are known to detect volatile pheromones (Dweck et al., 2015; van der Goes van Naters and Carlson, 2007), responded to any of the numerous fruit-derived compounds. Finally, none of the Irs, which are expressed in neurons of coeloconic sensilla, responded to any of the fruit volatiles.

## Identification of the Active Compounds for the Screened Larval Receptors and Adult Neurons

The active FID peaks (for a definition of activity, see Figure 2B) from each extract were identified using linked GC-mass spectrometry (MS), linked SPME-GC-MS, and synthetic standards. The identification of the active compounds was further confirmed physiologically by injecting the synthetic standards in GC-SSR measurements (Figure 2B). Based on the retention time, the 1,085 and 1,668 active FID peaks in larval and adult flies corresponded to 165 and 278 compounds, respectively. Of these compounds, only 59 and 102 could be unambiguously identified. The other compounds did not produce clear mass

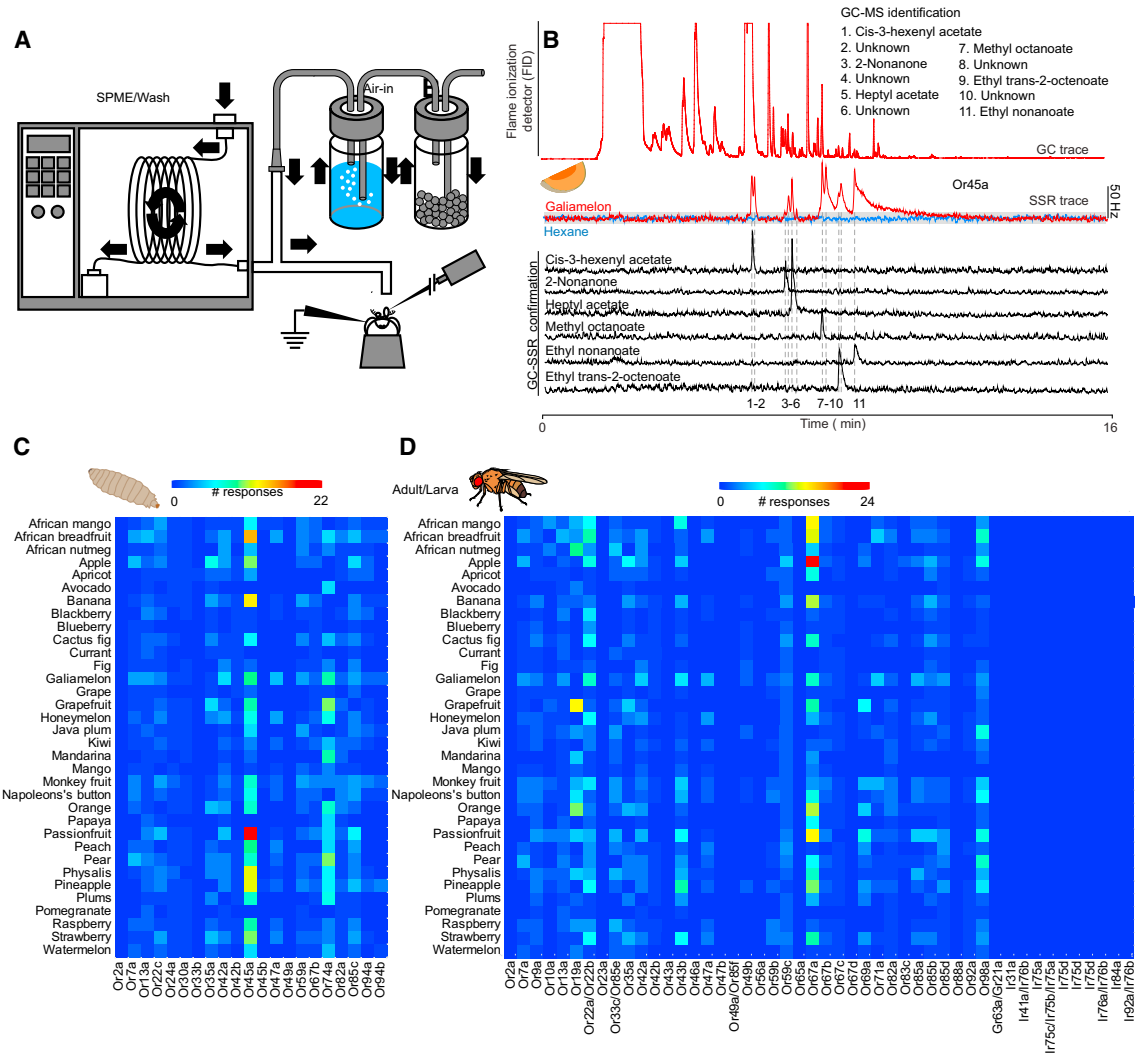



**Figure 2. Coding of Fruit-Headspace Extracts by Olfactory Receptors Expressed in Larvae and/or Adult *Drosophila***

(A) Schematic drawing of SPME-GC-SSR.

(B) Coupled GC-SSR from Δhalo neuron expressing the larval receptor Or45a with the headspace extracts of galiamelon (red), hexane (blue), and synthetic compounds (black). Synthetic compounds were used both for GC identification of headspace compounds and for identification of the receptor based on published receptor profiles. Neuronal activity was regarded as response when frequency reached double frequency of the average hexane response (gray bar).

(C and D) Heatmap of number (#) of responses elicited by each fruit headspace extract as determined via a system-wide GC-SSR screen from 21 larval Ors (C) and the 48 OSNs expressing Ors, Irs, or Grs of adult flies (D) (n = 3–5). For example, # = 24 indicates that 24 compounds of this fruit activated this specific Or.

spectra and, thus, remain unidentified. Fourteen compounds were larval specific, 57 were adult specific, and 45 were common between larval and adult flies (Table S1). Nineteen of these compounds were identified as natural ligands for the first time. While, in larvae, only 10% of the identified compounds activated multiple receptors (e.g., hexyl acetate activated the four receptors Or13a, Or35a, O45a, and Or47a), in adults, 25% of the identified compounds activated multiple Ors (e.g., ethyl hexanoate activated seven receptors, Or22a/Or22b, Or47a, Or43b, Or69a, Or19a, Or59c, and Or85d).

In both larvae and adult flies, the highest number of responses was observed among aliphatic esters (~52% in larvae and ~44% in adults). In larvae, this was followed by aromatics (~24%), aliphatic alcohols (12%), aliphatic ketones (~8%), and aliphatic aldehydes (~3%); while, in adult flies, it was followed by terpenes (~25%), aromatics (19%), aliphatic alcohols (~8%), aliphatic ketones (~6%), and aliphatic aldehydes (~2%).

We noted, with interest, that adult flies detected 23 terpenes, of which only three were detected by larvae, suggesting that terpenes might be of particular ecological significance to adult flies but not to larvae. We have previously shown that gravid flies prefer terpene-rich citrus fruit as an oviposition substrate and that, in turn, terpenes protect *Drosophila* offspring against endoparasitoid wasps (Dweck et al., 2013).

A cluster analysis of all activated receptors based on their response patterns to the 116 identified compounds in the fruit

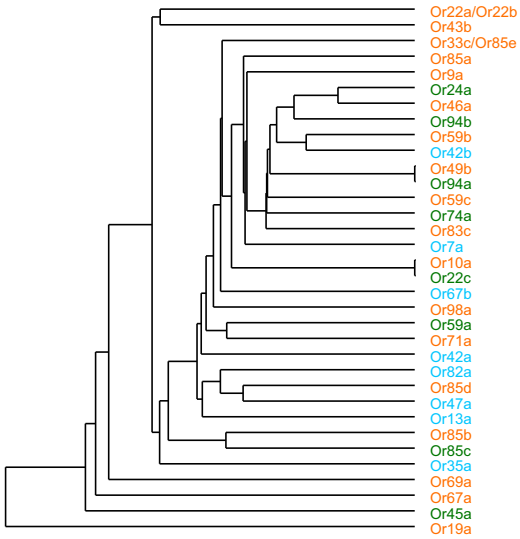



**Figure 3. Cluster Analysis of Receptors Based on Their Response Spectra**

Receptors are color coded according to their expression. Green indicates larvae; orange indicates adults; blue indicates both stages. Cluster analysis was performed using Ward's algorithm and Euclidian similarity index based on responses depicted in Table S1.

See also Table S1.

headspaces (Figure 3) revealed that the larval receptor Or22c shares its response pattern with the adult Or10a, while the larval Or94b responds to the same compounds as the adult Or49b. Interestingly, while the former two receptors are phylogenetically closely related, the similarly responding Or94b and Or49b show up at very distant places along a phylogenetic tree (Couto et al., 2005; Robertson et al., 2003). Why larvae and adults recruit different receptors for the same function remains open. However, the similar olfactory tuning of distantly related receptors might later lead to a better understanding of ligand receptor interactions.

## Correlation of Peripheral Input to Behavioral Output in Larval and Adult *Drosophila*

It is almost impossible to present identical stimuli in different behavioral and in physiological experiments. The odor presentation regimes in the electrophysiological and the larval and adult behavioral tests, therefore, differ regarding stimulus duration and probably also stimulus concentration. We, nevertheless, performed a principal-component analysis (PCA) for the 34 fruit odors based on the physiological responses they elicited (Figures 4A and 4B; see also Table S3). Interestingly, despite the lower number of expressed receptors, the larval system seems to be better suited to discriminate between the fruit odors (as seen from the higher spread of fruit odors in the PCA in Figure 4A as compared to Figure 4B). When comparing the physiology-based representation of fruit odors with the behavior they elicited in larval and adult flies, we found a correlation of attraction and the first principal component (PC1) of the PCAs in both cases, with attraction being positively correlated with neuronal activity in larvae (Figure 4C) and negatively correlated in flies (Figure 4D). PC1 (and, therefore, attraction) was mainly (but not exclusively) affected by Or45a in larvae (Figure 4E), signifying larval attraction to acetates to which this receptor mainly responds (Table S1). Interestingly, even the larval receptor Or74a, which responded to the described larval repellent 1-Nonanol (Cobb et al., 1992), revealed the second highest loading for the correlation with larval attraction (Figure 4E). In adult flies, the activation of Or67a was the main (but not exclusive) factor responsible for the negative correlation of neuronal activity and attraction (Figure 4F). Or67a, in our screen, responded to many compounds that, like the two reported *Drosophila* repellents acetophenone and benzaldehyde (Knaden et al., 2012), carry a phenol group (Table S1). Phenolic compounds have been shown to induce oviposition avoidance in *Drosophila* (Mansourian et al., 2016) via the activation of Or46a (i.e., a receptor that also responded to a phenolic compound in our screen; Table S1).

We next correlated the total number of either responses or activated receptors by each tested fruit odor from all larval or adult Ors to behavioral responses elicited by the same fruit odor in both larval and adult flies. We found that, in larvae, these correlations were strongly positive (Figure 4G), meaning that as the total number of elicited responses or activated Ors increases, larvae become more attracted. In adult flies, again, these correlations were weakly negative (Figure 4H).

A positive correlation between OSN activity and attraction in larvae was already suggested by Kreher et al. (2008), who correlated the sum of the action potentials from all the 21 larval receptors and behavioral responses elicited by a panel of monomolecular odors. Larvae do not have to localize food but grow on the substrate on which their mother chose to oviposit. This might be the reason why only a few of the 21 receptors seem to govern avoidance behavior (e.g., Or49a governing the avoidance of parasitoid wasps; Ebrahim et al., 2015). The remaining majority of receptors seem to be involved in detecting positive cues from the fruit, resulting in an overall positive correlation of OSN activity and attraction. Adult flies, however, do need to localize food and oviposition sites. Many of the receptors are tuned to low concentrations, allowing the fly to detect rotten fruit over distance. In addition, female flies need to judge the quality of a potential oviposition site. If, e.g., the stage of decay of a fruit is too advanced (which usually goes along with increased emission of odors), harmful bacteria might colonize the fruit and kill the offspring (Stensmyr et al., 2012). The preference for slightly, but not too rotten, fruit might explain the negative correlation of OSN activity and attraction in adult flies, as strong OSN activity might signal a ripening stage that is too advanced. Indeed, it was shown for several monomolecular odorants (Farhan et al., 2013; Strutz et al., 2014) and for the headspace of vinegar (Semmelhack and Wang, 2009) that attractive odors can turn aversive at high concentrations. Based on these findings, one can speculate that not only the increased emission induced by the ripening process but also the fruit-species related emission rate dictates which fruit is chosen by the fly, with less smelly fruit being preferred.

Obviously, although being equipped with overlapping sets of olfactory receptors, larval and adult flies have evolved different mechanisms for the detection and computation of fruit odors.

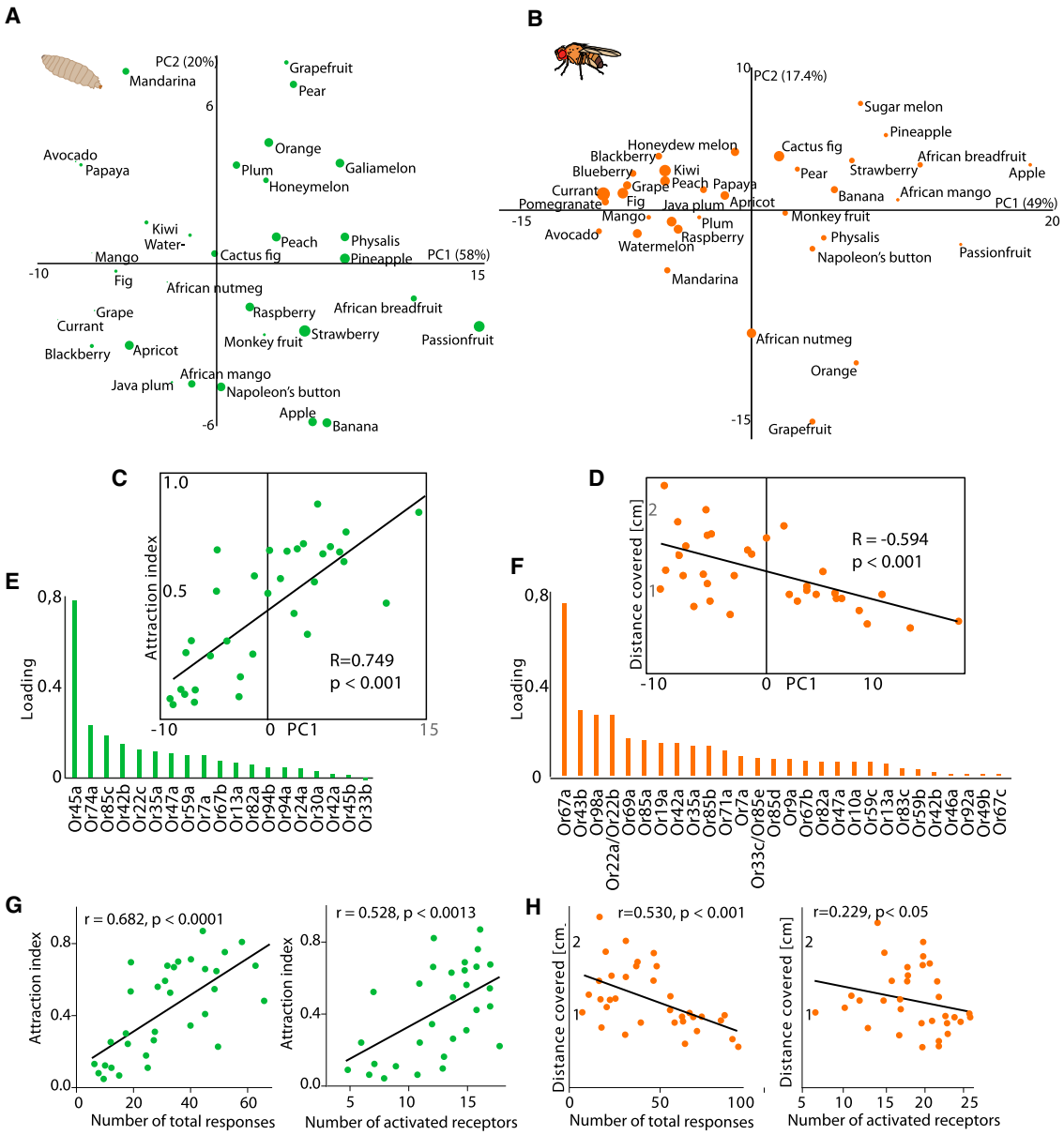



**Figure 4. Correlation of Peripheral Input to Behavioral Responses in Larval and Adult *Drosophila***

(A and B) PCA of all fruit headspaces based on the activation of receptors that are expressed in larvae (A) and adult flies (B). PCA in (A) is based on the responses of larval-specific Ors gained from mis-expression in the empty-neuron system and on responses of Ors expressed in both developmental stages obtained from recordings of adult OSNs. PCA in (B) is based on the responses of adult-specific Ors and of Ors expressed in both stages obtained from recordings of adult OSNs. Hence, identical data for Ors expressed in both stages were used in (A) and (B). Ors were regarded as activated, when firing rates upon peak arrival in GC-SSR reached at least twice the rates of the spontaneous activity (Figure 2B). Size of the dots depicts relative attraction of each fruit headspace in behavioral assays.

(C and D) Pearson's correlation of the first principal components from (A) and (B) and attraction measured in behavioral assays. (C) Attraction index. (D) Distance covered.

(E and F) Loadings of the individual receptors expressed in larvae (E) and in adult flies (F) for the PCAs shown in (A) and (B), respectively.

(G and H) Pearson's correlation of number of total responses (left) or activated receptors (right) elicited by each tested fruit and attraction measured in bioassays for larvae (G) and adult flies (H).

## EXPERIMENTAL PROCEDURES

### Fly Stocks

All experiments with wild-type (WT) *D. melanogaster* were carried out with the Canton-S strain. Δhalo;Or22a-GAL4/UAS-OrX lines were a gift from John R. Carlson (Yale University).

### Headspace Collections

The headspaces of the different samples were collected for 24 hr on a Super-Q filter (50 mg, Analytical Research Systems; www.ars-fla.com). The samples were placed individually in a l-L laboratory glass bottle that was halfway filled with samples and closed with a custom-made polyether ether ketone (PEEK) stopper. Airflow at 0.5 L/min was drawn through the flask by a pressure pump.

Filters were eluted with 300 μL hexane, and samples were stored at −20°C until analysis.

### SPME/SPME-GC-SSR/SPME-GC-MS

The samples were placed individually in 10-mL glass vials that were filled with 2 mL of each fruit sample and closed with a cap equipped with a polytetrafluoroethylene-lined silicone septum. After penetrating the septum of the cap with the SPME fiber holder, the SPME fiber coated with 100 μm polydimethylsiloxane (Supelco) was exposed to the headspace in each fruit-containing vial for 10 min at room temperature. Afterward, the SPME fiber was directly inserted into the inlet of a gas chromatograph for GC connected to either SSR or MS.

### GC-SSR/GC-MS

Adult flies were immobilized in pipette tips, and the palps or antennae were placed in a stable position onto a glass coverslip. Sensilla were localized under a binocular at 1,000× magnification, and the extracellular signals originating from the OSNs were measured by inserting a tungsten wire electrode into the base of a sensillum. The reference electrode was inserted into the eye. Signals were amplified (10×; Syntech Universal AC/DC Probe; www.syntech.nl), sampled (10,667 samples per second), and filtered (100–3,000 Hz with 50/60-Hz suppression) via a USB/IDAC (universal serial bus/inter-digital analog converter) connection to a computer (Syntech). Action potentials were extracted using Syntech Auto Spike 32 software. For GC-SSR, neuron activities were recorded for 1,220 s, the time of a single GC run. For GC stimulation, 1 μL of the odor sample was injected into a GC (Agilent 6890, column: DB-5, 30 m × 0.32 mm (id), 0.25-μL film thickness; inlet at 250°C, oven: 50°C for 2 min, then 15°C × $min^{-1}$ up to 250°C, held for 5 min; carrier gas: helium, 2.0 mL $min^{-1}$ constant flow). The GC was equipped with a 4-arm effluent splitter (Gerstel; www.gerstel.com), with a split ratio of 1:1 and $N_2$ (30.3 kPa) as makeup gas. One arm was connected with the FID of the GC, and the other arm was introduced into a humidified air stream (200 mL × $min^{-1}$). GC-separated components were directed toward the palps or the antennae of the mounted fly. Signals from OSNs and FID were recorded simultaneously. Headspace samples were analyzed by GC-MS (Agilent 6890GC and 5975bMS, Agilent Technologies [www.agilent.com]). During GC-SSR recordings, an increased firing rate of action potentials of at least twice the spontaneous activity was interpreted as a response. We converted the 16-min recording time into 250-ms bins and quantified the response strength as the maximum spike frequency (spikes $s^{-1}$; counted over 250-ms intervals) during the period of increased neuronal activity following stimulation. We only considered responses that were repeatable across different measurements. We excluded from our analysis bad contacts or contacts that were lost during measurements.

### Chemicals

All odorants tested were purchased from commercial sources (Sigma [http://www.sigma-aldrich.com] and TCI America [http://www.tcichemicals.com/en/us/]), except for 2-heptyl acetate, 2-heptyl butyrate, 2-heptyl hexanoate, and 2,3-butanediol diacetate, which were synthesized in house.

### Synthesis of 2-Heptyl Acetate, 2-Heptyl Butyrate, and 2-Heptyl Hexanoate

2-Heptanol (580 mg, 5 mmol) was dissolved in 15 mL dichloromethane, and 10 mmol of the corresponding carboxylic anhydride, 1.4 mL triethylamine, and 20 mg 4-dimethylaminopyridine were added. The mixture was stirred at room temperature for 3–4 hr, quenched with 20 mL ice water, and extracted with diethylether (3 × 30 mL). The combined organic layers were washed with water (40 mL) and brine (40 mL), dried with anhydrous sodium sulfate, filtered, and concentrated in vacuum. Purification with silica gel column chromatography (3:1 to 9:1 hexane:ethyl acetate) yielded racemic 2-heptyl esters as colorless liquids.

### Synthesis of 2,3-Butanediol Diacetate

2,3-Butanediol diacetate was synthesized from 2,3-butanediol with the procedure described for 2-heptyl acetate using 4 molar equivalents of acetic anhydride.

### Larval Two-Choice Assay

The larval olfactory two-choice assay is illustrated in Figure 1B. For the measurement of olfactory responses, 50 larvae were briefly dried on a filter paper before being placed in the center of a Petri dish (diameter, 9 cm) filled with 1% agarose. The Petri dish contained, on one side, a filter paper disc (diameter, 0.5 cm) loaded with 10 μL of one of the fruit extracts and, on the opposite side, a similar disc loaded with hexane. After 5 min of larvae placement and covering of the Petri dish, the number of larvae in respective zones was counted, and a response index was calculated: (O − C)/T, where O represents the number of larvae on the side of the dish containing fruit-headspace extracts, C represents the number of larvae on the hexane side, and T represents the total number of larvae.

### Flywalk Assay

Apart from few technical modifications on the behavioral setup (discussed laer), the Flywalk experiments were performed and analyzed as described previously (Steck et al., 2012; Thoma et al., 2014, 2015), with 7-day-old mated female flies starved for 24 hr before the start of the experiments. In short, 15 individual flies were placed in glass tubes (diameter, 0.8 cm). The glass tubes were aligned in parallel, and flies were monitored continuously by an overhead camera (HD Pro Webcam C920; Logitech). XY positions were recorded automatically at 20 fps using Flywalk Reloaded v1.0 software (Electricidade Em Pó; http://flywalk.eempo.net). Experiments were performed under red LED light (peak intensity at λ, 630 nm). During the experiments, flies were continuously exposed to a humidified airflow of 20 cm/s (70% relative humidity, 20°C). Flies were repeatedly presented with 500-ms pulses of various olfactory stimuli at interstimulus intervals of 90 s. Stimuli were added to the continuous airstream and thus traveled through the glass tubes at a constant speed. In brief, 100 μL of each fruit-headspace extract was prepared in 200-μL PCR tubes, which were placed into odor vials made of polyetheretherketone. The odor vials were tightly sealed and connected to the stimulus device via ball-stop check valves that allowed only unidirectional airflow through the odor-saturated headspace. Odor stimulation was achieved by switching an airflow otherwise passing through an empty vial (compensatory airflow) to the odor-containing vial. Tracking data were analyzed using custom-written routines programmed in R (www.r-project.org). Flies were assigned to individual glass tubes using the Y coordinates and, thus, could be unambiguously identified throughout the whole experiment. As flies were allowed to distribute freely within their glass tubes, they might have encountered the odor pulse at different times. This was compensated for by calculating the time of odor encounter for each individual tracking event based on the X position of the fly, system intrinsic delay, and airspeed. The time of encounter was set to 0, and the speed of movement was interpolated in the interval between 10 s before and 10 s after an encounter at 10 Hz. Because the tracking system does not capture the entire length of the glass tubes, not every fly was tracked for every stimulation cycle, and some entered or left the region of interest during the tracking event; thus, we decided to consider only complete trajectories in the interval between 1 s before and 7 s after odor encounter for further analysis.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and three tables and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.04.085.

## ACKNOWLEDGMENTS

We thank John R. Carlson for providing empty neuron fly lines and Kerstin Weniger and Silke Trautheim for technical support. This work was supported by the Max Planck Society.

## AUTHOR CONTRIBUTIONS

H.K.M.D., S.A.M.E., T.R., V.G., J.W., A.S., B.S.H., and M.K. designed all experiments. H.K.M.D., S.A.M.E., T.R., V.G., and J.W. performed all experiments and data analysis. H.K.M.D., B.S.H., and M.K. wrote the manuscript.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: December 19, 2017
Revised: February 22, 2018
Accepted: April 19, 2018
Published: May 22, 2018

## REFERENCES

Bell, J.S., and Wilson, R.I. (2016). Behavior reveals selective summation and max pooling among olfactory processing channels. Neuron *91*, 425–438.

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

Cobb, M., Bruneau, S., and Jallon, J.M. (1992). Genetic and developmental factors in the olfactory response of *Drosophila melanogaster* larvae to alcohols. Proc. Biol. Sci. *248*, 103–109.

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

Dobritsa, A.A., van der Goes van Naters, W., Warr, C.G., Steinbrecht, R.A., and Carlson, J.R. (2003). Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. Neuron *37*, 827–841.

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

Dweck, H.K.M., Ebrahim, S.A.M., Thoma, M., Mohamed, A.A.M., Keesey, I.W., Trona, F., Lavista-Llanos, S., Svatoš, A., Sachse, S., Knaden, M., and Hansson, B.S. (2015). Pheromones mediating copulation and attraction in *Drosophila*. Proc. Natl. Acad. Sci. USA *112*, E2829–E2835.

Ebrahim, S.A.M., Dweck, H.K.M., Stökl, J., Hofferberth, J.E., Trona, F., Weniger, K., Rybak, J., Seki, Y., Stensmyr, M.C., Sachse, S., et al. (2015). *Drosophila* avoids parasitoids by sensing their semiochemicals via a dedicated olfactory circuit. PLoS Biol. *13*, e1002318.

Farhan, A., Gulati, J., Groβe-Wilde, E., Vogel, H., Hansson, B.S., and Knaden, M. (2013). The CCHamide 1 receptor modulates sensory perception and olfactory behavior in starved *Drosophila*. Sci. Rep. *3*, 2765.

Fishilevich, E., Domingos, A.I., Asahina, K., Naef, F., Vosshall, L.B., and Louis, M. (2005). Chemotaxis behavior mediated by single larval olfactory neurons in *Drosophila*. Curr. Biol. *15*, 2086–2096.

Hansson, B.S., Knaden, M., Sachse, S., Stensmyr, M.C., and Wicher, D. (2010). Towards plant-odor-related olfactory neuroethology in *Drosophila*. Chemoecology *20*, 51–61.

Hernandez-Nunez, L., Belina, J., Klein, M., Si, G., Claus, L., Carlson, J.R., and Samuel, A.D.T. (2015). Reverse-correlation analysis of navigation dynamics in *Drosophila* larva using optogenetics. eLife *4*, e06225.

Hoare, D.J., McCrohan, C.R., and Cobb, M. (2008). Precise and fuzzy coding by olfactory sensory neurons. J. Neurosci. *28*, 9710–9722.

Knaden, M., Strutz, A., Ahsan, J., Sachse, S., and Hansson, B.S. (2012). Spatial representation of odorant valence in an insect brain. Cell Rep. *1*, 392–399.

Kreher, S.A., Kwon, J.Y., and Carlson, J.R. (2005). The molecular basis of odor coding in the *Drosophila* larva. Neuron *46*, 445–456.

Kreher, S.A., Mathew, D., Kim, J., and Carlson, J.R. (2008). Translation of sensory input into behavioral output via an olfactory system. Neuron *59*, 110–124.

Kwon, J.Y., Dahanukar, A., Weiss, L.A., and Carlson, J.R. (2007). The molecular basis of $CO_2$ reception in *Drosophila*. Proc. Natl. Acad. Sci. USA *104*, 3574–3578.

Mansourian, S., Corcoran, J., Enjin, A., Löfstedt, C., Dacke, M., and Stensmyr, M.C. (2016). Fecal-derived phenol induces egg-laying aversion in *Drosophila*. Curr. Biol. *26*, 2762–2769.

Mathew, D., Martelli, C., Kelley-Swift, E., Brusalis, C., Gershow, M., Samuel, A.D.T., Emonet, T., and Carlson, J.R. (2013). Functional diversity among sensory receptors in a *Drosophila* olfactory circuit. Proc. Natl. Acad. Sci. USA *110*, E2134–E2143.

Ramaekers, A., Magnenat, E., Marin, E.C., Gendre, N., Jefferis, G.S., Luo, L., and Stocker, R.F. (2005). Glomerular maps without cellular redundancy at successive levels of the *Drosophila* larval olfactory circuit. Curr. Biol. *15*, 982–992.

Robertson, H.M., Warr, C.G., and Carlson, J.R. (2003). Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA *100* (*Suppl 2*), 14537–14542.

Semmelhack, J.L., and Wang, J.W. (2009). Select *Drosophila* glomeruli mediate innate olfactory attraction and aversion. Nature *459*, 218–223.

Shanbhag, S.R., Müller, B., and Steinbrecht, R.A. (1999). Atlas of olfactory organs of *Drosophila melanogaster* 1. Types, external organization, innervation and distribution of olfactory sensilla. Int. J. Insect Morphol. Embryol. *28*, 377–397.

Steck, K., Veit, D., Grandy, R., Bermúdez i Badia, S., Mathews, Z., Verschure, P., Hansson, B.S., and Knaden, M. (2012). A high-throughput behavioral paradigm for *Drosophila* olfaction – the *Flywalk*. Sci. Rep. *2*, Article 361.

Stensmyr, M.C., Giordano, E., Balloi, A., Angioy, A.M., and Hansson, B.S. (2003). Novel natural ligands for *Drosophila* olfactory receptor neurones. J. Exp. Biol. *206*, 715–724.

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

Strutz, A., Soelter, J., Baschwitz, A., Farhan, A., Grabe, V., Rybak, J., Knaden, M., Schmuker, M., Hansson, B.S., and Sachse, S. (2014). Decoding odor quality and intensity in the *Drosophila* brain. eLife *3*, e04147.

Su, C.Y., Menuz, K., Reisert, J., and Carlson, J.R. (2012). Non-synaptic inhibition between grouped neurons in an olfactory circuit. Nature *492*, 66–71.

Thoma, M., Hansson, B.S., and Knaden, M. (2014). Compound valence is conserved in binary odor mixtures in *Drosophila melanogaster*. J. Exp. Biol. *217*, 3645–3655.

Thoma, M., Hansson, B.S., and Knaden, M. (2015). High-resolution quantification of odor-guided behavior in *Drosophila melanogaster* using the *Flywalk* paradigm. J. Vis. Exp. (*106*), e53394.

van der Goes van Naters, W., and Carlson, J.R. (2007). Receptors and neurons for fly odors in *Drosophila*. Curr. Biol. *17*, 606–612.

Wadhams, L.J. (1982). Coupled gas chromatography–single cell recording: a new technique for use in the analysis of insect pheromones. Zeitschrift für Naturforschung A *37C*, 947–952.

**Cell Reports, Volume *23***

## Supplemental Information

# The Olfactory Logic behind Fruit Odor Preferences in Larval and Adult *Drosophila*

**Hany K.M. Dweck, Shimaa A.M. Ebrahim, Tom Retzke, Veit Grabe, Jerrit Weißflog, Ales Svatoš, Bill S. Hansson, and Markus Knaden**

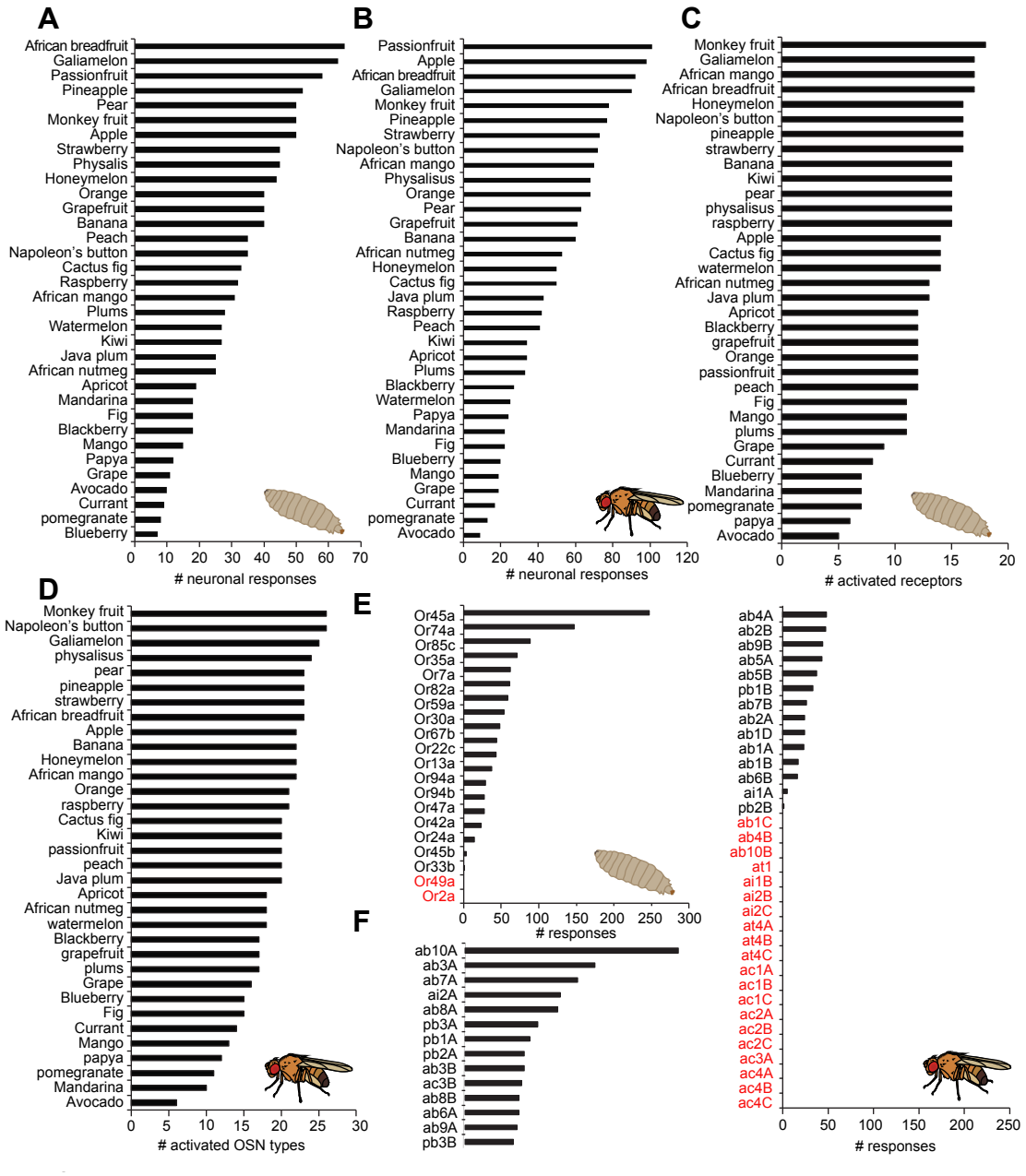



**Figure S1 . Number of fruit-specific neuronal responses and activated receptor types (related to Figure 2 )**

(A) Number of neuronal responses of OSNs expressing larval receptors when activated with headspaces of the different fruit. (B) Number of neuronal responses of adult OSNs when activated with headspaces of different fruit. (C) Number of activated receptor types in larvae when activated with headspaces of different fruit. (D) Number of activated receptor types in adults when activated with headspaces of different fruit. (E) Total number of responses of individual larval receptors when tested with all headspaces. (F) Total number of responses of individual adult receptors when tested with all headspaces. Receptors in red did not elicit a