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

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

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INTRODUCTION

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.

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 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 CO2 (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.

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.
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., 2006), 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., 2008), which reported recordings from up to 11 OSNs in a single preparation.

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-nonenone, 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 CO2; 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 a halo 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, Or45a, 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
elicited in larval and adult flies, we found a correlation of based representation of fruit odors with the behavior they saw from the higher spread of fruit odors in the PCA in Figure 4A to be better suited to discriminate between the fruit odors (as compared to Figure 4B). When comparing the physiology-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 odors (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.
EXPERIMENTAL PROCEDURES

Fly Stocks
All experiments with wild-type (WT) *D. melanogaster* were carried out with the Canton-S strain. Δhato;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 1-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.

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 larval (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).
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 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 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 larval 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 later), 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 (Electri- cidade Em Pó; http://flywalk.eemopo.net). Experiments were performed under red LED light (peak intensity at 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 a 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.

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**AUTHOR CONTRIBUTIONS**


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.
DEKLARATION DER INTERESES 

The authors declare no competing interests.

REFERENCES


