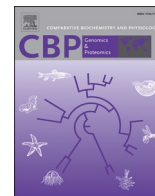




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## Comparative transcriptomic assessment of the chemosensory receptor repertoire of *Drosophila suzukii* adult and larval olfactory organs

William B. Walker III<sup>a,b,\*</sup>, Boyd A. Mori<sup>a,c</sup>, Alberto M. Cattaneo<sup>a</sup>, Francisco Gonzalez<sup>a,d</sup>, Peter Witzgall<sup>a</sup>, Paul G. Becher<sup>a</sup>

<sup>a</sup> Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden

<sup>b</sup> USDA-ARS Temperate Tree Fruit and Vegetable Research Unit, 5230 Konnowac Pass Road, Wapato, WA 98951, USA

<sup>c</sup> Department of Agricultural, Food and Nutritional Science, 4-10 Agriculture/Forestry Centre, University of Alberta, Edmonton, Alberta T6G 2P5, Canada

<sup>d</sup> Department of Research and Development, ChemTica Internacional S.A., Apdo. 640-3100, Santo Domingo, Heredia, Costa Rica

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### ABSTRACT

The spotted wing *Drosophila*, *Drosophila suzukii*, has emerged within the past decade as an invasive species on a global scale, and is one of the most economically important pests in fruit and berry production in Europe and North America. Insect ecology, to a strong degree, depends on the chemosensory modalities of smell and taste. Extensive research on the sensory receptors of the olfactory and gustatory systems in *Drosophila melanogaster* provide an excellent frame of reference to better understand the fundamentals of the chemosensory systems of *D. suzukii*. This knowledge may enhance the development of semiochemicals for sustainable management of *D. suzukii*, which is urgently needed. Here, using a transcriptomic approach we report the chemosensory receptor expression profiles in *D. suzukii* female and male antennae, and for the first time, in larval heads including the dorsal organ that houses larval olfactory sensory neurons. In *D. suzukii* adults, we generally observed a lack of sexually dimorphic expression levels in male and female antennae. While there was generally conservation of antennal expression of odorant and ionotropic receptor orthologues for *D. melanogaster* and *D. suzukii*, gustatory receptors showed more distinct species-specific profiles. In larval head tissues, for all three receptor gene families, there was also a greater degree of species-specific gene expression patterns. Analysis of chemosensory receptor repertoires in the pest species, *D. suzukii* relative to those of the genetic model *D. melanogaster* enables comparative studies of the chemosensory, physiology, and ecology of *D. suzukii*.

### 1. Introduction

Olfactory and gustatory chemosensory systems play a significant role in mediating a broad suite of insect behaviours. Food and host selection, mate finding and acceptance, as well as avoidance of natural enemies are all processes largely influenced by the detection and interpretation of chemosensory cues and signals. This central role of insect chemosensory systems demands a great degree of sensitivity to ecological and evolutionary pressures relevant to niche adaptation and speciation (Hansson and Stensmyr, 2011; Conchou et al., 2019). Chemosensory receptor proteins are typically expressed in the dendrites of

chemosensory neurons and are specifically responsible for interaction with chemical signals (Leal, 2013; Fleischer et al., 2018; Robertson, 2019). Odorant receptors (ORs), gustatory receptors (GRs) and ionotropic receptors (IRs) comprise the primary multi-gene families responsible for chemosensory detection in insects.

The molecular basis for olfactory and gustatory function is well characterized in *D. melanogaster*, in both adults and larvae, with respect to chemosensory receptor expression and functional activation (Hallem et al., 2004; Kreher et al., 2008; Benton et al., 2009; Kwon et al., 2011; Menz et al., 2014; Croset et al., 2016; Sanchez-Alcaniz et al., 2018). Conversely, functional capabilities of drosophilid chemosensory

**Abbreviations:** CO<sub>2</sub>, Carbon Dioxide; FPKM, Fragments per Kilobase per Million; GR, Gustatory Receptor; IR, Ionotropic Receptor; OR, Odorant Receptor; ORCO, Odorant Receptor Co-Receptor; OSN, Olfactory Sensory Neurons; RNA-Seq, RNA Sequencing; RPM, Reads per Million; RT-PCR, Reverse Transcriptase Polymerase Chain Reaction.

\* Corresponding author at: USDA-ARS, Temperate Tree Fruit and Vegetable Research Unit, 5230 Konnowac Pass Road, Wapato, WA 98951, USA.

E-mail addresses: [william.walker@usda.gov](mailto:william.walker@usda.gov) (W.B. Walker), [bmori@ualberta.ca](mailto:bmori@ualberta.ca) (B.A. Mori), [albertomaria.cattaneo@slu.se](mailto:albertomaria.cattaneo@slu.se) (A.M. Cattaneo), [francisco\\_gonzalez@chemtica.com](mailto:francisco_gonzalez@chemtica.com) (F. Gonzalez), [peter.witzgall@slu.se](mailto:peter.witzgall@slu.se) (P. Witzgall), [paul.becher@slu.se](mailto:paul.becher@slu.se) (P.G. Becher).

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receptors, across other drosophilid species, including expression patterns and functional assay of receptors have been more limited (de Bruyne et al., 2001; Hallem et al., 2004; Hallem and Carlson, 2006; de Bruyne et al., 2010; Crowley-Gall et al., 2016; Shaw et al., 2019; Auer et al., 2020).

The spotted wing drosophila, *Drosophila suzukii* has emerged during the last decade as a globally relevant, invasive species with significant economic impact on the cultivation of soft-fruits and berries (Walsh et al., 2011; Cini et al., 2014; Asplen et al., 2015). Owing largely to the evolution of a serrated ovipositor organ, *D. suzukii* is able to exploit fresh fruit as a larval host, whereas other closely related *Drosophila* species lay their eggs in soft over-ripe and fermenting fruit. The divergence of *D. suzukii*, with regards to this aspect of niche utilization provides an opportunity to explore the evolutionary consequences of ecological adaptation in comparison with the genetic model system, *Drosophila melanogaster*. For example, differences exist between *D. melanogaster* and *D. suzukii* in sensitivity to and in preference for odors emitted by ripe or decaying strawberries (Karageorgi et al., 2017). Furthermore, shifts in the olfactory tuning of some olfactory sensory neurons housed in antennal sensilla subtypes of *D. suzukii*, relative to *D. melanogaster*, have been characterized (Keeseey et al., 2015; Keeseey et al., 2022). A fundamental question concerns how these differences are manifested, with reference to the chemosensory receptors that directly mediate the olfactory response.

Complete chemosensory receptor repertoires present in the *D. suzukii* genome have been characterized (Crava et al., 2016; Hickner et al., 2016; Ramasamy et al., 2016). These reports confirm gene duplications of subsets of ORs, GRs and IRs relative to *D. melanogaster*. A recent report on a *D. suzukii* female antennal transcriptome examined the effect of mating on chemosensory gene expression and olfactory system function (Crava et al., 2019). Antennal expression of olfactory receptors in *D. suzukii* largely mirrored that of *D. melanogaster*. For example, only three OR orthologues present in *D. melanogaster* antennae were not found to be expressed in *D. suzukii* female antennae. Consistent with these findings, a recent report on the neuroethology of *D. suzukii* revealed functional conservation of olfactory responses in 86 % of olfactory sensory neuron (OSN) types between *D. suzukii* and *D. melanogaster* (Keeseey et al., 2022), suggesting a high degree of conservation of receptor function for most of the orthologues that are expressed in the antennae of both species. However, differences are apparent elsewhere, as *D. suzukii* shows a lower expression in several bitter-compound GRs in the labellum, which correlates with a reduced bitter deterrence during oviposition as compared to *D. melanogaster* (Dweck et al., 2021).

Similarities in chemosensory receptor genes and function facilitate both basic and applied research directions. At the basic level, exploration of chemosensory receptors may identify stage- or sex-specific differences that could influence physiological or ecological events. In addition, niche adaption may be driven by chemosensory receptors and further reflected throughout the olfactory system of adults and larvae (Chakraborty et al., 2022; Keeseey et al., 2022). At the applied level, exploration of chemosensory receptors may contribute to the use of semiochemicals for management of *D. suzukii* (Caballero-Vidal et al., 2021; Liu et al., 2022). Current semiochemical-based efforts are aimed at exploiting attraction to fermenting yeasts and fruit for population control and species-specific monitoring (Hamby and Becher, 2016; Schetelig et al., 2018; Wallingford et al., 2018; Noble et al., 2019; Rehmann et al., 2022). Attempts have been made to translate yeast attraction into synthetic attractants (Feng et al., 2018), where possible differences between larvae (Lewis and Hamby, 2019; Roubos et al., 2019) and adult flies (Clymans et al., 2019; Kleman et al., 2022), as well as the sexes (Mori et al., 2017; Piñero et al., 2019) are consequential.

To better understand the role of the *D. suzukii* olfactory system in niche exploitation and for developing semiochemical-based management strategies, we analyzed adult male and female antennal tissue and larval heads, as proxy for larval chemosensory systems. Using

transcriptomics, qualitative assessments were made concerning genes expressed in the different tissues, and larval head expression data was supported with reverse transcriptase end-point polymerase chain reaction (RT-PCR) profiling. Quantitative assessments of chemosensory receptor expression levels were also made, with transcript abundance estimates generated for preliminary comparisons within and across samples. Utilizing the vast knowledge of *D. melanogaster* chemosensory receptor expression and function, we analyzed expression profiles across these two species, comparing and contrasting differences between adult and larval chemosensory capabilities.

## 2. Materials and methods

### 2.1. Insect specimen and RNA-sequencing (RNA-Seq) sample preparation

The *D. suzukii* colony originated from Trento, Italy (courtesy of Gianfranco Anfora, C3A Center Agriculture Food Environment of University of Trento). The colony was derived from the same colony used for previous transcriptomic studies (Crava et al., 2019). Flies were reared on a standard cornmeal-based ('Bloomington *Drosophila* Stock Center' (BDSC) <https://bdsc.indiana.edu/information/recipes/bloomfood.html>) artificial diet at 22–24 °C, 35–60 % RH, and 12:12 h L:D photoperiod. Two- to five-day old male and female adults from a mixed population were used for antennal collections. Antennae were harvested from 500 males and 500 females separately into RNAlater (Sigma-Aldrich, St. Louis, MO, USA) and stored at 4 °C until shipment for RNA-Seq. The RNAlater samples were sent to LGC Genomics GmbH (Berlin, Germany) for further processing.

Third instar (six to seven days old) larvae were obtained by placing adult males and females from the colony in a vial containing standard BDSC cornmeal diet. After 24 h, the adults were removed, and the vials incubated for seven days. After seven days, larvae were removed from the vials, rinsed in sterile mQ-H<sub>2</sub>O, and dissected under a stereomicroscope. The three anterior (head) segments were removed and placed immediately into a 1.5 mL microcentrifuge tube containing TRIzol® reagent (Life Technologies, Carlsbad, CA, USA) on ice. Approximately 350 larvae were dissected and heads collected in a single tube of TRIzol. Total RNA was extracted and purified with a combined approach of TRIzol-based extraction followed by RNeasy® Mini spin column purification (Qiagen, Venlo, Netherlands), as previously described (Walker et al., 2016). RNA was eluted with supplied RNase-Free water and immediately assayed for quality and concentration with a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Purified RNA was sent to the Beijing Genomics Institute (BGI) facility in Hong Kong (BGI Hong Kong Co.) for sequencing.

### 2.2. RNA sequencing

Pure total RNA, from one sample each for both male and female antennae, was extracted by LGC Genomics and separate cDNA libraries were prepared using standard in-house protocols. Through Illumina MiSeq V3 and NextSeq 500 V2 sequencing, paired-end reads, 300 bp and 150 bp, respectively, were generated and saved in FASTQ format (Cock et al., 2010). Pre-processing of sequenced reads were carried out by LGC Genomics as follows: libraries were demultiplexed for each sequencing lane using the Illumina bcl2fastq 2.17.1.14 software; one or two mismatches or Ns were allowed in the barcode read when the barcode distances between all libraries on the lane allowed for it; sequencing adapter remnants were clipped from all raw reads; reads containing more than one N were discarded; removal of bases or complete reads with sequencing errors, via trimming of reads at 3'-end to get a minimum average Phred quality score of 10 over a window of ten bases; reads with final length < 20 bases were discarded; rRNA sequence reads were filtered out using RiboPicker 0.4.3.

For the single larval head sample, at BGI total RNA was converted to a cDNA library as previously described (Walker et al., 2019). With

Illumina HiSeq™ 2000 sequencing, paired-end reads (90 bp PE) were generated and saved in FASTQ format (Cock et al., 2010). Low quality reads that did not meet any of the following criteria were removed with proprietary BGI software: reads with sequenced adaptors, reads with >5 % unknown nucleotides and reads that have >50 % of nucleotide bases with PHRED quality scores <10 (Ewing et al., 1998; Ewing and Green, 1998).

## 2.3. Bioinformatic pipelines for transcriptome analyses

### 2.3.1. Adult antennal transcriptome

A single transcriptome comprised of sequenced libraries from both male and female antennal samples was assembled using Trinity v.2.2.0 (Grabherr et al., 2011); digitally normalized read pairs were used, all scaffolds larger than 200 bp were kept, low confidence contigs were filtered out using RSEM v.1.2.14 (Li and Dewey, 2011). To facilitate unambiguous read mapping of individual sample reads back to unique locations on the assembled transcriptome sequences for downstream quantitative analyses, the software CD-HIT-EST (v. 4.5.4-2011-03-07) was used to identify and remove redundant sequences that share 98 % or greater identity with other sequences (Li and Godzik, 2006). The transcriptome Trinity.fasta file was used as input, program parameters -c 0.98 -n 8 were specified. In cases where sequences shared >98 % identity but were of different sizes, the largest of the sequences were retained in the fasta file.

To assess the completeness of both the adult antennae and larval head transcriptomes, an Arthropoda BUSCO database, consisting of 1066 core genes that are highly conserved single-copy orthologues (Seppey et al., 2019; Waterhouse et al., 2019), was used to query the transcriptomes. For this process, the gVolante web server (<https://gvolante.riken.jp/>) was utilized with the following parameters: min\_length\_of\_seq\_stats: 1, assembly\_type: trans, Program: BUSCO\_v2/v3, selected\_reference\_gene\_set: Arthropoda (Nishimura et al., 2017).

For identification and characterization of chemosensory receptors, text files were compiled in fasta format with protein sequences obtained from the supplementary materials of the genomic analyses of ORs, GRs and IRs (Crava et al., 2016; Ramasamy et al., 2016). BLAST nucleotide databases were created from the Trinity.fasta file and were queried by the protein sequence fasta files for each of the chemosensory gene families. For this procedure, BLAST v.2.9.0+ was used to perform a tblastn query and a minimum e-score threshold of 1e-05 was required for hits; additional parameters included -num\_descriptions 50; and output format six (Camacho et al., 2009). For each of the previously annotated chemosensory genes, the top BLAST hit transcript cluster was manually extracted from the Trinity.fasta file. Nucleotide sequences were translated into protein sequence with the ExPASy web Translate tool (<https://web.expasy.org/translate/>; (Artimo et al., 2012)), and the protein sequences were aligned to reference annotations with the ClustalOMEGA web tool (<http://www.ebi.ac.uk/Tools/msa/clustalo/>; (Sievers et al., 2011)).

Read mapping of individual sample reads to the de novo transcriptome and subsequent expression level abundance estimations were carried out, as described (Haas et al., 2013) with the Trinity Perl script "align\_and\_estimate\_abundance.pl" in the release version of Trinity 2.8.4, using RSEM v.1.2.12 (Li and Dewey, 2011), Bowtie v.0.12.6 (Langmead et al., 2009) and samtools v.0.1.19 (Li et al., 2009). The CD-HIT-EST-modified Trinity.fasta file was used as reference transcripts input and the trimmed fastq adult antennal reads described above were used as mapping input. A gene\_trans\_map file was generated with an RSEM perl script, and used as input to assess relative expression levels for all transcripts within each relevant Trinity cluster. Estimated mapped reads for each gene were normalized to the total mapped reads in each sample, divided by one million to calculate reads per million reads mapped (RPM). To maintain consistency with relevant comparative studies on *Drosophila* antennal transcriptomes, genes were defined as antennal expressed if they were detected at RPM > 1 in both male and

female samples (Menuz et al., 2014; Crava et al., 2019). Expression was further normalized by gene length to calculate FPKM values (Li and Dewey, 2011) to facilitate estimation of relative gene expression abundance levels for genes within and across each sample.

### 2.3.2. Larval head transcriptome

For larval head sequenced reads, raw sequencing files were downloaded from the BGI server. The sequences were checked for quality with FastQC v.0.11.5 (Andrews, 2010). To remove low quality reads and adaptor contamination, Trimmomatic v.0.36 (Bolger et al., 2014) was used (Parameters: ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:10 TRAILING:20 MINLEN:36). The trimmed, paired reads were aligned to the *D. suzukii* genome v.1.0 (bioSample: SAMN02953868; Assembly: GCA\_000472105.1; (Chiu et al., 2013)) using Bowtie2 v.2.3.4.1 (parameters as specified within RSEM) run with RSEM v.1.3.1 (Li and Dewey, 2011). Briefly, the genome and annotation file (NCBI *Drosophila suzukii* Annotation Release 101) were used by *rsem-prepare-reference* to create transcriptome files, necessary internal RSEM files, and Bowtie2 index files. This was followed by *rsem-calculate-expression*, which then mapped the reads to the transcriptome and calculated expression levels.

Chemosensory receptor genes were identified based on genome annotations and, in addition, by tblastn searches of all the amino acid sequences of previously identified *D. suzukii* receptors (Crava et al., 2016; Hickner et al., 2016; Ramasamy et al., 2016). When more than one receptor was identified with a significant BLAST hit, the results were compared manually (based on query coverage, % identity, and e-value) to retain the best BLAST hit, and hence, chemosensory gene annotation. The gene annotations were then used to query the results of the RSEM file at the isoform level and gene expression levels (FPKM) were obtained.

## 2.4. cDNA synthesis and PCR assay of larval chemosensory receptor expression

### 2.4.1. cDNA synthesis

To create cDNA, the SuperScript III Reverse Transcriptase (Invitrogen) kit was used with the same purified RNA sample used for the RNA sequencing reaction. Ten micrograms of RNA were used in the reaction following the manufacturers protocol.

### 2.4.2. PCR primer design

To confirm the expression of ORs, GRs and IRs in larval heads, primers were designed (Eurofins Genomics, Ebersberg, Germany) based off genomic sequences (Crava et al., 2016; Ramasamy et al., 2016). To ensure full-length transcripts were expressed, most primer pairs were designed to amplify the entire coding sequence of each transcript (Supplementary Table S1).

### 2.4.3. RT-PCR confirmation of ORs, GRs and IRs

Each PCR was carried out in a 12.5 µL volume containing: 5.25 µL H<sub>2</sub>O, 5.75 Green master mix (Dreamtaq green PCR mastermix 2×, Thermo Fisher Scientific) 0.25 µL forward primer (10.0 µM), 0.25 µL reverse primer (10.0 µM) and 1.0 µL of the diluted cDNA sample. Temperature program with an initial 5-min step at 95 °C, and then 45 cycles of 95 °C for 1 min, primer melting temperature for 1 min, 72 °C for 1 min, and a final 7-min step at 72 °C was used.

Each PCR reaction was repeated twice, and no-template controls were used. PCRs were performed in parallel on genomic DNA (gDNA) templates extracted from adult insects (male and female), pupae and larvae (DNeasy Blood & Tissue, Qiagen). Amplification was performed using primers for the coding sequence of Orco. No amplification or amplifications products with different sizes were observed, indicating that no significant gDNA contamination occurred in our cDNA preparations (Supplementary Data S2). Amplifications of PCR products were analyzed by electrophoresis on a 1.5 % agarose gel, with the 1 kb GeneRuler ladder (Thermo Fisher Scientific), stained with GelRed



Nucleic Acid Stain (Biotium, Fremont, CA), and visualized using GeneFlash (Syngene Bio Imaging, <https://www.syngene.com/>).

### 2.5. Heatmap presentation of transcript expression

Heatmap plots were generated for the binary logarithm of raw FPKM-plus-1 values. These plots were made using the conditional formatting function in Microsoft Excel, with a three-color scale. For each plot, the minimum value was set to number type, with a value of one, and displayed as white; midpoint was set to percentile type, with a value of 75, and displayed as dark color; maximum was set to highest value type and displayed as bright color. For all gene families, the range was specified for each tissue type independently, such that the color gradient was set based upon the highest FPKM values within each tissue, not across all tissues.

### 2.6. Phylogenetic analysis of chemosensory receptors

Amino acid sequences for chemosensory receptors of each gene family for *D. suzukii* were obtained from previous reports on genomic characterizations of chemosensory receptors (Crava et al., 2016; Ramasamy et al., 2016); for *D. melanogaster*, protein sequences were obtained from NCBI-GenBank. Protein sequences were aligned using MAFFT online v.7.220 (<http://mafft.cbrc.jp/alignment/server/phylogeny.html>) through the FFT-NS-i iterative refinement method, with JTT200 scoring matrix, “leave gappy regions” set, and other default parameters (Katoh et al., 2019). Aligned sequences were used to build the phylogenies with MEGA7 software in command line (Kumar et al., 2012), with the following parameters: Maximum Likelihood Tree Method with the JTT-F' model, uniform rates, use all sites, nearest neighbor interchange heuristic method, very strong branch swap filter and default automatic NJ/BioNJ initial tree. The bootstrap consensus of each phylogenetic tree was inferred from 600 replicates. Consensus Newick format trees were compiled with MEGA6.06 software (Tamura et al., 2013) and edited with Adobe Illustrator.

## 3. Results

### 3.1. Transcriptome overview

Transcriptomes for *D. suzukii* adult antennae and larval heads were generated and analyzed separately. Male and female adult antennae sequenced samples were combined for a total of 99.1 million quality trimmed and filtered read pairs, digitally normalized to 1.1 million read pairs, and assembled de novo by Trinity Assembler (v. 2.2.0). After redundancy removal, a total of 260,000 transcript sequences remained, with a mean sequence length of 431 bp and an N50 sequence length of 451 bp. BUSCO analysis of the adult antennal transcriptome with the Arthropoda database of single-copy orthologues, resulted in hits for 99.4 % of queried sequences, with 85.27 % identified as complete.

For the larval heads, 61 million clean trimmed reads were utilized to create a genome-guided transcriptome. After redundancy removal, a total of 238,000 transcript sequences remained, with mean sequence length of 2410 bp and an N50 sequence length of 3470 bp. BUSCO analysis of the third-instar larval head transcriptome with the Arthropoda database of single-copy orthologues, resulted in hits for 98.78 % of queried sequences, with 97.56 % identified as complete.

### 3.2. Odorant receptors

In adult antennae, transcripts encoding 58 unique OR gene products across 52 genomic loci were identified, including one of two predicted OR46a splice variants and all five predicted OR69a splice variants (Ramasamy et al., 2016). Detectable expression levels were attributed to 40 OR genes encoding 44 gene products (Fig. 1). Sexually dimorphic expression patterns for ORs in adult antennae were generally not

observed (Pearson Correlation Coefficient = 0.996). The odorant receptor co-receptor (Orco) was the most highly expressed gene (male = 975.5 FPKM; female = 1166.4 FPKM). In both male and female antennae, DsuzOR92a and DsuzOR42b were the most highly expressed tuning ORs, with FPKM values at least two- to three-fold higher than all other individual ORs (Supplementary Data S3).

In larval heads, genome guided read-mapping revealed expression of 34 ORs, including Orco (1.78 FPKM), with FPKM values greater than zero (Fig. 1). These results were validated by RT-PCR for 27 ORs including Orco (Supplementary Fig. S4). Orco along with OR2a (3.81 FPKM), OR10a (1.14 FPKM) and OR43b (1.37 FPKM) were the most highly expressed OR family members in larval head tissue, all with FPKM values greater than one (Supplementary Data S3). Notably, 12 of the 27 ORs confirmed in *D. suzukii* larval heads are not expressed in adult antennae, while 15 ORs were detected in both larvae and adults (Fig. 1; Fig. 2).

For each of the three analyzed gene families, phylogenetic trees were generated for all genes present in the genomes of *D. melanogaster* and *D. suzukii* to facilitate comparisons of chemosensory receptor expression profiles in adult antennae and larval head tissues. As previously reported in adult antennae (Menuz et al., 2014; Crava et al., 2019), there is a broad conservation of expression of OR homologues in *D. melanogaster* and *D. suzukii* (Fig. 2). Orthologous pairs of 29 ORs are expressed in both species, while ORs found in complex clusters of five other lineages are also expressed in both species, including OR19a, OR23a, OR65b/OR65c, OR67a, and OR69a. These lineages often contain multiple paralogues in *D. suzukii*, where only one orthologue is present in *D. melanogaster*. Only OR85a, OR33a and OR33c are found expressed in the antennae of *D. melanogaster* but not in *D. suzukii*. Conversely, in the larval head, a different picture emerges. In *D. melanogaster* larval heads, 25 ORs are expressed (Fishilevich et al., 2005; Kreher et al., 2008) and the homologues of seven of these are not found expressed in *D. suzukii* larval heads, while for the 26 tuning ORs confirmed expressed in *D. suzukii* larval head, nine of the corresponding homologues are not expressed in *D. melanogaster* larval heads (Fig. 2; Supplementary Table S5).

### 3.3. Gustatory receptors

Transcripts encoding 36 GR proteins were identified in the antennal transcriptome, though only 10 GRs had expression levels >1 RPM. Eight of the GRs that were identified in our transcriptome, albeit with RPM values less than one in both male and female antennae (GR10a, GR28bD, GR58c, GR61a, GR64b, GR64c, GR77a and GR85a1) were previously reported as expressed (Crava et al., 2019). Among all expressed GRs, sexually dimorphic expression patterns were not observed (Fig. 3). The most highly expressed GRs include putative carbon dioxide (CO<sub>2</sub>) receptors, GR21a and GR63a (Jones et al., 2007; Kwon et al., 2007), as well as single putative sugar and bitter compound receptors, GR64f and GR66a, respectively (Moon et al., 2006; Jiao et al., 2008). Expression of these four receptors ranged from 22.9 to 290.0 FPKM. In both male and female antennae, GR66a was the most highly expressed GR, with FPKM values at 290.0 and 287.7 in male and female antennae, respectively. All other GR transcripts displayed low expression, with FPKM values below five (Supplementary Data S3).

In third instar larval heads, 24 GRs were identified as expressed with FPKM values greater than zero. Expression of 23 of these receptors was confirmed via RT-PCR (Supplementary Fig. S4). Putative bitter-compound receptors GR66a (Weiss et al., 2011), and GR58c were the most highly expressed GRs in the larval head sample, both with FPKM values greater than one (Supplementary Data S3). Five GRs were commonly expressed across adult antennae and larval head, including one of the two broadly conserved CO<sub>2</sub> receptors (GR63a), as well as the conserved candidate fructose receptor GR43a. More candidate bitter-compound receptor (Weiss et al., 2011) transcripts were detected in larval head (N = 10; GR8a/GR22e/GR28a/GR32a/GR33a/GR59b/GR66a/GR92a/GR93a/GR93b) than in adult antennae (N = 4; GR32a/

GR39b/GR66a/GR98b) (Fig. 3; Fig. 4).

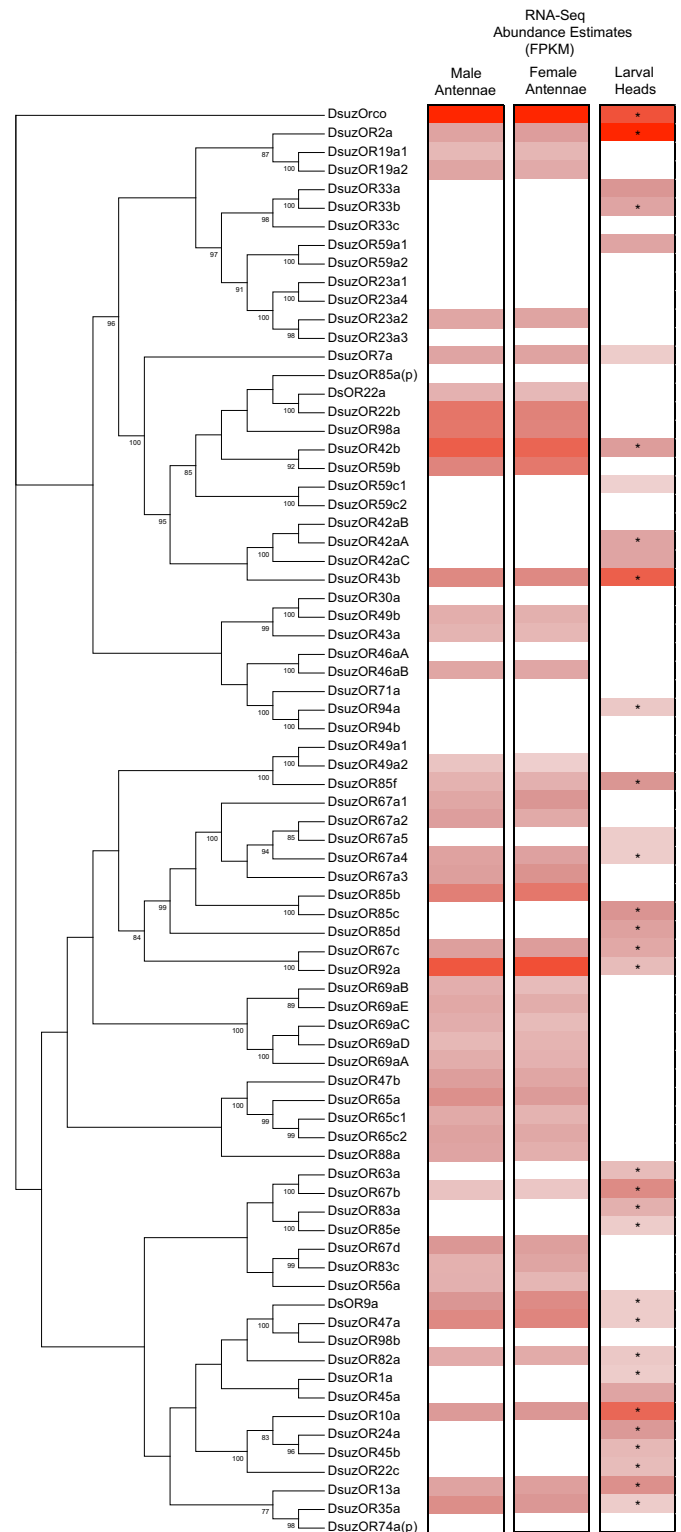
In both male and female adult antennae, as well as larval heads, notable differences in GR expression profiles exist when comparing *D. suzukii* with *D. melanogaster*. Using a transcriptomic approach supported with quantitative RT-PCR, 14 GRs were reported to display antennal expression in *D. melanogaster* (Menuz et al., 2014). This is similar to findings in support of expression of up to 18 GRs in the antennae of *D. suzukii* (results from this report and Crava et al., 2019). Of the 18 GRs, 10 homologues display expression in *D. melanogaster* antennae (Fig. 4). Four GRs in *D. melanogaster*, and up to nine GRs in *D. suzukii* were thus identified without corresponding orthologue expression in the adult antennae of the opposite species. In *D. melanogaster*, three of these are found within the sugar clade (GR64a/GR64d/GR64e), conversely, in *D. suzukii*, four of these GRs (GR28b.D/GR32a/GR39aB/GR98b) are orthologous to *D. melanogaster* GRs implicated in detecting bitter compounds (Weiss et al., 2011). In *D. melanogaster* larval head sensory organs, utilizing transgenic GR promoter-Gal4/GFP reporter detection systems, 39 GRs were identified as expressed (Kwon et al., 2011). In *D. suzukii* larval heads, of the 23 GRs confirmed expressed in this report, orthologues of 15 of these were identified in *D. melanogaster* larval head sensory systems, while the remaining eight, including three putative sugar receptors (GR61a/GR64a/GR64c), were not expressed in *D. melanogaster* (Fig. 4).

### 3.4. Ionotropic receptors

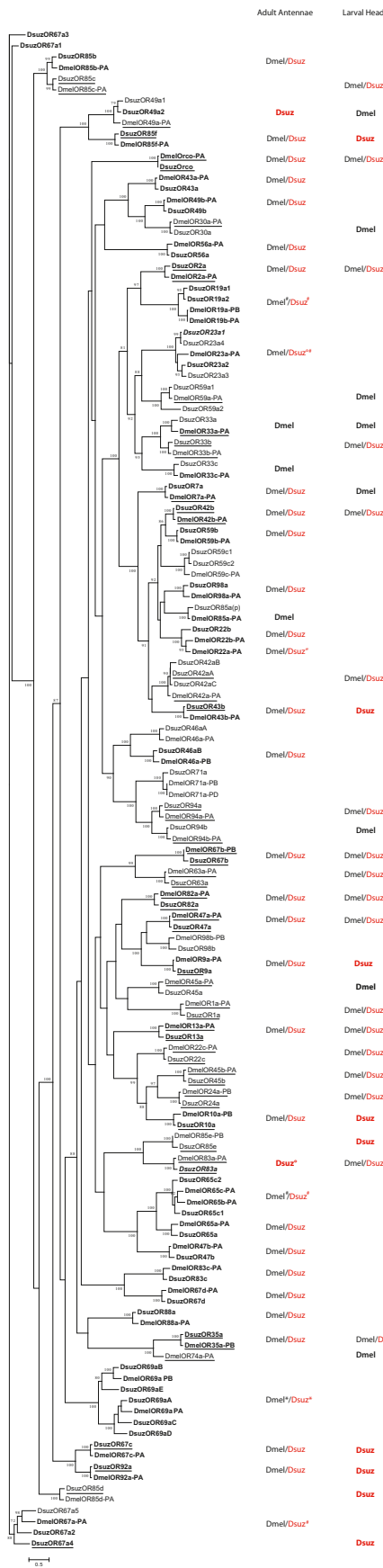
Gene transcripts encoding 38 IRs were identified in the antennal transcriptome, with 14 having expression levels >1 RPM in both male and female. Notably, among these 14, all are phylogenetically grouped with either the IR co-receptors (IR8a/IR25a/IR76a) or the antennal IRs, (Croset et al., 2010), except for IR62a, which is part of the divergent IR subfamily. Four additional IRs that were identified here, albeit with expression values below 1 RPM in both male and female antennae (IR40a, IR51a, IR52c2 and IR100a) were previously reported as expressed (Crava et al., 2019). As with the ORs and GRs, sexually dimorphic expression patterns were generally not observed (Fig. 5), though exceptions to this are noted for IR21a (male-bias) and IR76a (female-bias). All putative IR co-receptors were the most highly expressed members of this family. Among the remaining, IR75d and IR64a were the most highly expressed in both male and female antennae samples (Supplementary Data S3).

In larval heads, 29 IRs were identified with FPKM values greater than zero; expression was confirmed for 20 of these by RT-PCR (Supplementary Fig. S4), including the co-receptors IR25a and IR76b. The divergent IR, IR62a displayed the highest expression value, as the only IR with an FPKM value above one (Supplementary Data S3). In contrast to IRs found expressed in adult antennal samples, which are predominantly of the antennal IR subfamily, most IRs expressed in larval head are members of the divergent IR subfamily.

As with the odorant receptors, the IR expression profile in *D. suzukii* male and female antennae largely reflected that of *D. melanogaster*. In *D. melanogaster* expression of 16–20 IRs was confirmed via RNA-Seq (Menuz et al., 2014) and Gal4/GFP promoter-reporter detection (Sanchez-Alcaniz et al., 2018). Likewise, in *D. suzukii*, 16–20 IRs show antennal expression (here and Crava et al., 2019). Orthologues of all co-receptors and antennal IRs are expressed in both species, apart from IR68a, for which expression evidence exists in *D. melanogaster* (Sanchez-Alcaniz et al., 2018), but not *D. suzukii*. One divergent IR, IR62a, was observed to be expressed in the antennae of both species as well. In *D. melanogaster* larval-head sensory organs, utilizing transgenic IR promoter-Gal4/GFP reporter detection systems, 33 IRs were identified as expressed (Sanchez-Alcaniz et al., 2018). Of the 20 IRs confirmed expressed in *D. suzukii* larval heads, the orthologues of 12 of these were identified in *D. melanogaster* larval head sensory systems, while the remaining eight (IRs 64a/75d/76a/7c/10a/56b/62a/85a/) were unique to *D. suzukii* larval heads, including three antennal IRs (Fig. 6).



**Fig. 1.** Heat-plot of relative expression values for *D. suzukii* odorant receptors (ORs). Estimation of abundance values determined by read mapping. White indicates no expression or transcripts not identified in transcriptome; for adult antennal samples specifically, expression values have been suppressed for transcripts with RPM values less than one in both samples. Lighter colors indicate relatively lower expression, brighter colors indicate relatively higher expression. Color plots represent binary log of FPKM plus one for each gene (See Supplementary Data S3 for raw data). For larval head data, asterisk “\*” indicates expression has been validated by RT-PCR assay. Color scales for each tissue type are independent of other tissue types. Range of values for Male Antenna: 0.26–9.93; Female Antennae: 1.02–10.18; Larval Heads: 0.042–2.26.



**Fig. 2.** Summary of OR expression profiles across *D. suzukii* and *D. melanogaster* antennae and larval heads. ORs ordered in phylogenetic tree, with bold highlight indicative of antennal expression and underline indicative of larval head expression. For *D. suzukii*, antennae expression is indicated based upon data from this report and that of Crava et al. (2019), with confirmed expression based upon RPM values greater than one. ° symbol indicates gene was determined to be expressed in Crava et al. (2019), but not in this report. # is indicative of expression of multiple genes within subfamily, \* is indicative of expression of multiple isoforms. ° symbol is indicative of expression of putative pseudogene DsuzOR22a, which is not shown in phylogenetic tree due to poor alignment with full length ORs. Larval expression is indicated if RNA-Seq data was validated by RT-PCR Assay. For *D. melanogaster*, antennae expression is reported based upon Menz et al. (2014), larval head expression profile is derived from Kreher et al. (2008) and Mathew et al. (2013).

**4. Discussion**

**4.1. Summary of findings**

Expression profiles of ORs, GRs and IRs have been characterized in *D. suzukii* male and female antennae and third instar larval heads. Expression of 40 ORs, 10 GRs, and 14 IRs has been identified in our adult antennal transcriptome. In our larval head transcriptome, 28 ORs, 23 GRs, and 20 IRs have been identified as expressed and confirmed with RT-PCR. In consideration of the fact that sequencing of our samples was not replicated, we clarify that our results are supported by multiple lines of evidence. BUSCO analysis suggests a high degree of completeness of both transcriptomes. In consideration of low abundance estimates for chemosensory receptors in larval heads, expression was validated by PCR assay for most transcripts detected in the transcriptome. Expression in adult antennae is verified in two independent antennal RNA-Seq samples reported here (male and female), and in comparison, with a replicated RNA-Seq study on chemosensory receptors expressed in female antennae of the same strain of *D. suzukii* as reported herein (Crava et al., 2019). Using the same threshold of expression indicated by RPM values greater than one, Crava et al. (2019) found a similar number of chemosensory receptor genes expressed in adult female antennae: 43 ORs, 19 GRs, 20 IRs. Differences here may be attributed to parameters such as sequencing depth or abundance estimation methodology. Indeed, some receptors previously reported to be expressed, but not here in this study, all had low expression values ca. FPKM of one or less ((Crava et al., 2019), Fig. 3).

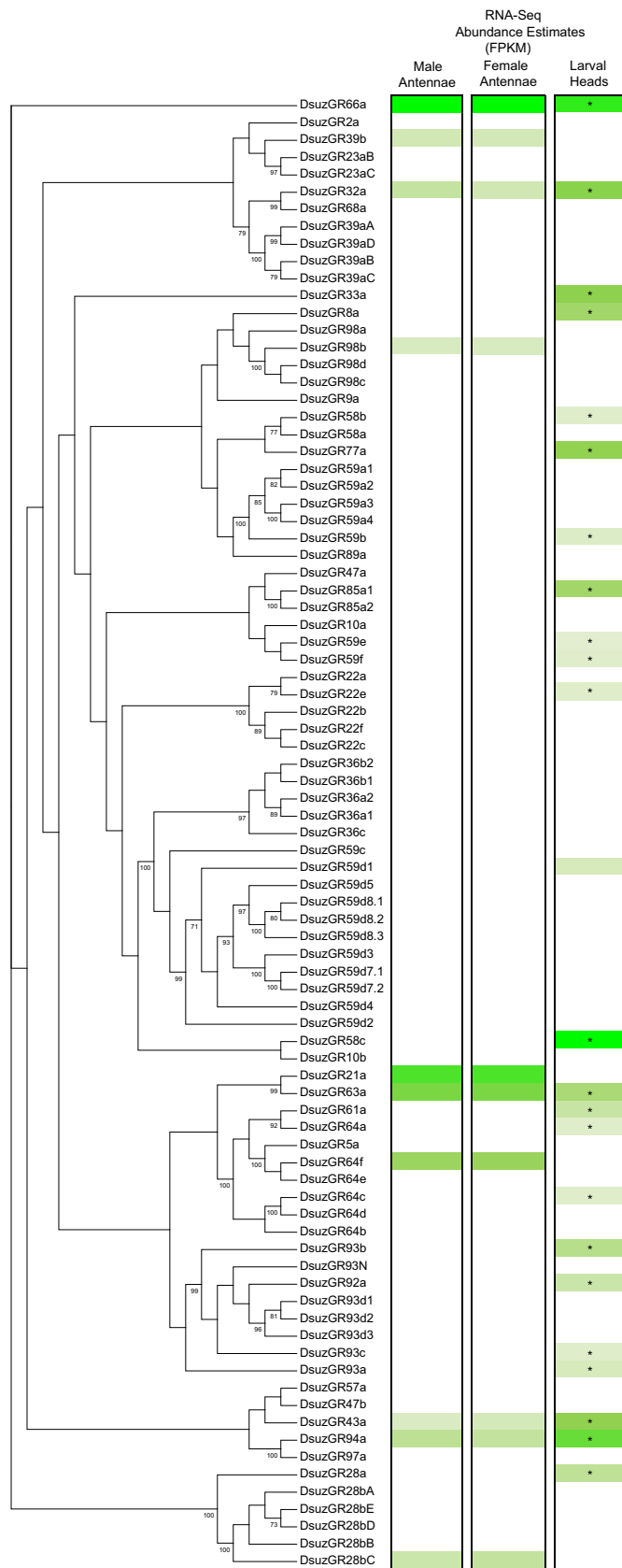
**4.2. Odorant receptors**

Expression patterns of ORs in *D. suzukii* male and female antennae are consistent with previous observations in female antennae (Crava et al., 2019). All previously reported antennal expressed ORs were observed expressed in our antennal transcriptome except for OR23a1 and OR83a, though it was noted that these two ORs displayed low expression estimates <1 FPKM (Crava et al., 2019).

No pattern of sex-biased expression was observed for odorant receptors in adult antennae in this report. This contrasts with a recent transcriptomic study on *D. suzukii* antennae, which reported female-biased expression for ten ORs (Ahn et al., 2020). Variables across these studies, such as geographical population, insect age, mating status or other factors may account for differences in observed results. Lending support to this, it must be noted that a mating-effect on expression was reported (Crava et al., 2019) for a majority of the ORs reported to display female-bias (Ahn et al., 2020). While Ahn et al. (2020) sampled only non-mated insects, insects of mixed mating-status were utilized for this report.

While ORs in *D. melanogaster* have been thoroughly functionally characterized in both adult (Hallem et al., 2004; Hallem and Carlson, 2006; Galizia et al., 2010; Stensmyr et al., 2012; Dweck et al., 2013; Dweck et al., 2015a; Dweck et al., 2015b; Ebrahim et al., 2015; Münch

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**Fig. 3.** Heat-plot of relative expression values for *D. suzukii* gustatory receptors (GRs). Estimation of abundance values determined by read mapping. White indicates no expression or transcripts not identified in transcriptome for adult antennal samples specifically, expression values have been suppressed for transcripts with RPM values less than one in both samples. Lighter colors indicate relatively lower expression, brighter colors indicate relatively higher expression. Color plots represent binary log of FPKM plus one for each gene (See Supplementary Data S3 for raw data). For larval head data, asterisk “\*” indicates expression has been validated by RT-PCR assay. Color scales for each tissue type are independent of other tissue types. Range of values for Male Antenna: 0.98–8.18; Female Antennae: 1.21–8.17; Larval Heads: 0.042–1.64.

and Galizia, 2016; Lebreton et al., 2017; Auer et al., 2020;) and larvae (Kreher et al., 2005; Kreher et al., 2008; Mathew et al., 2013; Dweck et al., 2015a), few *D. suzukii* tuning ORs have been functionally characterized to date (Cattaneo et al., 2022). The development of *D. suzukii* transgenic, as well as CRISPR, lines targeting Orco (Karageorgi et al., 2017) highlight the potential for direct study of *D. suzukii* olfactory receptors in vivo. A recent report, however, has thoroughly characterized olfactory response profiles of *D. suzukii* antennal and maxillary palp OSNs relative to *D. melanogaster* using the same odorant test panel for both species (Keeseey et al., 2022).

Consistent with findings on antennal OR expression profiles reported here and previously (Crava et al., 2019), functional conservation of olfactory response was observed in 86 % of OSN types, with differences in *D. suzukii* reported for only five analogous OSN types (ab2B, ab3A, ab9B, ab10A, ai3a, (Keeseey et al., 2022)). If conservation of OR expression underlies the conservation of olfactory response in the OSN types, those with differences may likely be attributed to gene duplications or deletions in *D. suzukii*. (Hickner et al., 2016; Ramasamy et al., 2016; Keeseey et al., 2022).

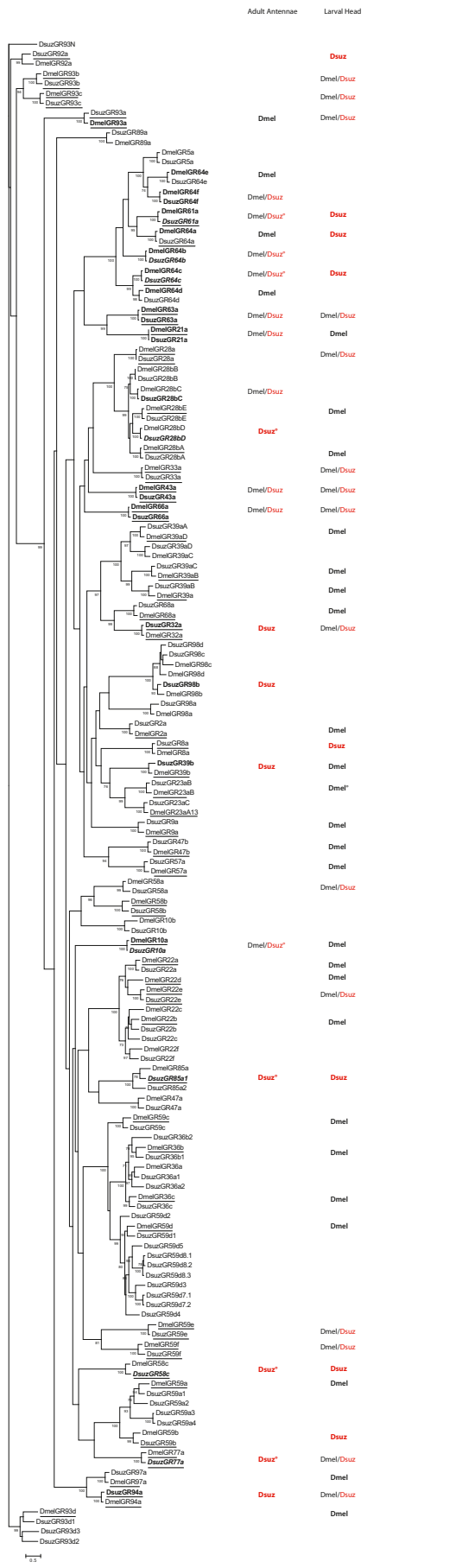
A similar number of tuning ORs were confirmed to be expressed in *D. suzukii* larval heads (26) as has been reported for *D. melanogaster* (25). In *D. melanogaster* larval heads, olfactory function, and indeed OR expression, is restricted to the dorsal organ (Fishilevich et al., 2005). In the dorsal organ of *D. melanogaster*, 21 OSNs are present (Python and Stocker, 2002), each typically expressing Orco and one tuning OR, though two instances of tuning OR co-expression have been observed (Fishilevich et al., 2005). The morphology and physiology of the *D. suzukii* larval olfactory system has yet to be investigated.

In *D. melanogaster*, 16 of the 25 ORs expressed in the dorsal organ displayed larval specificity, in that they are not expressed in the adult olfactory system (Fishilevich et al., 2005; Menuz et al., 2014). Similarly, we observe here that 12 of the 27 ORs expressed in *D. suzukii* larval heads are not expressed in adult antennae, though three of these larval expressed ORs are orthologues to *D. melanogaster* palp expressed genes (OR42a, OR85d and OR85e). In *D. melanogaster*, it was reported that 90 % of larval dorsal organ-expressed ORs, but only 53 % of adult antennal-expressed ORs responded to fruit odorants, underlying behavioral differences in response to these odorants during the different life stages (Dweck et al., 2018). Given the partially divergent expression patterns between larval head and adult antennae, similar dynamics may be apparent in *D. suzukii*.

Notably, as with *D. melanogaster*, none of the adult OR-family pheromone receptor homologues (OR47b, OR65a, OR67d, OR69a, OR88a) display larval expression (Kurtovic et al., 2007; van der Goes van Naters and Carlson, 2007; Dweck et al., 2015b; Lebreton et al., 2017). Larval pheromones have been described in *D. melanogaster* (Farine et al., 2014; Mast et al., 2014), though the receptors for some of these have been identified, not as ORs but rather members of the pickpocket family (Mast et al., 2014). Knowledge on larval pheromones in *D. suzukii* is currently lacking.

While antennal expression profiles of orthologous ORs are largely conserved between *D. melanogaster* and *D. suzukii*, a greater degree of divergence is observed for larval heads. Specifically, nine ORs are observed expressed in *D. suzukii* larval head for which no homologues





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**Fig. 4.** Summary of GR expression profiles across *D. suzukii* and *D. melanogaster* antennae and larval heads. GRs ordered in phylogenetic tree, with bold high-light indicative of antennal expression and underline indicative of larval head expression. For *D. suzukii*, antennae expression is indicated based upon data from this report and that of Crava et al. (2019), with confirmed expression based upon RPM values greater than one. ° symbol indicates gene was determined to be expressed in Crava et al. (2019), but not in this report. Larval expression is indicated if RNA-Seq data was validated by RT-PCR Assay. For *D. melanogaster*, antennae expression is reported based upon Menuez et al. (2014), larval head expression profile is derived from Kwon et al. (2011).

are expressed in *D. melanogaster* larval head. Comparative investigations on the functionality of these ORs in *D. suzukii* would shed light on the potential of these molecular gatekeepers of the olfactory system to influence ecological interactions at the larval stage.

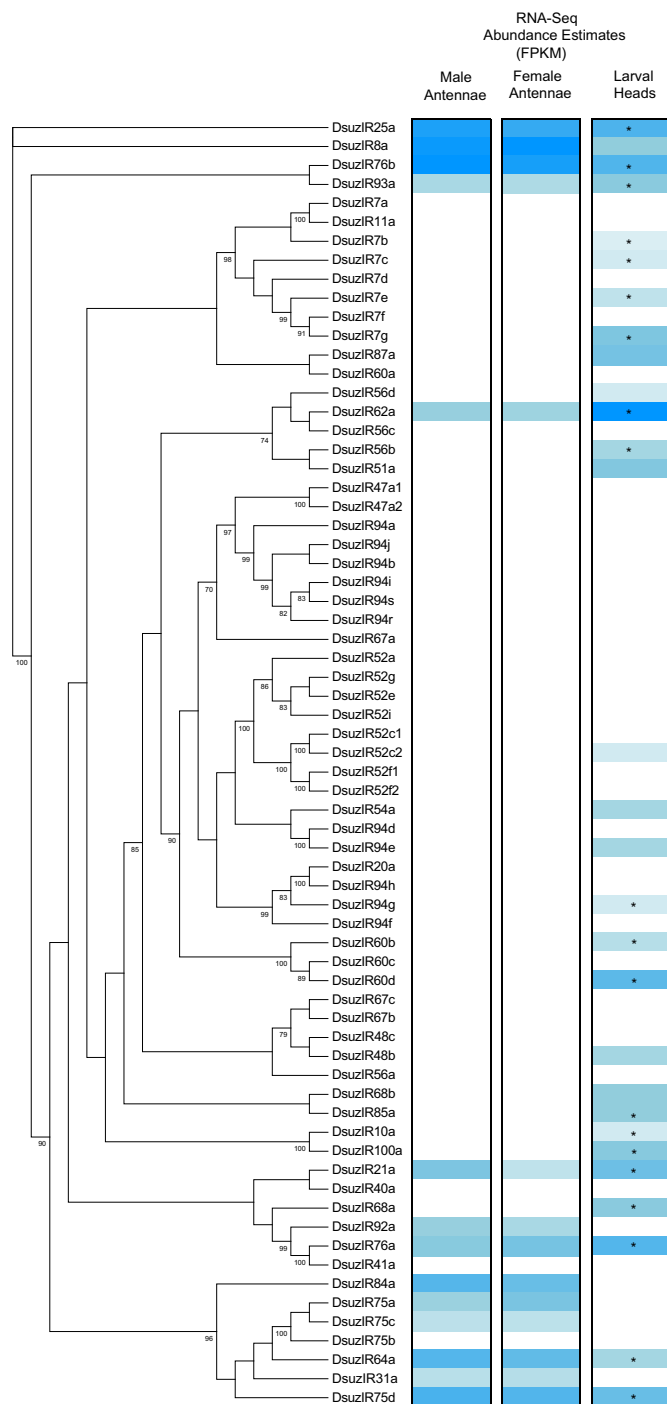
The chemical ecology of *D. melanogaster* has been synthesized (Mansourian and Stensmyr, 2015), reviewing functional significance of numerous odorant compounds that specifically activate different chemosensory receptors that are expressed in fly chemosensory systems. Taking into consideration the nine ORs that are expressed in larval heads of *D. suzukii* but not *D. melanogaster*, insights can be made on the evolution of olfactory capabilities to meet the ecological demands in each species. Notably, three ORs (OR9a, OR67c and OR92a) found in larval heads of *D. suzukii* but not *D. melanogaster* are reported to primarily respond, in adult *D. melanogaster*, to odorants that represent alcoholic fermentation scents (Mansourian and Stensmyr, 2015). Evidence is provided for functional conservation of these receptors across *D. melanogaster* and *D. suzukii* (Keeseey et al., 2022, see Fig. 1). An enrichment of ORs in *D. suzukii* putatively responsive to scents from alcohol fermentations may possibly underlie an increased sensitivity to ethanol in *D. suzukii* relative to *D. melanogaster*. This may be especially important when it is considered that *D. suzukii* displays much reduced tolerance for surviving higher doses of ethanol compared to *D. melanogaster* (Gao et al., 2018; Kim et al., 2018; Chakraborty et al., 2022). Additionally, the increased sensitivity to ethanol may be reflective of known associations to yeasts, such as *Hanseniaspora uvarum*, in which alcoholic fermentation is reduced (Mestre Furlani et al., 2017; Spitaler et al., 2020).

Of the 70 *D. suzukii* OR genes reported by Ramasamy et al. (2016), 55 were expressed in either adult antennae or the third instar larval head in this report or previously (Crava et al., 2019). Of the remaining 15 ORs, three of these (OR74a, OR85a, OR98b) are pseudogenes in *D. suzukii* (Hickner et al., 2016; Ramasamy et al., 2016). Four (OR71a, OR33c, OR59c1, OR59c2) are homologues of *D. melanogaster* ORs expressed in the maxillary palp. Taking into consideration the conservation of maxillary palp OSN olfactory response profiles in *D. melanogaster* and *D. suzukii* (Keeseey et al., 2022), one would expect the *D. suzukii* OR homologues to also be expressed in the palps. Four (OR33a, OR45a, OR59a1, OR59c1) were identified in our larval transcriptome but were not validated by RT-PCR, while three (OR30a, OR59a2 and OR94b) are orthologues of ORs expressed in *D. melanogaster* larval heads, but not found likewise in *D. suzukii* larval heads. Finally, five of the ORs not observed expressed in either the adult antennae or larval heads (OR23a4, OR49a1, OR59a2, OR59c2, OR67a5) are all representative of loci in *D. suzukii* that have experienced expansion or contraction consistent with the birth and death model of multi-gene family evolution (Nei et al., 1997).

### 4.3. Gustatory receptors

Beyond the carbon dioxide receptors, GR21a and GR63a, the ecological role of GRs expressed in *Drosophila* antennae remain unknown. Relative to *D. melanogaster*, an abundance of putative bitter GRs has been observed to be expressed in the antennae of *D. suzukii*. In this report and elsewhere (Crava et al., 2019), a renowned bitter-compound





**Fig. 5.** Heat-plot of relative expression values for *D. suzukii* ionotropic receptors (IRs). Estimation of abundance values determined by read mapping. White indicates no expression or transcripts not identified in transcriptome; for adult antennal samples specifically, expression values have been suppressed for transcripts with RPM values less than one in both samples. Lighter colors indicate relatively lower expression, brighter colors indicate relatively higher expression. Color plots represent binary log of FPKM plus one for each gene (See Supplementary Data S3 for raw data). For larval head data, asterisk "\*" indicates expression has been validated by RT-PCR assay. Color scales for each tissue type are independent of other tissue types. Range of values for Male Antenna: 1.58–6.94; Female Antennae: 1.95–7.61; Larval Heads: 0.028–1.44.

receptor, GR66a, is among the most highly expressed antennal GRs, whereas in *D. melanogaster* GR66a was only faintly detected (Menuez et al., 2014). In *D. melanogaster*, the carbon dioxide receptors are functionally expressed in ab1C OSNs (Jones et al., 2007; Kurtovic et al., 2007), while a bitter-compound receptor, GR10a, (Rimal and Lee, 2019) has been mapped to ab1D OSNs (Fishilevich and Vosshall, 2005). No other GRs have been mapped to antennal chemosensory neurons in *Drosophila*, neither via GAL4-based promoter-reporter assays nor in situ hybridization (Scott et al., 2001; Couto et al., 2005). The localization and function of additional antennal expressed GRs largely remain unknown.

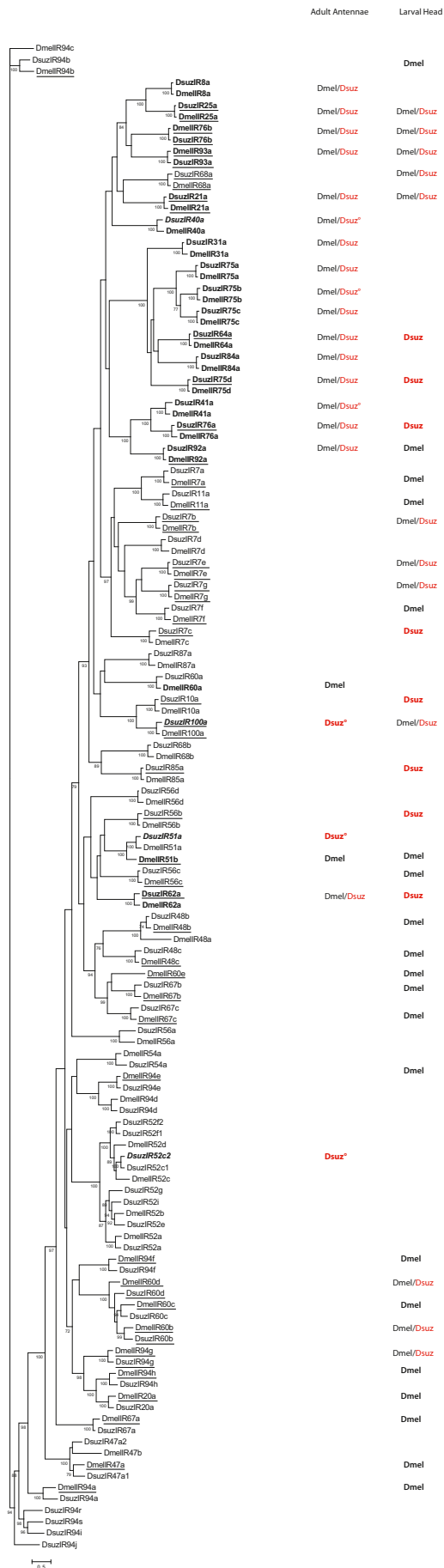
A comprehensive study on gustatory receptor expression patterns in *D. melanogaster*, utilizing gustatory receptor promoter GFP lines, identified 39 GRs expressed in various larval head sensory organs (Kwon et al., 2011). In contrast, only 23 GRs have been confirmed to be expressed in *D. suzukii*, here. This difference may indeed be biological, reflective of differences in ecology between the two species. This idea is supported further by our antennal expression data, in which more distinct species-specific patterns were observed for GRs than for ORs and IRs, and also a recent study that reported reduced expression of bitter-compound GR genes in the labellum of *D. suzukii* relative to *D. melanogaster* (Dweck et al., 2021). Differences in methods may also account for the observed difference in number of larval head GRs. Promoter-Gal4 mediated expression of GFP may result in unfaithful reporting of GR expression profiles. Alternatively, the transcriptomic approach utilized here may result in an under-representation of GR genes expressed. Lack of sufficient sequencing depth, and also sequencing of whole larval head RNA, as opposed to olfactory tissue specific profiling may hinder the detection of low-expressed transcripts.

Unlike with *D. melanogaster* (Kwon et al., 2011; Mishra et al., 2013), we observed expression of putative sugar receptors (beyond Gr43a) in *D. suzukii* larval head, namely GR61a, GR64a and GR64c. It may be hypothesized that *D. suzukii* larvae require greater sensitivity towards the detection and discrimination of sugars given the proclivity of female adults to lay eggs in fresh and ripening fruits, as compared to *D. melanogaster*, with its preference for overripe and fermenting fruits. Expression of a broader array of sugar receptors in *D. suzukii* larval heads would facilitate this. In *D. melanogaster* expression mapping profiles have been generated using Gal4 driver lines (Kwon et al., 2011), providing precise cellular localization in each of the sensory organs in third instar larval heads. Such maps are lacking for *D. suzukii* and would be required to facilitate greater understanding of the functional significance of each of the specific GRs expressed in the larval head.

Based upon their role in bitter sensing neurons in *D. melanogaster* adult chemosensory systems (Weiss et al., 2011; Delventhal and Carlson, 2016), many larval-expressed GRs are presumed to have a similar function in mediating larval sensitivity to bitter compounds. In *D. suzukii* larval heads, expression was observed for no less than nine GRs that are homologous to *D. melanogaster* GRs identified in adult bitter-sensitive sensilla (Weiss et al., 2011). Among these, in *D. suzukii*, are homologues of the broadly expressed GR32a, GR33a and GR66a, which have been identified in all classes of bitter-sensitive sensilla in adult *D. melanogaster*. Indeed, in the gustatory terminal organ of *D. melanogaster* larval heads, GR33a and GR66a were observed to be co-expressed in six of eight chemosensory neurons, supporting a hypothetical role of these GRs as bitter-compound GR co-receptors (Weiss et al., 2011). At the same time, most of the GRs reported in *D. melanogaster* larval head (Kwon et al., 2011) that we did not detect in *D. suzukii* larval head were previously characterized by their presence in bitter sensitive neurons (Weiss et al., 2011).

#### 4.4. Ionotropic receptors

Detection of antennal-expressed IRs in this report is consistent with previous antennal transcriptomic studies on both *D. suzukii* (Crava et al., 2019) and *D. melanogaster* (Menuez et al., 2014). All categorized antennal



**Fig. 6.** Summary of IR expression profiles across *D. suzukii* and *D. melanogaster* antennae and larval heads. IRs ordered in phylogenetic tree, with bold highlight indicative of antennal expression and underline indicative of larval head expression. For *D. suzukii*, antennae expression is indicated based upon data from this report and that of Crava et al. (2019), with confirmed expression based upon RPM values greater than one. ° symbol indicates gene was determined to be expressed in Crava et al. (2019), but not in this report. Larval expression is indicated if RNA-Seq data was validated by RT-PCR Assay. For *D. melanogaster*, antennae expression is reported based upon Menuz et al., 2014, larval head expression profile is derived from Sanchez-Alcaniz et al. (2018).

IRs have been detected by RNA-Seq as expressed in the antennae of *D. suzukii* and *D. melanogaster*, except for IR68a. This receptor, which has been characterized in *D. melanogaster* to mediate moist-air sensing (Frank et al., 2017; Knecht et al., 2017) has been clearly shown to function, together with IR93a and IR25a, in a small population of neurons in the sacculus of the antenna. Low-level expression in a restricted number of cells seems to be below the threshold of detection via the transcriptomic RNA-Seq approach (Menuz et al., 2014). Similarly, IR40a, implicated in dry- and cold-air sensing in a likewise small subset of sacculus neurons (Enjin et al., 2016; Knecht et al., 2016), was identified in the antennal transcriptome of this report though at expression levels below our threshold definition of expression.

It was recently reported in a replicated antennal transcriptomic study that DsuIR21a displays male-biased expression while DsuIR76a displays female-biased expression, though sex-biased differences were not confirmed through qRT-PCR assay (Ahn et al., 2020). Interestingly, in our study, similar patterns of bias were observed (Supplementary Data S3). In *D. melanogaster*, IR21a has been demonstrated to have a role in thermosensation (Knecht et al., 2016; Ni et al., 2016), while IR76a functions as an olfactory receptor that detects polyamines (Abuin et al., 2011; Silbering et al., 2011). Further research is required to investigate roles for these and perhaps other IRs in mediating sex-specific physiology or behavior in *D. suzukii*.

While the antennal-expressed IRs are largely of the antennal IR subfamily, most IRs expressed in the larval head cluster within the divergent IR subfamily, including multiple genes from the IR7 and IR20a sub-families, which both have been implicated in taste function (Croset et al., 2010; Koh et al., 2014; Stewart et al., 2015). Given that larval *Drosophila* heads include olfactory and taste organs, it is not surprising to identify an abundance of candidate taste IRs in *D. suzukii* larval heads relative to adult antennae. A recent comparative study has thoroughly examined chemosensory receptor expression in the *D. melanogaster* and *D. suzukii* adult gustatory organ, the labellum, which would be a more suitable comparison of adult and larval gustatory capabilities (Dweck et al., 2021).

A single divergent IR, IR62a was determined to be expressed in the antennae of *D. suzukii*, here and previously (Crava et al., 2019), and also in *D. melanogaster* (Menuz et al., 2014). IR62a was also identified as the most highly expressed IR in our larval head transcriptome (FPKM = 1.73). IR62a, together with IR76b and IR25a, has recently been implicated for its role in calcium ion (Ca<sup>2+</sup>) detection in gustatory neurons of the labellum and elsewhere, and mediating avoidance of high calcium levels, which can have adverse effects on survival (Lee et al., 2018). However, it has been noted that IR62a resides in the intron of a gene that is highly expressed in the antennae of *D. melanogaster* (Menuz et al., 2014), and that its presence in antennal transcriptomes may be an artifact, reflective of the transcriptomic detection of a low proportion of unspliced transcripts. In fact, a similar genomic arrangement is present for IR62a in *D. suzukii*, identified within an intron of the gene “increased minichromosome loss 1 (iml1)”. Notably, RSEM analysis of iml1 expression levels indicates higher expression of this gene, relative to IR62a, in both the antenna as well as larval head transcriptomes (Supplementary Data S3). It is undetermined whether the observed IR62a expression values are thus only reflective of RNA sequencing capture of unspliced iml1 transcripts, or whether IR62a expression has biological

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relevance in the chemosensory tissues described herein. Consistent with the former, it should be noted that in *D. melanogaster*, promoter-reporter analysis of IR62a expression patterns did not find evidence for antennal, nor larval-head, presence of this gene (Sanchez-Alcaniz et al., 2018).

Similar to the larval-head expressed GRs in *D. melanogaster* (Kwon et al., 2011), expression of IRs in larval heads have been assessed by promoter-reporter assays (Sanchez-Alcaniz et al., 2018). As with the GRs, clear differences in the number of larval-head expressed IRs were observed between *D. melanogaster* and *D. suzukii*, with 33 identified in *D. melanogaster*, and 20 confirmed in this report for *D. suzukii*. IR co-receptors IR25a and IR76b were detected in the larval head transcriptome, which was confirmed by RT-PCR assay. Conversely the co-receptor, IR8a, was not detected by RT-PCR despite sequenced reads that map to IR8a in the larval head transcriptome. This pattern mirrors that of *D. melanogaster*, in which IR25a and IR76b, but not IR8a, were detected in larval head sensory organs (Sanchez-Alcaniz et al., 2018).

In *D. melanogaster*, antennal IRs expressed in larval head are known to mediate cool temperature sensing (IR25a + IR21a + IR93a; (Knecht et al., 2016; Ni et al., 2016)), moist air sensing (IR25a + IR68a + IR93a; (Frank et al., 2017; Knecht et al., 2017)), and olfactory detection of ammonia (IR25a + IR76b + IR92a; (Benton et al., 2009; Min et al., 2013)). Except for IR92a, conserved orthologues of all of these antennal IRs were observed to be expressed in *D. suzukii* larval heads. Notably, expression of IR75a, which is reported as an acetic acid receptor in *D. melanogaster* (Prieto-Godino et al., 2016), was detected in adults only in both species. Conversely, other antennal IRs known to mediate acid (IR64a) and amine sensing (IR75d/IR76a) respectively (Ai et al., 2010; Silbering et al., 2011; Ai et al., 2013), have been identified in larval heads of *D. suzukii*, but not *D. melanogaster* (Sanchez-Alcaniz et al., 2018), possibly suggesting increased sensitivity towards detection of specific acid and amine compounds in the former.

Interestingly IR64a is one of the IRs with which IR8a forms cation channels (Ai et al., 2013). Given the lack of confirmation of IR8a expression by RT-PCR in this report, and indeed the general lack of IR8a expression beyond the adult antennae in *D. melanogaster* (Sanchez-Alcaniz et al., 2018), it may be speculated that for IR64a, more complex heteromerization with other co-receptors may occur, expanding on findings showing broad overlap of co-receptor subunits of various chemosensory receptor subfamilies (Task et al., 2022).

## 5. Conclusions

Transcriptomic profiling of adult and larval expressed chemosensory receptors of *D. suzukii* provides a solid foundation towards a better understanding of the olfactory physiology and chemosensory ecology of this organism. Leveraging the vast amount of information on the molecular underpinnings of olfaction and gustation in *D. melanogaster* yields an appreciation for ecological similarities and differences with *D. suzukii* including their differences in preference for unripe and overripe fruit, respectively (Keeseey et al., 2022). Moreover, direct comparisons of expression patterns and profiles of chemosensory receptors within *D. suzukii* and *D. melanogaster* facilitates future studies aimed at better understandings of genetic phenomena relevant to multigene families such as alternative splicing, birth and death evolution, receptor co-expression and receptor choice by sensory neurons. Conversely, as it relates to applied perspectives, some of the components of attractive semiochemical blends (Cha et al., 2013, 2014; Kleman et al., 2022) match candidate ligands of male and female antennal expressed *D. suzukii* receptors, based on response profiles of *D. melanogaster* receptor orthologues (Galizia et al., 2010; Münch and Galizia, 2016). In consideration of the general conservation of olfactory detection capabilities of the antennae of *D. melanogaster* and *D. suzukii* (Keeseey et al., 2022), further functional research on *D. suzukii* chemosensory receptors where differences do exist may contribute to future improvements in semiochemical-based control of the spotted wing drosophila.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbd.2022.101049>.

## CRedit authorship contribution statement

P.G.B., P.W., and W.B.W. conceived and designed the experiments. W.B.W. and B.A.M. dissected tissues, prepared the RNA, B.A.M., A.M.C., and F.G. designed primers and conducted PCR experiments. W.B.W. and B.A.M. conducted gene annotations and bioinformatics analyses. W.B.W., B.A.M. and A.M.C. analyzed the data, W.B.W. designed figures and tables and prepared and submitted sequence files for NCBI deposit. P.W. and P.G.B. contributed to material support. W.B.W., B.A.M. and A.M.C. wrote the manuscript. All authors edited and approved the final version of the manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

All data generated and analyzed in this study are included in the published article and its supplementary files. Transcriptome raw reads sequence data are available through the NCBI Sequence Read Archive, (BioProject: PRJNA848010, SRA Accession Numbers SRR19627177-SRR19627179 (3 samples)).

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