Loss of Drosophila pheromone reverses its role in sexual communication in Drosophila suzukii

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The Drosophila pheromone cis-11-octadecenyl acetate (cVA) is used as pheromone throughout the melanogaster group and fulfils a primary role in sexual and social behaviours. Here, we found that Drosophila suzukii, an invasive pest that oviposits in undamaged ripe fruit, does not produce cVA. In fact, its production site, the ejaculatory bulb, is atrophied. Despite loss of cVA production, its receptor, Or67d, and cognate sensillum, T1, which are essential in cVA-mediated behaviours, were fully functional. However, T1 expression was dramatically reduced in D. suzukii, and the corresponding antennal lobe glomerulus, DA1, minute. Behavioural responses to cVA depend on the input balance of Or67d neurons (driving cVA-mediated behaviours) and Or65a neurons (inhibiting cVA-mediated behaviours). Accordingly, the shifted input balance in D. suzukii has reversed cVA’s role in sexual behaviour: perfuming D. suzukii males with Drosophila melanogaster equivalents of cVA strongly reduced mating rates. cVA has thus evolved from a generic sex pheromone to a heterospecific signal that disrupts mating in D. suzukii, a saltational shift, mediated through offsetting the input balance that is highly conserved in congeneric species. This study underlines that dramatic changes in a species’ sensory preference can result from rather ‘simple’ numerical shifts in underlying neural circuits.

1. Introduction

Melanogaster-clade species generically use male-produced cis-11-octadecenyl acetate (cVA) as sex pheromone [1]. The underlying cVA circuitry is arguably the best-studied pheromone communication system, from production to detection, and from processing to resulting muscle output [2–4]. Different from ‘typical’ sex pheromones in insects, cVA acts at short range and not only fulfils a role in orientation and sexual attraction, but also most prominently in a variety of sexual and social behaviours in Drosophila melanogaster: it increases male mate acceptance by females [5,6], reduces attractiveness of newly mated females, reduces male–male courtship, while increasing aggression between males [7,8]. cVA is a sex pheromone found throughout and basal to the melanogaster group species, such as in the obscura and immigrans group species [1], underlining its primal role in Drosophila.

The fact that all melanogaster group species studied thus far use cVA as volatile sex pheromone [1] would preclude a role of cVA in species recognition, a function that is otherwise typical for insect sex pheromones [9]. Moths in particular are well known for their species-specific pheromone blends, which are used to discriminate between conspecific and heterospecifics [10]. Whereas males are finely tuned to their conspecific female-produced pheromone, they often also display acute sensitivity to pheromones of other, often sympatric, species. In the latter case, such pheromones elicit responses on sensory neurons...
that mediate behavioural antagonism, and typically disrupt orientation to conspecifics [11].

Perhaps owing to the complex role that cVA fulfills in a range of sexual and social behaviours in Drosophila, evolution of the signal to convey species-specificity may be constrained. Besides, other, non-volatile Drosophila pheromones fulfill a function in species recognition instead [12]. These are non-volatile pheromones, produced by enocytes and embedded in the cuticular hydrocarbons (CHs), and sensed through the taste sensilla on the legs and proboscis [13–15].

However, in spite of the evolutionary constraints cVA may have, here we report how this conserved pheromone has undergone a radical functional reversal in Drosophila suzukii, a melanogaster group species. Drosophila suzukii does not produce cVA. Here, we describe the changes that are at the basis of the inversion of the role of cVA from a broadly used sex pheromone into a behavioural antagonist in this species.

2. Material and methods

(a) Flies

A Drosophila suzukii colony was established in 2010 from around 1000 individuals emerging from infested blueberries and raspberries collected in Valsugana, Trentino Province, Italy. The US strain of D. suzukii was derived from a colony established by D. Walsh (Washington State University, Prosser, WA, USA). Drosophila biarmipes (14023-0561.02) and Drosophila subpulchrella (14023-0401.01) were obtained from the San Diego stock centre. For D. melanogaster, we used the wild-type Dalby strain [16,17]. Flies were reared in a quarantine facility on a standard cornmeal–yeast–agar medium at 21 °C under 12 L:12 D conditions.

(b) Behavioural assays

Sexes of D. melanogaster and D. suzukii (Trentino strain) were separated within 1 h post emergence and placed in groups of five (females) and seven (males). They were placed in standard food vials and flipped after three days to new food vials. After four days, seven males were placed into the vial containing five conspecific females. We used groups of individuals to increase the mating incidence of D. suzukii, which had a lower mating rate compared with D. melanogaster. The higher ratio of males to females was there to offset any potentially negative effect of the perfuming of the flies (see §2c, below) on the ‘availability’ of male mates, although no clear negative effects of the experimental procedures were observed. No individuals died during the mating experiments. As individuals could not be reliably recognized and followed during the course of the experiments, we scored mating rates only. Matings were noted every 15 min. We observed the mating behaviour for both species during five consecutive hours. A total of 21 and 30 groups of 12 flies (five females, seven cVA-perfumed males) were perfumed D. melanogaster and D. suzukii, respectively, against a total of 19 and 26 control groups, respectively. Flies only mated once during our assay.

(c) Perfuming flies

Three millilitre vials were coated on the inside with a total of 50 μg cVA. The procedures were roughly similar to those described in Billette et al. [13]. Fifty microlitres of hexane with or without 1 μg ml⁻¹ of cVA were pipetted into a 3-ml glass screw cap vial. The hexane was slowly evaporated while the vial was placed in a horizontal position and slowly rotated. Seven males were briefly immobilized on ice (1 min) and placed in a 3-ml treatment or control vial. Vials, coated with cVA or hexane-treated only and containing seven flies, were placed on a rotator and rotated at 4500 rotations min⁻¹ for 2 min, in a cycle of 6 s on, 4 s off. In D. melanogaster, cVA deposits are mostly found on the abdominal segments. However, perfuming the fly with cVA results in more equal distribution of pheromone across the body. We therefore increased the total amount of cVA slightly to compensate for lower local concentrations on the fly. After shaking, the group of seven treatment (+cVA) or seven control (−cVA) male flies, both shaken on the rotator, were introduced into the food vial containing five virgin females.

(d) Chemical analysis

Pheromones were extracted from the fly cuticle (n = 5, 6 for male and female D. suzukii, respectively) by leaving individual flies in 100 μl of hexane for 5 min at room temperature. One hundred nanograms of heptadecenyl acetate were added as an internal standard. These extracts were analysed on a gas chromatograph coupled with a mass spectrometer (GC-MS; 6890 GC and 5975 MS, Agilent technologies Inc., Santa Clara, CA, USA). Extracts were concentrated to ca 10 μl, and 2 μl were injected into a HP-5MS silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness; Agilent technologies Inc.) that was temperature-programmed from 30 °C (2 min), 8 °C min⁻¹ to 300 °C (5 min).

Compounds of interest were identified based on their mass spectrum, retention time, comparison with previously published works on D. melanogaster CHs [18] and injection of synthetic corresponding compound (for cVA). They were quantified by peak integration and comparison with the response of the internal standard. CH extracts of both the Trentino and US strains of D. suzukii were analysed (indicated in §3).

(e) Sensory physiology

Using a strong airflow, flies were pushed head-first into a truncated pipette tip. The pipette tip was cut distally from the head and the fly was gently pushed forward until the head protruded from the narrow end. The pipette tip was placed on a wax surface on a microscope slide, and using a glass micropipette the right antenna was gently bent backwards and stably positioned on a coverslip. The fly was placed under a microscope (Olympus BX51WI), with a magnification less than or equal to 1500×. Via a glass tube, a 1 min⁻¹ coal-purified and humidified airflow was constantly blown over the fly head. Tungsten microelectrodes, sharpened in a KNO₃-solution, were used for recording of action potentials of antennal sensory neurons. A motor-controlled micromanipulator (Märzhäuser DC-3K, Wetzlar, Germany) equipped with a piezo unit (Märzhäuser PM-10) was used for fine positioning. A reference electrode was inserted into the eye with a manually controlled micromanipulator (Narishige MM33, Tokyo, Japan). Touch stimulation was performed using an additional piezo unit to move the electrode at micrometre scale towards the sensillum. A glass electrode drawn to a sharp tip was dipped briefly in a 1 μg ml⁻¹ cVA solution, air dried and used for stimulation. After A/D conversion (Syntech IDAC PCI card), spikes were visualized and stored on a PC. Analysis was done using AureaSnz v. 3.2 software (Syntech, Kirchzarten, Germany). For our sensory physiological analysis of D. suzukii, we used the Trentino strain. For each species, six individual replicates were used.

(f) Counts of sensilla trichoea

Antenna of WT Dalby (n = 8) and D. suzukii (n = 9, Trentino strain) were mounted using spacer rings (Secure-Seal TM imaging spacers, Sigma-Aldrich) and Vectashield mounting medium Hard Mount (Vector Laboratories, Burlingame, CA, USA). High-resolution confocal scans (Zeiss LSM 510 confocal microscope, Carl Zeiss, Jena, Germany), using a 20× objective, were made through the antennae. The contrast of the relatively thick-walled
trichoid sensilla permitted identification and counting of trichoid sensilla on the entire antennal surface.

(g) Ejaculatory bulb

*Drosophila suzukii* (Trentino strain) and *D. melanogaster* (WT Dalby) adult males were collected two days after emergence. For each species, a total of 10 males were selected. After brief anaesthesia in the freezer, individuals were dissected in phosphate-buffered saline (PBS). The abdomen was clipped and immersed in 5% KOH for 5 h to remove soft tissues and expose hard cuticular structures, washed in distilled water and partly dehydrated in 70% ethanol. Afterwards, the ejaculatory bulb (EB) ([19,20]) was separated from the rest of the male genitalia and mounted on a glass slide with glycerine. Observations were made with a microscope (Leica LMD7000, Wetzlar, Germany). The EB dimensions were measured using a Leica LMD7000 microscope with Leica Application Suite Image Analysis Software (n = 10). As individuals within and among species differed in size and in order to obtain values comparable from one animal to the others, the estimates of EB dimensions of *D. melanogaster* were compensated for by the smaller body length compared to *D. suzukii* (multiplication of the dimensions by the ratio (average *D. suzukii* length/average *D. melanogaster* body length)). Volumes were calculated by assuming a sphere (\(4/3 \times \pi \times (a/2) \times (b/2) \times (d/2)\)). Measurements from 10 individuals were averaged.

(h) Immunocytochemistry

We verified antennal lobe projection patterns of T1 neurons in *D. suzukii* (Trentino strain and US strain, as indicated in §3) using anterograde-neurobiotin (Molecular Probes, Carlsbad, CA, USA) backfills. Neurobiotin is readily taken up by neurons and transported throughout the neuron, including its axonal targets in the antennal lobes. A glass microelectrode with a 0.25 M KCl + 2% neurobiotin was placed over the tip of a T1 neuron. Neurobiotin was allowed to diffuse into the sensillum and taken up by the neuron for 1 h. Preparations were then fixed in 0.1 M PBS with 0.25% Triton-X for 3.5 h at 4 C, dissected, washed 3× with PBS containing 0.25% Triton-X (PBST) and incubated with fluorescein-avidin 488. Ten per cent mouse α-synapsin antibody (HybriDNA, University of Iowa, Iowa, IA, USA) was included to identify targeted glomeruli in the antennal lobes. After 24 h at room temperature on the rotator, brains were washed 3× with PBST and incubated with anti-mouse conjugated with AlexaFluor 546 (Molecular Probes). After another 24 h on the rotator at room temperature, brains were washed 3× with PBST and mounted in Vectashield Hard Mount (Vector Laboratories), 0.12 mm thick, using spacer rings (Secure-Seal TM imaging spacers, Sigma-Aldrich). The above-described technique for mouse α-synapsin antibody staining was also used for overview stainings and reconstructions of antennal lobes of *D. suzukii*, *D. biarmipes* and *D. subpulchrella*.

(i) Confocal microscopy and reconstructions

Whole-mount brains were viewed in a Zeiss LSM 510 confocal microscope (Carl Zeiss) equipped with a 40×, 1.4 oil-immersion DIC objective lens. Structures labelled with Fluorescein Avidin were excited with an Argon laser at 488 nm with detection of reflected light in the range of 505–515 nm. Alexa 546-labelled structures were excited with a HeNe laser at 543 nm and detected using a 560 nm long pass filter. Stacks of 50–200 confocal images were scanned and the images were stored at a size of 1024 × 1024 pixels. The three-dimensional reconstructions, volumetric measurements of the glomeruli were done using AMIRA v. 3.0 software (Visage Imaging, Berlin, Germany). In every optical section, the contours of glomeruli were demarcated by hand (i.e. image segmentation) and interpolated. Volumes were obtained from AMIRA, based on reconstructed images.

(j) Bioinformatics and phylogenetics

Orthologues searches and assembly. We downloaded various types of genome data (gene, transcript and protein sequences) for odorant receptors and other cVA-related genes (desaturases, elongases, Fruitless, Transformer) of *D. melanogaster*, *Drosophila simulans*, *Drosophila sechellia*, *Drosophila erecta* and *D. ananassae* from Flybase [21] and OrthoDB [22] repositories. For *D. biarmipes* and *D. suzukii*, the protein sequences of *D. melanogaster* were used for queries to identify the corresponding orthologues through exhaustive blast searches. First, TBlastN (BLOSUM 62 matrix with an e-value threshold of 10\(^{-5}\)) was applied for preliminary search of all the gene families. For the elongases superfamily alone, orthologues were searched using HHMER (http://hmmer.janelia.org/) with an e-value threshold of 10\(^{-5}\) and the following specifications: profile ‘hmmbuild’ was constructed from GNS1/SUR4 (ELO/PPF01151) HMM profile of the PFAM database [24], the result of which was run against *D. suzukii* and *D. biarmipes* genomes using ‘hmmsearch’; the output was manually checked for all the positive hits, specifically looking for the presence of elo-specific domain HXHH and hydrophobic transmembrane regions. For fruitless, we underwent a complex manual curation directly from *D. suzukii* genome contigs, using the 11 main isoforms of *D. melanogaster* to construct putative exons in *D. suzukii*. This is because the *fru* gene spans over a long genome region, contains long introns and codes for various different isoforms, which are unlikely to be present in transcriptomic data or being correctly annotated in gene models.

(k) Phylogenetics

For each of the gene families (Desaturases, Elongases, Fruitless and Odorant receptors), we constructed nucleotide datasets and checked for the correct reading frame. The translated protein datasets were aligned using MUSCLE v. 3.8.31 [25], and preliminary tree searches were done to assess the actual orthology of genes. These preliminary analyses led to recognition of a few wrong orthologies (false positives owing to gene loss) and characterization of new orthologues in unannotated genomes. After this manual curation, a second round of MUSCLE alignment using option—refine was done followed by phylogenetic reconstruction using PHYML [26] and employing 100 non-parametric bootstrap replicates and the LG + G model [27] of replacement.

(l) Statistics

Behavioural data (mating rate) were analysed using a Kaplan–Meier survival probability estimator and curves were statistically compared using a log-rank test. Correlation between cVA depletion after application (measured through GC-MS analysis, see above) and the relative mating rate in *D. suzukii* were analysed with a Pearson product–moment correlation coefficient, with the relative mating rate of cVA-perfumed *D. suzukii* being the fraction of the mating rate of cVA perfumed over control flies. Anatomical data (EB and glomerular volumes) were analysed using independent two-sample t-tests. Sensory physiological data (spiking rate) were analysed with a one-way ANOVA, followed by Tukey’s HSD post hoc test.

3. Results and discussion

(a) cVA production is lost in *Drosophila suzukii*, and its production site atrophied

Using gas chromatography-coupled mass spectrometry (GC-MS), we analysed the CH profile of *D. suzukii*. Unlike the CH profile of *D. melanogaster*, *D. suzukii*’s CH profile was isomorphic, with just small quantitative differences between sexes (figure 1a,b). This was also true for its sibling species *D. biarmipes* and *D. subpulchrella* (electronic supplementary material, figure S2). Unlike *D. melanogaster*, the antennal lobes of *D. suzukii* are atrophied (figure 1c). This is also true for *D. biarmipes* and *D. subpulchrella* (electronic supplementary material, figure S2).
material, figure S1c). We note that D. suzukii and D. biarmipes lacked the desaturase desat2 and the elongases eloF, which have been implicated in the biosynthesis of species-specific and sexually dimorphic CH profiles in Drosophila ([28,29]; electronic supplementary material, figure S2). However, more striking was the lack of cVA in the chemical profile of D. suzukii (figure 1a, arrow, verified with D. suzukii from the USA: electronic supplementary material, figure S1d). The lack of cVA in D. suzukii casts the question of whether this pheromone might have been replaced by another compound similar to cVA. However, no other cVA-type compounds (including the C20 variant present in some species [1]) were found in D. suzukii cuticular extractions (figure 1a,b and electronic supplementary material, figure S1d). In fact, the EB, the production site of cVA [30], was dramatically reduced in volume in D. suzukii compared with that of D. melanogaster (by a factor of 7.0, p < 0.001, unpaired t-test, n = 10, d.f. = 18, t = 10.1, d = 3.22, figure 2a). Intact, conserved homologues of corresponding genes for cVA production, eloB and desat1, were nevertheless present in the genome of D. suzukii (table 1 and electronic supplementary material, figure S2), as were miRNA-124 binding sites upstream of transformer, a factor involved in cVA production in male D. melanogaster (electronic supplementary material, figure S3e).

(b) The relative volume of glomeruli tuned to cVA is reversed in Drosophila suzukii

We then determined how loss of cVA might have influenced its corresponding olfactory circuitry. In D. melanogaster, the trichoid sensillum T1 and its cognate Or67d-expressing neuron [5] are key in cVA-mediated behaviours. T1s project to a large glomerulus, DA1 [31,32]. T1s are the most abundant sensillum type in D. melanogaster [33]. Although T1s were fully functional (figure 1c), and its receptor, Or67d, highly conserved (table 1 and figure 3), T1 sensilla were rare in D. suzukii antennae: we identified only around 7–10 T1s per individual, compared to 55–60 found in D. melanogaster [33]. Accordingly, the relative volume of DA1 was minute in both sexes of D. suzukii when compared with D. melanogaster (p < 0.0001, two-tailed independent t-test, t = 10.5, n = 8, d.f. = 6, d = 3.7, figure 4 and electronic supplementary material, figure S4).

At close range, cVA also induces responses in Or65a-expressing OSNs housed in antennal trichoid T4 [32,34,35]. Or65a neurons counteract behaviour induced through Or67d OSNs, and reduce cVA-mediated male–male aggression [8], as well as male attraction to recently mated females [35]. In D. suzukii, Or65a was highly conserved (table 1), and T4 sensilla were abundantly present and responded to cVA touch stimulation [34] (figure 2c). The neuron may be slightly more sensitive to cVA in D. suzukii than in D. melanogaster, responding prior to contact, unlike in D. melanogaster (less than 1 mm distance). The cognate antennal lobe glomerulus, DL3, which receives input from Or65a neurons, was enlarged in D. suzukii (two-tailed independent t-test, t = 12.9, d.f. = 6, p < 0.001, d = 4.6, figure 4) and 19% larger in male than female D. suzukii (two-tailed independent t-test, t = 3.13, d.f. = 6, p = 0.01, d = 1.1). Other glomeruli innervated by sensilla trichodea and sensilla intermedia neurons [32,33] were similar in volume between the two species (except for DA4m) and sexually isomorphous (figure 4). The total number of trichoid sensilla was similar between species (figure 2b).

Figure 1. (a) Chromatograms showing female and male D. suzukii CHs. Arrow indicates retention time where cVA would elute. Inset: chromatogram of D. melanogaster with the arrow indicating cVA (see also electronic supplementary material, figure S1). Note the dominance of tricosenes in both sexes. Tricosenes are more abundant in male D. melanogaster’s CH profile. IS internal standard (heptadecenyl acetate, 17:OAc). Asterisks (*) indicate significantly higher amount in females (p < 0.05). (b) Comparison of the CH profile of male and female D. suzukii (n = 6 and n = 5, respectively). Stars indicate significant differences between males and females (Mann–Whitney test, α < 0.05). x = unknown double bond position. (Online version in colour.)
Glomerular volume and reversal from pheromone to antagonist

Or65a and DL3 provide context-dependent suppression of cVA responses in D. melanogaster, putatively by suppressing output of DA1 via antennal lobe inhibitory interneuronal connections [8, 17]. We therefore hypothesized that the reversal of glomerular volume ratios in D. suzukii's cVA circuit (DA1/DL3 from 3.3 in D. melanogaster to 0.41 in D. suzukii, figure 4) would favour Or65a-mediated behavioural responses to cVA, generating opposite behavioural outputs to those observed in D. melanogaster. We tested this by applying cVA to male D. suzukii cuticle with doses equivalent to those found on male D. melanogaster and assaying the effect on conspecific courtship behaviour. Because mating rates are low in D. suzukii, and because applied cVA rapidly decreases over time on the cuticle (figure 5, red line), we grouped seven virgin males and five virgin females to ensure sufficiently...
high courtship and mating rates in the bioassay. As we predicted, application of cVA on male D. suzukii strongly suppressed mating (figure 5 insets, Kaplan–Meier estimator, \( Z = 4.45, p < 0.001, n = 150 \)). By contrast, perfuming male D. melanogaster with cVA did not affect the mating rate

Figure 3. cVA odorant receptors are conserved in D. suzukii. Phylogenetic tree of Or67d and Or65a in D. suzukii and other Drosophila. These genes are extremely conserved in D. suzukii and the other species sampled. This suggests that these genes are indeed expressed in neurons of T1 and T4 sensilla and are structurally constrained to recognize cVA, as indicated by the electrophysiological responses. The tree is rooted with the Or coreceptor Orco. Abbreviations and supports are as in electronic supplementary material, figure S2. (Online version in colour.)

Figure 4. Volume of DA1 and other trichoid glomeruli, relative to the total volume of all glomeruli receiving input from sensilla trichodea and intermedia. VL2a, which receives input from coeloconic Ac4a neurons, is included as it is part of the fru circuitry. Red-outlined bars: DA1 of D. melanogaster and D. suzukii ♂. Insets are reconstructions of the antennal lobes, with DA1 in bright red, and other glomeruli that receive input from sensilla trichodea and sensilla intermedia neurons in light red. *, \( p < 0.05 \). Scale bar, 20 \( \mu \text{m} \).

Figure 5. Effect of cVA perfuming on mating in D. suzukii and D. melanogaster. The relative mating rate increased (blue line, \( \alpha \) values see below) with a decrease in cVA levels on the male flies over time (\( n = 5 \) per data point). Insets: cumulative mating in Dm and Ds in response to the perfuming with cVA (+cVA, red lines, \( n = 21 \), 30 for Dm and Ds, respectively) or hexane (control, −cVA, grey lines, \( n = 19 \), 26 for Dm and Ds, respectively). ***, \( p < 0.001 \).

\begin{align*}
\text{Relative mating rate (treatment/ctrl)} & = 2.30 \times 10^{-12} \times \text{cVA dose} + 0.44, \quad \text{Pearson's } r = 0.927, p < 0.025. \\
\text{The down-regulation of T1 sensilla expression, the volume decrease of DA1 and the suppression of mating in cVA-perfumed D. suzukii support a model in which Or65a and DL3 suppress Or67d-mediated behaviours similar to observations in D. melanogaster [7,8,17,35]. Apparently, in D. suzukii, the balance of DA1 and DL3 input and output has shifted through evolution, resulting in a chronic suppression of cVA-induced behaviours in this species, when compared with other melanogaster group species. This effectively has reversed the role of cVA from a pheromone to a heterospecific signal that inhibits mating.}
\end{align*}

The concurrent miniaturization of DA1 volume and the behavioural shift in response to cVA are reminiscent of observations in Drosophila’s coding of general odour preference [36,37] and preference coding of pheromones in moths [38]. In these studies, changes in relative glomerular volume were associated with shifts in olfactory preference. Increased glomerular volume is associated with an increased preference for the ligand of these glomeruli. In this study, however, we observed the opposite: a severe reduction in glomerular volume converts attraction into inhibition, suppressing the cascade of behaviours associated with its ligand (figure 6a).

What factors underlie the reduced T1 expression and diminution of DA1 are unknown. An important factor in driving sexual behaviours in D. melanogaster is the transcription factor fruitless (fru). A male-specific splicing variant of fru, FruM, causes sex-specific neuronal growth, targeting and corresponding behaviour [31,39–42]. Our gene annotations show nevertheless that the fru region, spanning 100 kb of genomic DNA, contains all putative exons to build the various isoforms found in D. melanogaster, including FruM (electronic supplementary material, figure S3; [31]). Other well-known genes involved in sexual dimorphism of the olfactory circuitry, such as sexlethal (sxl [43,44]), transformer (tra [43,45]) and...
The loss of doublesex (compared to *D. melanogaster*) reduction in volume of DA1 in *D. suzukii* that Fru is translated in brains of both sexes of *c* also be simply the result of drift, although the presence of *fru* in the two other glomeruli receiving input from sensory neurons (electronic supplementary material, figure S3). However, we noted that the volume of DA1 is sexually isomorphic in *D. suzukii* (figure 4). This contrasts with *D. melanogaster*, where Fru causes a substantial dimorphism in volume and expression pattern in its new niche, or reflect this species’ opposite behavioural and ecological function, mediated by off-setting the balance of sensory input. Although this study does

**Figure 6.** (a) Schematic overview of the change in the circuitry of *D. suzukii* compared to *D. melanogaster*. Based on earlier work [6], we conclude that reduction in volume of DA1 in *D. suzukii* lead to the observed chronically depresses DA1 output and the observed inhibition of sexual behaviour. OSNs, olfactory sensory neurons; PNs, antennal lobe projection neurons. (b) Reconstructions of the antennal lobes of *D. subpulchrella* and *D. biarmipes*. In bright red DA1, which received input from T1 neurons, and in light red other glomeruli receiving input from sensilla trichodea neurons. Note: the small volume of DA1 in *D. subpulchrella*, which was comparable to *D. suzukii* (see figure 4).

**4. Conclusion**

Sex pheromones are intraspecific signals involved in influencing behaviours of conspecifics during sexual communication. cVA is a sex pheromone produced by male *Drosophila* species and fulfils a complex role in intraspecific communication. We show that, in spite of the fact that cVA signalling fulfils a primal role and is highly conserved in the *melanogaster* group, it has been lost in *D. suzukii*. We furthermore demonstrate that its underlying circuit can rapidly evolve to serve a radically opposite behavioural and ecological function, mediated by off-setting the balance of sensory input. Although this study does
not exclude the existence of other volatile pheromones in D. suzukii, the CH extracts and the ‘relictual’ size of the EB indicate otherwise.

The results are of significance for our understanding of how sensory circuits can mediate, through numerical changes, radical changes in preference that may support the speciation process. In more practical terms, loss of eVA in this pestiferous species may be used in designing new control tools.

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