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The maxillary palps of Tephritidae are selectively tuned to food volatiles and diverge with ecology

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ABSTRACT

The maxillary palp is an auxiliary olfactory organ in insects, which, different from the antennae, is equipped with only a few olfactory sensory neuron (OSN) types. We postulated that these derived mouthpart structures, positioned at the base of the proboscis, may be particularly important in mediating feeding behaviors. As feeding is spatio-temporally segregated from oviposition in most Tephritidae, this taxonomic group appears quite suitable to parse out sensory breadth and potential functional divergence of palps and antennae. Scanning electron microscopy and anterograde staining underlined the limited palpal olfactory circuit in Tephritidae: only three morphological subtypes of basiconic sensilla were found, each with two neurons, and project to a total of six antennal lobe glomeruli in *Bactrocera dorsalis*. Accordingly, the palps detected only few volatiles from the headspace of food (fermentation and protein lures) and fruit (guava and mango) compared to the antennae (17 over 77, using gas-chromatography coupled electrophysiology). Interestingly, functionally the antennae were more tuned to fruit volatiles, detecting eight times more fruit than food volatiles (63 over 8), whereas the number of fruit and food volatile detection was more comparable in the palps (14 over 8). As tephritids diverge in oviposition preferences, but converge on food substrates, we postulated that the receptive ranges of palpal circuits would be more conserved compared to the antennae. However, palpal responses of three tephritid species that differed in phylogenetic relatedness and ecologically niche, diverged across ecological rather than phylogenetic rifts. Two species with strongly overlapping ecology, *B. dorsalis* and *Ceratitis capitata*, showed inseparable response profiles, whereas the cucurbit specialist *Zeugodacus cucurbitae* strongly diverged. As *Z. cucurbitae* is phylogenetically placed between *B. dorsalis* and *C. capitata*, the results indicate that ecology overrides phylogeny in the evolution of palpal tuning, in spite of being predisposed to detecting food volatiles.

1. Introduction

Insects rely on olfactory cues in most of their behaviors, such as the search for and selection of mates, food and oviposition sites. Their olfactory circuits thus need to accommodate multipartite behavioral contexts and switches between tasks depending on their internal state. Some olfactory cues may be intimately connected with certain behaviors, whereas others are more generic. In either case, it may be required to temporally adjust the circuitry to fit the task at hand through, for example, regulating the sensitivity of sensory neurons using for instance neuromodulators (Kim et al., 2017). Defined olfactory tasks may also lead to a subdivision in the olfactory circuitry. For instance, male moths have devoted a limited set of overrepresented sensory neurons and much enlarged glomeruli to the detection of female pheromones and used in mate searching only (Hansson and Anton, 2000).

In Diptera, the olfactory sensory organs are subdivided into the antennae, expressing most of olfactory sensory neuron (OSN) types, and the maxillary palps, which contain only a few types (de Bruyne et al., 1999). Evidence suggested some functional subdivision, but the difference is disputed. However, since the palps are derived mouthparts, a particular role in food and feeding behaviors has been suggested (Oh et al., 2021; Shiraiwa, 2008). Much of the research on the maxillary

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palps has been done on *Drosophila melanogaster* (de Bruyne et al., 1999; Dweck et al., 2016). The disadvantage of drosophilids for studying a potential segregation of tasks between antennae and palps is, however, that most species are saprophilic (Markow and O'Grady, 2008; Starmer, 1981). This means that mating, feeding and oviposition behaviors coincide on the same decaying substrate, which makes parsing the roles of the palps and its OSNs in defined behaviors very difficult.

In Tephritidae, a distantly related dipteran taxa, feeding and oviposition are much more segregated in time and space. Although saprophily is ancestral in tephritids, oviposition in fruiting bodies of plants is a derived trait in this speciose family (Díaz-Fleischer et al., 1999; Drew and Yuval, 1999). Yet, adult Tephritidae still orient to and feed on decomposing, protein-rich substrates on which they depend for sexual maturation and oogenesis (Nash and Chapman, 2014; Pascacio-Villafán et al., 2023), and indeed this trait is at the basis of many fruit fly control techniques. This segregation of behaviors in space and time in Tephritidae offers the possibility to study a potential functional behavioral significance of the antennae and palps. We therefore described the sensory circuitry in *B. dorsalis* from palps to antennal lobes, and mapped out the tuning breadth (the diversity of odors to which the two organs respond) of the antennae and maxillary palps to assess a differential affinity for food and fruit odors. In addition, we compared the tuning curves of palps of tephritid species that differed in phylogenetic relatedness and ecology to assess whether palpal responses were conserved or divergent, similar to what has previously been done for the antennae (Biasazin et al., 2019). We used gas chromatography (GC) coupled whole mount sensory recordings, GC-EPD (electro-palpal detection). Data were compared to a database of antennal responses (i.e., GC-EAD, electro-antennographic detection) in Tephritidae (Biasazin et al., 2019). We hypothesized that being a mouthpart positioned on the proboscis, combined with the fact they are exposed mostly when feeding, the maxillary palps fulfill a more prominent role in saprophytic feeding behaviors and are accordingly more tuned to fermentation volatiles than plant and fruit volatiles. In addition, since saprophily is an ancestral trait, we also conjectured that the receptive ranges of the palps would be more conserved than the antennae and follow phylogeny rather than ecology. The species used for this study include two species with overlapping niches and broadly oviposits in fruits from diverse fruit trees, including mango (*B. dorsalis* and *C. capitata*), as well as a species which infests primarily cucurbitaceous plants (*Z. cucurbitae*), a trait common in its genus.

2. Material and methods

2.1. Insects

Pupae of *B. dorsalis, C. capitata and Z. cucurbitae* were obtained from the International Atomic Energy Agency (IAEA) division of nuclear techniques in food and agriculture, Austria, Vienna, in several batches over the years. Adults were reared as previously described (Biasazin et al., 2019). Briefly, adults were kept in polyester netting bugdom cages (32.5 x 32.5 x 32.5 cm^2) in a climate controlled room at 26 ± 3 °C, 60–65 % RH and 12:12 L: D. Adult flies were fed on an artificial diet composed of three-parts sugar:one-part yeast, while larvae were reared on a carrot based diet (Ekesi et al., 2007).

2.2. Experimental fruits and volatile collection

We used volatiles that had been previously collected from two important hosts of *B. dorsalis*, mango, *Mangifera indica*, and guava, *Psidium guajava* (for details see (Biasazin et al., 2019)). We used a previously collected extract of food volatiles used to assess antennal sensitivity to food odors (Biasazin et al., 2018) collected from brewer's waste, torula yeast, baker's yeast, GF-120 and Anamed, a commercial bait (ISCA, Inc., Riverside, USA), using Porapak Q, extracted with n-hexane and stored at −80 °C until use.

2.3. Electrophysiology

Gas Chromatography coupled Electro-Palpographic Detection (GC-EPD) was used to identify compounds that elicit response to the maxillary palp. Experimental animals were obtained from the stock colony from IAEA in Austria (see above) and reared for several generations to cover the GC-EPD recordings needed. GC-EPD recordings were carried out from the tip of maxillary palp of male and female flies. Briefly, 10–15 day old *B. dorsalis* flies were inserted in a 200 μL micropipette tip allowing maxillary palps to protrude from its narrow end. Glass capillaries filled with beadle Ephrussi-Ringer solution (7.5 g NaCl, 0.35 g KCl, 0.29 g $CaCl_2$, dissolved in 1 L of distilled water) were connected to the reference electrode inserted into the head and recording electrode connected to the tip of the palp. The recording electrode was connected to a pre-amplifier then to a high impedance GC amplifier interface box (IDAC-2; Syntech, Kirchzarten, Germany). Aliquots of 3 μL of headspace volatiles in hexane (from (Biasazin et al., 2018; Biasazin et al., 2019), stored at −80 °C) were injected into the GC, Agilent 6890/5975. DB-Wax 30 m, 0.25 mm id, and 0.25 μm film thickness and helium was used as carrier gas. The injector, in splitless mode, and flame ionization detector (GC-FID) were kept at 250 °C and 270 °C respectively. The oven was kept at 40 °C for 3 min, then rose to 150 °C at a rate of 3 °C min^{-1} then raised again to a final temperature of 240 °C at a rate of 15 °C min^{-1} and the final temperature was maintained for 5 min. The effluent of the GC was split 1:1 between the GC flame ionization detector and, via a heated transfer line, a humidified airstream (1500 ml min^{-1}) that passed over a fly's antennae.

To obtain single sensillum traces from the three sensillum types found on the maxillary palps, flies were mounted as described above. The pipette tip was placed on a wax surface on a microscope slide, and using a glass micropipette one of the palps was fixed onto the coverslip. The fly was placed under a microscope (Olympus BX51W1), with a magnification of up to 1500. Via a glass tube, a 1 L min^{-1} charcoal-purified and humidified airflow was constantly blown over the fly head. Tungsten microelectrodes, sharpened in a KNO_2-solution, were used for recording of action potentials of antennal sensory neurons. For fine positioning we used a motor-controlled micromanipulator (Märzhauser DC-3 K, Wetzlar, Germany) equipped with a piezo unit (Märzhauser PM-10). A reference electrode was inserted into the eye with a manually controlled micromanipulator (Narishige MM33, Tokyo, Japan). After A/D conversion (Syntech IDAC PCI card), spikes were visualized and stored on a PC.

2.4. Chemical analysis

Compounds that elicited electrophysiological responses were identified using a gas chromatography-mass spectrometry (GC–MS), Agilent 6890/5975 equipped with a DB-Wax column of 60 m, 0.25 mm id, and 0.25 μm film thickness. Helium was used as carrier gas. The oven programme was the same as for GC-EAD/EPD analyses. The peaks that elicited EPD responses in *B. dorsalis* were identified by comparison of their mass spectra and their Kovats retention index (KI) with a custom-built library at SLU and the NIST-14 Library, and calculated retention indices were compared with published Kovats retention indices and confirmed using synthetic standards.

2.5. Antennal lobe reconstruction

The technique of immunostaining the brain of *Drosophila melanogaster* has been described previously (Dekker et al., 2015; Rybak et al., 2016) and was adapted to *B. dorsalis*. Flies were anesthetized with carbon dioxide, constrained in a pipette tip with the head protruding along with part of the proboscis to provide access to the maxillary palps of the flies. Using anterograde-neurobiotin (Molecular Probes, Carlsbad,CA, USA) maxillary sensory neurons were backfilled. 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 þ 2 % neurobiotin was placed over the maxillary palp, stabilized and allowed to diffuse into the sensory neurons for 3 h. Preparations were then fixed in 4 % paraformaldehyde phosphate solution (PFA) on the shaker in the dark at room temperature (RT). Following fixation, brains were washed 6 times for each 10 min in phosphate buffered saline (PBS) containing 0.1 % Triton X-100 (PBST), and blocked in 5 % normal goat serum (NGS) in PBST for 2 h on the shaker at RT. After 3 10 min washing in PBS, brains incubated (overnight on the shaker at RT) with fluorescein-avidin 488 to which a 1:30 dilution of the monoclonal mouse antibody nc82 in 5 % NGS was added to identify targeted glomeruli in the antennal lobes. Following 6 10 min washes in PBST, brains were incubated in 1:200 goat anti mouse antibody Alexa Fluor 586 for a day on the shaker at 4 °C. Afterwards the brains were washed 3 times each 10 min in phosphate solution (PBST) and mounted in Vectashield (hard set, Vector Labs, Burlingame, CA).

Whole-mount brains were scanned in a Zeiss LSM 510 confocal microscope (Carl Zeiss, Jena, Germany) equipped with a 40x, 1.4 oil-immersion DIC objective lens. Structures were excited with a Argon laser at 488 nm (fluorescein Avidin labelling) and a HeNe 543 nm laser (Alexa 546 labelling) and detected using a 505–515 bandpass and a 560 nm long pass filter, respectively. Stacks of around 50 confocal images were scanned, and the images were stored at a size of 1024 × 1024 pixels. AMIRA 5.0 software (Visage Imaging, Berlin, Germany) was used as platform for 3D reconstructions.

2.6. Scanning electron microscopy

Individuals of both sexes of *B. dorsalis* were used for SEM. Flies were anesthetized using CO_2, decapitated and fixed for 3 hrs in a 0.1 M Phosphate Buffer Saline (PBS) containing 2.5 % glutaraldehyde at pH of 7.2 on a shaker. After fixation the heads were washed three times for 15 min each in 0.01 M PBS, followed by dehydration in a series of 15 min steps in 30, 50, 70 and 90 % ethanol, respectively. The dehydrated heads were kept for ≈ 45 min in 99.6 % ethanol before critical point drying (CPD). After CPD the palps of the flies were carefully removed and mounted on two-sided carbon tape on stubs and thereafter sputtered. SEM was performed on a SU3500 (Hitachi, Japan) at the Microscopy Facility at the Department of Biology, Lund University. Single scans were merged into high-resolution stitched pictures using Adobe ® Photoshop ® CS6.

2.7. Statistical analysis

Electrophysiological data generated from GC-EAD/GC-EPD were normalised for each recording. This was done by dividing each individual response by a weighted average responsiveness of that trace (back-transformed average of all log-transformed EAD responses within a trace). The response to a given compound was then averaged across recordings and represents a relative sensitivity of the sensory organ to that compound (Biasazin et al., 2019). Averaging is thus made independent of overall sensitivity differences between antennae tested under the same conditions (same sample, species, and other variables), but not across different ones. Since the same extracts were used as in previous studies on the antennae (Biasazin et al., 2018; Biasazin et al., 2019) the GC-EPD data obtained here could be analyzed together with the previously obtained raw GC-EAD data to assess tuning overlap between these two olfactory sensory organs. For each species, sample, sex and organ, at least three recordings were used for each analysis. Each trace was obtained from a separate individual. Chemical compounds were grouped into major groups such as esters, terpenoids, ketones etc. For producing principle component analysis (PCA) plots, the package 'Tidymodels' (Kuhn and Wickham, 2020) was used and the data was scaled and centered. Compounds that didn't elicit a response were treated as zeroes. The percentage explained for each principal component was calculated as the variance of the principal component divided with the sum of the variance across all principal components. Nonmetric multidimensional scaling (NMDS) was performed with package Vegan (Oksanen et al., 2019), using a Jaccard's dissimilarity index and centering data before analysis. Also, Permutational Multivariate Analysis of Variance was performed using Distance Matrices from the function adonis 2 in package Vegan to determine if there was a significant effect between species, organs and species:organs. For annotating ellipses around data points in both NMDS and PCA plots, a Khachiyan algorithm was used through the package ggforce (Pedersen, 2020). For producing heatmaps the average response across all recorded individuals was taken and divided with the average response so that the average response was one. Pairwise comparisons between all species were performed using linear models based on the response to each compound. All data analysis was done using R (R Core Team, 2020), and data handling and graphing used 'Tidyverse' (Wickham, 2017).

3. Results

3.1. The palpal olfactory neuroanatomy

SEM images show a single olfactory sensillum type, sensillum basiconica, on the maxillary palps of *B. dorsalis*. Putatively, three subtypes were distinguished based on shape and length (Fig. 1), which could also be tentatively distinguished under a light microscope based on length and diameter. Their distribution appeared confined mostly to the distal portion of the palp and with subtypes appearing more or less randomly distributed within this area. Consistent with three sensilla basiconica subtypes and two sensory neurons in each, anterograde fills with neurobiotin from the palp consistently labeled six glomeruli in the ventromedial portion of the antennal lobes (Fig. 2, Fig S1). Traces from single sensillum recordings demonstrate indeed, three subtypes, each inhabited by 2 neurons each (Fig. 1).

3.2. B. dorsalis palps tuned to food odors, antenna to fruit odors, particularly esters

Whereas food volatiles were roughly equally detected by the palpi and antennae (Fig. 3), the antennae dominated the sensory responses to fruit volatiles, detecting more than four times as many fruit volatiles (68, 96 % of the total number of fruit volatiles that gave a response) as the palps (14, 20 %). Headspace from fruit induced more than eight times more responses in the antenna than the headspace from food, whereas in the palp this relative difference was less than a factor two. Accordingly, only 16 % of the antennal responses to fruit volatiles overlapped with those of the palp, whereas this overlap was 50 % for food volatiles. Interestingly, the 16 % overlap was largely due to an overlap in fruit and food volatiles (5 out of 11 volatiles). The antennae responded particularly well to the large diversity of aliphatic esters that dominated the fruit headspaces (46 or 65 % of the responses), which increased the number of antennal responses to esters in fruit by a factor of 9 compared to those from food. All food esters that gave a response in both palp and antennae were also present in fruits. The response strength in the palpi and antennae was uncorrelated between fruits (Fig. S2). Pyrazines were only found in food odor and predominantly induced responses in the palpi.

The palpal responses to volatiles of mango and guava strongly overlapped, whereas antennal responses to these fruit volatiles diverged: of the 14 fruit volatiles detected by the palp, 57 % (8) were shared between guava and mango. In addition, half of these compounds, all esters, overlapped with food volatiles. Conversely, of the 68 guava and mango fruit volatiles that gave antennal responses, only 22 % (15) were shared between guava and mango. Both the PCA and NMDS reflected this: palpal recordings grouped closely compared to antennal recordings (Fig. 4 and Fig. S3). Sexes did not differ in the palpal responses.

The 18 fruit and food volatiles that were detected by the palps included several distinct groups. Fig. 3 lists the compounds detected by

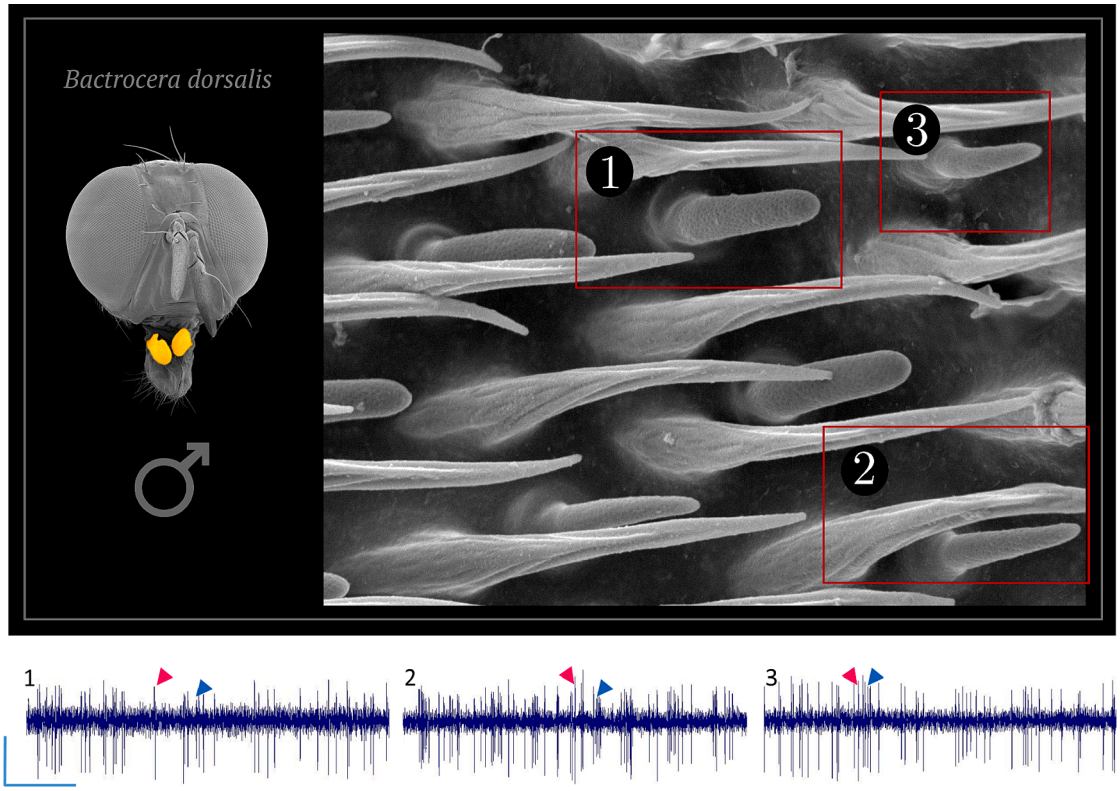

Fig. 1. Top: scanning electron microscopy (SEM) pictures of the head of *B. dorsalis* with the maxillary palp in yellow (left panel), on which three (1–3) putative morphological subtypes of sensilla basiconica can be distinguished. Type I, which is most abundant, is thick and not tapering. Type II is more slender and cone shaped and tapering toward the tip. Type III is similar to type II but slightly thinner and smaller. Bottom: sample traces of the tree types, each indicating an A and B neuron. The spike amplitude differences of A (red) and B (blue) neurons in type II are most distinct, while more close together in the other sensillum types. Horizontal and vertical scale bars represent 0.5 s and 80 μV, respectively.

the palps of *B. dorsalis*, color coded by their chemical group. Based on the diversity of compounds, we postulate that at least four distinct olfactory receptors are needed to account for the diversity of responses noted here.

3.3. Palpal tuning of *C. capitata* and *B. dorsalis* are convergent, *Z. cucurbitae* is divergent

Analysis of GC-EPD and GC-EAD recordings shows that palpi and antennae responded to roughly an equal number of food-associated volatiles in *B. dorsalis* (8 EPD and 8 EAD responses), *C. capitata* (8 and 10 resp.) and *Z. cucurbitae* (6 each, see Fig. 5). Three of these volatiles were detected by the palpi and antenna of all species (butyl acetate, 3-methylbutyl acetate and ethyl hexanoate). Across all species 50 % of the compounds detected by the palps were also detected by the antennae. Five compounds, 2-methylpyrazine, 2,-dimethylpyrazine, 2-ethyl-6-methylpyrazine, heptan-2-one and hexyl acetate, were detected by the palps of *B. dorsalis* and *C. capitata*, but not *Z. cucurbitae*. Instead, only *Z. cucurbitae* detected three other compounds, limonene, ethyl *cis*-4-hexanoate and 2,3-dimethylpyrazine.

Comparison between species further showed that the receptive ranges and the strength of palpal responses were significantly ($p < 0.001$) correlated (Adj. $R^2 = 0.84$) between *B. dorsalis* and *C. capitata* (Fig. 6). In contrast, *Z. cucurbitae* palpal responses were divergent and did not correlate with either *B. dorsalis* or *C. capitata* (Fig. 6). Accordingly, *B. dorsalis* and *C.capitata* either fully overlapped (palps) or grouped closely (antennae) in a PCA analysis using normalised responses, whereas *Z. cucurbitae* was an outlier in both (Fig. 7). A NMDS analysis, where presence/absence standardisation was performed, grouped the palpal responses between *B. dorsalis* and *C. capitata* completely overlapping, whereas, similar to the PCA analysis, *Z. cucurbitae* was an outlier in regards to both the palp and antennae (Fig S3).

4. Discussion

The maxillary palps are a set of mouthpart appendages that in dipterans fulfill an olfactory function. Yet, their olfactory sensitivity has been little explored, except for *Drosophila* and limited studies on Tephritidae (de Bruyne et al., 1999; Dweck et al., 2016; Noushini et al., 2020) and mosquitoes (Ghaninia et al., 2019; Grant et al., 1995; Jones et al., 2007; Lu et al., 2007; Majeed et al., 2017). Further, it is largely unknown whether the palps are a mere auxiliary olfactory organ to the antennae, or serve a particular function in an insect's life history. For instance, as modified mouthpart structures positioned on the proboscis it has been postulated that the palps are particularly used in feeding behaviors (Shiraiwa, 2008), although recent work contradicts this (Oh et al., 2021). In mosquitoes, CO_2 detection, which is critical in host finding (Dekker et al., 2005), is mediated through neurons in the sensilla basiconica in the palps (Grant et al., 1995), with other neurons in this sensillum responding to 1-octen-3-ol and acetone (Ghaninia et al., 2019; Grant et al., 1995; Lu et al., 2007). In *Drosophila*, the maxillary palps express six olfactory sensory neurons that combinedly respond to a diverse set of compounds that broadly mediate short and long-range attraction (Dweck et al., 2016). However, in saprophilic *Drosophila* feeding, mating, oviposition and other behaviors are confluent, making it tricky to discern the 'olfactory mode' of an individual. In fact, the behaviors often occur interspersed with each other and may not be much separated in time and space, which may also have faded over evolutionary time. In contrast, in most Tephritidae, feeding (particularly for sexual maturation and oogenesis) and oviposition have diverged from a saprophilic past to become largely spatio-temporally segregated

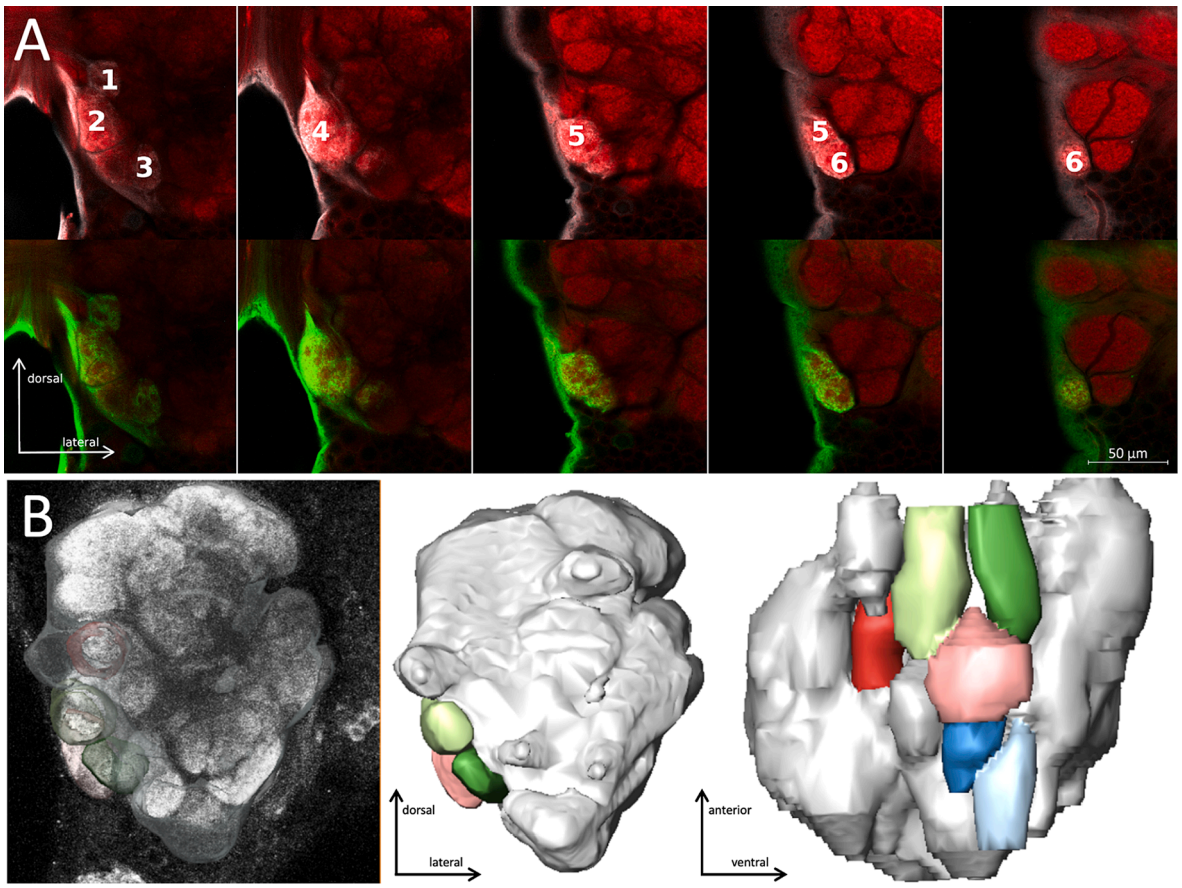

Fig. 2. A. Neurobiotin backfill from the maxillary palps to the antennal lobes of *B. dorsalis*. Top panel: overview staining with numbered glomeruli innervated by sensory neurons from the palpi. Red = synapsin background staining, green = neurobiotin backfill. B. Draft antennal lobe reconstruction of *B. dorsalis* highlighting the six ventro-medial glomeruli targeted by palpal OSNs (colored glomeruli). Panels, left: anterior view of an confocal image with faded palpal glomeruli as overlay; middle: anterior view of antennal lobe in grayscale with medio-ventral palpal glomeruli highlighted in color; right: medial view of the antennal lobe in greyscale, with reconstructed palpal glomeruli in color.

behaviors. Here we show that the receptive range of the palps is overlapping but distinct from the antennae, and appears predisposed to the detection of food, whereas the antennae capture a wide variety of fruit volatiles.

4.1. Tephritidae and Drosophila palps both have 3 sensillum and 6 OSN types

Previous studies on Tephritidae have reported a single olfactory sensillum type, sensilla basiconica, with the numbers ranging from 52 to 117 (Oh et al., 2019; Park et al., 2018; Zhang et al., 2011). Our SEM analyses and backfills confirm that the only olfactory sensillum type on the maxillary palp of Tephritidae was sensilla basiconica. Three subtypes could be distinguished by SEM, consistent with two sensory neurons per sensillum and 6 antennal lobe glomeruli. The latter was confirmed by single sensillum recordings, which show two neurons per sensillum (Fig. 1). Interestingly, *D. melanogaster* maxillary palps has an similar basic setup, i.e. three basiconic sensillum subtypes, each with two OSNs that project to a total of 6 medioventral glomeruli (de Bruyne et al., 1999; Dweck et al., 2016), in spite of phylogenetic distance between Drosophilidae and Tephritidae (within the same subsection, acalyptrata of higher Diptera), possibly indicating a high conservancy in maxillary palp neuroanatomy.

4.2. Palps tuned to food odors, antennae disproportionately tuned to fruit odors

Comparative olfactomics can surface patterns across odor sources (fruit and food), organs (antennae and palps) and species. Our analysis demonstrates that antennae of Tephritidae are broadly tuned to a wide diversity of fruit volatiles (see also Biasazin et al., 2019). In contrast, the maxillary palps detected food volatiles and fruit odors to a comparable degree. This is remarkable as the headspace of food (yeast and protein lures) contained relatively few odors, and stochastically one would expect a similar skew in palpal sensitivity for fruit odors as observed for the antennae. Similarly, with the low number of OSNs, the palps detected almost as many food volatiles as the antennae. This indicates a predisposition of the palpal neurons for detecting food, and a low suitability of the palps in distinguishing between fruits (underlined by PCA and NMDS separation of sensory responses to fruit by antennae, but not by palps, Fig. 4).

Of further interest is that three esters that are typically associated with fermentation substrates (isoamyl acetate, ethyl hexanoate, and ethyl butyrate), were detected by maxillary palps and antennae of all species. The overlap in detection does, however, not necessarily reflect redundancy, as the circuitry underlying palpal and antennal OSNs may well be responsible for inducing separate behaviors. In *D. melanogaster*, defined behaviors may be induced by the activation of specific OSNs (Dweck et al., 2016; Lebreton et al., 2014; Shaw et al., 2021), and thus detection of the same compound by different classes of OSNs may be needed if these OSNs steer different behaviors. A partition of behaviors

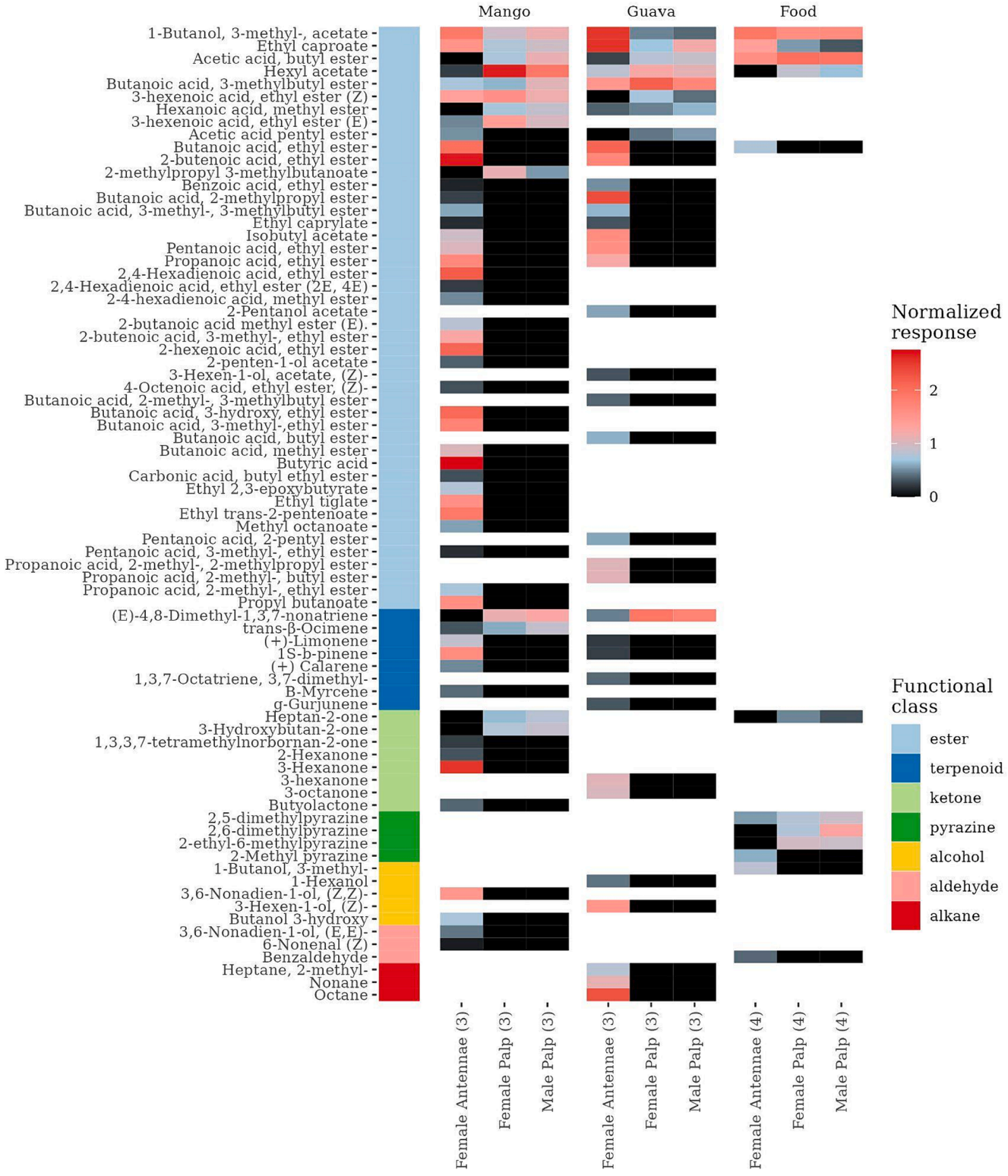

Fig. 3. A heatmap depicting antennal and palpal responses produced through GC-EAD and GC-EPD using fruit (guava and mango) and food headspace extracts (yeast and protein baits). From left to right: name of the compound identified, functional classes, heatmaps of female antenna, and female and male palps of *B. dorsalis*. The compounds are organized according to functional class and within these in decreasing order of detection frequency. The response strength from 0 (black) to 3 dark red is first normalised within a run, before being averaged across runs. The strength of a response is therefore always relative to the average responsiveness of the organ to all compounds within the same column. In parenthesis are the number of replicates. Color intensity coding can therefore only be compared within a columns and not across.

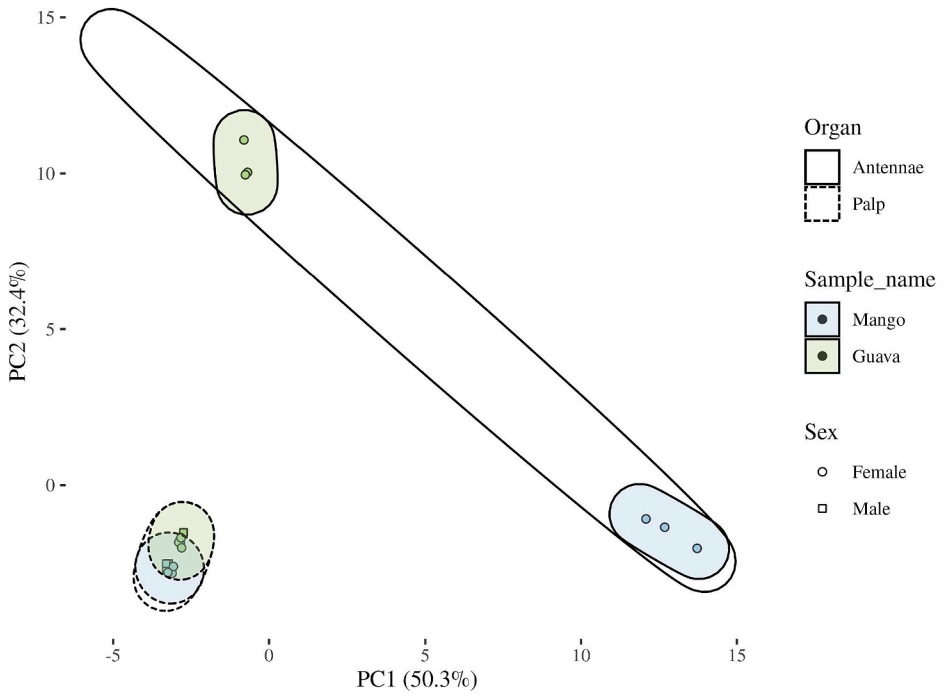

Fig. 4. A principle component analysis (PCA), with colors representing the different samples (mango and guava) used in the analysis. Filled and dashed lines represent the antennae and the palps, respectively. Recordings on the antennae were done on male flies, whereas recordings on the palps were done in both males (square dots) and females (circles), with no discernible difference, for all recordings three replications were done. Note: for GC-EAD (antennae) data we used raw data that has been previously used in Biasazin et al. (2019).

between antennal and palpal circuits seems likely considering that during flight the maxillary palps of Tephritidae are retracted in the fold in which the proboscis retracts and largely unexposed, but are fully exposed when landing and extending their proboscis. Finally, the fact that esters that are typically associated with fermentation are detected by palps of all species underlines the importance of the palps in food odor detection. Similarly, consistent with a predisposition of the palpal circuitry for feeding is the fact that palps detect male attractants ('parapheromones') and are required for ensuing behaviors (Chieng et al., 2018). Male attractants induce compulsive feeding, are used in sexual communication Tephritidae (Noushini et al., 2020; Park et al., 2018; Verschut et al., 2018), and are detected primarily by the maxillary palps. Of interest is the finding that in the blowfly, *Phormia regina*, some OSN as well as mechanosensory neurons from the palpae project to the suboesophageal ganglion and may directly interact with gustatory information (Maeda et al., 2014). While we did not find palpal efferents projecting to the SOG in our study, Maeda et al. (2014)'s finding suggests a possible intimate connection between the palps and gustation.

4.3. Ecology rather than phylogeny determines palpal tuning

If the maxillary palps mediate close range, saprophilic behaviors, an ancestral trait, one would expect a conserved olfactory coding in this auxiliary olfactory organ. However, our analyses, although limited to three species, show instead that the phylogenetically most distant, but ecologically very similar and frequently competing species *B. dorsalis* and *C. capitata*, had inseparable palpal sensitivities, whereas, the phylogenetically intermediate (Segura et al., 2006), but ecologically distant *Z. cucurbitae* exhibited divergent palpal sensitivities. It may be that the palpal circuitry has considerable degree of freedom in 'defining' food, and as such evolves along with the oviposition niche of the species, i.e., cucurbit-related fermentation volatiles for, for instance, *Z. cucurbitae*.

Several compounds contributed to the divergence in palpal sensitivities. Among these is a group of nitrogen-containing compounds, pyrazines. Pyrazines are produced by bacteria in the midgut ecosystem of Tephritidae (Hadapad et al., 2016; Robacker and Bartelt, 1997) and are released by calling males in other Tephritidae such as *Anastrepha serpentina* and *Toxotrypana curvicauda* (Robacker et al., 2009; Robledo et al., 2014). What role pyrazines play in behavior is unknown, but since they are strongly related to food and digestion and are detected by the palpae, they may play a significant role in feeding behaviors.

Further in depth studies are needed to decipher the evolutionary ecological dynamics of olfactory coding in the maxillary palps of Tephritidae. Such studies should include single sensillum studies to verify the number and tuning breadth of OSNs underlying palpal responses noted here, description of palpal olfactory receptors and their evolutionary dynamics across species, and deorphanization studies to functionally characterize these ORs and their evolution. These studies should also include how maxillary palp input steers odor-mediated behaviors, and how this could be used to enhance the attractiveness of lures by mediating for instance close range behaviors. Such information could be of use in the development of novel, sustainable control techniques for these important horticultural pests, such as mass trapping or attract-and-kill.

5. Conclusion

The maxillary palp in Tephritidae is an auxiliary olfactory organ consisting of six OSN classes that exhibits a higher sensitivity to feeding cues compared to the antennae. In spite of its tuning to saprophilic cues, the receptive range of maxillary palps is less conserved than we expected, and accordingly does not follow phylogeny more closely than the antennae. Whether its tuning follows the odors associated with its ecological niche needs further study. The limited number of receptors and sensilla with functional divergences makes them the ideal target for future in-depth physiological and molecular studies on the evolutionary ecological dynamics of olfaction in Tephritidae.

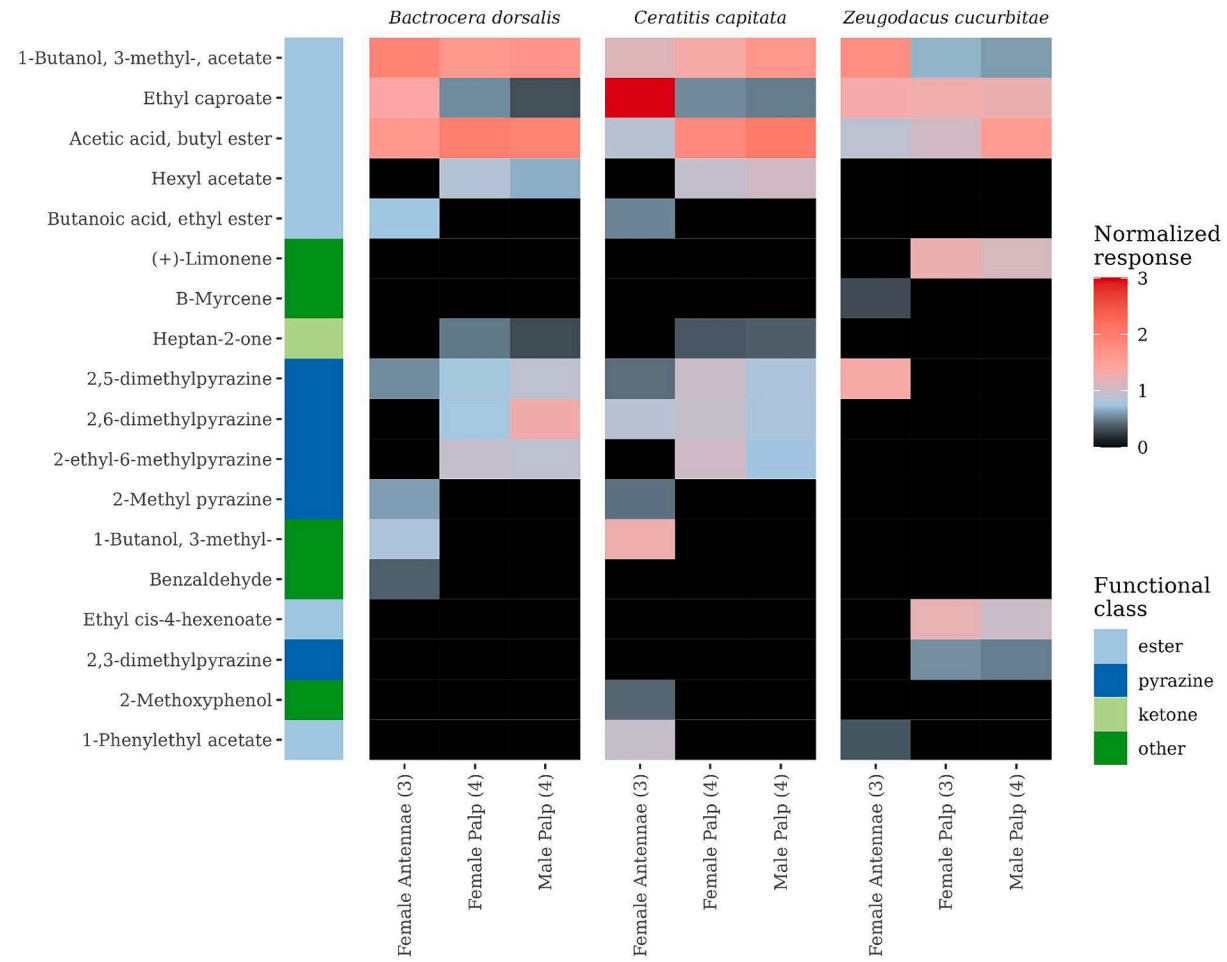

Fig. 5. A heatmap depicting antennal and palpal responses of three tephritid species. Food headspace extracts were presented through GC-EAD and GC-EPD. From left to right: name of the compound identified, functional classes, heatmaps of female antenna, and female and male palps of *B. dorsalis*. The compounds are organised according to functional class and within these in decreasing order of detection frequency. In parenthesis are the number of replicates. The response strength from 0 (black) to 3 dark red was first normalized within a run, before being averaged across runs. The strength of a response is therefore always relative to the average responsiveness of the organ to all compounds in the same column. Color intensity coding can therefore only be compared within a column and not across and do not represent absolute values. Note: for GC-EAD (antennae) data we used raw data that has been previously used in Biasazin et al. (2018).

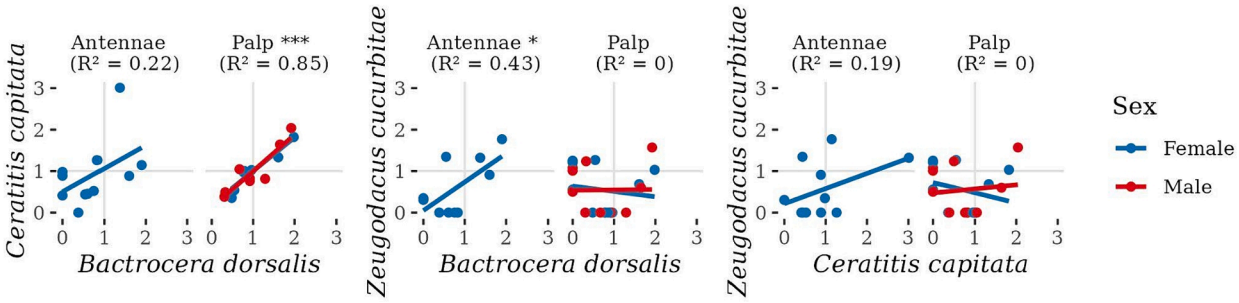

Fig. 6. Pairwise regression analysis between response strengths of species to food volatiles, for antennae and maxillary palp between each of the fruit fly species. Stars after the organ denotes the significance (* < 0.05, ** > 0.01, *** < 0.001). Note: for GC-EAD (antennae) data we used raw data that has been previously used in Biasazin et al. (2018).

CRediT authorship contribution statement

Sebastian Larsson Herrera: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Fikira Kimbokota:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sohel Ahmad:** Writing – review & editing, Resources, Investigation. **Katharina Heise:** Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Tibebe Dejene Biasazin:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Teun Dekker:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

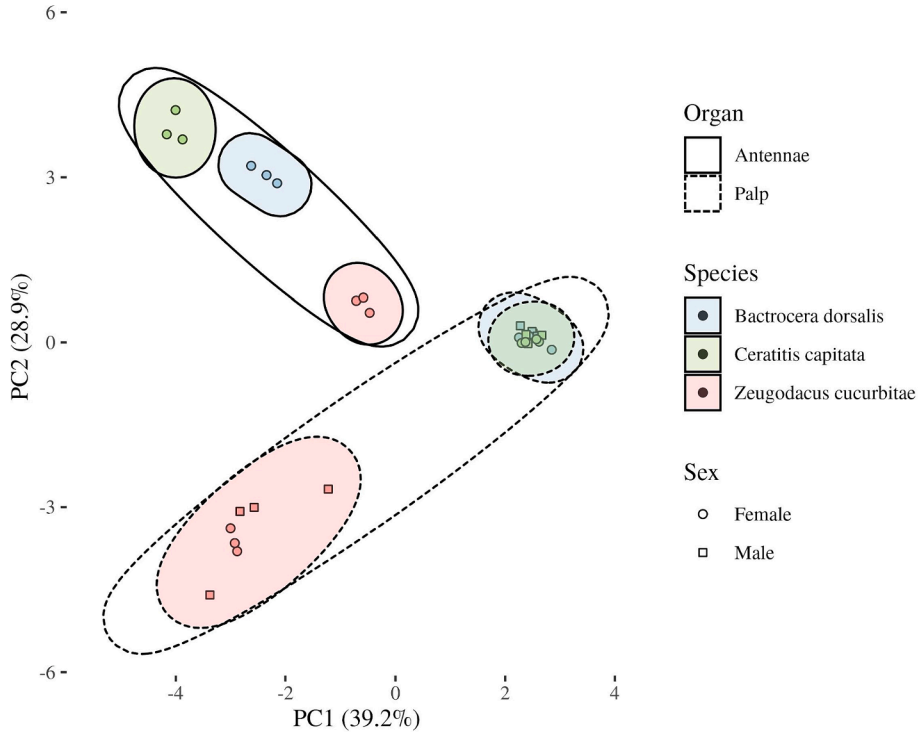

Fig. 7. A principle component analysis (PCA) of the responses to food-related volatiles, with colors representing the different species (*B. dorsalis*, *C. capitata* and *Z. cucurbitae*). Filled and dashed lines represent the antennae and the palps, respectively. Recordings on the antennae were done on females, and those on the palps on both sexes. No differences between sexes were observed. Note: for GC-EAD (antennae) data we used raw data that has been previously used in Biasazin et al. (2018).

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 used to for this manuscript and the scripts needed to make the figures are available for review at: https://zenodo.org/records/10845546?token=eyJhbGciOiJIUzUxMiJ9.eyJpZ-CI6ImQ3N2M2MTk4LTEzNjMtNGY1OC04MWZmLTQ1YWM1ODR-mY2ZjNyIsImRhdGEiOnt9LCJyYW5kb20iOiI-wYWFiMTBmMTIyZDY1MjIwYTkyYTI0NDA5ZmZjN2FlMCJ9.6U23E-Lo9KEqmMo7l3CWhGVWhI9AviKg10BVzQ3XWg_M8b_vngoOuW8BZ-ey9lsqxIqUYb7PPvseC0FWFgIJ05g and will be made publicly available at doi: https://doi.org/10.5281/zenodo.10845546 upon publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2024.104632.

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