

1 **Functional characterization of olfactory receptors in the Oriental**
2 **fruit fly *Bactrocera dorsalis* that respond to plant volatiles**

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24 ABSTRACT

25 The Oriental fruit fly, *Bactrocera dorsalis*, is a highly destructive pest of various
26 fruits. The reproductive and host-finding behaviors of this species are affected by
27 several plant semiochemicals that are perceived through chemosensory receptors.
28 However, the chemosensory mechanisms by which this perception occurs have not
29 been fully elucidated. We conducted RNA sequencing analysis of the chemosensory
30 organs of *B. dorsalis* to identify the genes coding for chemosensory receptors. We
31 identified 60 olfactory receptors (ORs), 17 gustatory receptors and 23 ionotropic
32 receptors—including their homologs and variants—from the transcriptome of male
33 antennae and proboscises. We functionally analyzed ten ORs co-expressed with the
34 obligatory co-receptor ORCO in *Xenopus* oocytes to identify their ligands. We tested
35 24 compounds including attractants for several *Bactrocera* species and volatiles from
36 the host fruits of *B. dorsalis*. We found that BdorOR13a co-expressed with ORCO
37 responded robustly to 1-octen-3-ol. BdorOR82a co-expressed with ORCO responded
38 significantly to geranyl acetate, but responded weakly to farnesenes (a mixture of
39 isomers) and linalyl acetate. These four compounds were subsequently subjected to
40 behavioral bioassays. When each of the aforementioned compound was presented in
41 combination with a sphere model as a visual cue to adult flies, 1-octen-3-ol, geranyl
42 acetate, and farnesenes significantly enhanced landing behavior in mated females, but
43 not in unmated females or males. These results suggest that the ORs characterized in
44 the present study are involved in the perception of plant volatiles that affect host-
45 finding behavior in *B. dorsalis*.

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47 **Keywords:** Chemosensory receptor; *Bactrocera dorsalis*; Plant semiochemical;

48 Functional analysis; *Xenopus* oocyte; Behavioral bioassay

49 1. Introduction

50

51 Plant semiochemicals play a crucial role in insect–plant interactions, because they
52 affect insect physiology and behavior (Reddy and Guerrero, 2004). Many
53 phytophagous insects use plant semiochemicals as cues to find their feeding, mating,
54 and oviposition sites. Moreover, some insects specifically recognize host plant
55 chemicals via chemosensory organs to acquire or sequester those chemicals as
56 defensive substances, sex pheromones, or sex pheromone precursors (Nishida, 2002;
57 Opitz and Müller, 2009). Therefore, an elucidation of the basic mechanisms
58 underlying the chemoreception of plant semiochemicals is essential for an
59 understanding of the adaptation of phytophagous insects to plants as sources of
60 essential substances for growth and reproduction.

61 The tephritid fruit fly species, which include destructive horticultural pests in
62 both tropical and temperate regions, provide a good model for understanding how
63 insects adapt to plant chemicals, because their life cycles involve response to several
64 characteristic semiochemicals (Metcalf, 1990; Shelly, 2010). Several such species
65 belonging to two Dacinae genera, *Bactrocera* and *Zeugodacus*, exhibit striking
66 behaviors towards certain semiochemicals that contribute to the floral fragrances of
67 several orchid species. For example, male Oriental fruit flies (*Bactrocera dorsalis*) are
68 strongly attracted to a specific phenylpropanoid, methyl eugenol (ME). This leads to
69 voracious consumption of the compound by the male flies, and its subsequent
70 utilization as a sex pheromone precursor (Howlett, 1912; Nishida et al., 1988; Tan and
71 Nishida, 2012). Furthermore, volatiles derived from host fruits play a crucial role in
72 the search for oviposition sites by gravid females. Electrophysiological studies using
73 gas chromatography-flame ionization detection coupled with electroantennographic

74 detection (GC-EAD) have shown that a number of compounds including terpenes and
75 phenylpropanoids derived from host fruits elicit female antennal responses in *B.*
76 *dorsalis* (Siderhurst and Jang, 2006a; Siderhurst and Jang, 2006b; Kamala Jayanthi et
77 al., 2012; Kamala Jayanthi et al., 2014; Damodaram et al., 2014).

78 Although the perception of plant semiochemicals is so important in the
79 diverse life history of tephritid fruit flies, as described above, the mechanisms by
80 which chemoreception occurs have not been fully elucidated. The major molecular
81 components of insect chemoreception have been identified mainly from studies on
82 *Drosophila melanogaster* (Fleischer et al., 2017); chemosensory receptors are
83 essential for the recognition of ligands at peripheral neurons. Insect chemosensory
84 receptors consist of three types of insect-specific superfamilies: olfactory receptors
85 (ORs), gustatory receptors (GRs), and ionotropic receptors (IRs) (Vosshall and
86 Stocker, 2007; Touhara and Vosshall, 2009; Rytz et al., 2013). These receptors are
87 thought to form ligand-gated ion channels and/or to function as G-protein-coupled
88 receptors (GPCRs). Among these insect receptor superfamilies, ORs have been
89 relatively well characterized as heteromeric ligand-gated ion channels that consist of a
90 specific OR and a highly conserved co-receptor ORCO (Sato et al., 2008; Wicher et
91 al., 2008). Because a specific odorous ligand is tuned to a specific OR in this system,
92 the identification of the ligands for uncharacterized ORs could provide clues to the
93 essential chemicals involved in the life cycle of *Bactrocera* species.

94 A previous study has shown that the odorant receptor co-receptor, ORCO, is
95 involved in the perception of ME in *B. dorsalis*, suggesting that specific ORs are
96 required for the chemoreception of ME and its metabolites (Zheng et al., 2012).
97 Furthermore, chemosensory genes that code for ORs, IRs, and GRs have been
98 identified in the transcriptome of *B. dorsalis* (Wu et al., 2015; Liu et al., 2016).

Importantly, it is possible to access the reference genome sequences of *B. dorsalis* at the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/genome/10754>). Although the sequence data for *B. dorsalis* has been obtained, no chemosensory receptors responding to semiochemicals have been characterized. In the present study, we conducted RNA sequencing (RNA-seq) analysis of the chemosensory organs of *B. dorsalis* to identify genes coding for chemosensory receptors. We identified 60 ORs, 17 GRs, and 23 IRs—including their homologs and variants—from the transcriptome of male antennae and proboscises. We characterized the functional properties of two ORs that respond to plant semiochemicals in a heterologous expression system comprising *Xenopus* oocytes. We further assessed the attraction of both female and male flies to four volatiles recognized as ligands for the two ORs, and found that when used in combination with visual cues, certain plant volatiles had a significant effect on the landing behavior of mated females. In the present study, we demonstrated the functional identification of specific ligands for chemosensory receptors, which should provide clues to the identity of chemicals that influence insect behaviors.

2. Materials and methods

2.1. Insects

For preparation of total RNA, we obtained a strain of *B. dorsalis* from a colony maintained by the Naha Plant Protection Station in Okinawa, Japan. The strain—originating in Okinawa, Japan—was reared with the permission of the Minister of Agriculture, Forestry, and Fisheries of Japan (permit No. 56Y-1882). For the behavioral bioassay, we used a laboratory-reared colony of *B. dorsalis* from the

Department of Biology, the Faculty of Science, Universiti Putra Malaysia. The flies were kept at 25–29°C and 83–90% relative humidity, and subjected to a 12-h light/12-h dark photoperiod regimen. The adult flies were given *ad libitum* access to water and a mixture of sugar and hydrolyzed protein (3:1 w/w). Males and females were separated within 3 days of emergence to prevent mating, and kept in cages (30 cm × 30 cm × 30 cm) until required for the bioassay, which took place 16–19 days after emergence. Mated flies were obtained in the following manner. Two days before the bioassay, virgin males and females were placed together in a cage in the morning. The mated pairs (> 30 min of non-stop copulation) were gently collected in the late evening using a glass vial (2 mm diameter × 8.5 cm), and placed in a separate cage containing food and water. This was done to ensure that the copulating pairs did not separate from each other. Those flies were then segregated again by sex into separate cages (40 cm × 40 cm × 40 cm), and allowed to acclimatize in a sheltered outdoor bioassay area that received sunlight from the east before the experiment commenced the next morning. The virgin, males and females were also separated and allowed to acclimatize in the bioassay area prior to the behavioral trials.

2.2. RNA sequencing and assembly

The male flies were staged at 0–2, 2–4, 3–5 and 5–7 days after eclosion. Approximately 150 males were collected from each adult stage. Their antennae and proboscises were dissected and homogenized in TRIzol Reagent (GIBCO-BRL, Gaithersburg, MD, USA). Total RNAs were extracted from the homogenates and purified using NucleoSpin RNA (Macherey-Nagel, Germany). Sequence libraries were prepared using the TruSeq RNA Sample Preparation Kit v2 (Illumina, Inc., San Diego, CA, USA) as described previously (Yang et al., 2015). RNA sequencing was

performed on an Illumina MiSeq system using the MiSeq Reagent Kit v3 600 cycle (Illumina, Inc., San Diego, CA, USA). The reads were preprocessed with Trimmomatic v0.33 (Bolger et al., 2014) for quality trimming using the following parameters: LEADING: 10; TRAILING: 10; SLIDINGWINDOW: 4:20; MINLEN: 150. The resulting clean reads data have been deposited in the DNA Data Bank of Japan (DDBJ) Sequence Read Archive under accession number PRJDB6798. The pass-through reads were subjected to *de novo* assembling using the Trinity, Bowtie, eXpress, and DEGseq (PE) programs (Grabherr et al., 2011) implemented in the maser pipeline of the Cell Innovation Program at the National Institute of Genetics (http://cell-innovation.nig.ac.jp/index_en.html). Fragments per kilobase of exon per million (FPKM) values were calculated to estimate the expression levels of the transcripts.

2.3. Screening and characterization of sequences of candidate chemosensory receptors

We identified candidate chemosensory receptor genes from the Trinity contigs using the Pfam database. For this purpose, we obtained four protein domain families of *D. melanogaster* from the Pfam database (<http://pfam.xfam.org>): 7tm odorant receptor (PF02949), 7tm chemosensory receptor (PF08395), trehalose receptor (PF06151), and ligand gated ion channel (PF00060). We screened the Trinity contigs by similarity to these protein domain families using a BLASTX search at an Expect value (E-value) threshold of 1e-5. In parallel, we analyzed the Trinity contigs using a TBLASTN search against protein databases consisting of chemosensory receptors of *D. melanogaster* at the same E-value threshold. We obtained open reading frames (ORFs) of the extracted contigs using EMBOSS Transeq

(https://www.ebi.ac.uk/Tools/st/emboss_transeq/), and used them as queries in a BLASTP search against the NCBI non-redundant protein database. Contigs that ranked highly with ORs, GRs, or IRs were considered candidate genes coding for insect chemosensory receptors. Overlapping variants with identical ORFs were merged at this step by selecting the longest as the representative transcript of a variant group. Full-length ORFs of several ORs were predicted from genome sequences in the genome sequencing and assembly project, and were cloned into a vector, as described in Section 2.4. Candidate chemosensory receptors were named according to the following criteria. i) Chemosensory receptors were named as described in a previous paper (Wu et al., 2015) when their amino acid sequences were identical. ii) Orthologs of chemosensory receptors uncharacterized from *B. dorsalis* were named according to those of *D. melanogaster*. iii) For homologous chemosensory receptors with amino acid similarities of less than 80%, the names of the homologs were differentiated with a numerical postscript, e.g., *BdorOR7a-1* and *BdorOR7a-2*. iv) In cases where the amino acid similarities were 80% or more, version numbers were assigned to the receptors, e.g., *BdorOR67c-v1* and *BdorOR67c-v2*. v) In cases where multiple partial sequences of a candidate chemosensory receptor were identified, each sequence was labeled *-part1*, *-part2*, etc., e.g., *BdorOR2a-part1* and *BdorOR2a-part2*. To compare amino acid sequences of chemosensory receptors identified from *B. dorsalis* between the present study and previous studies, we performed BLASTP searches with an E-value cutoff of 1e-100.

2.4. Cloning of full-length coding sequences of candidate ORs into an expression vector

198 We cloned full-length coding sequences of candidate ORs into a pCS2P+ vector
199 kindly provided by Prof. Marc Kirschner (<https://www.addgene.org/17095/>). The
200 primers were designed from the predicted ORFs based on the assembled contigs or
201 reference genome sequences of *B. dorsalis* at the NCBI web site
202 (<https://www.ncbi.nlm.nih.gov/genome/?term=JFBF01>). The ORFs of *BdorORCO*,
203 *BdorOR94b-1*, and *BlatOR59a* were amplified by PCR from cDNA prepared from
204 male antennae using primers including untranslated regions based on the contig
205 sequences. The PCR products were then cloned into a pGEM-T vector (Promega, WI,
206 USA). The ORFs were modified with a Kozak consensus sequence (5' -GCCGCC-
207 3') and the appropriate restriction site by PCR amplification using the following
208 primers. The forward primer included the Kozak consensus sequence followed by a
209 BamHI restriction site, and the reverse primer included an XbaI restriction site. The
210 PCR products were cloned into a pCS2P+ vector using the restriction sites. The ORFs
211 of the other ORs were amplified by PCR as described above, except that the primers
212 included a part of the pCS2P+ sequence to enable cloning into the vector using an In-
213 Fusion HD cloning Kit (Takara, Otsu, Japan). The PCR reactions were performed
214 using AmpliTaq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA)
215 according to the manufacturer's protocol. The primers used for construction are listed
216 in Table S1.

217

218 **2.5. Phylogenetic analysis**

219 Deduced amino acid sequences of candidate ORs were aligned using the Clustal W
220 2.1. program (Thompson et al., 1994). Prior to this process, we merged the partial
221 sequences of *BdorOR2a*, *BdorOR7a-8*, *BdorOR24a*, *BdorOR45a*, and *BdorOR63a1-*
222 *v1*. We selected candidate ORs with sequences of more than 150 amino acid for

phylogenetic analysis, and constructed a phylogenetic tree from the aligned sequences. We applied the maximum likelihood method with the Jones–Taylor–Thornton (JTT) model with among-site rate heterogeneity according to gamma distribution with invariant sites (G + I) using MEGA5 software (Tamura et al., 2011). We performed 1000 bootstrap replicates.

2.6. Expression analyses of the candidate receptors by RT-PCR and quantitative RT-PCR (qPCR)

Total RNAs were prepared from various tissues of the staged adults within 2 days of eclosion, as described above. Reverse transcription was performed using the ReverTra Ace qPCR RT Master Mix (TOYOBO, Tsuruga, Japan). The generated cDNAs were subjected to PCR amplification with gene-specific primers using the GoTaq Green Master Mix (Promega, WI, USA). The PCR conditions were: 94°C for 1 min; and 35 or 40 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1 min, and 72°C for 2 min. Alternatively, the cDNA were used as a template for qPCR using the THUNDERBIRD SYBR qPCR Mix (TOYOBO, Tsuruga, Japan) on a Thermal Cycler Dice Real Time System (Takara, Shiga, Japan). We investigated five or six independent biological samples to quantify the levels of transcription. The transcription levels were normalized with *rpS3* transcription levels in the same samples. The primers used for RT-PCR and qPCR are listed in Table S2.

2.7. Chemicals

The chemicals used for the functional analysis of the BdorORs are listed in Table S3, and their structures are shown in Fig. S1. We synthesized 3-oxo-7,8-dihydro- α -ionone (P3) according to the method described in a previous paper (Enomoto et al., 2010).

We synthesized 4-propionyloxyisophorone (E0P) from 4-oxoisophorone (TCI, Tokyo, Japan) (Nishida and Tan, 2016). Briefly, the carbonyl function of 4-oxoisophorone at C-1 was protected by converting it into a ketal group using ethylene glycol. The carbonyl function at C-4 was then reduced to a hydroxyl moiety, and the ketal at C-1 was simultaneously deprotected using NaBH₄. The product was then propionylated into E0P using anhydrous propionic acid.

2.8. Receptor expression in *Xenopus* oocytes and two-electrode voltage-clamp recording

The preparation of *Xenopus laevis* oocytes, the microinjection of receptor gene RNAs, and the recording of whole-cell currents were performed as described previously with minor modifications (Mitsuno et al., 2008). In brief, complementary RNAs (cRNAs) were synthesized from linearized pCS2P+ vectors containing the full-length coding sequences of the ORs using a mMACHINE T7 Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA). Stage V to VII *Xenopus* oocytes treated with collagenase in Ca²⁺-free saline solution were microinjected with a mixture comprising *OR* and *BdorORCO* cRNAs (2.5 ng each). Using a two-electrode voltage clamp (OC-725, Warner, Hamden, CT, USA), we recorded whole cell currents from injected oocytes after incubation for 5–7 days at 20°C in an assay buffer comprising 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1.6 mM MgCl₂, 2.5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 2.5 mM 2-(*N*-morpholino)ethanesulfonic acid MES (pH 7.5). The inward current was monitored at a holding potential of –80 mV. Each ligand was diluted with the assay buffer to a specific concentration containing 0.1% dimethyl sulfoxide (DMSO). The assay buffer containing 0.1% DMSO was used as a negative control. Data acquisition and analyses

were carried out using Digidata 1322A and pCLAMP software (Axon Instruments, Foster City, CA, USA).

2.9. Behavioral bioassays

We evaluated the attraction of the flies to 1-octen-3-ol, geranyl acetate, farnesenes, and linalyl acetate, in combination with white or green sphere models as visual cues. Ethanol was used as a control. We used a total of four spheres (one green with a test compound and one green with ethanol; one white with a test compound and one white with ethanol) for attraction in each of the four groups of flies (Fig. S2A). Sexually mature adult flies (50 virgin females, 50 virgin males, 50 mated females, or 50 mated males) were transferred to a meshed cage (40 cm × 40 cm × 40 cm; placed in a sheltered outdoor bioassay area) in the morning 1 day prior to commencement of the experiment for acclimatization. On the day of the experiment (08:00–11:00), we impregnated pieces of Whatman® No. 1 filter paper (15 mm × 3 mm) with 1 mg of each of the test compounds dissolved in 5 µL of ethanol, and dried them at room temperature. Each filter paper was then placed in a clean 0.2-mL clear microtube (with the cap removed) (Labchem, Malaysia), which was positioned facing up in one of the holes of a polyethylene sphere that consisted of 26 holes (sphere diameter 40 mm; hole diameter 6 mm; Catalog No. GV0310, Tabata Co., Ltd., Tokyo, Japan). Each sphere was placed on a plastic petri dish (diameter 5 cm) to prevent rolling on the cage floor during the bioassay (Fig. S2B). Ethanol was used as a control. Each of the four spheres was then placed 10 cm from its respective corner in the meshed cage. The position of the spheres based on color and compound combination was re-randomized in each of the 4–6 replicates used, with different cohorts of flies tested

each time. Fruit flies landing on the spheres were counted and rapidly removed by aspiration during the 15-min bioassay.

2.10. Statistical analysis

Statistical analyses were conducted using R software (www.r-project.org). Dose responses were analyzed using the four-parameter log-logistic model of the *drc* extension package (Ritz et al., 2015). For the behavioral bioassay, we used a generalized linear model (GLM) with binomial distribution to determine whether the volatile compound or the color of the sphere significantly affected the number of fruit flies landing on the sphere. The most parsimonious model was identified using the Akaike information criterion (AIC). The likelihood ratio test (LRT) with chi-square distribution was used to determine the difference between the nested models.

3. Results

3.1. RNA sequencing and identification of chemosensory receptors

We obtained 1,675,116 and 2,159,685 raw reads from the transcriptomes of the male antennae and proboscises, respectively, using the Illumina MiSeq system (Table 1). After removing the low-quality, adaptor, and contaminating sequence reads, the male antennae and proboscises yielded 1,159,879 and 1,383,389 clean reads, respectively, which were assembled into 71,766 contigs (S1 text). We identified chemosensory receptors—namely, ORs, GRs, and IRs—by a BLASTX search of the contigs against amino acid sequences and Pfam domains of chemosensory receptors in *D. melanogaster* (Table 2).

321 A homology search based on the Pfam domains of the 7tm odorant receptor
322 (PF02949) and the amino acid sequences of the *Drosophila* ORs revealed 60
323 candidate ORs. The full-length coding sequences of 13 ORs were expected by *de*
324 *novo* assembly. We also used a BLASTN search to predict the full-length coding
325 sequences of three ORs—*BdorOR13a*, *BdorOR63a-2-v1*, and *BdorOR67c-v1*—and
326 determined their sequences by RT-PCR. Interestingly, we found multiple homologous
327 genes for several ORs including *BdorOR7a* and *BdorOR67d* (Table 2), whereas the
328 corresponding *Drosophila* ORs have only one gene. The divergence of the *BdorOR7a*
329 subfamily in the phylogenetic tree is remarkable (Fig. 1).

330 We identified four GRs that are homologous to sugar receptors such as the
331 *GR5a* and *GR64* subfamilies by a homology search based on the Pfam domains of
332 trehalose receptors (PF06151) (Freeman and Dahanukar, 2015). We identified another
333 13 GRs using the Pfam domains of 7tm chemosensory receptors (PF08395). We
334 identified two GRs as *BdorGR21a* and *BdorGR63a*, which are carbon dioxide
335 receptors and are highly conserved in insect species (Jones et al., 2007; Kwon et al.,
336 2007). We also found four homologous genes of *BdorGR21a*. Apart from the
337 trehalose and carbon dioxide receptors, we identified eight GRs from the Trinity
338 contigs, one of which was a homolog of the fructose receptor *GR43a* (Sato et al.,
339 2011).

340 We identified ligand-gated ion channels by a homology search based on the
341 Pfam domains of ligand-gated ion channels (PF00060) and *Drosophila* IRs. Of those,
342 we identified the candidate IRs by a BLASTP search based on translated protein
343 sequences. Among the IRs, we identified two—*BdorIR8a* and *BdorIR25a*—as
344 ionotropic co-receptors (Benton et al., 2009; Abuin et al., 2011). We also found a

homologous gene of *BdorIR8a*, and a pair of homologous genes in *BdorIR31a*,
BdorIR64a, *BdorIR75a*, *BdorIR76a*, *BdorIR92a*, and *BdorIR93a*.
Previous studies have identified candidate chemosensory receptors in *B.*
dorsalis (Wu et al., 2015; Liu et al., 2016). A comparison of those receptors with the
chemosensory receptors found in the present study indicated the novel ORs, GRs, and
IRs listed in Table 2. The coding sequences of the candidate chemosensory receptor
genes and their accession numbers are shown in the S2 text and Table S4,
respectively.

353

3.2. Expression profiles of the ORs

Sex-specific behaviors—including attraction and oviposition responses to
plant semiochemicals—have been observed in *B. dorsalis* (Howlett, 1912; Nishida et
al., 1988; Siderhurst and Jang, 2006a; Siderhurst and Jang, 2006b; Kamala Jayanthi et
al., 2012; Kamala Jayanthi et al., 2014; Damodaram et al., 2014). Therefore, we used
qPCR to compare the transcription levels of 15 ORs with known full-length coding
sequences in female and male antennae to determine if expression was sex-specific.
All the ORs tested were expressed in both sexes, although we did observe a
significant difference in the transcription level of *BdorOR35a* between the female and
male antennae (Fig. 2).

The expression of candidate chemosensory receptors in the male antenna and
proboscises was predicted by FPKM analysis (Table S5). Whereas most of the
transcripts coding ORs had various FPKM values in the antennae, some including
BdorORCO also had relatively low FPKM values in the proboscises. With regard to
the GRs, the transcripts coding carbon dioxide receptors—namely *BdorGR21a* and its
variants—had high FPKM values in the antennae. Some of the transcripts coding

GRs—including the sugar receptor subfamilies *BdorGR5a* and *BdorGR64*—only had high FPKM values in the proboscises, whereas the transcripts coding *BdorGR28b* and *BdorGR8a* had FPKM values in the antennae, but not in the proboscises. Most of the transcripts coding IRs had various FPKM values in the antennae, and some had FPKM values in the proboscises. In contrast, the transcripts coding *BdorIR56c* and *BdorIR93a-2* only had FPKM values in the proboscises.

We used RT-PCR to examine the transcription profile of *BdorORCO* in various tissues including olfactory and gustatory organs (Fig. 3A). *BdorORCO* was expressed primarily in the olfactory organs, antennae, and maxillary palps, although we observed marginal expression in the proboscises. We used qPCR to quantitatively compare the transcription levels of this gene in female and male antennae, proboscises, and tarsi (Fig. 3B). *BdorORCO* was highly expressed in both female and male antennae in accordance with its role as an obligatory co-receptor. In contrast, the transcription levels of *BdorORCO* were extremely low in both female and male proboscises (less than one thousandth of those in the antennae). The transcription levels of *BdorORCO* in both female and male tarsi were as low as one tenth those in the proboscises.

We also used RT-PCR to analyze the expression profiles of the selected ORs *BdorOR13a* and *BdorOR82a* (Fig. 3A). The PCR products of these genes were detected by 40 cycles of amplification, but not by 35 cycles, probably owing to the low transcription levels. We observed *BdorOR13a* expression in both female and male antennae, and in male maxillary palps and gustatory organs, i.e., the proboscises and foreleg tarsi. We observed *BdorOR82a* expression in both female and male antennae, and in male maxillary palps and female foreleg tarsi. We used qPCR to compare transcription levels in females and males to determine if *BdorOR13a* and

BdorOR82a were expressed in a sexually dimorphic pattern. There were extremely low *BdorOR13a* and *BdorOR82a* transcription levels in both the proboscises and foreleg tarsi (Fig. 3C). There were no significant differences in the transcription levels of these genes between female and male proboscises or foreleg tarsi ($p > 0.05$, Student's *t*-test).

3.3. Identification of ligands for *BdorOR13a* and *BdorOR82a* by two-electrode voltage-clamp recording

To identify the ligands for the ORs in *B. dorsalis*, we co-expressed each of the ten receptor proteins—*BdorOR7a-4*, *BdorOR7a-7*, *BdorOR13a*, *BdorOR35a*, *BdorOR43a-2-v1*, *BdorOR63a-2-v1*, *BdorOR67c-v1*, *BdorOR67d-1*, *BdorOR74a* and *BdorOR82a*— with the obligatory co-receptor *BdorORCO* in *Xenopus* oocytes. We tested 24 compounds including host plant chemicals, male attractants, and sex pheromones for *Bactrocera* species, as shown in Table S3 and Fig. S1. Of the ten receptor proteins tested, *BdorOR13a* responded to one compound and *BdorOR82a* responded to three compounds. The oocyte co-expressing *BdorOR13a* with *BdorORCO* responded significantly to 1-octen-3-ol at a concentration of 100 μ M (Fig. 4A-C). The current value induced by 1-octen-3-ol was significantly higher than that induced by the control (DMSO). The current induced by 1-octen-3-ol increased in a dose-dependent manner, and the EC_{50} value was 52.0 μ M (Fig. 4D, E). The oocyte co-expressing *BdorOR82a* and *BdorORCO* responded significantly to geranyl acetate, and responded weakly to farnesenes and linalyl acetate at a concentration of 100 μ M (Fig. 5A-C). Further experiments revealed significant differences between the current values of these compounds and that of the control (Fig. 5D). The current induced by

geranyl acetate increased in accordance with an increase in concentration, but we did not observe a plateau at the maximum concentration tested (Fig. 5E, F). We compared the amino acid sequences of the characterized ORs—BdorOR13a and BdorOR82a—with those of the *Drosophila* ORs because the properties of these ORs have been well characterized in *D. melanogaster*. BLASTP analysis indicated that the deduced amino acid sequences of BdorOR13a and BdorOR82a were similar to the sequences of DmOR13a (GenBank: AAF48549.2) and DmOR82a (GenBank: AAN13335.1), with 51% and 43% amino acid identities, respectively. Alignments of these ORs revealed that the amino acids within the transmembrane domains are well conserved (Fig. S3).

3.4 Behavioral bioassay

We examined the effect of volatiles characterized as ligands for BdorOR13a and BdorOR82a on the landing behavior of *B. dorsalis*. To determine whether there were behavioral differences between the sexes in terms of mating, we tested both females and males individually with four volatiles—1-octen-3-ol, geranyl acetate, farnesenes, and linalyl acetate—before and after mating. Because hardly any flies were attracted to the volatile-treated filter papers when used alone, we placed two sets of green and white spheres in the cage as visual cues, each containing microtubes with filter papers treated with or without the aforementioned volatiles (Fig. S2). We attempted to determine whether volatiles or color affected the number of flies landing on each sphere using the GLM model. When the fruit flies were exposed to 1-octen-3-ol—the ligand for BdorOR13a—the volatile factor but not the color factor alone significantly affected the numbers of mated females, and the numbers of both virgin and mated males landing on the spheres (Table 3). The number of mated females landing on the

spheres increased by the exposure to 1-octen-3-ol (Fig. 6A). Furthermore, we observed a few of the mated females probing the surface of the sphere with abdominal bending and aculeus extension, which are typical oviposition behaviors. However, the numbers of both virgin and mated males decreased by the exposure to 1-octen-3-ol (Fig. 6A). When the fruit flies were exposed to geranyl acetate or farnesenes—the ligands for BdorOR82a—the volatile factor but not the color factor alone significantly affected the behavior of the mated females, and more of them landed on the spheres emitting the volatiles (Table 3, Fig. 6A). In contrast, the number of mated males landing on the spheres decreased by the exposure to farnesenes. When the fruit flies were exposed to linalyl acetate, the volatile factor did not affect the number of fruit flies landing on the spheres, but color alone significantly increased the number of mated females (Table 3). Taken together, these results indicate that exposure to each of the volatiles—namely 1-octen-3-ol, geranyl acetate and farnesenes—significantly affected the landing behavior of the mated *B. dorsalis* females, whereas the males seemed to avoid the spheres that emitted 1-octen-3-ol or farnesenes. We confirmed that exposure to ethanol as a solvent did not affect the landing behavior of the mated females (Fig. S4A). Although the total number of mated females landing on the four spheres emitting 1-octen-3-ol, geranyl acetate, or farnesenes was significantly higher than in the other groups (Fig. 6B), there was no significant difference in the number of females landing on the spheres when the volatile-emitting and ethanol-emitting spheres (the control) were compared (Fig. S4B). This suggests that mated females frequently landed on spheres regardless of whether they were emitting a volatile.

4. Discussion

4.1. Repertoires of chemosensory receptor families in B. dorsalis

We identified multiple candidate chemosensory receptors in *B. dorsalis*—including novel ORs, GRs, and IRs not reported in previous studies—by transcriptome analysis. We found divergent homologs and variants in several ORs from *B. dorsalis*, suggesting that ancient genes have diverged by gene duplication in these OR families during adaptation to environmental odorants such as plant volatiles. The physiological roles of the highly divergent BdorOR7a family are of particular interest because *B. dorsalis* seems to require homologous ORs to detect specific odorants or sets of similar odorants. With regard to GRs, we identified two highly conserved carbon dioxide receptors, sugar receptors, and several other receptors. We identified 23 IRs including the homologs of *Drosophila* ionotropic co-receptors IR8a and IR25a. We found several IRs with two variants—e.g., BdorIR75a, BdorIR76a and BdorIR93a—suggesting that gene duplication has occurred in these IR families, as in the ORs. It is interesting to note that *Drosophila* OR67d and GR32a have been characterized as receptors for volatile and contact pheromones, respectively (Kohl et al., 2015). OR67d functions as a receptor for 11-*cis*-vaccenyl acetate (Ha, 2006; Kurtovic et al., 2007); this compound acts as an anti-aphrodisiac pheromone in males to avoid male–male courtship (Zawistowski and Richmond, 1986), but also acts as an aggregation signal in both sexes (Bartelt et al., 1985). The aggregation behavior of males—known as lek formation—has been observed in *B. dorsalis* and related species (Iwahashi and Majima, 1986; Tan and Nishida, 1996). Therefore, it would be intriguing if the divergent receptors (i.e., members of the BdorOR67d family) were involved in such social behavior.

4.2. Expression profiles of chemosensory receptors of *B. dorsalis*

493 In *Drosophila*, OR genes are expressed exclusively in olfactory organs (Vosshall et
494 al., 2000), and GR genes are mainly expressed in gustatory organs, with some
495 exceptions such as the expression of *GR21a*, *GR63a*, and *GR22e* in antennae (Scott et
496 al., 2001; Dunipace et al., 2001; Thorne and Amrein, 2008). In contrast, we acquired
497 sequences coding multiple ORs from reads derived from the proboscises, although the
498 FPKM values of these transcripts were low. Likewise, we found transcripts of several
499 GR genes in the sequencing reads from the antennae. Among these, *BdorGR8a* and
500 *BdorGR28b* had relatively high FPKM values in the antennae, but no FPKM values in
501 the proboscises. Therefore, differences in the expression profiles of several OR and
502 GR genes in the olfactory and gustatory organs are more obscure in *B. dorsalis* than in
503 *D. melanogaster*. Because the males of many *Bactrocera* species are strongly
504 attracted to specific compounds—e.g., *B. dorsalis* to ME—and subsequently feed
505 voraciously on the compounds, the perception of attractants probably involves both
506 olfactory and gustatory stimulation. A previous study demonstrated that ORCO is
507 required for the attraction of *B. dorsalis* to ME (Zheng et al., 2012), suggesting the
508 involvement of ORs in ME reception. It is also possible that IRs mediate the detection
509 of male attractants in *B. dorsalis*, because IRs function as both olfactory and gustatory
510 receptors (Rytz et al., 2013; Fleischer et al., 2017). In the present study, although we
511 found most transcripts of *BdorIRs* in sequencing reads from the antennae, we found
512 others in reads from both antennae and proboscises, or from proboscises only. The
513 question of whether ORs, GRs, IRs, or some combination of them is required for the
514 chemoreception of the attractants is very interesting.

515 Although sexually dimorphic behavior in response to semiochemicals has been
516 reported in *B. dorsalis* (Howlett, 1912; Nishida et al., 1988; Tan and Nishida, 2012),
517 in the present study we found no distinction between females and males in terms of

the transcription levels of almost all the ORs tested. Conversely, in lepidopteran species there is sex-specific expression of the chemosensory receptors required for sexually dimorphic behavior such as mating or oviposition in chemosensory organs. Since the characterization of a sex pheromone receptor from the silkworm *Bombyx mori* (Sakurai et al., 2004; Nakagawa et al., 2005), lepidopteran receptors that perceive female pheromones, all of which are specifically expressed in male antennae, have been identified in various genera including *Plutella*, *Mythimna*, *Diaphania*, *Antheraea*, and *Ostrinia* (Mitsuno et al., 2008; Forstner et al., 2009; Miura et al., 2009; Miura et al., 2010; Wanner et al., 2010). Other than sex pheromone receptors, the female-specific expression of lepidopteran gustatory receptor for the detection of an oviposition stimulant contained in host plant leaves has been reported in the swallowtail butterfly *Papilio xuthus* (Ozaki et al., 2011). These findings suggest that the sex-specific expression of chemosensory receptors is closely related to sexually dimorphic behavior in lepidopteran species. In *D. melanogaster*, however, chemosensory receptor genes are mostly expressed in both sexes, whereas the gustatory pheromone receptor GR68a is specifically expressed in male taste neurons in the foreleg tarsi (Bray and Amrein, 2003). Therefore, the chemosensory information involved in sexually dimorphic behavior triggered by semiochemicals is processed by the central nervous system, rather than the peripheral, in dipteran species including *B. dorsalis*.

4.3. Binding properties of ORs that respond to plant volatiles

In the present study, we characterized two ORs that respond to plant volatiles using *Xenopus* oocytes as a heterologous expression system. BdorOR13a responded strongly to 1-octen-3-ol, as reported in its homologs in *D. melanogaster* and the

543 mosquito species *Anopheles gambiae* and *Aedes aegypti* (Lu et al., 2007; Kreher et
544 al., 2008; Bohbot and Dickens, 2009). BdorOR82a responded to geranyl acetate, as
545 reported in its homologs in *D. melanogaster* and *A. gambiae* (Hallem et al., 2004;
546 Wang et al., 2010). BdorOR82a also responded significantly to farnesenes and linalyl
547 acetate, whereas a response to these compounds by the homologous OR in *D.*
548 *melanogaster* has not been reported, to the best of our knowledge. Whereas the
549 response of BdorOR13a to 1-octen-3-ol reached a plateau at an approximate
550 concentration of 100 μ M, the response of BdorOR82a to geranyl acetate failed to
551 reach a plateau up to 10 mM, the maximum concentration tested. Furthermore,
552 BdorOR82a responded weakly to farnesenes and linalyl acetate at 100 μ M. Whereas
553 1-octen-3-ol contains a hydroxyl group, geranyl acetate, the isomers of farnesenes and
554 linalyl acetate lack hydrophilic groups, suggesting that the latter three compounds are
555 relatively insoluble in buffer solutions owing to their hydrophobicity. The sparing
556 solubility of geranyl acetate in buffer solution may explain the weak response of
557 BdorOR82a, even at 1 mM. Generally, odorant-binding proteins allow hydrophobic
558 ligands to access the receptor neurons of insect chemosensilla, which are surrounded
559 by an aqueous lymphatic fluid (Fleischer et al., 2017). Therefore, geranyl acetate,
560 farnesenes, and linalyl acetate could effectively access BdorOR82a on receptor
561 neurons mediated by binding proteins *in vivo*. An alternative explanation for the weak
562 response is the low affinity of BdorOR82a for geranyl acetate. Because the amino
563 acid identity between BdorOR82a and DmOR82a is not necessarily high, differences
564 in amino acid residues may affect the sensitivity and specificity of the heteromeric
565 insect OR82a/ORCO complex.

566 BdorOR82a responded to the three hydrophobic compounds. Geranyl acetate
567 and linalyl acetate are both isomeric monoterpenes, but linalyl acetate is branched in a

different way. Farnesenes are sesquiterpene hydrocarbons that differ from geranyl acetate and linalyl acetate in both size and nature. Of the compounds that have been tested, the *D. melanogaster* DmOR82a homolog responds exclusively to geranyl acetate (Hallem et al., 2004; Hallem and Carlson, 2006), but it is unclear whether DmOR82a also responds to linalyl acetate and farnesenes. Although BdorOR82a has a low E-value ($4e-98$) against DmOR82a according to a BLASTP search, the amino acid sequences of BdorOR82a and DmOR82a have only 43% identity. Therefore, it would be interesting if DmOR82a responds to linalyl acetate and farnesenes as does BdorOR82a. A comparison of the binding affinities and amino acid sequences of BdorOR82a and DmOR82a would provide information about the specificities of these receptors.

4.4. Biological function of volatiles characterized as ligands for BdorORs

We found that three ligands for BdorORs—namely, 1-octen-3-ol, geranyl acetate and farnesenes—significantly affect the landing behavior of adult flies. The results suggest that adult flies respond to plant volatiles via the ORs characterized in the present study. However, it should be noted that at least one other OR may be involved in information processing at the peripheral and/or central nervous system level during the response to these volatiles.

Interestingly, the ligands had different effects depending on the sex and mating status of the flies. For example, 1-octen-3-ol increased the number of mated females landing on the spheres but reduced the numbers of virgin and mated males landing on the spheres. It should be noted that 1-octen-3-ol has been identified as an oviposition stimulant of *B. dorsalis* in studies on the volatile components of a host fruit (mango; *Mangifera indica*); gravid females laid more eggs on discs treated with

593 1-octen-3-ol in binary choice tests (Kamala Jayanthi et al., 2014). We also found that
594 a few of the mated females exhibited oviposition behavior on the surfaces of the
595 spheres emitting 1-octen-3-ol. Our findings suggest that in tephritids, mating causes a
596 switch from normal to oviposition-related behavior, as observed in *B. dorsalis*. A
597 study on female Mediterranean fruit flies (*Ceratitis capitata*) has also demonstrated
598 that mating confers a preferential switch; mated females choose host fruit odor over
599 male pheromones (Jang, 1995). The negative effects on the landing behavior of males,
600 regardless of mating experience, suggest that information processing after the
601 perception of volatiles differs between the sexes. It is worth noting that various
602 dipteran behavioral responses to 1-octen-3-ol have been reported, e.g., its ability to
603 attract *D. melanogaster* larvae (Kreher et al., 2008), and its ability to repel adult
604 females of the spot wing drosophila *D. suzukii* (Wallingford et al., 2016). *Anopheles*
605 *gambiae* and *Aedes aegypti* mosquitoes are attracted to 1-octen-3-ol in the breath of
606 animals (Kline, 1994). It would be interesting to know if OR13a homologs are
607 associated with these different behaviors in dipteran species, or if another receptor is
608 involved.

609 Geranyl acetate, farnesenes, and linalyl acetate, which are ligands for
610 BdorOR82a, have been detected in the tropical almond fruit *Terminalia catappa*, one
611 of the hosts of *B. dorsalis* (Siderhurst and Jang, 2006b); geranyl acetate and methyl
612 eugenol elicited the largest electroantennogram detection (EAD) responses from the
613 antennae of *B. dorsalis* among the volatiles collected from *T. catappa* (Siderhurst and
614 Jang, 2006b). Linalyl acetate and the isomers of farnesenes also elicited EAD
615 responses to some extent (Siderhurst and Jang, 2006b). In accordance with the EAD
616 responses, exposure to geranyl acetate or farnesenes had a significant effect on the
617 landing behavior of *B. dorsalis* in our experiments, and we observed different

618 responses to 1-octen-3-ol with regard to gender and mating experience. These results
619 suggest that the electrophysiological responses to geranyl acetate and farnesenes are
620 linked to their effects on the landing behavior of fruit flies.

621 Geranyl acetate also seems to be important for *D. melanogaster*, because
622 DmOR82a responds to its analog geranyl acetone, probably as a signal for fruit
623 ripening (Mansourian and Stensmyr, 2015). It would be interesting to see if OR82a
624 homologs, which respond to geranyl acetate and its analogs, commonly mediate
625 semiochemical information released from host fruits in fruit flies of the families
626 Drosophilidae and Tephritidae. Recently, ORs that respond to geranyl acetate and an
627 isomer of farnesene have been identified in aphid and moth species (Liu et al., 2014;
628 Zhang et al., 2017), although these receptors do not belong to the OR82a family.
629 ApisOR5 from the aphid *Acyrtosiphon pisum* responds to the alarm pheromone (*E*)-
630 β -farnesene and the repellent geranyl acetate, even though there is no homology
631 between ApisOR5 and OR82a at the amino acid level. Instead, ApisOR5 most closely
632 resembles OR85f, with an E-value of 1.6e-4 according to a BLASTP search against
633 *Drosophila* ORs. Similarly, SexiOR3, which has been identified in the beet
634 armyworm moth *Spodoptera exigua*, responds to (*E*)- β -farnesene, but most closely
635 resembles OR13a, with an E-value of 7.9e-6 according to a BLASTP search against
636 *Drosophila* ORs. The relationships between the binding properties and structures of
637 these ORs, which share common ligands, are intriguing.

638 To the best of our knowledge, the present study was the first attempt to functionally
639 characterize the ORs of tephritid fruit flies using *Xenopus* oocytes as a heterologous
640 expression system. The results will enable us to further characterize the orphan
641 receptors of Tephritidae. Furthermore, the identification of ligands for chemosensory
642 receptors will provide information about the important chemicals that affect the life

643 cycles of fruit flies. Despite the characterization of the BdorORs, we have not
644 provided direct evidence to link the properties of ORs with behavior as an output of
645 signal processing mediated by these receptors. Insect genome engineering using the
646 CRISPR/Cas9 system is now available for *Bactrocera* species (Choo et al., 2017), and
647 the loss of the function of specific chemosensory receptors of interests will clarify
648 their roles *in vivo*. This will lead to the elucidation of the mechanisms underlying the
649 chemoreception of various semiochemicals, including plant volatiles, male-specific
650 attractants, and sex pheromones.

651

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Figure captions

Fig. 1. Phylogenetic tree of candidate olfactory receptors (ORs) identified in *Bactrocera dorsalis*. Branch length is proportional to genetic distance estimated by the maximum likelihood method. The values shown at the nodes of the branches are bootstrap values (%) from 1000 replicate samplings. The numbers in parentheses indicate the numbers of amino acids in the ORs. “F” or “P” in parentheses indicate full or partial determination of the coding sequence of an OR, respectively. ORs expressed in *Xenopus* oocytes are indicated in bold.

Fig. 2. Transcription levels of olfactory receptors (ORs) identified in *Bactrocera dorsalis*. FA: female antennae; MA: male antennae. Each value is plotted as a dot ($n = 6$). The box plot shows 25–75% (box), median (band inside), and minima to maxima (whiskers). Student’s t -test: $*p < 0.05$.

Fig. 3. Expression patterns of olfactory receptors (ORs) identified in *Bactrocera dorsalis*. (A) Expression of ORs in various tissues detected by RT-PCR. PCR amplifications were performed in 35 cycles for *BdorORCO* and *rpS3*, and 40 cycles for *BdorOR13a* and *BdorOR82a*. The lanes are as follows: AT: antennae; MP: maxillary palps; PB: proboscises; FT: foreleg tarsi; ML: midlegs; HL: hindlegs. (B) Transcription levels of *BdorORCO* in chemosensory organs. (C) Transcription levels of *BdorOR13a* and *BdorOR82a* in gustatory organs. (B, C) FA: female antennae; MA: male antennae; FP: female proboscises; MP: male proboscises; FT: female tarsi; MT: male tarsi. Each value is plotted as a dot ($n = 5-6$). The box plot shows 25–75% (box), median (band inside), and minima to maxima (whiskers).

683

684 Fig. 4. Responses of *Xenopus* oocytes expressing BdorOR13a with BdorORCO to
685 various compounds. (A) Current trace of an oocyte upon successive exposures to 25
686 samples including DMSO (the control). Each chemical was applied at the time
687 indicated by the arrowhead. (B) Currents measured in the oocytes. The structure of
688 each compound and its corresponding abbreviation is shown in Fig. S1. The number
689 in parentheses after each compound corresponds to the number on the arrowhead in
690 (A). Error bars indicate SE ($n = 3$). Student's t -test: $*p < 0.05$. (C) Structure of a
691 ligand for BdorOR13a. (D) Responses of an oocyte expressing BdorOR13a with
692 BdorORCO to 1-octen-3-ol at various concentrations. (E) Dose–response curve of
693 oocytes responding to 1-octen-3-ol. Each point represents the mean current value.
694 Error bars indicate SE ($n = 9–11$).

695

696 Fig. 5. Responses of *Xenopus* oocytes expressing BdorOR82a with BdorORCO to
697 various compounds. (A) Current trace of an oocyte upon successive exposures to 25
698 samples including DMSO (the control). Each chemical was applied at the time
699 indicated by the arrowhead. (B) Currents measured in the oocytes. The structure of
700 each compound and its corresponding abbreviation is shown in Fig. S1. The number
701 in parentheses after each compound corresponds to those on the arrowheads in (A).
702 Error bars indicate SE ($n = 3$). Student's t -test: $*p < 0.05$. (C) Structures of ligands for
703 BdorOR82a. The structure of one of the farnesene isomers is shown. (D) Currents
704 measured in oocytes responding to L-OAc (linalyl acetate), FRN-mix (a mixture of
705 farnesene isomers), and G-OAc (geranyl acetate) at 100 μ M. Each value is plotted as
706 a dot ($n = 9–10$). The box plot shows 25–75% (box), median (band inside), and
707 minima to maxima (whiskers). Student's t -test: $*p < 0.05$, $**p < 0.01$. (E) Responses

of an oocyte expressing BdorOR82a with BdorORCO to geranyl acetate at various concentrations. (F) Dose–response curve of oocytes responding to geranyl acetate. Each point represents the mean current value. Error bars indicate SE ($n = 8–9$).

Fig. 6. Effects of volatiles and visual cues on landing behaviors of *Bactrocera dorsalis*. The box plot shows 25–75% (box), median (band inside), and minima to maxima (whiskers). Virgin-F, Mated-F, Virgin-M, and Mated-M indicate virgin females, mated females, virgin males, and mated males, respectively. (A) Numbers of virgin or mated females or males landing on green or white spheres. The numbers of flies are plotted as dots ($n = 5–6$). Significant effects of volatiles or colors indicated in Table 3 are shown in the categories of fruit flies as V or C with p -values (< 0.05), respectively. T-G, T-W, C-G, and C-W indicate volatile-treated green balls, volatile-treated white balls, control (volatile-untreated) green balls, and control white balls, respectively. (B) Total numbers of fruit flies landing on the spheres calculated from (A). Boxes with letters are significantly different at $p < 0.05$ according to Tukey's HSD test.

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Fig. 1

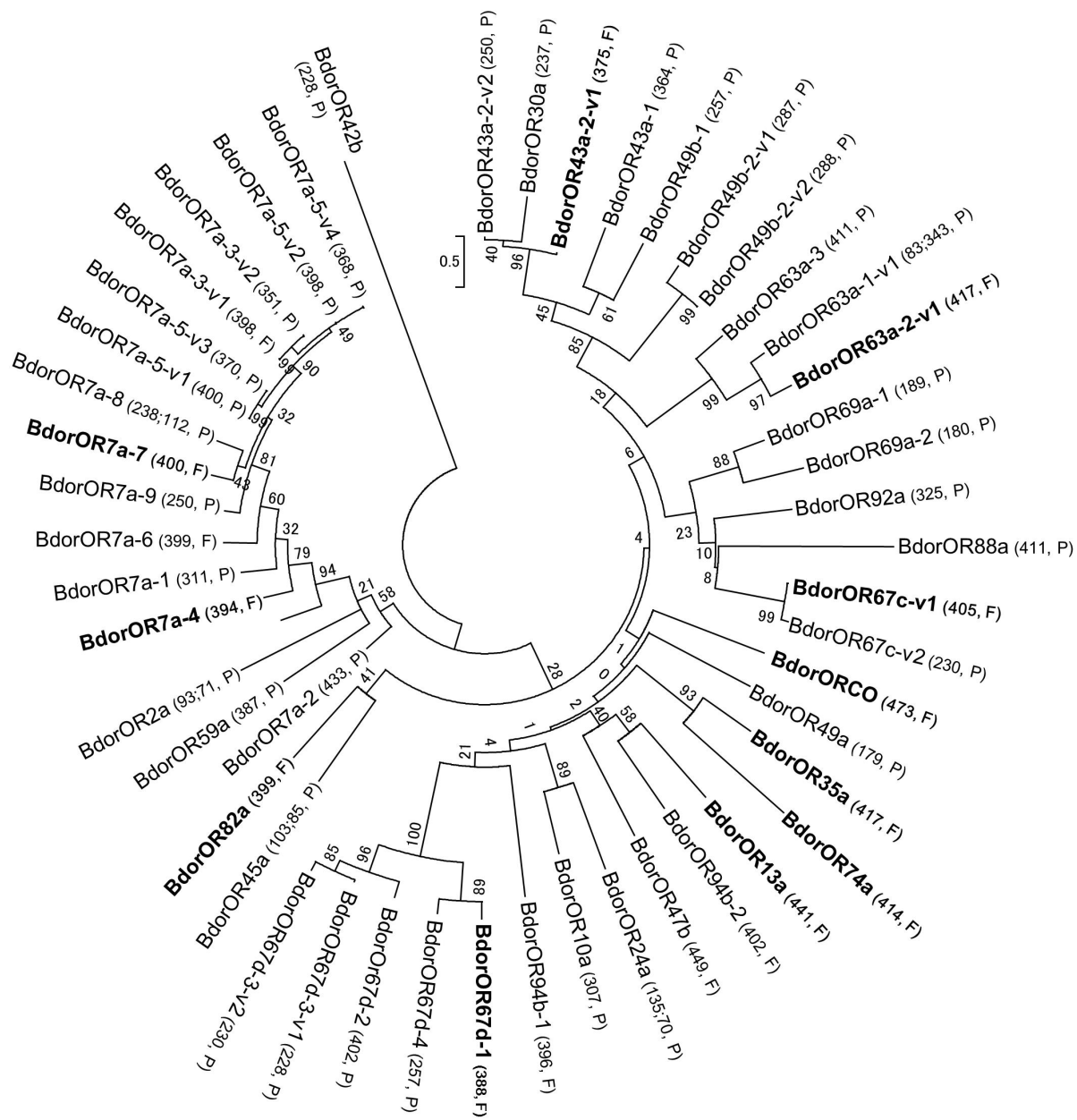


Fig. 2

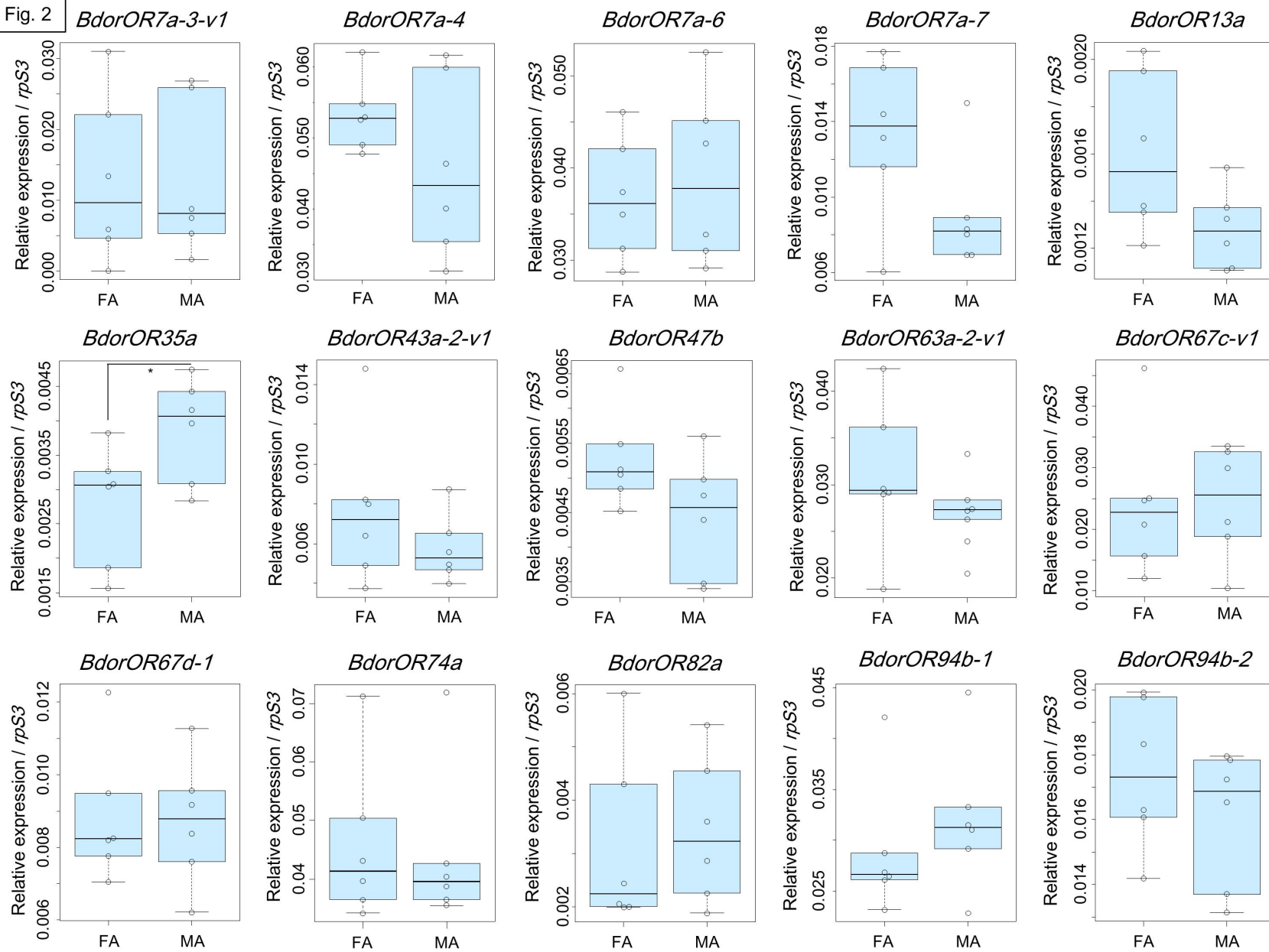
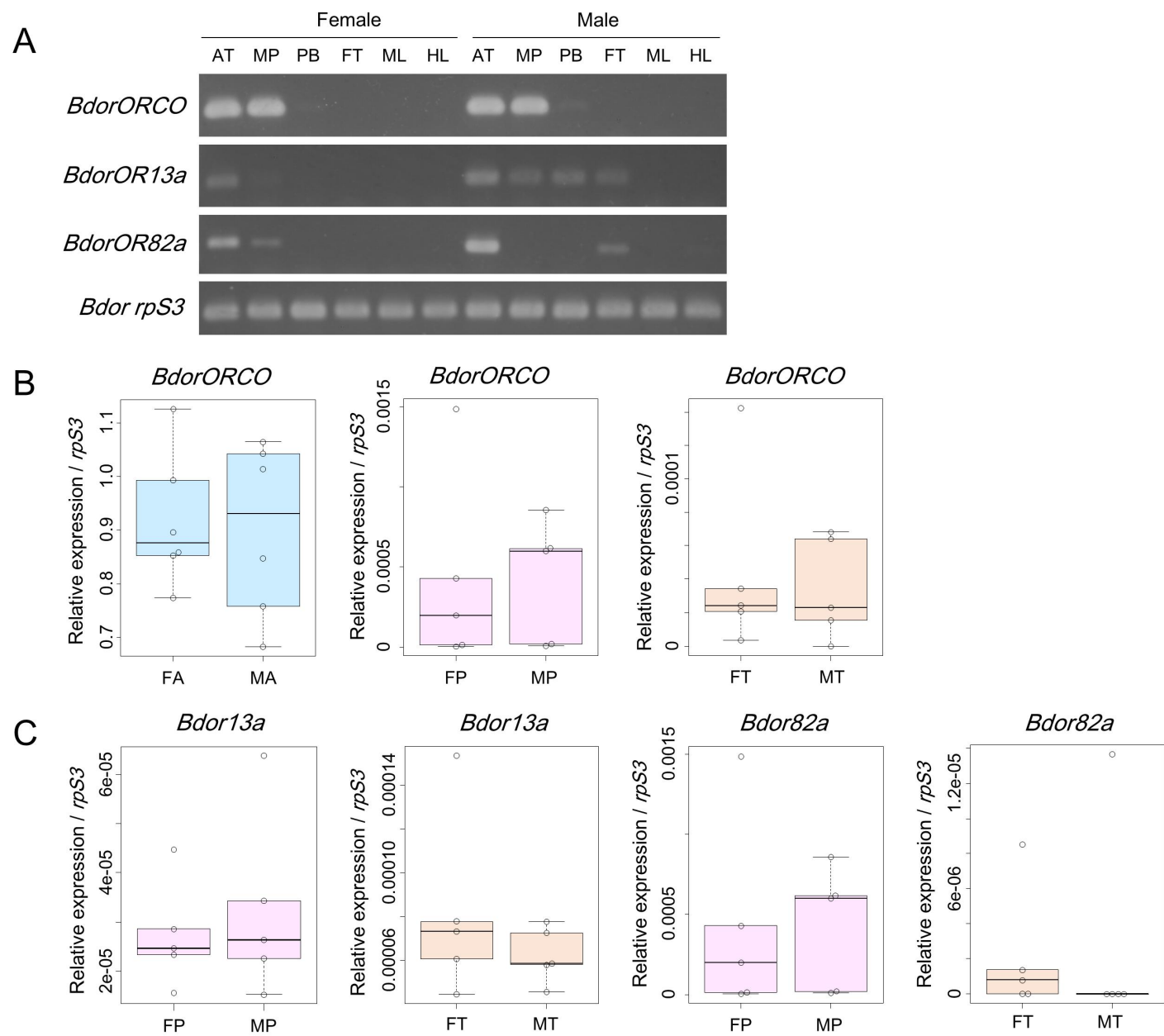
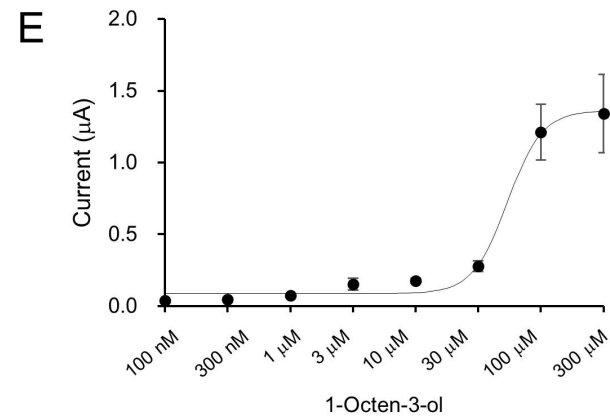
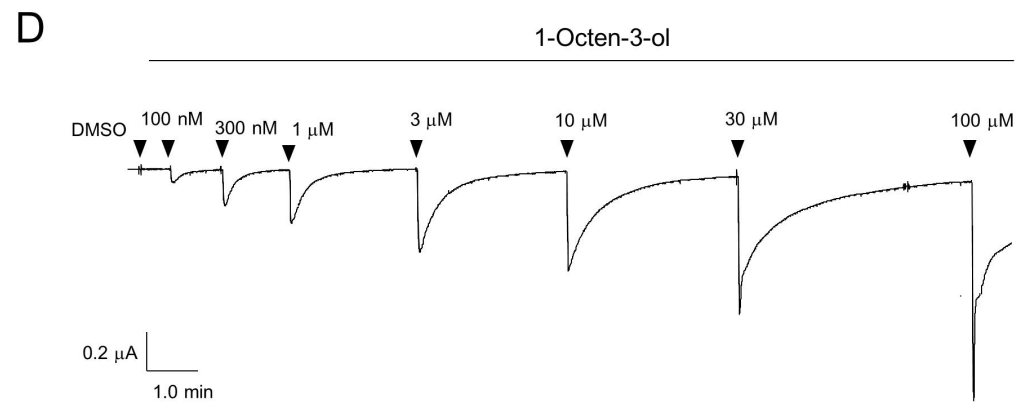
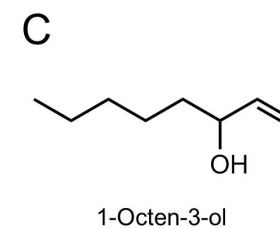
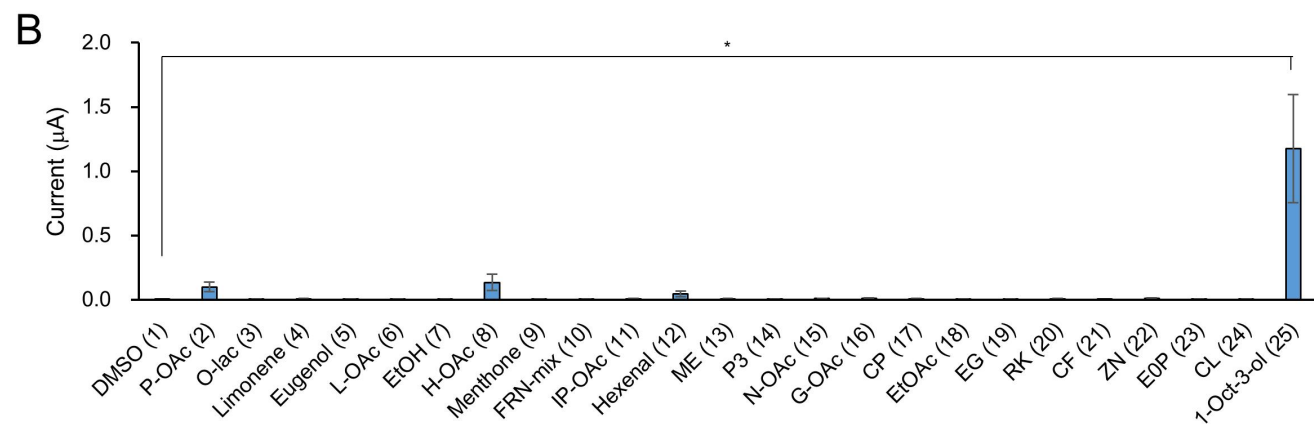
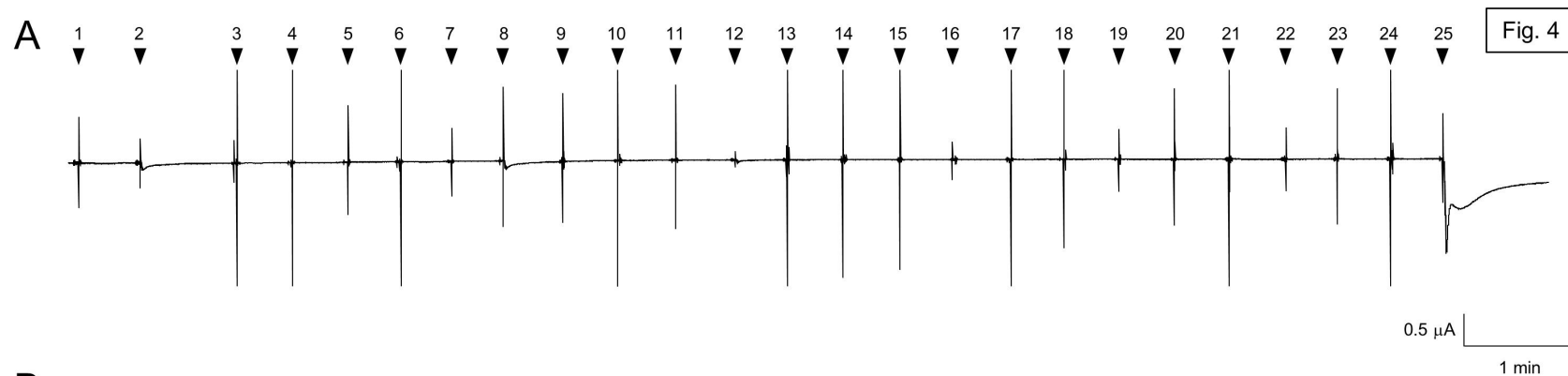


Fig. 3





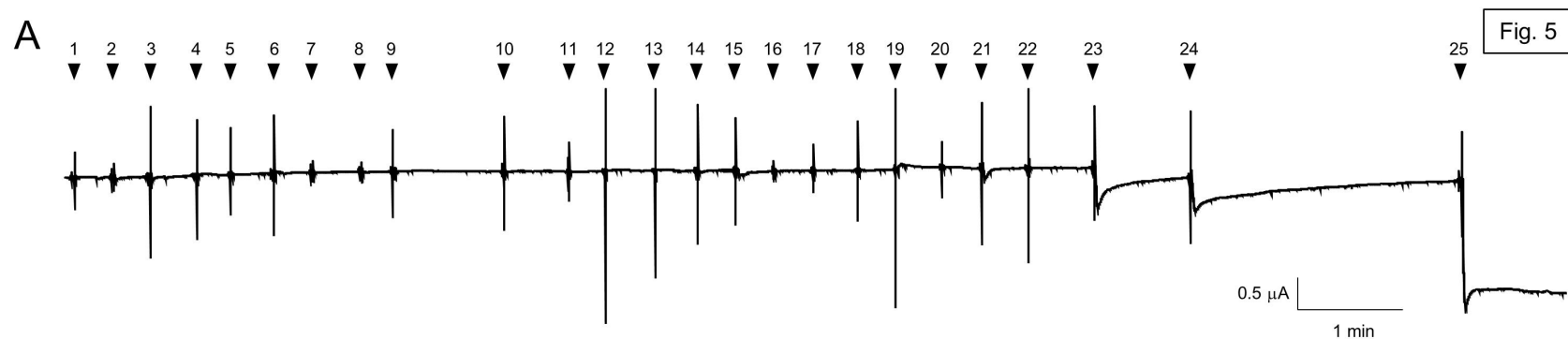
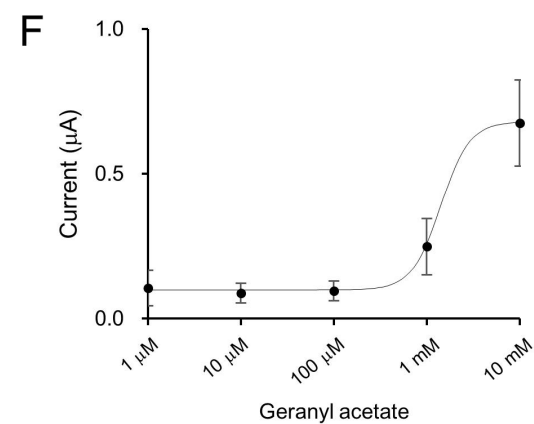
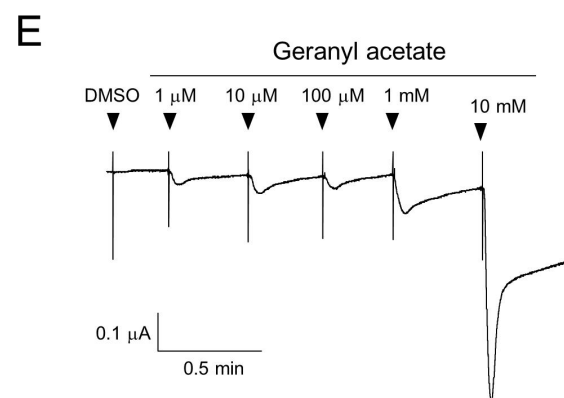
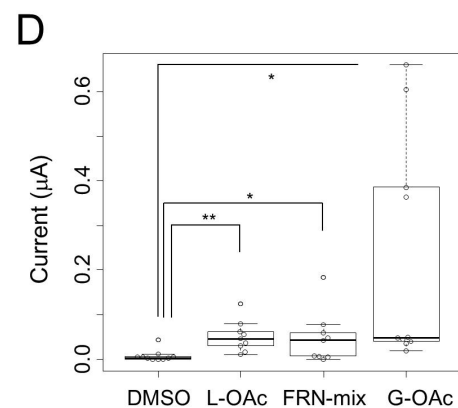
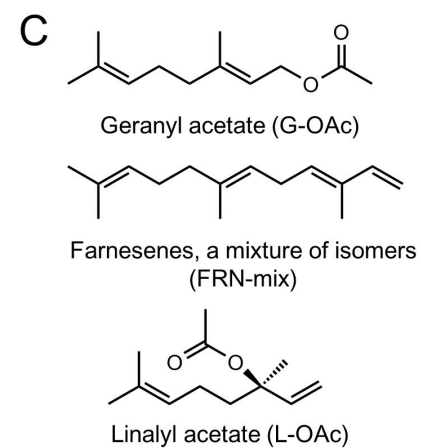
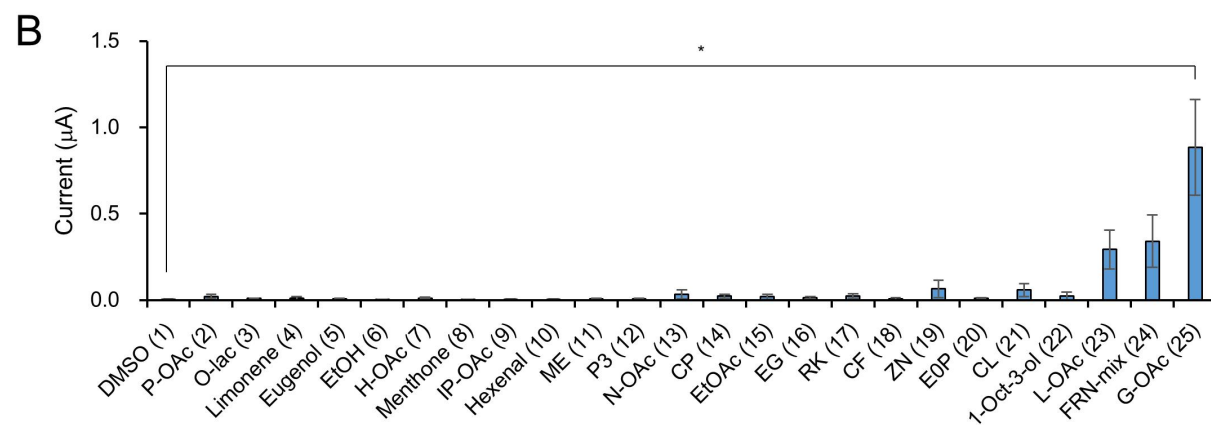


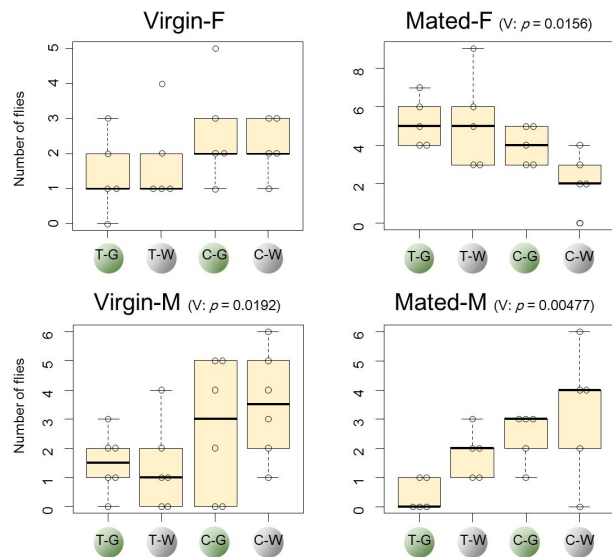
Fig. 5



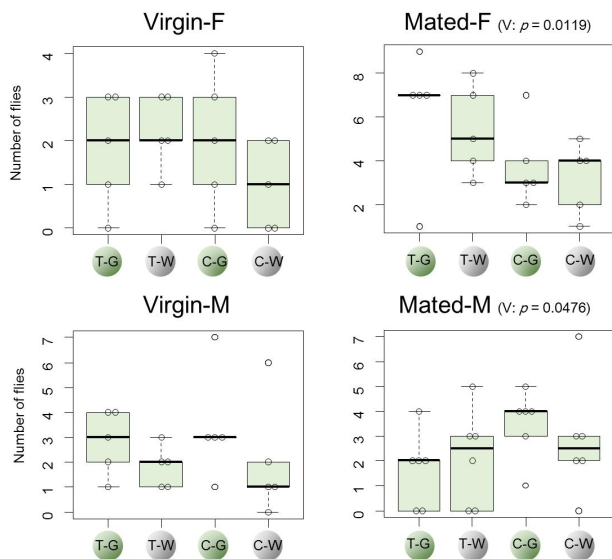
A

Fig. 6

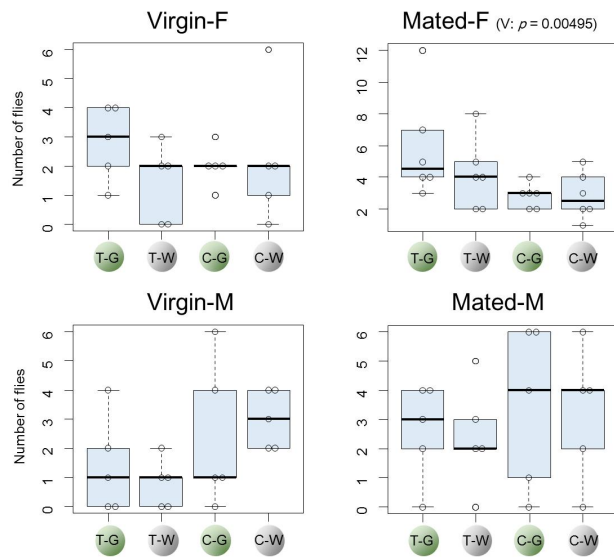
1-Octen-3-ol



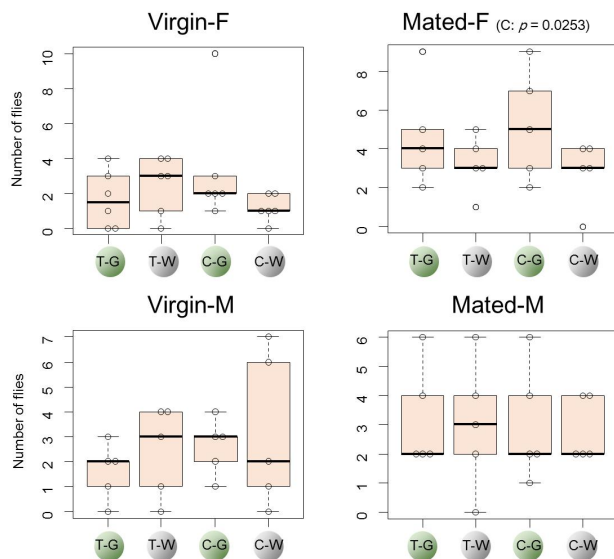
Farnesenes



Geranyl acetate

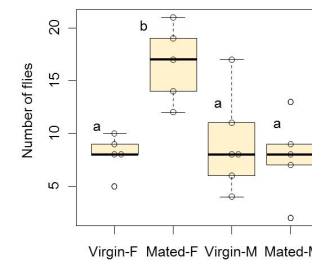


Linalyl acetate

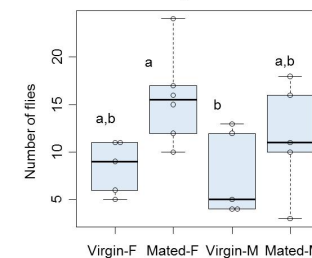


B

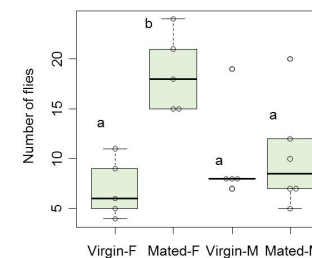
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Geranyl acetate



Farnesenes



Linalyl acetate

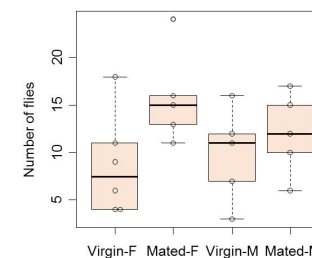


Table 1. Summary of sequence data analysis.

	Antenna	Proboscis
Number of raw reads	1,675,116	2,159,685
Number of clean reads	1,159,879	1,383,389
Number of assembled contigs	71,766	
Mean length of contigs (bp)	486	

Table 2. Candidate chemosensory receptor genes identified from the transcriptome.

Gene name	Length (AA)	CDS	E-value	BLASTP best hit (Accession number; Name; Species)	Reference ¹ (Accession number or reference name)
ORs					
<i>BdorORCO</i>	473	Full	0	ADK97803.1; Or83b (ORCO) <i>Zeugodacus cucurbitae</i>	Ref. 1: KP743711; Ref. 2: CL538C2
<i>BdorOR2a</i>	93; 71	Partial	2e-61; 2e-41	XP_011198390.1; OR2a-like; <i>Bactrocera dorsalis</i>	
<i>BdorOR7a-1</i>	311	Partial	0	XP_019847175.1; OR7a-like; <i>Bactrocera dorsalis</i>	Ref. 1: KP743712; Ref. 2: U14846
<i>BdorOR7a-2</i>	433	Partial	0	XP_019847361.1; OR7a-like; <i>Bactrocera dorsalis</i>	Ref. 1: KP743713; Ref. 2: U16745
<i>BdorOR7a-3-v1</i>	398	Full	0	AKI29030.1; OR7a-3; <i>Bactrocera dorsalis</i>	Ref. 1: KP743714
<i>BdorOR7a-3-v2</i>	351	Partial	0	AKI29030.1; OR7a-3; <i>Bactrocera dorsalis</i>	
<i>BdorOR7a-4</i>	394	Full	0	XP_011198720.1; OR7a-like; <i>Bactrocera dorsalis</i>	Ref. 1: KP743715; Ref. 2: U15921
<i>BdorOR7a-5-v1</i>	400	Partial	0	XP_019845111.1; OR7a-like; <i>Bactrocera dorsalis</i>	Ref. 1: KP743716; Ref. 2: C2326C1
<i>BdorOR7a-5-v2</i>	398	Partial	0	XP_019845111.1; OR7a-like; <i>Bactrocera dorsalis</i>	
<i>BdorOR7a-5-v3</i>	370	Partial	0	XP_019845111.1; OR7a-like; <i>Bactrocera dorsalis</i>	
<i>BdorOR7a-5-v4</i>	368	Partial	0	XP_019845111.1; OR7a-like; <i>Bactrocera dorsalis</i>	
<i>BdorOR7a-6</i>	399	Full	0	XP_011208901.1; OR59b-like; <i>Bactrocera dorsalis</i>	Ref. 2: C1686C3
<i>BdorOR7a-7</i>	400	Full	0	XP_019846037.1; OR7a-like; <i>Bactrocera dorsalis</i>	Ref. 1: KP743721; Ref.2: C3971C1
<i>BdorOR7a-8</i>	238; 112	Partial	2e-156; 5e-69	XP_018798519.1; OR59b-like; <i>Bactrocera latifrons</i>	Ref. 2: C788C3
<i>BdorOR7a-9</i>	250	Partial	3e-106	XP_019845111.1; OR7a-like; <i>Bactrocera dorsalis</i>	Ref. 2: C2460C2
<i>BdorOR7a-10</i>	188	Partial	4e-130	AKI29029.1; OR7a-2; <i>Bactrocera cucurbitae</i>	Ref. 2: U4218
<i>BdorOR7a-11</i>	90	Partial	2e-56	XP_019846037.1; OR7a-like; <i>Bactrocera dorsalis</i>	
<i>BdorOR10a</i>	307	Partial	0	XP_018791769.1; OR10a; <i>Bactrocera latifrons</i>	Ref. 2: C4154C1
<i>BdorOR13a</i>	441	Full	0	AKI29033.1; OR13a; <i>Bactrocera dorsalis</i>	Ref. 1: KP743717; Ref. 2: C739C1
<i>BdorOR24a</i>	135; 70	Partial	9e-91; 4e-40	XP_011199522.1; OR24a; <i>Bactrocera dorsalis</i>	
<i>BdorOR30a</i>	237	Partial	5e-128	XP_019847596.1; OR30a-like; <i>Bactrocera dorsalis</i>	
<i>BdorOR35a</i>	417	Full	0	XP_019844437.1; OR35a-like; <i>Bactrocera dorsalis</i>	Ref. 1: KP743718; Ref. 2: U10148
<i>BdorOR42b</i>	228	Partial	4e-151	XP_011210512.1; OR7a-like; <i>Bactrocera dorsalis</i>	Ref. 2: U4218
<i>BdorOR43a-1</i>	364	Partial	0	AKI29035.1; OR43a-1; <i>Bactrocera dorsalis</i>	Ref. 1: KP743719

<i>BdorOR43a-2-v1</i>	375	Full	0	AKI29036.1; OR43a-2; <i>Bactrocera dorsalis</i>	Ref. 1: KP743720
<i>BdorOR43a-2-v2</i>	250	Partial	4e-179	XP_019847608.1; Or2-like; <i>Bactrocera dorsalis</i>	Ref. 2: C3544C1
<i>BdorOR43a-3</i>	72	Partial	1e-37	XP_014097484.1; Or2-like; <i>Bactrocera oleae</i>	
<i>BdorOR45a</i>	103; 85	Partial	8e-66; 6e-54	XP_011212447.2; OR45a-like; <i>Bactrocera dorsalis</i>	
<i>BdorOR47b</i>	449	Full	0	XP_019847427.1; OR47b; <i>Bactrocera dorsalis</i>	
<i>BdorOR49a</i>	179	Partial	1e-126	XP_011212431.1; OR49a-like; <i>Bactrocera dorsalis</i>	Ref. 2: U11993
<i>BdorOR49b-1</i>	257	Partial	0	XP_019845516.1; OR49b; <i>Bactrocera dorsalis</i>	Ref. 1: KP743723; Ref. 2: C6087C2
<i>BdorOR49b-2-v1</i>	287	Partial	2e-170	XP_019847679.1; OR49b-like; <i>Bactrocera dorsalis</i>	
<i>BdorOR49b-2-v2</i>	288	Partial	0	XP_019847679.1; OR49b-like; <i>Bactrocera dorsalis</i>	Ref. 1: KP743724
<i>BdorOR49b-3</i>	105	Partial	2e-67	AKI29039.1; OR49b-1; <i>Bactrocera dorsalis</i>	
<i>BdorOR49b-4</i>	83	Partial	4e-50	XP_019847607.1; OR2-like; <i>Bactrocera dorsalis</i>	
<i>BdorOR59a</i>	387	Partial	0	AKI29041.1; OR59a; <i>Bactrocera dorsalis</i>	Ref. 1: KP743725; Ref. 2: U350
<i>BdorOR63a-1-v1</i>	83; 343	Partial	1e-49; 0	AKI29042.1; OR63a-1; <i>Bactrocera dorsalis</i>	Ref. 1: KP743726
<i>BdorOR63a-1-v2</i>	52	Partial	2e-21	AKI29042.1; OR63a-1; <i>Bactrocera dorsalis</i>	
<i>BdorOR63a-1-v3</i>	82	Partial	6e-48	XP_018787905.1; OR63a-like; <i>Bactrocera latifrons</i>	
<i>BdorOR63a-2-v1</i>	417	Full	0	AKI29043.1; OR63a-2; <i>Bactrocera dorsalis</i>	Ref. 1: KP743727; Ref. 2: U11167
<i>BdorOR63a-2-v2</i>	139	Partial	4e-70	XP_019847162.1; OR63a-like; <i>Bactrocera dorsalis</i>	
<i>BdorOR63a-3</i>	411	Partial	0	XP_018783180.1; OR63a; <i>Bactrocera latifrons</i>	Ref. 2: U1859
<i>BdorOR67c-v1</i>	405	Full	0	XP_011200400.1; OR67c-like; <i>Bactrocera dorsalis</i>	Ref. 1: KP743728; Ref. 2: C173C1
<i>BdorOR67c-v2</i>	230	Partial	2E-152	XP_011200401.1; OR67c-like; <i>Bactrocera dorsalis</i>	
<i>BdorOR67d-1</i>	388	Full	0	XP_011203703.1; OR67d-like; <i>Bactrocera dorsalis</i>	Ref. 1: KP743729; Ref. 2: C8295C1
<i>BdorOR67d-2</i>	402	Partial	4e-178	XP_017473047.1; OR67d-like; <i>Rhagoletis zephyria</i>	
<i>BdorOR67d-3-v1</i>	228	Partial	2e-133	XP_017473047.1; OR67d-like; <i>Rhagoletis zephyria</i>	Ref. 2: U33
<i>BdorOR67d-3-v2</i>	230	Partial	6e-138	XP_017473047.1; OR67d-like; <i>Rhagoletis zephyria</i>	
<i>BdorOR67d-4</i>	257	Partial	0	XP_011203704.2; OR67d-like; <i>Bactrocera dorsalis</i>	Ref. 2: U3061
<i>BdorOR69a-1</i>	189	Partial	3e-133	AKI29046.1; OR69a; <i>Bactrocera dorsalis</i>	Ref. 1: KP743730
<i>BdorOR69a-2</i>	180	Partial	3e-103	XP_011191113.1; OR69a isoformA; <i>Bactrocera cucurbitae</i>	Ref. 2: U12022
<i>BdorOR69a-3</i>	69	Partial	5e-42	XP_011209369.1; putative OR69a; <i>Bactrocera dorsalis</i>	
<i>BdorOR74a</i>	414	Full	0	XP_011201924.2; OR74a-like; <i>Bactrocera dorsalis</i>	Ref. 1: KP743731

<i>BdorOR82a</i>	399	Full	0	XP_011208732.1; OR82a; <i>Bactrocera dorsalis</i>	Ref. 2: U803
<i>BdorOR83a</i>	47	Partial	3e-16	XP_011184142.1; OR83a-like; <i>Zeugodacus cucurbitae</i>	
<i>BdorOR85d</i>	85	Partial	3e-37	XP_018801738.1; OR85d; <i>Bactrocera latifrons</i>	
<i>BdorOR88a</i>	411	Partial	0	AKI29048.1; OR88a; <i>Bactrocera dorsalis</i>	Ref. 1: KP743732; Ref. 2: 5300C1
<i>BdorOR92a</i>	325	Partial	0	XP_011208819.1; OR92a; <i>Bactrocera dorsalis</i>	
<i>BdorOR94b-1</i>	396	Full	0	XP_019847876.1; OR94b-like; <i>Bactrocera dorsalis</i>	Ref. 1: KP743733; Ref. 2: U3077
<i>BdorOR94b-2</i>	402	Full	0	XP_018801531.1; OR94b-like; <i>Bactrocera latifrons</i>	Ref. 2: U3948
GRs					
<i>BdorGR5a</i>	79; 100	Partial	3e-47; 2e-63	XP_011213356.2; GR5a; <i>Bactrocera dorsalis</i>	
<i>BdorGR8a</i>	75; 43	Partial	8e-41; 0.014	XP_011185249.1; GR8a-like; <i>Zeugodacus cucurbitae</i>	
<i>BdorGR21a-1</i>	456	Full	0	XP_011204023.1; GR21a; <i>Bactrocera dorsalis</i>	Ref. 1: KP743664; Ref. 2: U13527
<i>BdorGR21a-2-v1</i>	432	Partial	0	AOE48126.1; GR6; <i>Scaeva pyrastris</i>	
<i>BdorGR21a-2-v2</i>	424	Partial	0	AOE48126.1; GR6; <i>Scaeva pyrastris</i>	
<i>BdorGR21a-2-v3</i>	339	Partial	7e-136	AOE48126.1; GR6; <i>Scaeva pyrastris</i>	
<i>BdorGR21a-2-v4</i>	184	Partial	2e-62	AOE48126.1; GR6; <i>Scaeva pyrastris</i>	
<i>BdorGR28b</i>	110	Partial	8e-69	XP_011180327.1; putative GR28b; <i>Zeugodacus cucurbitae</i>	
<i>BdorGR32a-1</i>	353	Partial	0	XP_019847005.1; GR32a; <i>Bactrocera dorsalis</i>	Ref. 1: KP743666
<i>BdorGR32a-2</i>	76	Partial	1e-40	XP_018792133.1; uncharacterized protein LOC108970891; <i>Bactrocera latifrons</i>	
<i>BdorGR39b</i>	106	Partial	3e-46	XP_004536482.1; GR39b; <i>Ceratitis capitata</i>	
<i>BdorGR43a</i>	67; 75	Partial	6e-38; 3e-43	XP_019845196.1; GR43a-like; <i>Bactrocera dorsalis</i>	
<i>BdorGR63a</i>	485	Full	0	XP_011212836.1; GR63a; <i>Bactrocera dorsalis</i>	Ref. 1: KP743667
<i>BdorGR64b</i>	67	Partial	3e-39	XP_011213352.1; GR64b; <i>Bactrocera dorsalis</i>	
<i>BdorGR64e</i>	59; 71; 62	Partial	2e-32; 3e-42; 3e-26	XP_011213347.1; GR64e; <i>Bactrocera dorsalis</i>	
<i>BdorGR64f</i>	278	Partial	5e-147	XP_018783853.1; uncharacterized protein LOC108965721; <i>Bactrocera latifrons</i>	
<i>BdorGR98b</i>	69	Partial	7e-42	XP_011205406.1; putative GR98b; <i>Bactrocera dorsalis</i>	
IRs					
<i>BdorIR8a</i>	104; 673	Partial	1e-54; 0	XP_011211753.1; glutamate receptor ionotropic, kainate 2; <i>Bactrocera dorsalis</i>	Ref. 2: C8433C2
<i>BdorIR8a-2</i>	153	Partial	5e-100	XP_014100759.1; glutamate receptor ionotropic, kainate 2-like; <i>Bactrocera oleae</i>	

<i>BdorIR25a</i>	940	Full	0	XP_011207795.1; IR25a; <i>Bactrocera dorsalis</i>	Ref. 1: U215
<i>BdorIR31a-1</i>	108	Partial	1e-54	XP_018804290.1; uncharacterized protein LOC108978446; <i>Bactrocera latifrons</i>	
<i>BdorIR31a-2</i>	83	Partial	1e-27	XP_012162538.1; LOC101456253; <i>Ceratitis capitata</i>	
<i>BdorIR40a</i>	128; 265; 83	Partial	6e-85; 0; 1e-47	XP_011212457.2; uncharacterized protein LOC105232474; <i>Bactrocera dorsalis</i>	Ref. 1: KP743669; Ref. 2: U9427
<i>BdorIR41a</i>	135; 87; 213	Partial	5e-91; 1e-48; 8e-147	AKI28986.1; IR41a; <i>Bactrocera dorsalis</i>	Ref. 1: KP743670
<i>BdorIR56c</i>	118; 216	Partial	1e-12; 4e-132	XP_018794909.1; uncharacterized protein LOC108972669; <i>Bactrocera latifrons</i>	
<i>BdorIR64a-1</i>	322	Partial	0	XP_018799073.1; uncharacterized protein LOC108975188; <i>Bactrocera latifrons</i>	Ref. 2: U7132
<i>BdorIR64a-2</i>	96	Partial	1e-58	XP_019845172.1; uncharacterized protein LOC105224490; <i>Bactrocera dorsalis</i>	
<i>BdorIR75a-1</i>	342	Partial	0	XP_019845038.1; uncharacterized protein LOC109579404; <i>Bactrocera dorsalis</i>	Ref. 2: U14774
<i>BdorIR75a-2</i>	140; 232	Partial	2e-79; 2e-164	XP_019845037.1; glutamate receptor; <i>Bactrocera dorsalis</i>	
<i>BdorIR75b</i>	95	Partial	2e-49	XP_014088428.1; uncharacterized protein LOC106616338; <i>Bactrocera oleae</i>	
<i>BdorIR75d</i>	162; 105;	Partial	5e-110; 3e-64; 0	XP_019844868.1; uncharacterized protein LOC105223467; <i>Bactrocera dorsalis</i>	Ref. 1: KP743671
<i>BdorIR76a-1</i>	137; 147	Partial	7e-92; 3e-100	XP_011204763.1; uncharacterized protein LOC105227219; <i>Bactrocera dorsalis</i>	
<i>BdorIR76a-2</i>	286	Partial	0	XP_014086277.1; uncharacterized protein LOC106614874; <i>Bactrocera oleae</i>	
<i>BdorIR76b</i>	659	Full	0	AKI28988.1; IR76b; <i>Bactrocera dorsalis</i>	Ref. 1: KP743672; Ref. 2: C1154C3
<i>BdorIR84a</i>	703	Partial	0	XP_011193628.1; glutamate receptor 1; <i>Zeugodacus cucurbitae</i>	Ref. 1: KP743673
<i>BdorIR92a-1</i>	140; 116; 246	Partial	3e-85; 4e-53; 4e-177	AKI28990.1; IR92a; <i>Bactrocera dorsalis</i>	Ref. 1: KP743674; Ref. 2: C2923C2
<i>BdorIR92a-2</i>	146	Partial	2e-92	XP_019845172.1; uncharacterized protein LOC105224490; <i>Bactrocera dorsalis</i>	
<i>BdorIR93a-1</i>	93; 602	Partial	7e-55; 0	XP_011214752.1; glutamate receptor ionotropic, delta-1; <i>Bactrocera dorsalis</i>	Ref. 2: U7132
<i>BdorIR93a-2</i>	76	Partial	1e-38	XP_014095980.1; uncharacterized protein LOC106621575; <i>Bactrocera oleae</i>	
<i>BdorIR94f</i>	95	Partial	3e-58	XP_011199185.1; uncharacterized protein LOC105223232; <i>Bactrocera dorsalis</i>	

¹Homologs representing more than 90% amino acid identities with chemosensory receptors identified in the present study are listed as references. Ref. 1: Wu et al., 2015; Ref.2: Liu et al., 2016.

Table 3. Results of data analysis using a generalized linear model with a binomial logit fit to identify the factors that influenced the numbers of fruit flies landing on the spheres.

Volatile	Fruit fly	Factor	AIC	Deviance	<i>P</i>
1-Octen-3-ol	Virgin female	Null	64.9	—	—
		Volatile (V)	65.2	1.68	0.195
		Color (C)	66.9	0.00	1.000
		V × C	67.2	1.68	0.432
	Mated female	Null	85.0	—	—
		Volatile (V)	81.2	5.85	0.016*
		Color (C)	86.0	1.07	0.302
		V × C	82.1	6.92	0.031*
	Virgin male	Null	79.3	—	—
		Volatile (V)	75.8	5.48	0.019*
		Color (C)	81.3	0.00	1.000
		V × C	77.8	5.48	0.065
	Mated male	Null	73.9	—	—
		Volatile (V)	67.9	7.97	0.005**
		Color (C)	72.6	3.27	0.071
		V × C	66.6	11.26	0.004**
Geranyl acetate	Virgin female	Null	71.4	—	—
		Volatile (V)	73.4	0.00	1.000
		Color (C)	72.5	0.90	0.343
		V × C	74.5	0.90	0.638
	Mated female	Null	105.6	—	—
		Volatile (V)	99.7	7.90	0.005**
		Color (C)	106.4	1.16	0.282
		V × C	100.5	9.06	0.011*
	Virgin male	Null	77.4	—	—
		Volatile (V)	72.1	7.22	0.007**
		Color (C)	79.4	0.00	1.000
		V × C	74.1	7.22	0.027*
	Mated male	Null	88.5	—	—
		Volatile (V)	89.4	1.17	0.278
		Color (C)	90.5	0.07	0.787
		V × C	91.3	1.25	0.536
Farnesenes	Virgin female	Null	65.3	—	—

Linalyl acetate	Mated female	Volatile (V)	66.5	0.74	0.389
		Color (C)	67.0	0.27	0.606
		V × C	68.3	1.01	0.604
		Null	92.7	—	—
		Volatile (V)	88.4	6.33	0.012*
		Color (C)	94.2	0.58	0.446
	Virgin male	V × C	89.8	6.92	0.031*
		Null	77.0	—	—
		Volatile (V)	78.6	0.34	0.562
		Color (C)	75.9	3.06	0.080
		V × C	77.6	3.40	0.183
	Mated male	Null	98.4	—	—
		Volatile (V)	96.5	3.92	0.048*
		Color (C)	100.4	0.02	0.895
		V × C	98.5	3.94	0.139
	Virgin female	Null	98.6	—	—
		Volatile (V)	100.5	0.08	0.777
		Color (C)	99.3	1.29	0.256
		V × C	101.3	1.37	0.504
	Mated female	Null	90.2	—	—
		Volatile (V)	92.2	0.01	0.907
		Color (C)	87.2	5.01	0.025*
		V × C	89.2	5.02	0.081
	Virgin male	Null	81.8	—	—
		Volatile (V)	82.0	1.75	0.186
		Color (C)	82.7	1.06	0.304
		V × C	83.0	2.80	0.246
	Mated male	Null	77.4	—	—
		Volatile (V)	79.3	0.07	0.790
		Color (C)	79.3	0.07	0.790
		V × C	81.3	0.14	0.932

* $p < 0.05$, ** $p < 0.01$.

1 Supplemental figure captions

2

3 Fig. S1. Chemical structures of tested compounds for the functional analysis of
4 candidate olfactory receptors (ORs).

5

6 Fig. S2. Behavioral bioassay to evaluate the attractiveness of the volatiles. (A) The
7 meshed cage (40 cm × 40 cm × 40 cm) used for the behavioral bioassay. We placed
8 one sphere 10 cm from each corner of the cage. (B) To test each compound, we
9 impregnated a piece of filter paper (15 mm × 3 mm) with 1 mg of the compound
10 dissolved in 5 µL of ethanol, and dried the paper at room temperature. Each filter
11 paper was then placed in a clean 0.2-mL clear microtube, which was positioned facing
12 up in one of the holes of a polyethylene sphere that consisted of 26 holes. Each sphere
13 was placed on a plastic petri dish (diameter 5 cm) to prevent rolling on the cage floor
14 during the bioassay.

15

16 Fig. S3. Comparison of amino acid sequences of olfactory receptors (ORs).
17 Alignments of BdorOR13a and DmOR13a (A), and BdorOR82a and DmOR82a (B)
18 are shown. Seven transmembrane domains (TM1–TM7) are indicated by solid lines.

19

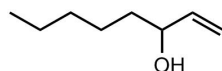
20 Fig. S4. Effects of ethanol and visual cues on the landing behavior of mated
21 *Bactrocera dorsalis* females. The box plot shows 25–75% (box), median (band
22 inside), and minima to maxima (whiskers). (A) Numbers of mated females landing on
23 the green or white spheres. The numbers of flies are plotted as dots ($n = 7$). T-G, T-W,
24 C-G, and C-W indicate volatile-treated green balls, volatile-treated white balls,
25 control (volatile-untreated) green balls, and control white balls, respectively. The

26 table shows the results of data analysis using a generalized linear model with a
27 binomial logit fit to identify the factors that influenced the number of fruit flies
28 landing on the spheres. Neither the volatile nor the color of the spheres had a
29 significant effect (p -values > 0.05). (B) Comparison of the total numbers of mated
30 females landing on the spheres calculated from Fig. 6A and S4A. We detected no
31 significant differences between the treatments at $p < 0.05$ according to Tukey's HSD
32 test.
33

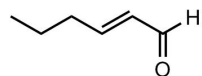
Fig. S1



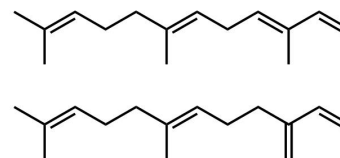
Ethanol (EtOH)



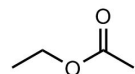
(±)-1-Octen-3-ol (1-Oct-3-ol)



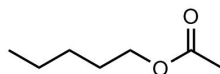
(*E*)-2-Hexenal (Hexenal)



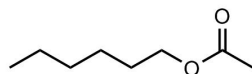
Farnesenes, mixture of isomers (FRN-mix)



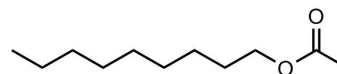
Ethyl acetate (EtOAc)



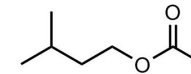
Pentyl acetate (P-OAc)



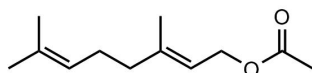
Hexyl acetate (H-OAc)



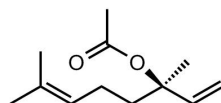
Nonyl acetate (N-OAc)



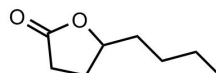
Isopentyl acetate (IP-OAc)



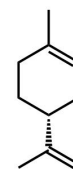
Geranyl acetate (G-OAc)



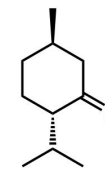
Linalyl acetate (L-OAc)



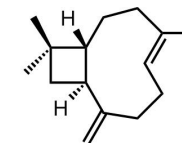
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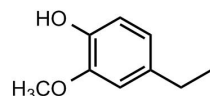
(*R*)-(+)-Limonene
(Limonene)



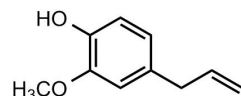
(-)-Menthone
(Menthone)



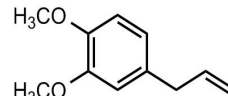
β-Caryophyllene (CP)



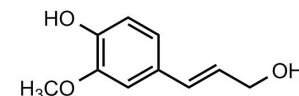
4-Ethylguaiacol (EG)



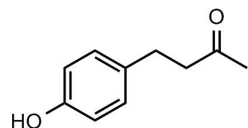
Eugenol



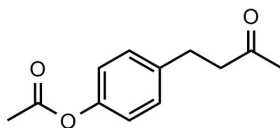
Methyl eugenol (ME)



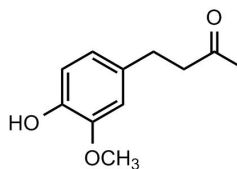
(*E*)-Coniferyl alcohol (CF)



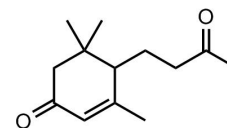
Raspberry ketone (RK)



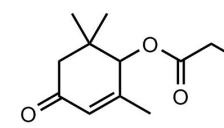
Cue-lure (CL)



Zingerone (ZN)



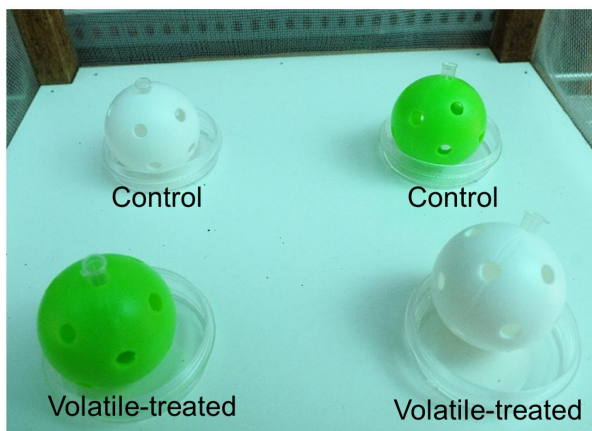
(±)-3-Oxo-7,8-dihydro-α-ionone
(P3)



(±)-4-Propionyloxyisophorone
(E0P)

Fig. S2

A

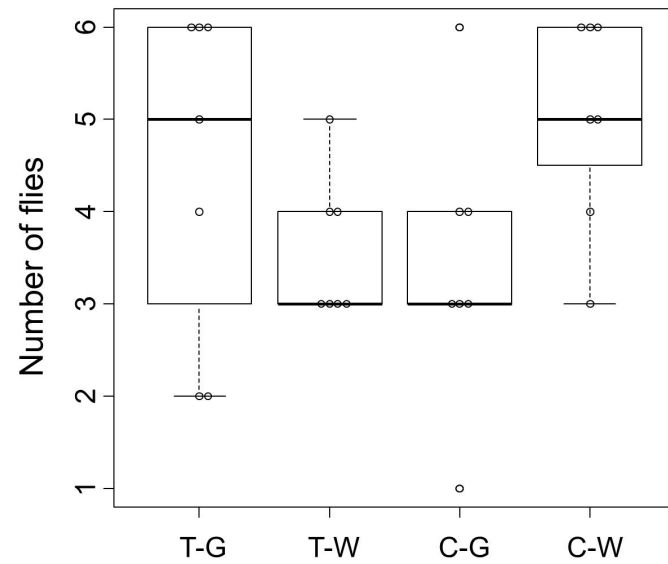


B



Fig. S4

A



Volatile	Factor	AIC	Deviance	<i>P</i>
Ethanol	Null	105.9	-	-
	Volatile (V)	107.8	0.0853	0.770
	Color (C)	107.7	0.237	0.626
	V X C	109.6	0.322	0.851

B

