Full Title: *Aedes aegypti* (Dipetera: Culicidae) odorant receptors respond to floral and plant derived volatiles: an expansive deorphanization of odorant receptors expressed in male and female mosquitoes.

Short Title: Mosquitos sense plant derived volatiles

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Abstract:

Adult mosquitoes require regular sugar meals, usually in the form of floral nectar, to survive in natural habitats. Both males and females locate potential sugar sources using sensory proteins called odorant receptors, that are activated by plant volatiles and facilitate orientation toward flowers or honeydew. The yellow fever mosquito, *Aedes aegypti* (Linnaeus, 1762), possesses a large gene family of odorant receptors, many of which are likely to be involved in floral odor detection. Using a heterologous expression system and a technique called two-electrode voltage clamping, we are able to identify environmentally relevant chemical cues that produce responses in specific odorant receptors. In this study, we have uncovered ligand-receptor pairings for a suite *Aedes aegypti* receptors that are likely to mediate appetitive or aversive behavioral responses. This information can be used to assess mosquito foraging behavior and develop novel control strategies, especially those that incorporate mosquito bait-and-kill technologies.

Introduction:

Arthropods rely on sensory receptors to interpret and interact with their environments. Arguably, olfaction is the most important sensory modality, conveying the ability to sense volatile chemical cues over large distances [1-3]. Olfaction is responsible for many arthropod behaviors, including host-seeking [4, 5], oviposition [6, 7], mate selection [8, 9], and nectar foraging [10, 11]. Odorant receptors (ORs) comprise the largest family of volatile odor detectors in mosquito genomes [12]. Another family of proteins called ionotropic receptors (IRs) also functions in *Aedes aegypti* olfaction, especially in organic acid detection [13, 14]. Odorant receptors are transmembrane proteins expressed by odorant receptor neurons housed in hairlike projections called sensilla [15, 16]. Sensilla on sensory appendages such as antennae, maxillary palps, labella, and leg tarsi constitute the primary olfactory appendages in insects [17, 18, 19]. ORs are ligand-gated ion channels that respond to diverse, environmentally relevant volatile odorants [20]. ORs most likely form multimers that include co-expression of a single type of tuning OR complexed with an odorant receptor coreceptor (Orco), which is highly conserved across most insect taxa [21-23]. Chemical volatiles enter the sensilla through tiny pores on their surfaces and either diffuse through the sensilla lymph

37 or are shuttled by odorant binding proteins to the neuronal membrane, where they can interact with the
38 OR/Orco complex [24, 25]. Most arthropod species have between 50 and 100 different ORs, which allow
39 for complex and robust interactions with their respective environments through the perception of various
40 volatile compounds, though some species have as few as five or as many as 300 [26, 27]. The diversity of
41 receptor proteins underlies distinct behavioral phenomena across arthropod species.

42 The prominent disease vector *Aedes aegypti* (Linnaeus, 1762) has 133 putative odorant receptors
43 that vary across sex, life stage, and tissue [28-30]. Despite the significant impact of this species on human
44 health, few ORs have been characterized, particularly ones presumed to function in female host-seeking
45 behaviors [31-34]. However, significant knowledge gaps remain in our understanding of the molecular
46 mechanisms that underpin nectar foraging in this species. As both male and female mosquitoes engage in
47 this behavior, the ORs that mediate this may provide vital information about potential attractants or
48 repellants that can be used in trapping or control systems [35-37].

49 In this study, we investigated ORs in *Ae. aegypti* that are conserved in several disease vector
50 mosquito species and are closely related to other ORs that function in floral odor sensing. These odorant
51 receptors are expressed mainly in male and female antennae [28-30], suggesting they may function in a
52 shared behavior such as nectar foraging. To complete this study, we used a heterologous expression system
53 and a technique called two-electrode voltage clamping (TEVC) to identify essential ligands [38]. After
54 synthesizing cRNA for odorant receptors of interest, we injected unfertilized *Xenopus laevis* oocytes with
55 the cRNA for the OR of interest (called the tuning receptor) and cRNA for *Ae. aegypti* Orco. After allowing
56 time for protein expression, we used TEVC to record changes in membrane potential as a library of 147
57 potential ligands was perfused past. Using this method, we identified probable ligands for ten ORs, eight
58 of which were novel or revealed a previously unknown ligand. Many odorant receptors responded to indole-
59 like compounds that affect *Ae. aegypti* behavior [39, 40]. These studies highlight the importance of
60 characterizing ORs as a method of improving our understanding of the molecular basis for *Ae. aegypti*
61 nectar seeking or other behaviors.

While we have demonstrated that these receptors and ligands interact on a molecular basis, more research is needed to determine how the ligands might influence a mosquito's behavior. Future studies will include olfactometry with wild-type and gene-knockout mosquitoes to verify the role of these ORs and research into the expression patterns of these genes in response to ligand exposure. These studies help to identify specific attractants or repellants that can be leveraged in surveillance systems or bait traps to help control mosquito populations and combat the spread of disease.

Materials and Methods

Phylogenetic Analysis

A conserved clade of genes was hypothesized to be activated by floral or nectar odors and utilized by mosquitoes in foraging. These genes were identified via BLAST via tBLASTn or BLASTp using the NCBI database. These identified genes were sequenced and cloned for experimental purposes.

Gene cloning and Sequencing

The coding regions of ten genes of interest (Supplemental File 1) and AaegOrco were synthesized by Twist Bioscience (San Francisco, CA, USA) and cloned into the pENTR plasmid. The Gateway LR directional cloning system (Invitrogen Corp., Carlsbad, CA, USA) was utilized to subclone AaegOR coding regions into the pSP64t-RFA oocyte expression plasmid. Plasmids were purified using the GeneJet Plasmid Miniprep Kit (ThermoFisher Scientific, Waltham, MA, USA). Purified plasmids were sequenced in both directions to confirm coding regions.

OR Gene Expression Analysis and Transcriptome Assembly

RNAseq reads were downloaded from the NCBI database and mapped to the *Aedes aegypti* annotated transcript (*Aedes aegypti*_63_LVP_AGWG) obtained from VectorBase. We used the Sailfish alignment-free software in the Galaxy bioinformatics platform (usegalaxy.org) to map the reads and quantify expression levels in transcripts per million reads (TPM).

Chemical Reagents

86 Chemical compounds (Supplemental File 2) used in the deorphanization of AaegORs were purchased from
87 Acros Organics (Morris, NJ, USA), Alfa Aesar (Ward Hill, MA, USA), and ThermoFisher Scientific
88 (Waltham, MA, USA) at the highest purity available. Compounds were stored as 1M stocks in 100%
89 DMSO. Serial dilutions of each compound were prepared in 10% DMSO and blended by chemical class.
90 *Two-electrode voltage clamping*

91 pSP64t-RFA:AaegOR clones were linearized using Xbal and cRNAs were synthesized using the
92 mMESSAGE mMACHINE ® SP6 kit (Life Technologies, Carlsbad, CA, USA). Xenopus laevis stage V-
93 VII oocytes purchased from Xenopus1 were stored at 18^0C in Incubation medium (ND96 96 with 5%
94 dialyzed horse serum,50µg/mL gentamycin,100µg/mL streptomycin, 100µg/mL penicillin, and 550µg/mL
95 sodium pyruvate). 30nL of cRNA was injected into multiple oocytes (Microinjection Syringe Pump, World
96 Precision Instruments, Sarasota, FL, USA) maintained at 18C for 48-72 hrs. Odorant blends were perfused
97 across individual oocytes for 10-15 seconds held at a resting membrane potential of -80 mV using an OC-
98 725C oocyte clamp (Warner Instruments, LLC, Hamden, CT, USA). Induced currents were measured
99 indirectly using the two-electrode voltage clamp technique (TEVC). Following each stimulus, currents were
100 allowed to return to baseline before further stimulation. Data was captured and recorded using the Digidata
101 1550 B digitizer and Pclamp10 software (Molecular Devices, San Jose, CA, USA). Concentration-response
102 curves were generated by perfusing unitary odorants over oocytes at increasing concentrations from [10^{-3M}]
103 to [10^{-9}M]. Data were analyzed using GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA, USA). At
104 least two biological replicates were conducted at each concentration and for each compound. Data for at
105 least seven oocytes that responded to each compound was pooled for analysis (Supplemental File 3.
106

107 **Results:**

108 1. *Aedes aegypti* odorant receptors are expressed in male and female antennae and conserved in other
109 disease vector mosquitoes.

Analysis of previously published [41] RNAseq data demonstrated that these ORs are expressed in both male and female sensory appendages (Table 1). Except for AaegOR8, which is expressed in the maxillary palps [28]. ORs were most highly expressed in the antennae of both sexes (Table 1; [29]). Phylogenetic analysis of possible floral volatile sensing odorant receptors in three major disease vectors revealed several different groupings, which may indicate shared or similar ligands (Figure 1). Orco serves as an outgroup. This analysis revealed previously deorphanized receptors that share ligands cluster together, as in the case of CquiOR21 and T2, which are skatole receptors [42]. The cladogram also provides insights for future functional characterization in other species. AaegOR71 clusters with AalbOR71 and CquiOR32, AaegOR6, AaegOR13, and AaegOR15 with AalbOR6, AalbOR13, and AalbOR15 respectively, AaegOR28 with CquiOR117, and AaegOR31 with CquiOR96. The phylogenetic analysis reveals that newly deorphanized genes are closely related to Ors in other species and can provide helpful information in future studies with *Culex quinquefasciatus* (Say, 1823) and *Aedes albopictus* (Skuse, 1894).

Table 1: *Ae. aegypti* OR Expression in Sensory Appendages proxied via RNAseq

Odorant receptor gene expression profiles in *Ae. aegypti* male and female antennae and proboscis. Darker shading indicated elevated transcripts per million.

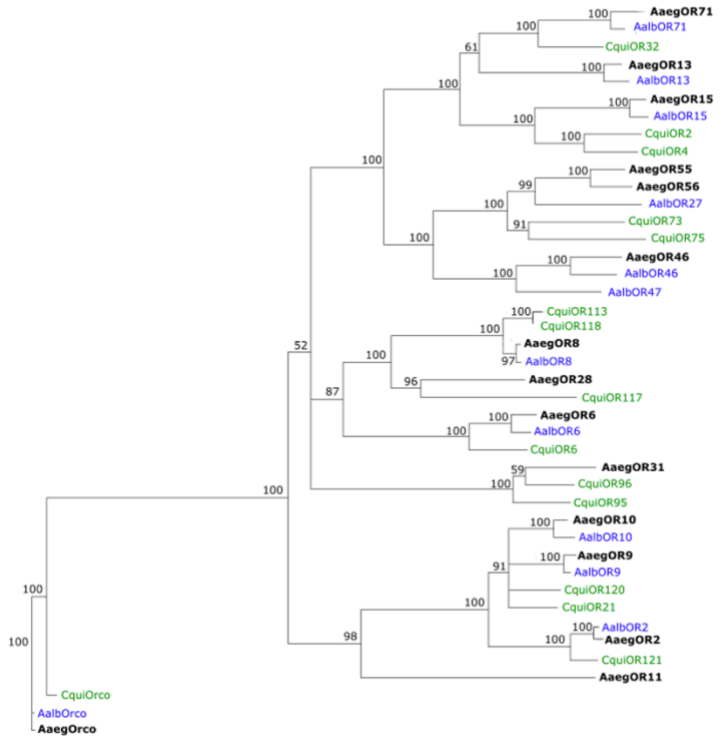
			Transcripts per million (+/- SE) in *Ae. aegypti* head appendages				
AaegOR	**VectorBase ID**	**NCBI ID**	**female ant.**	**male ant.**	**female palp**	**male rost.**	**female prob.**
Orco	AAEL005776	NM_001358471	2545.4 (146.6)	385.1 (9.2)	985.6 (87.3)	80.6 (4.2)	35.7 (2.1)
Or6	AAEL017548	NM_001358467	5.3 (1.0)	6.6 (0.5)	0.1 (0.1)	2.6 (0.4)	5.8 (0.7)
Or8	AAEL012254	XM_001662305.3	0 (0.0)	0 (0.0)	387.7 (37.8)	25.7 (1.3)	0.2 (0.2)
Or10	AAEL006003	NM_001358339	21.0 (3.0)	11.5 (0.6)	0 (0.0)	0.5 (0.0)	0.4 (0.1)
Or11	AAEL011583	NM_001358172	146.1 (8.9)	37.0 (1.1)	0 (0.0)	0.2 (0.1)	2.5 (0.5)
Or13	AAEL008368	NM_001358180	20.9 (1.5)	3.8 (0.6)	0 (0.0)	0.3 (0.0)	0.6 (0.1)
Or15	AAEL008448	NM_001358181	6.7 (1.2)	3.3 (0.2)	0 (0.0)	2.2 (0.2)	4.0 (0.3)
Or28	AAEL027053	NM_001358166	4.0 (0.4)	3.4 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
Or31	AAEL013217	NM_001358160	19.0 (1.5)	10.4 (0.7)	0.2 (0.1)	0.5 (0.0)	1.0 (0.3)
Or55	AAEL010415	NM_001358184	4.4 (0.3)	1.4 (0.3)	0 (0.0)	0.5 (0.1)	1.3 (0.5)
Or71	AAEL017564	NM_001358112	43.1 (2.7)	14.6 (0.3)	0.9 (0.3)	2.9 (0.4)	9.4 (0.7)

Data from VectorBase.org (Matthews *et al.* 2016)

TPM scale

0 ---------- 2545

125 **Figure 1: Phylogenetic Analysis of Possible Floral Volatile Sensing ORs in Major Disease Vectors**



Neighbor-joining tree showing relationships of floral volatile sensing OR conceptual peptides for three major disease vector species of mosquitoes *Ae. aegypti* (Aaeg, Black), *Ae. albopictus* (Blue), and *Cu. quinquefasciatus* (green). Node values signify bootstrap values from 1000 pseudoreplicates.

2. Odorant receptors respond to environmentally

138 relevant compounds in a concentration dependent manner

139 *Ae. aegypti* ORs responded to blends of chemical compounds (Figure 2). While there is some
140 responsiveness to multiple blends by specific ORs, all demonstrated a pronounced specificity for one blend
141 over the others, as indicated by highly positive kurtosis values (Table 2). The blends that were most
142 frequently responded to were indole or alcohol blends. The unitary compounds for the most responsive
143 blend for each OR was tested individually. We determined the primary ligand each OR responded to from
144 our chemical library and noted other secondary ligands (Table 2, Figure 3). As with the blends, ORs had a
145 pronounced responses to a single ligand, again supported by highly positive kurtosis values. Additionally,
146 a defining feature of OR responsiveness is that it is concentration based, we also investigated responses to
147 increasing concentrations with that compound. From this, we were able to determine the half maximal
148 effective concentration (EC_{50}) for each primary ligand (Table 2, Figure 4).

Figure 2: Heat Map of *Ae. aegypti* odorant receptor responses to blends of chemical compounds

Blends of chemical compounds used for receptor screening are listed as rows on the lefthand side of the figure while receptor identities are listed as columns below the figure. Heat map gradation is listed on the right of the figure and spans from hot (100, yellow) to cold (0, blue).

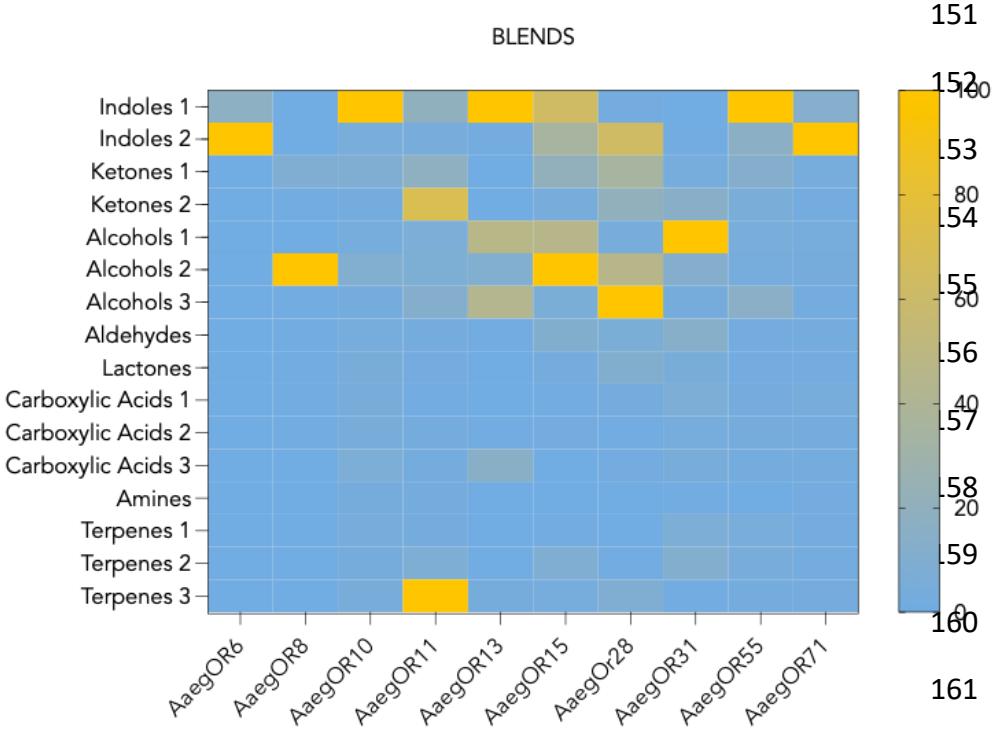


Table 2: Functional responses of *Ae. aegypti* odorant receptors

163 Summarization table of 10 *Ae. aegypti* odorant receptors and the corresponding responsiveness to blends
164 of chemical compounds and unitary compounds. Kurtosis values are indicated for both blend and unitary
165 compounds. EC50 values (μM) are indicated for primary ligands. Secondary ligands are presented if
166 identified during the functional characterizations.

Odorant Receptor	Blend	Kurtosis	Primary Ligand (s)	Kurtosis	EC_{50} (μM)	Secondary Ligand(s)
AaegOr6	Indoles 2	14.86	4-chromanone	10.85	6.84	
AaegOr8	Alcohols 2	15.71	1-octen-3-ol	8.75	2.14	
AaegOr10	Indoles 1	15.61	3-methylindole	5.87	0.16	
AaegOr11	Terpenes 3	5.68	cis-deca-hydro-1-napthol	6.85	68.40	fenchone
AaegOr13	Indoles 1	6.24	4-ethyl-phenol	9.11	0.17	
AaegOr15	Alcohols 2	3.63	1-hexanol	5.23	1.07	phenethyl propionate
AaegOr28	Alcohols 2	15.96	tetrahydrolinalool	7.70	44.13	
AaegOr31	Alcohols 1	14.58	Farnesol	10.70	14.32	
AaegOr55	Indoles 1	14.50	2,3-dimethylindole	0.22	8.65	
AaegOr71	Indoles 2	15.56	4-quinolinecarboxaldehyde	3.08	0.96	

167

168

169

170 **Figure 3: *Ae. aegypti* odorant receptor responses to blend components**

171 Normalized mean current responses of AaegORX/Orco expressing oocytes (n>7) to the individual
172 components of various blends (A) 4-chromanone (B) 1-octen-3-ol, (C) 3-methylindole (D) cis-decahydro-
173 1-napthol, (E) 4-ethyl-phenol, (F) 1-hexanol, (G) tetrahydrolinalool, (H) farnesol, (I) 2,3-dimethylindole,
174 and (J) 4-quinolinecarboxyaldehyde. The structure of each compound is shown in figure inserts.

175

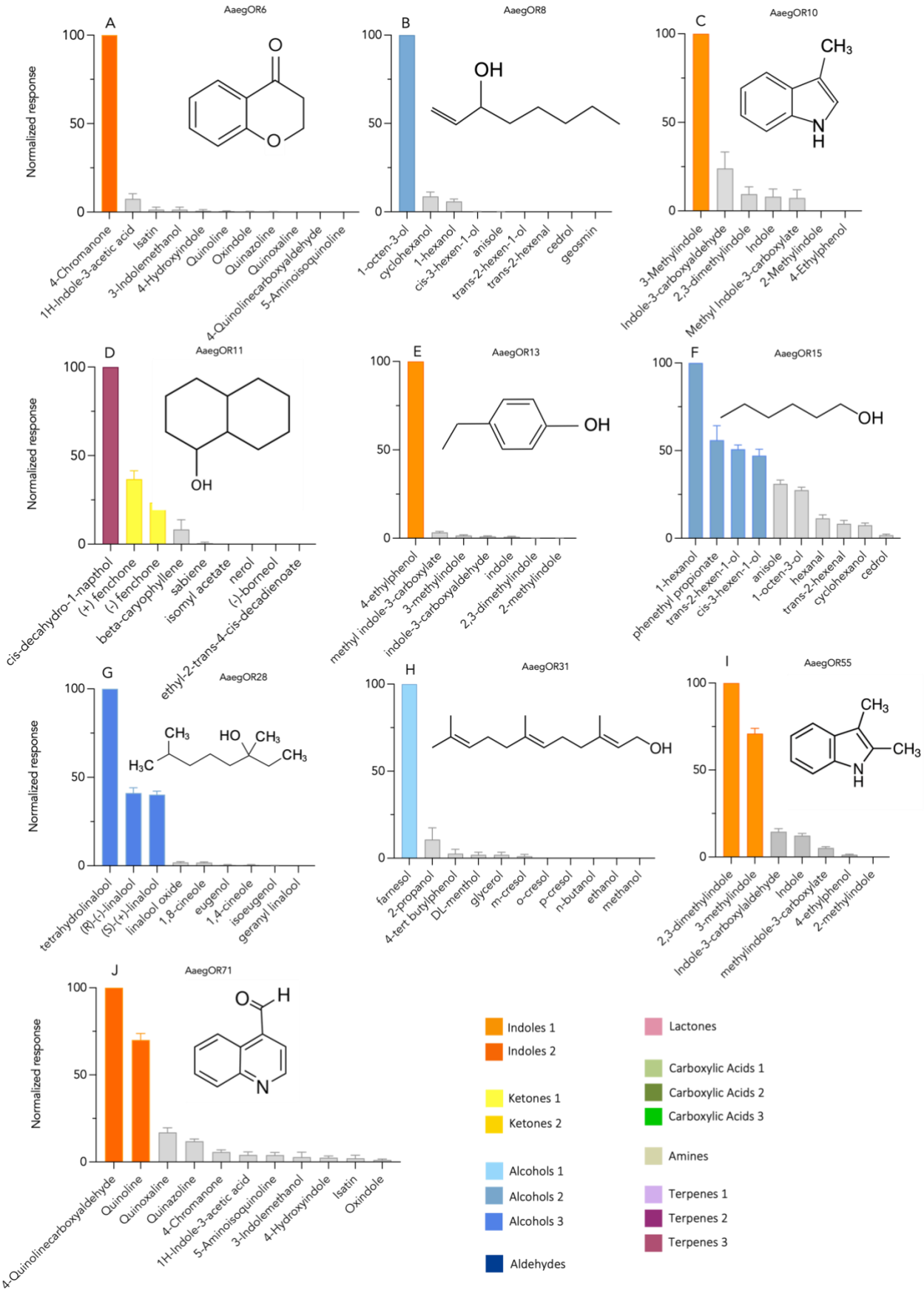
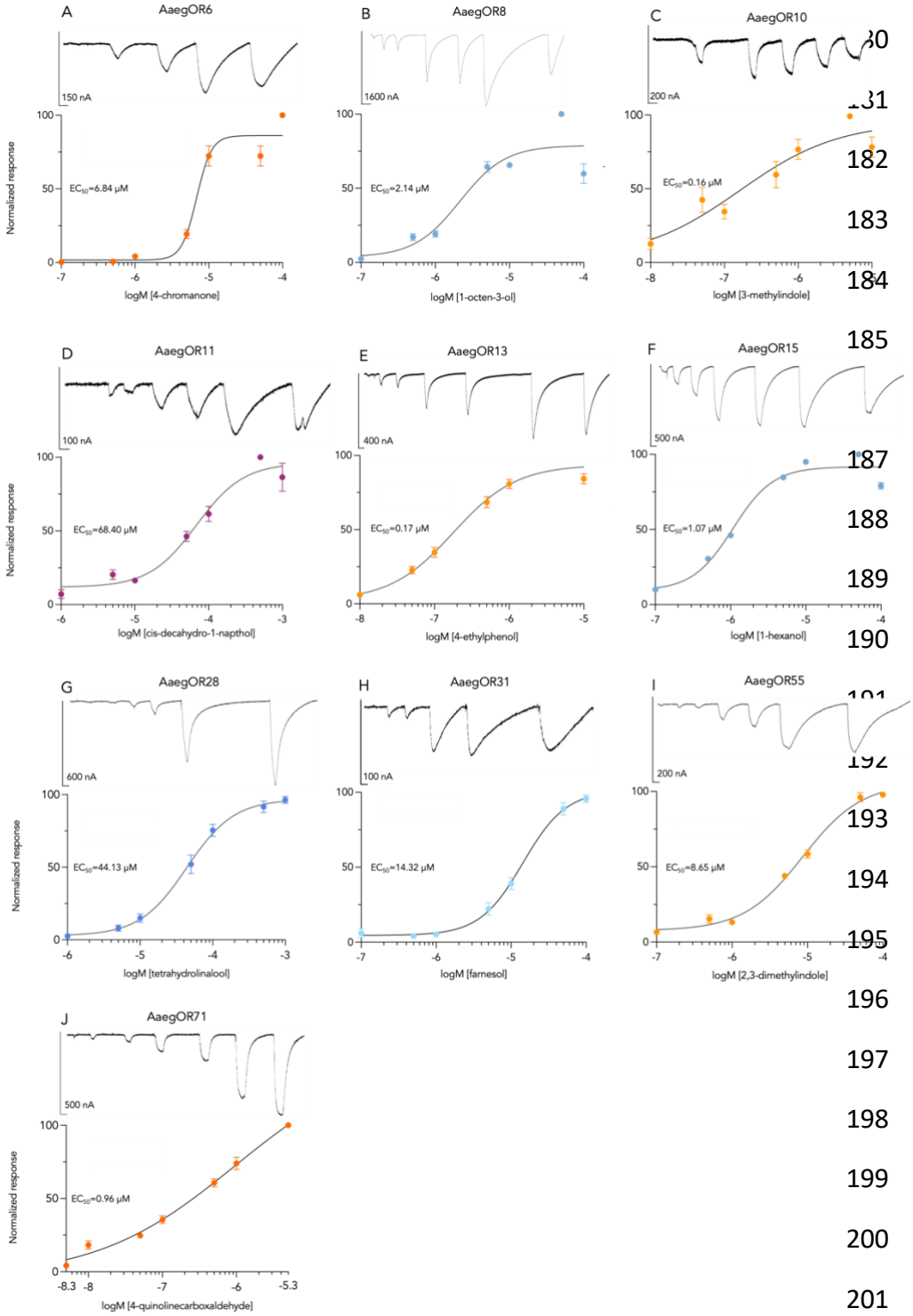

A
AaegOR6
Normalized response
100
50
0
4-Chromanone
1H-Indole-3-acetic acid
Isatin
3-Indolemethanol
4-Hydroxyindole
Quinoline
Oxindole
Quinazoline
Quinoxaline
4-Quinolinecarboxyaldehyde
5-Aminoisoquinoline
B
AaegOR8
OH
1-octen-3-ol
cyclohexanol
1-hexanol
cis-3-hexen-1-ol
anisole
trans-2-hexen-1-ol
trans-2-hexenal
cedrol
geosmin
C
AaegOR10
CH3
N
H
3-Methylindole
Indole-3-carboxyaldehyde
2,3-dimethylindole
Indole
Methyl Indole-3-carboxylate
2-Methylindole
4-Ethylphenol
D
AaegOR11
OH
cis-decahydro-1-napthol
(+) fenchone
(-) fenchone
beta-caryophyllene
sabiene
isomyl acetate
nerol
(-)-borneol
ethyl-2-trans-4-cis-decadienoate
E
AaegOR13
OH
4-ethylphenol
methyl indole-3-carboxylate
3-methylindole
indole-3-carboxyaldehyde
indole
2,3-dimethylindole
2-methylindole
F
AaegOR15
OH
1-hexanol
phenethyl propionate
trans-2-hexen-1-ol
cis-3-hexen-1-ol
anisole
1-octen-3-ol
hexanal
trans-2-hexenal
cyclohexanol
cedrol
G
AaegOR28
CH3
HO
CH3
H3C
CH3
tetrahydrolinalool
(R)-(-)-linalool
(S)-(+)-linalool
linalool oxide
1,8-cineole
eugenol
1,4-cineole
isoeugenol
geranyl linalool
H
AaegOR31
OH
farnesol
2-propanol
4-tert butylphenol
DL-menthol
glycerol
m-cresol
o-cresol
p-cresol
n-butanol
ethanol
methanol
I
AaegOR55
CH3
CH3
N
H
2,3-dimethylindole
3-methylindole
Indole-3-carboxylaldehyde
Indole
methylindole-3-carboxylate
4-ethylphenol
2-methylindole
J
AaegOR71
O
H
N
4-Quinolinecarboxyaldehyde
Quinoline
Quinoxaline
Quinazoline
4-Chromanone
1H-Indole-3-acetic acid
5-Aminoisoquinoline
3-Indolemethanol
4-Hydroxyindole
Isatin
Oxindole
Indoles 1
Indoles 2
Ketones 1
Ketones 2
Alcohols 1
Alcohols 2
Alcohols 3
Aldehydes
Lactones
Carboxylic Acids 1
Carboxylic Acids 2
Carboxylic Acids 3
Amines
Terpenes 1
Terpenes 2
Terpenes 3

Figure 4: *Ae. aegypti* odorant receptor concentration response curves

Normalized mean current responses of AaegORX/Orco expressing oocytes (n>10) to (A) 4-chromanone (B) 1-octen-3-ol, (C) 3-methylindole (D) cis-decahydro-1-napthol, (E) 4-ethyl-phenol, (F) 1-hexanol, (G) tetrahydrolinalool, (H) farnesol, (I) 2,3-dimethylindole, and (J) 4-quinolinecarboxyaldehyde.



Representative traces from oocyte recordings are shown above each curve. EC50 values are shown in figure insets.

Three ORs responded most robustly to the Indole 1 blend. OR10, OR13, and OR55 responded to 3-methylindole (0.16 μM) , 4-ethylphenol (0.17 μM), and 2,3-diemthylindole (8.65 μM), respectively. OR6 responded to 4-

202 chromanone (6.81 μM) from the Indole 2 blend. The other receptor that responded to this blend was OR71
203 which responded to 4-quinoline carboxaldehyde (0.96 μM). From the Alcohol 1 blend, OR31 responded to
204 farnesol (14.32 μM), while from the Alcohols 2 blend, OR8 and OR15 responded to 1-octen-3-ol (2.14
205 μM) and 1-hexanol (1.07 μM), respectively. A previously identified ligand of OR15, phenethyl propionate,
206 demonstrated an EC_{50} of 20.16 μM. OR28 responded to tetrahydrolinalool (44.13 μM) from the Alcohols 3
207 blend. Finally, the only receptor to respond to a compound not belonging to an indole or alcohol blend was
208 OR11, which responded to cis-decahydro-1-naphthal (68.40 μM) from the Terpenes 3 blend. We found that
209 OR11 has an EC_{50} of about 77.10 μM to a previously identified ligand, fenchone.

210 **Discussion:**

211 Nectar foraging is crucial for mosquito species to intake sugar meals for reproduction and survival
212 [43-46]. Previous studies have shown that several mosquito species, including *Ae. aegypti,* exhibit
213 preferences for certain nectar sources [47-52]. ORs provide essential details about the surrounding
214 environment and allow mosquitoes to identify and select nectar sources based on the information provided
215 by their chemical signals [3]. Here, we assess the responsiveness of various ORs to a library of ecologically
216 relevant ligands, focusing on receptors that could be related to floral odor sensing.

217 We began by looking at phylogeny and RNAseq data. This allowed us to select potential targets
218 based on expression patterns and determine relatedness between ORs within *Ae. aegypti* and between two
219 other disease vectors, *Cu. quinquefasciatus* and *Ae. albopictus,* assuming that functionally related ORs
220 would cluster together [53]. Specifically, we selected specific ORs to study based on their relatedness to
221 other ORs that respond to floral odors [54] or had previously been deorphanized. AaegOR10, AaegOR9,
222 AaegOR2, and AaegOR8 have been thoroughly investigated previously, and many of the ORs we selected
223 for study were closely related to them. When looking at RNAseq data, we were interested in ORs expressed
224 in antennae. While AaegORs are expressed in various chemosensory tissues, antennae are the primary
225 structures involved in odor sensing [1]. Additionally, OR expression in the antennae of both males and

226 females indicates that the receptors are used for a shared behavior, such as nectar feeding. These initial
227 analyses allowed us to predict and select receptors that would respond to our chemical library.

228 Using two-electrode voltage clamping in conjunction with the heterologous expression system
229 *Xenopus laevis* oocytes, we initially assessed the responses of the AaegORs to our chemical library, which
230 contains 147 environmentally significant compounds organized into 14 blends. This revealed that many of
231 the AaegORs responded most robustly to the "Indole 1" and "Indole 2" blends, which contain indole or
232 indole-like compounds (Supp. Table 1). Indoles are relatively ubiquitous in natural settings and are present
233 in potential nectar sources. AaegOR1 responds to 4-quinoline carboxaldehyde, which has been found in
234 honey samples and endophytic bacteria via gas chromatography [55-57]. However, indole and its related
235 compounds have many other sources and influence behaviors across species [58], so it is uncertain if these
236 ORs are directly related to floral odor sensing. The specific indoles that several ORs respond to are not
237 typical plants, and their sources may suggest other functions, such as oviposition or host-seeking. AaegOR
238 responds to 4-chromanone, of which there is little literature regarding its occurrence in natural settings.
239 However, it is a key element of flavonoid skeletons [59]. AaegOR10 responds to 3-methylindole (skatole),
240 which is found in animal feces and produces behavioral responses in other Dipteran species [60-63]. 4-
241 ethylphenol, the primary ligand of AaegOR13, is a volatile constituent in wine and animal waste and has
242 been linked to oviposition in *Culex pipiens* [64-66]. While there is little evidence about natural sources of
243 2,3-dimethylindole, the AaegOR55 ligand it is closely related structurally to 3-methylindole. Other ORs
244 responded to compounds from the "Alcohols 1", "Alcohols 2", or "Alcohols 3" blends. AaegOR8 responds
245 to 1-octen-3-ol, a well-characterized fungal volatile and insect attractant [67]. A green-leaf volatile, 1-
246 hexanol, is another compound with well-characterized physiological effects in many arthropods [68-71].
247 Our study discovered that 1-hexanol activates AaegOR15 with a low threshold (EC_{50} ~1.07 μM).
248 AaegOR28 responds to tetrahydrolinalool, which is structurally related to linalool, another plant-derived
249 ligand to mosquito ORs [72]. AaegOR31 responds to farnesol, a significant constituent of the honeybee
250 Nasanov pheromone [73]. Lastly, the AaegOR11 ligand, cis-decahydro-1-napthol, is structurally related to

251 geosmin, a known oviposition attractant [74]. However, our compound library needs to be more
252 comprehensive, as more efficacious or potent ligands for AaegOR11 and AaegOR28 may exist, which had
253 the highest EC_{50} values in this set of ORs. We will continue to expand our library by adding compounds
254 structurally related to known ligands.

255 While four (AaegOR8, AaegOR10, AaegOR11, and AaegOR15) of the ORs tested in this study
256 already have identified ligands, we assessed them to uncover more potentially behaviorally relevant ligands,
257 as our chemical library differs from others tested [40, 75-77]. For AaegOR8 and AaegOR10, our data
258 supports what has previously been demonstrated. For AaegOR11 and AaegOR15, our data both supports
259 and adds to previous studies. For AaegOR11, we found that cis-decahydro-1-napthol produced a greater
260 magnitude of response than (-)-fenchone or (+)-fenchone, and we identified 1-hexanol as a potent activator
261 of AaegOR15 in addition to phenethyl propionate. This demonstrates that even for deorphanized ORs,
262 assessing their function with expanded libraries can aid in further understanding receptor-ligand
263 interactions.

264 *Ae. Aegypti* and other vector mosquitoes continue to threaten human health and flourish worldwide.
265 By continuing to elucidate receptor-ligand pairings, we can identify potential attractants for behavioral
266 control or targets for genetic knockout. By investigating floral-odor sensing receptors in particular, we can
267 identify compounds that are potentially behaviorally relevant to both sexes and can be used for attractive
268 toxic sugar bait systems. OR deorphanization is a valuable first step in understanding the sensory systems
269 of arthropods and can inform future experiments and control measures.

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