LETTER

doi:10.1038/nature10438

# A natural polymorphism alters odour and DEET sensitivity in an insect odorant receptor

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**Blood-feeding insects such as mosquitoes are efficient vectors of human infectious diseases because they are strongly attracted by body heat, carbon dioxide and odours produced by their vertebrate hosts. Insect repellents containing DEET (*N,N*-diethyl-*meta*-toluamide) are highly effective, but the mechanism by which this chemical wards off biting insects remains controversial despite decades of investigation[1–11]. DEET seems to act both at close range as a contact chemorepellent, by affecting insect gustatory receptors[12], and at long range, by affecting the olfactory system[1–11]. Two opposing mechanisms for the observed behavioural effects of DEET in the gas phase have been proposed: that DEET interferes with the olfactory system to block host odour recognition[1–7] and that DEET actively repels insects by activating olfactory neurons that elicit avoidance behaviour[8–11]. Here we show that DEET functions as a modulator of the odour-gated ion channel formed by the insect odorant receptor complex[13,14]. The functional insect odorant receptor complex consists of a common co-receptor, ORCO (ref. 15) (formerly called OR83B; ref. 16), and one or more variable odorant receptor subunits that confer odour selectivity[17]. DEET acts on this complex to potentiate or inhibit odour-evoked activity or to inhibit odour-evoked suppression of spontaneous activity. This modulation depends on the specific odorant receptor and the concentration and identity of the odour ligand. We identify a single amino-acid polymorphism in the second transmembrane domain of receptor OR59B in a *Drosophila melanogaster* strain from Brazil that renders OR59B insensitive to inhibition by the odour ligand and modulation by DEET. Our data indicate that natural variation can modify the sensitivity of an odour-specific insect odorant receptor to odour ligands and DEET. Furthermore, they support the hypothesis that DEET acts as a molecular 'confusant' that scrambles the insect odour code, and provide a compelling explanation for the broad-spectrum efficacy of DEET against multiple insect species.**

Previous work has shown that the odour of *Drosophila* food potently attracts adult *D. melanogaster* vinegar flies and that DEET blocks this attraction[5,7]. The behavioural effects of DEET require an intact olfactory system and the olfactory co-receptor ORCO[7]. These results implicated the olfactory system in the observed behavioural effects but failed both to distinguish between the two competing models of action for DEET and to determine whether DEET acts on the odour-specific odorant receptors, ORCO or both. We carried out electrophysiological recordings of *Drosophila* olfactory sensory neurons (OSNs) to test these competing possibilities.

In response to the suggestion that DEET and odours may interact in the vapour phase[9,10], we first quantified the respective amounts of vapour-phase 1-octen-3-ol emitted from the stimulus pipette in the presence and absence of DEET, using solid-phase microextraction (SPME) followed by gas chromatography mass spectroscopy analysis (GC–MS). The SPME measurements coupled to GC-MS (Fig. 1a) showed that the addition of a second filter paper containing pure DEET in the stimulus pipette had no significant effect on the release of 1-octen-3-ol ($10^{-2}$ dilution). Thus, we can rule out any fixative role of DEET under the conditions used here.

We next performed extracellular recordings to measure the effect of DEET on responses elicited by odours in *Drosophila* OSNs housed within the ab2 (Fig. 1a and Supplementary Fig. 1) or ab3 (Supplementary Fig. 2) olfactory hairs, or sensilla, on the fly antenna. Each of these sensilla houses two OSNs expressing different odorant receptors with unique odour response profiles[17]. We measured the activity of these OSNs simultaneously and compared their responses to odour with and without co-presentation of DEET (Fig. 1b, c).

The effect of DEET on four OSNs stimulated with ten structurally diverse odours was complex and dependent on odorant receptor, odour and concentration. In some OSNs, DEET suppressed odour-mediated inhibition (Fig. 1d, f and Supplementary Fig. 1a), in others it decreased odour-induced activation (Fig. 1e, Supplementary Fig. 1b, d, e and Supplementary Fig. 2a–g) and in others it had no effect (Fig. 1g and Supplementary Figs 1c and 2h–j). Moreover, the effects of DEET were strongly concentration dependent, such that high odour concentrations often overcame the effects of DEET (Fig. 1 and Supplementary Figs 1 and 2). DEET presented alone, without odour stimuli, elicited no response above that evoked by solvent in ab2A and ab3A neurons, slightly activated ab2B neurons and slightly inhibited ab3B neurons; but responses were considerably smaller than those elicited by cognate odour ligands (Supplementary Fig. 3). Therefore, DEET alone has a negligible effect on olfactory responses in ab2 and ab3 neurons.

Notably, 1-octen-3-ol presented in a dilution of $10^{-2}$ had opposite effects on the two neurons housed in ab2 sensilla, inhibiting the ab2A neuron expressing OR59B–ORCO (Fig. 1d) and activating the ab2B neuron expressing OR85A–ORCO (Fig. 1e). Co-application of DEET inverted OSN responses to odour, leading to activation of the ab2A neuron (Fig. 1d) and suppressing the odour-induced activation of the ab2B neuron (Fig. 1e). Similar opposite effects of DEET were observed when the ab2 sensillum was stimulated with a different odour, 1-octanol (Supplementary Fig. 1a, b).

Taken together, our results support the hypothesis that DEET acts as a molecular confusant, scrambling the *Drosophila* odour code by direct modulation of odorant receptor activity dependent on the type of odour and its concentration (Fig. 1h). Recent work examining the effect of DEET on mosquito odorant receptors in heterologous cells supports this hypothesis[18].

Because the effects of DEET varied with the specific OSN and odour tested, it seems unlikely that DEET acts directly and solely on the conserved co-receptor ORCO, which is co-expressed in all the OSNs examined here. To determine whether DEET acts on the odour-specific odorant receptor subunit, we focused on the pharmacology of the OR59B–ORCO complex in ab2A OSNs. 1-octen-3-ol inhibits basal activity of OR59B–ORCO at low concentrations but acts as an agonist at high concentrations (Fig. 1d). DEET interfered with inhibition of

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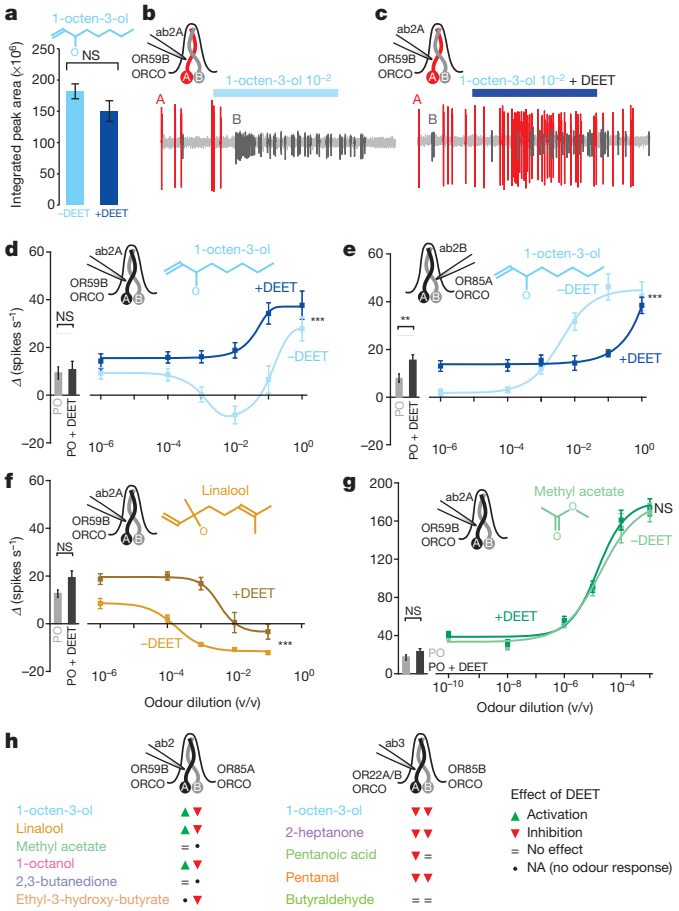


**Figure 1 | DEET scrambles the *Drosophila* odour code.** **a**, SPME and GC–MS quantitation of $10^{-2}$ 1-octen-3-ol emitted from the stimulus pipette in the absence (cyan bar) or presence (blue bar) of pure DEET. Data represent peak area (NS, not significant; *t*-test; mean ± s.e.m., $n = 5$). **b–c**, Representative traces of single-sensillum recordings from OR59B–ORCO in the ab2A OSN (red spikes) and OR85A–ORCO in the ab2B OSN (black spikes), stimulated by $10^{-2}$ 1-octen-3-ol with (**b**) and without (**c**) DEET, were recorded simultaneously and subsequently separated using spike-sorting algorithms. Bars represent 1-s odour stimulus. The delayed onset of odour response is a function of the odour delivery system. **d–g**, Dose–response curves of OR59B–ORCO in ab2A (**d**, **f**, **g**) and OR85A–ORCO in ab2B (**e**), stimulated with increasing concentrations of 1-octen-3-ol (**d**, **e**), linalool (**f**) and methyl acetate (**g**) in the absence (light colour) or presence (dark colour) of DEET. Bar plots next to the dose–response curves represent responses to the solvent paraffin oil (PO) in the absence (grey bar) or presence (black bar) of DEET (**$P < 0.01$, ***$P < 0.001$; *F*-test with Bonferroni correction; mean ± s.e.m., $n = 8–22$). *Δ*, relative response (Methods); v/v, volume concentration. **h**, Summary of effects of DEET on the *Drosophila* ab2 and ab3 odour code derived from dose–response curves in **d–g** and Supplementary Figs 1 and 2. The significance of the change in response due to co-application of odorant and DEET was assessed using an *F*-test. NA, not applicable.

OR59B–ORCO by 1-octen-3-ol, 1-octanol and linalool, but had no effect on odour-dependent activation by methyl acetate and 2,3-butanedione (Fig. 1g and Supplementary Fig. 1c). Notably, DEET had no effect on the OR59B–ORCO activation seen at higher concentrations of 1-octen-3-ol. This selective effect on inhibition might be explained by the presence on the OR59B receptor of distinct 1-octen-3-ol-interaction sites, a high-affinity site that inhibits the odorant receptor complex and is modulated by DEET and a low-affinity DEET-independent site that activates the odorant receptor complex.

To investigate the mechanistic basis of OR59B modulation by DEET, we turned to analysis of this receptor in *D. melanogaster* strains collected around the world. Polymorphisms in natural populations have been previously connected to different sensitivity to odours in humans[19,20], and oxygen and carbon dioxide sensing in the nematode *Caenorhabditis elegans*[21]. We reasoned that naturally occurring polymorphisms in insect odorant receptors might modify odorant receptor/odorant interaction sites and affect their sensitivity to DEET. To search for putative polymorphisms that affect DEET responses, we assessed responses of ab2A neurons to 1-octen-3-ol in $10^{-2}$ dilution in the absence or presence of DEET in 18 wild-type *D. melanogaster* strains from locations around the world, and compared these responses with those obtained in the $w^{1118}$ laboratory control strain (Fig. 2a, b and Supplementary Fig. 4a). In each strain, ab2 sensilla were identified by the characteristic size and location of the sensilla and responses of the ab2A cell to its cognate ligand, methyl acetate (data not shown). In 17 of the 18 strains, DEET increased responses of ab2A neurons to $10^{-2}$ 1-octen-3-ol (Fig. 2b). However, ab2A neurons in the Brazilian strain Boa Esperança were not inhibited by 1-octen-3-ol at any concentration tested and were therefore insensitive to modulation by DEET (Figs 2c and 3a, b and Supplementary Fig. 4b). In addition to the loss of inhibition by 1-octen-3-ol, the ab2A cell in the Brazilian strain showed robust activation by 1-octanol and ethyl hexanoate, odours that normally inhibit the ab2A cell in wild-type strains. Inhibition by linalool was equivalent in wild-type and Boa Esperança strains (Fig. 3e). Excitatory responses to methyl acetate, ethyl acetate and 2,3-butanedione, both in the absence and presence of DEET, did not differ when compared with the corresponding $w^{1118}$ neuron (Fig. 3c, d and Supplementary Fig. 5; data not shown). In control experiments, we confirmed that the odour response profiles of ab2A and ab2B OSNs in the Brazilian strain are otherwise similar to that of our $w^{1118}$ control strain (Fig. 3f and Supplementary Fig. 5).

We proposed that a genetic polymorphism in *Or59b* in the Boa Esperança strain may account for the changed responses to odour and DEET. We therefore sequenced and compared the coding region of *Or59b* in the 19 strains with the published *Or59b* sequence (NCBI reference sequence, NP_523822.1), and found seven missense polymorphisms and 36 silent polymorphisms among all strains (Supplementary Table 1 and Supplementary Fig. 6). The protein sequence of OR59B in Boa Esperança is referred to as $OR59B^{Boa}$ and varies from the NCBI reference at four amino-acid residues (Val41Phe, Val91Ala, Tyr376Ser and Val388Ala). Among these, two are unique to this strain: Val41Phe, located in the amino terminus near transmembrane domain 1 (TM1), and Val91Ala, located within TM2 (Fig. 4a, b and Supplementary Fig. 6). On the basis of our within-strain sampling, we detected only one protein variant per strain except for the $w^{1118}$ control strain, for which we identified two sequences: one identical to the published OR59B sequence ($OR59B^{NCBI\ REF}$), and one containing two missense changes ($OR59B^{M352I\ T376S}$; Fig. 4a and Supplementary Table 1). We analysed electrophysiological recordings obtained from the $w^{1118}$ control strain for each odour tested and found no evidence that the responses sort into two phenotypically separable clusters. Therefore, we assume that the $OR59B^{NCBI\ REF}$ and $OR59B^{I352\ S376}$ haplotypes are functionally equivalent, at least for the odours tested in this study. The coding sequences of *Orco* in the $w^{1118}$ and Boa Esperança strains did not differ from the NCBI reference (data not shown), which suggests that the protein sequence variations in the odour-specific subunit OR59B, rather than the co-receptor ORCO, eliminate inactivation by low concentrations of 1-octen-3-ol and thereby render the odorant receptor complex insensitive to modulation by DEET.

To test the functional consequences of the four OR59B missense changes in the Boa Esperança strain, we generated transgenic flies carrying receptor variants each containing one of the four changes (Val41Phe, Val91Ala, Tyr376Ser or Val388Ala), a combination of the two unique to Boa Esperança (Val41Phe and Val91Ala) or those shared with other strains (Tyr376Ser and Val388Ala), based on the $OR59B^{NCBI\ REF}$ backbone. OR59B variants were selectively expressed in the *Drosophila Δhalo* 'empty neuron' system[17,22], in which the endogenous odour-specific odorant receptors in ab3A OSNs were replaced

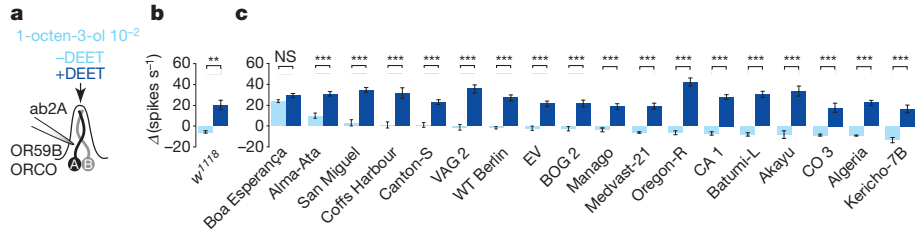


**Figure 2 | OR59B–ORCO sensitivity to DEET varies across wild-type *D. melanogaster* strains.** **a**, Schematic of the screening protocol: $10^{-2}$ 1-octen-3-ol was delivered in the absence and presence of DEET. **b**–**c**, Bar plots of odour-evoked responses of the $w^{1118}$ strain (**b**) and 18 wild-type strains (**c**) to $10^{-2}$ 1-octen-3-ol in the absence (light blue) or presence (dark blue) of DEET (*t*-test with Bonferroni correction; mean ± s.e.m., $n = 10$–17).

with our OR59B mutants (Fig. 4c and Supplementary Fig. 7). As expected, $10^{-2}$ 1-octen-3-ol caused inhibition of ab3A neurons expressing $OR59B^{NCBI\ REF}$ and activation of ab3A neurons expressing $OR59B^{Boa}$ (Fig. 4c). Whereas $OR59B^{T376A}$, $OR59B^{V388A}$ and $OR59B^{T376A\ V388A}$ showed normal inhibition to this odour, any variant of OR59B containing the Val91Ala change showed a loss of odour inhibition by 1-octen-3-ol and insensitivity to DEET (Fig. 4c). This demonstrates that the Val91Ala change is sufficient to phenocopy the electrophysiological properties of the endogenous Boa Esperança OR59B (Fig. 4c). It has previously been shown that responses of OR59B expressed in the empty neuron faithfully recapitulate receptor function measured in the endogenous ab2A neuron[23]. We therefore assume that a strain carrying only the $OR59B^{V91A}$ polymorphism would have the same phenotype as Boa Esperança.

DEET shows behavioural efficacy in insects as diverse as *Drosophila*[5,7] and mosquitoes[1–4,6,8–11]. We have shown that a single, naturally occurring polymorphism in an odour-specific odorant receptor can modify receptor interactions with an inhibitory odour and render the receptor insensitive to modulation by DEET. These results provide compelling evidence that DEET interacts directly with an odour-specific odorant receptor. Consistent with this, recent work showed that an odour-specific OR subunit is required for the behavioural effects of DEET on mosquito larvae[11]. Our data imply a complexity in ligand-binding interactions within a single insect odorant receptor complex that bears further investigation. The Val91Ala polymorphism is located in the second predicted transmembrane domain but little is known about which domains of this novel class of odour-gated ion channels contribute to ligand binding or ion channel function[13,14]. A recent study implicated the third predicted transmembrane

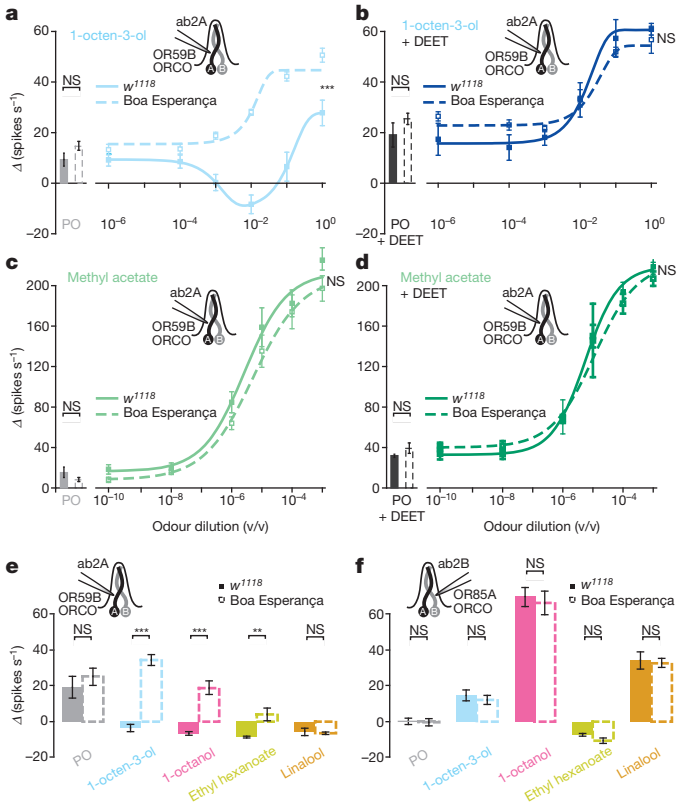


**Figure 3 | OR59B–ORCO neurons in the Boa Esperança strain are insensitive to modulation by DEET.** **a**–**d**, Dose–response curves of the OR59B–ORCO ab2A OSN in wild-type $w^{1118}$ (solid line) and Boa Esperança (dashed line) strains stimulated with increasing concentrations of 1-octen-3-ol (**a**, **b**) or methyl acetate (**c**, **d**), with (**b**, **d**) or without (**a**, **c**) DEET (*F*-test with Bonferroni correction; mean ± s.e.m., $n = 5$–14). The dose–response curve of $w^{1118}$ to 1-octen-3-ol in **a** and **b** is reproduced from Fig. 1d for comparison. Bar plots next to the dose–response curves represent responses to the solvent paraffin oil in the absence (grey bar) or presence (black bar) of DEET (*F*-test with Bonferroni correction; mean ± s.e.m., $n = 5$–11). **e**, **f**, Bar plots comparing responses of OR59B–ORCO in ab2A (**e**) and OR85A–ORCO in ab2B (**f**) in $w^{1118}$ (solid bar) and Boa Esperança (dashed bar) strains to $10^{-2}$ 1-octen-3-ol, $10^{-1}$ 1-octanol, $10^{-1}$ ethyl hexanoate and $10^{-1}$ linalool (*t*-test with Bonferroni correction; mean ± s.e.m., $n = 9$–11).

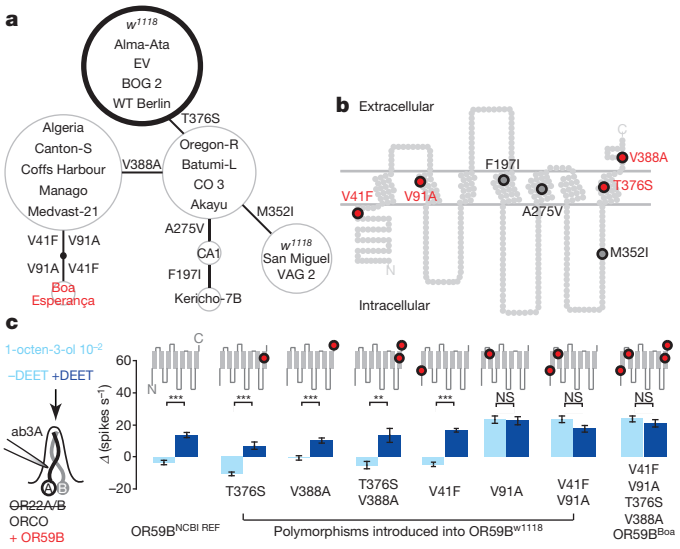


**Figure 4 | A single natural polymorphism in OR59B confers insensitivity to DEET.** **a**, Haplotype network of OR59B protein variants. Each circle represents a unique OR59B protein variant, its size proportional to the number of strains containing each variant. Connecting lines show the amino-acid substitutions that separate each variant. The bold circle represents the $OR59B^{NCBI\ REF}$ variant with NCBI accession code NP_5238822.1. The Boa Esperança strain is shown in red. **b**, Snake plot of OR59B showing the location of missense polymorphisms. Changes that differentiate Boa Esperança from the NCBI reference are shown in red. **c**, Bar plots show the responses of *Or59b* variants ectopically expressed in ab3A neurons lacking endogenous OR22A and OR22B to $10^{-2}$ 1-octen-3-ol in the absence (light blue) or presence (dark blue) of DEET. The locations of variant amino acids in OR59B are depicted in the cartoon snake plot on top of each set of bar graphs (*t*-test with Bonferroni correction; mean ± s.e.m., $n = 7$–11).

domain of an insect odorant receptor in ligand interactions[24], and additional structure–function work of this nature will ultimately reveal how these membrane proteins interact with odorants and modulators including DEET. Although Val and Ala are both amino acids with small aliphatic side chains, Val–Ala substitutions have been shown to affect other cation channels[25]. It therefore is plausible that this change would affect the function of the odour-gated ion channel subunit encoded by OR59B. We speculate that the Val91Ala polymorphism inactivates a high-affinity binding site for 1-octen-3-ol that locks the receptor into a closed configuration at low odour concentration. A separate site on the receptor would have a low-affinity binding site that would lead to activation. In this model, DEET would selectively interfere with the high-affinity binding site. Future investigation of the structure–function relation of this receptor is needed to test these ideas. Genetic insensitivity to DEET has previously been shown to exist in both *Drosophila* flies[5] and *Aedes aegypti* mosquitoes[10] but the genes responsible remain unknown. It will be interesting to investigate whether accumulated odorant receptor polymorphisms contribute to these phenotypes.

It has recently been proposed that DEET directly activates behavioural repulsion through the activation of odorant receptors that mediate avoidance behaviours[8–10]. The insect odorant receptor repertoire is highly diverse with very low protein similarity across insect species[26–28]. Furthermore, different species respond very selectively to host odour cues that meet disparate ecological needs[29,30]. It seems unlikely that a single molecule like DEET would activate a different yet similarly potent repulsive behaviour in all insects tested. Instead, our data support the hypothesis that DEET is a broad-selectivity insect odorant receptor modulator that alters the fine-tuning of the insect olfactory system. DEET-mediated scrambling of the odour code would interfere with behavioural responses as diverse as mosquitoes orienting to host odours produced by humans[29] and the attraction of *Drosophila* to yeast on rotting fruit[30].

## METHODS SUMMARY

**Fly strains and molecular biology.** *D. melanogaster* stocks were maintained on conventional cornmeal–agar–molasses medium in a 12-h-light, 12-h-dark cycle at 25 °C. Details of molecular biology manipulations, all primers and fly strains are in Methods.

**Single-sensillum extracellular recordings.** Recordings of female fly antennae were performed as described previously[7] and are detailed in Methods. The respective amounts of 1-octen-3-ol emitted from the stimulus pipettes with and without DEET was investigated through SPME and linked GC–MS analysis as detailed in Methods.

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

**Received 17 September 2010; accepted 8 August 2011.**
**Published online 21 September 2011.**

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

**Acknowledgements** We thank C. Bargmann, K. Lee, K. Scott, L. Stowers and members of the Vosshall lab for discussion and comments on the manuscript; and K. Weniger for technical assistance with the SPME and GC–MS experiments. This work was funded in part by a grant to R. Axel and L.B.V. from the Foundation for the National Institutes of Health through the Grand Challenges in Global Health Initiative and by a grant to L.B.V. from the NIH (RO1 DC008600). L.B.V. is an investigator of the Howard Hughes Medical Institute. M.C.S. and B.S.H. are supported by the Max Planck Society.

**Author Contributions** M.P. carried out all the experiments and analysed the data. N.S. contributed to sequencing *Or59b* in the 19 strains and generated the *Or59b* mutants. M.C.S. and B.S.H. designed and supervised the SPME collections and GC–MS analysis in Fig. 1a. M.P. and L.B.V. together designed the experiments, interpreted the results, produced the figures and wrote the paper.

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## METHODS

**Genomic DNA.** DNA was prepared according to the Quick Fly Genomic DNA Prep protocol from the Berkeley Drosophila Genome Project (http://www.fruitfly.org/about/methods/inverse.pcr.html). DNA (1.5 µl) was used for amplification using the KOD PCR Kit (Novagen). For *Or59b*, primers were designed to anneal to the 5′ and 3′ untranslated regions of the $w^{1118}$ *Or59b* locus: 5′-gaattcTCCGGG TATAAAGTGCAGGTGCTGGCACCG-3′ (forward); 5′-ctcgagGCTCTTTTTT GCGGGGGCTCATGGGTGCAG-3′ (reverse).

*Orco* was amplified using primers that amplify the complete coding region: 5′-gaattcATGACAACCTCGATGCAG-3′ (forward); 5′-caattgCTTGAGCTGCA CCAGCACCA-3′ (reverse).

PCR products were cloned into pGEM-T Easy (Promega Corporation,), sequenced (GENEWIZ, Inc.) and analysed using SeqMan software (DNASTAR, Inc.). For each strain, at least four independent samples were analysed, derived from at least two different genomic preparations and two different PCR reactions. These were sequenced and compared to NCBI reference sequences for each gene (*Or59b*: NM_079098.1; *Orco*: NM_079511.4).

**Complementary DNA preparation and transgenic flies.** Total RNA was extracted from $w^{1118}$ and Boa Esperança antennae using the RNeasy Mini Kit (QIAGEN).

Complementary DNA (cDNA) synthesis was performed according to the SuperScript III First-Strand Synthesis System for RT–PCR (Invitrogen) using oligo(dT) primers. *Or59b* cDNA from both $w^{1118}$ and Boa Esperança was amplified using these gene-specific primers: 5′-gaattcATGGCGGTGTTCAAGCT AATCAAACCG-3′ (forward); 5′-ctcgagTTACTGGAACTGCTCGGCCAGATT CA-3′ (reverse).

PCR products representing full-length $w^{1118}$ $Or59b^{NCBI\ REF}$ and $Or59b^{Boa}$ cDNAs were cloned into pGEM-T Easy, completely sequenced and subcloned into the pUAST attB vector[31] using EcoRI and XhoI restriction sites.

Single point mutations were introduced into the $w^{1118}$ $Or59b^{NCBI\ REF}$ cDNA by directed PCR mutagenesis. Two independent reactions were prepared: one contained the forward primer with the desired mutation and the reverse SP6 vector primer (5′-ATTTAGGTGACACTATAG-3′). The second contained the reverse mutating primer and the forward T7 vector primer (5′-TAATACGACTCAC TATAGGG-3′). PCR products from the reactions were purified and 1 µl of each was used as a template and mixed in a second round of amplification with T7 and SP6 primers to obtain the full gene. For each mutagenesis, the final PCR product was purified and subcloned in pGEM-T Easy, and the complete *Or59b* cDNA carrying the induced mutations was sequenced for verification and compared with the $Or59b^{NCBI\ REF}$ sequence.

The double mutants $Or59b^{V41F\ V91A}$ and $Or59b^{T376S\ V388A}$ were generated using $Or59b^{V41F}$ or $Or59b^{T376S}$ as a template and a second round of mutagenesis was implemented with the corresponding primers.

The following primers were used. $Or59b^{V41F}$: 5′-CCGCCGAAGGAGGGATT CCTGCGCTACGTGT-3′ (forward); 5′-ACACGTAGCGCAGGAATCCCTCC TTCGGCGG-3′ (reverse). $Or59b^{V91A}$: 5′-AGGTGTGCATCAATGCGTATGGC GCCTCGG -3′ (forward); 5′-CCGAGGCGCCATACGCATTGATGCACACCT -3′ (reverse). $Or59b^{T376S}$: 5′-TGAACAGCAACATAAGCGTGGCCAAGTTC GC-3′ (forward); 5′-GCGAACTTGGCCACGCTTATGTTGCTGTTCA-3′ (reverse). $Or59b^{V388A}$: 5′-GCATCATTACAATAGCGCGACAAATGAATCT-3′ (forward); 5′-AGATTCATTTGTCGCGCTATTGTAATGATGC-3′ (reverse).

Transgenic animals were generated in the $w^{1118}$ genetic background (Genetic Services, Inc.) using the phiC31-based integration system[31] targeted at the attP2-docking site on chromosome II (ref. 32).

**Fly stocks.** *Drosophila melanogaster* stocks were maintained on conventional cornmeal–agar–molasses medium in a 12-h-light, 12-h-dark cycle at 25 °C. The $w^{1118}$ strain was used as wild-type control.

The following wild-type strains were used: Akayu [*Drosophila* Genetic Resource Center (DGRC) #103389; origin, Japan]; Algeria (isogenic for II and III chromosomes, DGRC #103390; origin, Algeria); Alma-Ata (DGRC #103391; origin, Kazakhstan); Canton-S (isogenic for II and III, lab stock; origin, Ohio, USA); CA1 (Bloomington *Drosophila* Stock Center #3846; origin, Cape Town, South Africa); Coffs Harbour (DGRC #103411; origin, New South Wales, Australia); Kericho-7B (DGRC #103428; origin, Kericho, Kenya); Manago (isogenic for II and III, DGRC #103433; origin, Hawaii, USA); Oregon-R (isogenic for II and III, lab stock; origin, Oregon, USA); San Miguel (isogenic for II and III, DGRC #103450; origin, Buenos Aires, Argentina); WT Berlin (isogenic for II and III, Heisenberg laboratory, Würzburg, Germany; origin, Berlin, Germany); Batumi-L (DGRC #103396; origin, Batumi, Georgia); Boa Esperança (DGRC #103400; origin, Minas Gerais, Brazil); BOG2 (Bloomington #3842; origin, Bogota, Colombia); CO3 (Bloomington #3848; origin, Commack, New York, USA); EV (Bloomington #3851; origin, Ellenville, New York, USA); Medvast-21 (DGRC #103435; origin, Finland); VAG 2 (Bloomington #3876; origin, Athens, Greece).

The following mutant alleles and transgenic flies were used: $Or22a/b^{\Delta halo}$ (ref. 33) and *Or22a-Gal4* (ref. 34). The genotypes of the flies used for Fig. 4c and Supplementary Fig. 8 are as follows: $Or22a/b^{\Delta halo}$;*Or22a-Gal4*/*UAS-Or59b* (labelled $Or59b^{NCBI\ REF}$ in the figure), $Or22a/b^{\Delta halo}$;*Or22a-Gal4*/*UAS-*$Or59b^{V41F}$ (V41F), $Or22a/b^{\Delta halo}$;*Or22a-Gal4*/*UAS-*$Or59b^{V91A}$ (V91A), $Or22a/b^{\Delta halo}$;*Or22a-Gal4*/*UAS-*$Or59B^{V41F\ V91A}$ (V41F V91A), $Or22a/b^{\Delta halo}$;*Or22a-Gal4*/*UAS-*$Or59b^{T376S}$ (T376S), $Or22a/b^{\Delta halo}$;*Or22a-Gal4*/*UAS-*$Or59b^{V388A}$ (V388A), $Or22a/b^{\Delta halo}$;*Or22a-Gal4*/*UAS-*$Or59b^{T376S\ V388A}$ (T376S V388A) and $Or22a/b^{\Delta halo}$;*Or22a-Gal4*/*UAS-*$Or59b^{V41F\ V91A\ T376S\ V388A}$ (V41F V91A T376S V388A).

**SPME quantification of emitted volatiles.** The effect of DEET on the amount of 1-octen-3-ol emitted from the stimulus pipettes was investigated through SPME and linked GC-MS analysis. Stimulus pipettes, prepared as per the electrophysiology experiments, were loaded either with one filter strip impregnated with 5 µl of 1-octen-3-ol ($10^{-2}$) and with a second strip containing 5 µl of paraffin oil, or with the second strip impregnated with 5 µl of pure DEET. The pipettes were connected to a stimulus controller (Syntech CS 55; www.syntech.nl) and volatiles emitted from the pipettes during ten puffs, of 2-s duration each, delivered with 1-s intervals, were trapped on a SPME fibre (Supelco blue fibre; 57310-U; polydimethylsiloxane/divinylbenzene, 65-µm coating; http://www.sigmaaldrich.com), inserted 2 cm into the pipette tip. After completion of the stimulus cycle, the SPME fibres were immediately retracted and injected into a GC–MS device for quantification. This device (Agilent GC6890N fitted with MS5975B unit; http://www.agilent.com) was equipped with a HP5-MS column (Agilent Technologies) and operated as follows. The inlet temperature was set to 250 °C. Desorption time was 1 min. The temperature of the gas chromatography oven was held at 70 °C for 2 min and then increased by 20 °C $min^{-1}$ to 280 °C, with the final temperature held for 2 min. For mass spectroscopy, the transfer line was held at 280 °C, the source at 230 °C and the quad at 150 °C. Mass spectra were taken in EI mode (at 70 eV) in the range from 33$m/z$ to 350$m/z$, with a scanning rate of 4.42 scans per second. GC–MS data were processed with the MDS-CHEMSTATION software (Agilent Technologies), and peak areas were autointegrated. Five replicates were collected for each condition and data were plotted as mean ± s.e.m. Statistical significance was assessed using a *t*-test.

**Electrophysiology and odorants.** Female transgenic flies were recorded at 5 d after adult eclosion. All other flies were recorded at 5–10 d after adult eclosion. Single-sensillum recordings were performed as described previously[35,36]. For each experiment in which we recorded OR59B variants expressed in the ab3A neuron, we verified that responses of endogenous OR59B in the native ab2A neuron showed normal inhibition by $10^{-2}$ 1-octen-3-ol (data not shown). Odorants were obtained from Sigma-Aldrich at high purity and diluted (v/v) in paraffin oil as indicated. DEET was obtained from Alfa Aesar and was applied undiluted. Chemical Abstracts Service (CAS) numbers are as follows: paraffin oil (8012-95-1); 1-octen-3-ol (3391-86-4); pentanal (110-62-3); pentanoic acid (109-52-4); 2-heptanone (110-43-0); 1-octanol (111-87-5); (−)linalool (126-91-0); methyl acetate (79-20-9); 2,3-butanedione (431-03-8); ethyl hexanoate (123-66-0); butyraldehyde (123-72-8); ethyl-3-hydroxybutyrate (5405-41-4); ethyl acetate (141-78-6); hexanol (111-27-3); DEET (134-62-3).

The desired odour dilution (30 µl) was pipetted onto a filter paper strip (3 mm × 50 mm) and 30 µl of undiluted DEET or paraffin oil solvent was pipetted onto a second filter paper strip. Both filter paper strips were then carefully inserted into a glass Pasteur pipette. Before any recordings, charcoal-filtered air was forced through the pipette for 1–3 s to remove dead space in the odour delivery system. For actual recordings, charcoal-filtered air was continuously applied to the insect antenna, with odour delivered through the pipette to the fly antennae for 1 s. Each pipette was used at most three times and no more than three sensilla were tested per animal. Sensilla types were identified by size, location on the antenna and responsiveness to known preferred odorants[37].

Data were collected using AUTOSPIKE (Syntech) and analysed by custom spike-sorting algorithms[35]. Responses were initially classified as excitatory or inhibitory by visual inspection of the responses after odour application. An odour was classified as excitatory if it increased the spontaneous firing rate and inhibitory if it decreased the spontaneous firing rate. The data were then analysed by subtracting average spontaneous activity (expressed as spikes per second) in the 15 s before odour application from activity either in the first 600 ms after odour delivery, for excitatory odorants, or in the first 1 s, for inhibitory odorants. This value is referred to as $\Delta$, and will typically have a negative value for inhibitory odorants and a positive value for excitatory odorants. The onset of odour-evoked responses varied owing to slight variations in the position of the odour delivery system relative to the sensillum being recorded. To correct for this, we calibrated the inferred odour onset on the basis of excitatory responses elicited by control stimuli applied at the beginning of each trial (ab2, $10^{-5}$ methyl acetate; ab3, $10^{-5}$ 2-heptanone).

**Statistical analysis.** Dose–response curves were fitted with ORIGINPRO 8 (OriginLab) using a logistic function, except for responses to 1-octen-3-ol in Fig. 1d, which used a biphasic function.

Comparisons of paired dose–response curves in Figs 1 and 3 and Supplementary Figs 1, 2 and 4 used an *F*-test to assess the statistical significance of differences between the two curve fits. A two-tailed *t*-test was performed for all comparisons in Fig. 1i (non-paired), Figs 2–4 and Supplementary Figs 3, 4 and 7 (paired). Type I errors were addressed by using a Bonferroni correction for multiple comparisons applied to each set of experiments. Data in Supplementary Fig. 6 were fitted using a linear regression analysis.

The OR59B snake plots in Fig. 4 and Supplementary Fig. 7 were hand-composed on the basis of transmembrane domain predictions generated with the PredictProtein algorithm[38].


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