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

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Abstract

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 appears to act both at close range as a contact chemorepellent by acting on insect gustatory receptors[12] and at long range by acting on 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] or that DEET actively repels insects by activating olfactory neurons that elicit avoidance behaviour[8-11]. Here we show that the insect repellent DEET functions as a modulator of the odour-gated ion channel formed by the insect odorant receptor (OR) complex[13, 14]. The functional insect OR complex consists of a common co-receptor, Orco (ref. [15], formerly called Or83b, ref[16]), and one or more variable OR 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 OR and the concentration and identity of the odour ligand. We identify a single amino acid polymorphism in the second transmembrane domain of Or59b in a *Drosophila melanogaster* strain from Brazil that renders this

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receptor insensitive to inhibition by the odour ligand and modulation by DEET. These data provide the first evidence that natural variation can modify the sensitivity of an odour-specific insect OR to odour ligands and DEET. Our data 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 *Drosophila 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 to distinguish between the competing models of action for DEET or whether DEET acts on the odour-specific ORs, the olfactory co-receptor Orco, or both. We carried out electrophysiological recordings of *Drosophila* olfactory sensory neurons (OSNs) to test these competing hypotheses.

In response to the suggestion that DEET and odours may interact in the vapour phase[9,10], we first quantified the amounts of vapour-phase 1-octen-3-ol emitted from the stimulus pipette, in the presence or absence of DEET, using solid phase microextraction (SPME) and subsequent 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 employed 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; 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 ORs 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 10 structurally diverse odours was complex and OR-, odour-, and concentration-dependent. In some OSNs, DEET suppressed odour-mediated inhibition (Fig. 1d, f; Supplementary Fig. 1a), in others it decreased odour-induced activation (Fig. 1e, Supplementary Fig.1b, d, e; Supplementary Fig. 2a-g), and in others it had no effect (Fig. 1g; Supplementary Fig. 1c; Supplementary Fig. 2h-j). Moreover, the effects of DEET were strongly concentration dependent, such that high odour concentrations often overcame the effects of DEET (Fig. 1, Supplementary Figs. 1-2). DEET presented alone without odour stimuli elicited no response above that evoked by solvent in ab2A and ab3A neurons, slightly activated ab2B and slightly inhibited ab3B, 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.

Interestingly, 1-octen-3-ol presented at a dilution of 10^{-2} had opposing 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 opposing 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 via direct modulation of OR activity dependent on the type of odour and its concentration (Fig. 1h). Recent work from Bohbot and Dickens examining the effect of DEET on mosquito ORs 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 Orco co-receptor, which is co-expressed in all the OSNs examined here. To ask if DEET acts on the odour-specific OR 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 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). Interestingly, DEET had no effect on 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 OR complex and is modulated by DEET and a low affinity DEET-independent site that activates the OR complex.

To investigate the mechanistic basis of Or59b modulation by DEET, we turned to analysis of this receptor in *Drosophila 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 ORs might modify OR-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 10^{-2} 1-octen-3-ol in the absence or presence of DEET in 18 wild type *Drosophila melanogaster* strains originating from locations around the world and compared these responses to those obtained in the w^{1118} laboratory control strain (Fig. 2a-b; 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 (Fig. 2c; Fig. 3a-b; 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; Supplementary Fig. 5; data not shown). In control experiments, we confirmed that the odour response profiles of both ab2A and ab2B OSNs in the Brazilian strain have odour response profiles otherwise similar to our w^{1118} control strain (Fig. 3f; Supplementary Fig. 5).

We hypothesised 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 number NP_5238822.1), and found seven missense polymorphisms and 36 silent polymorphisms among all strains (Supplementary Table 1, Supplementary Fig. 6). The protein sequence of Or59b in Boa Esperança varies from the NCBI reference at four amino acid residues (V41F V91A T376S V388A; referred to as $Or59b^{Boa}$). Among these, two are unique to this strain: V41F, located in the N-terminus near TM1, and V91A, located within TM2 (Fig. 4a-b and Supplementary Fig. 6). Based on our within-strain sampling, we detected only one protein variant per strain, with the exception of 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 analyzed 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 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 and not the Orco co-receptor eliminate inactivation by low concentrations of 1-octen-3-ol, and thereby render the OR 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 containing each one of the four changes (V41F, V91A, T376S, or V388A), a combination of the two unique to Boa Esperança (V41F V91A), or those shared with other strains (T376S V388A), based on the $Or59b^{NCBI\ REF}$ backbone. Or59b variants were selectively expressed in the *Drosophila* Δ*halo* "empty neuron" system[17, 22] in which the endogenous odour-specific ORs in ab3A OSNs were replaced with our Or59b mutants (Fig. 4c; Supplementary Fig. 7). As expected, 10^{-2} 1-octen-3-ol caused inhibition of ab3A neurons expressing $Or59b^{NCBI\ REF}$ and activation when expressing $Or59b^{Boa}$ (Fig. 4c). While $Or59b^{T376A}$, $Or59b^{V388A}$, and $Or59b^{T376A\ V388A}$ showed normal inhibition to this odour, any variant of Or59b containing the V91A change showed a loss of odour inhibition by 1-octen-3-ol and insensitivity to DEET (Fig. 4c). This demonstrates that the V91A 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], but the mechanism by which this insect repellent acts on the olfactory system remains highly controversial. In this study, we show that a single naturally occurring polymorphism in an odour-specific OR 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 OR. Indeed, recent work from Zwiebel and co-workers showed a dependence of not only the conserved Orco subunit but also an odour-specific subunit for behavioural effects of DEET on mosquito larvae[11]. Our data imply a complexity in ligand-binding interactions within a single insect OR complex that bears further investigation. The V91A 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 contributes to ligand binding or ion channel function[13, 14]. A recent study implicated the third predicted transmembrane domain of an insect OR 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 V and A are both amino acids with small aliphatic side chains, V/A 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 V91A 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 structure-function investigation 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 gene(s) responsible remain unknown. It will be interesting to investigate if accumulated OR polymorphisms contribute to these phenotypes.

It has recently been proposed that DEET directly activates behavioural repulsion through the activation of ORs that mediate avoidance behaviours[8-10]. The insect OR repertoire is highly diverse with very low protein similarity across insects 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 OR 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] or the attraction of *Drosophila* flies to yeast on rotting fruit[30].

Methods Summary

Fly strains and molecular biology

Drosophila melanogaster stocks were maintained on conventional cornmeal-agar-molasses medium under a 12 hour light:12 hour dark cycle at 25°C. Details of molecular biology manipulations, all primers, and fly strains are in the online Methods.

Single sensillum extracellular recordings

Recordings of female fly antennae were performed as described[7] and detailed in online Methods. The amount of 1-octen-3-ol emitted from the stimulus pipettes with and without DEET was investigated through solid phase microextraction (SPME) and linked gas chromatography mass spectroscopy (GC-MS) analysis as detailed in the online Methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix

Online 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). 1.5 μl of DNA were used for amplification using the KOD PCR Kit (Novagen, Madison, WI, USA). For *Or59b*, primers were designed to anneal to the 5′ and 3′ UTR of the w^{1118} *Or59b* locus:

Forward: 5′-gaattcTCCGGGTATAAAGTGCAGGTGCTGGCACCG-3′

Reverse 5′-ctcgagGCTCTTTTTTGCGGGGGCTCATGGGTGCAG-3′

Orco was amplified using primers that amplify the complete coding region:

Forward: 5′-gaattcATGACAACCTCGATGCAG-3′

Reverse: 5′-caattgCTTGAGCTGCACCAGCACCA-3′

PCR products were cloned into pGEM-T Easy (Promega Corporation, Madison, WI, USA), sequenced (GENEWIZ, Inc., South Plainfield, NJ, USA), and analyzed using SeqMan software (DNASTAR Inc., Madison, WI, USA). For each strain, at least 4 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).

cDNA preparation and transgenic flies

Total RNA was extracted from w^{1118} and Boa Esperança antennae using the RNeasy Mini Kit (QIAGEN, Valencia, CA, USA). cDNA synthesis was performed according to the SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA,

USA) using oligo(dT) primers. *Or59b* cDNA from both w^{1118} and Boa Esperança was amplified using these gene-specific primers:

Forward: 5′-gaattcATGGCGGTGTTCAAGCTAATCAAACCG-3′

Reverse: 5′-ctcgagTTACTGGAACTGCTCGGCCAGATTCA-3′

PCR products representing full-length w^{1118} $Or59b^{NCBI\ REF}$ and $Or59b^{Boa\ Esperança}$ cDNAs were cloned into pGEM-T Easy, completely sequenced, and subcloned into the pUAST attB vector[1] 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′-TAATACGACTCACTATAGGG-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, subcloned in pGEM-T Easy, and the complete *Or59b* cDNA carrying the induced mutations was sequenced for verification and compared to 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: $Or59b^{V41F}$

Forward: 5′-CCGCCGAAGGAGGGATTCCTGCGCTACGTGT-3′

Reverse: 5′-ACACGTAGCGCAGGAATCCCTCCTTCGGCGG-3′

$Or59b^{V91A}$

Forward: 5′-AGGTGTGCATCAATGCGTATGGCGCCTCGG-3′

Reverse: 5′-CCGAGGCGCCATACGCATTGATGCACACCT-3′

$Or59b^{T376S}$

Forward: 5′-TGAACAGCAACATAAGCGTGGCCAAGTTCGC-3′

Reverse: 5′-GCGAACTTGGCCACGCTTATGTTGCTGTTCA-3′

$Or59b^{V388A}$

Forward: 5′-GCATCATTACAATAGCGCGACAAATGAATCT-3′

Reverse: 5′-AGATTCATTTGTCGCGCTATTGTAATGATGC-3′

Transgenic animals were generated in the w^{1118} genetic background (Genetic Services Inc., Cambridge, MA, USA) using the phiC31-based integration system[1] targeted at the attP2 docking site on chromosome II[2].

Fly stocks

Drosophila melanogaster stocks were maintained on conventional cornmeal-agar-molasses medium under a 12 hour light:12 hour 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: Kazakstan); 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).

Mutant alleles and transgenic flies used: *Or22a/b*$^{\Delta halo}$ (ref. [3]), *Or22a-Gal4* (ref. [4]). Genotypes of the flies used for Fig. 4c and Supplementary Fig. 8: *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), *Or22a/b*$^{\Delta halo}$*; Or22a-Gal4*/*UAS-Or59b*$^{V41F\ V91A\ T376S\ V388A}$ (V41F V91A T376S V388A).

Solid Phase Microextraction (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 Gas-Chromatography Mass Spectroscopy (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 10 puffs, of 2 sec duration each, delivered with 1 sec intervals, were trapped on a SPME fiber (Supelco blue fiber; 57310-U; polydimethylsiloxane /divinylbenzene, 65 µm coating; www.sigmaaldrich.com), inserted 2 cm into the pipette tip. After completion of stimulus cycle, the SPME fibres were immediately retracted and injected into a GC-MS for quantification. The GC-MS (Agilent GC6890N fitted with MS5975B unit; 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 GC-oven was held at 70°C for 2

min and then increased by 20°C min^{-1} to 280°C with final temperature held for 2 min. The MS transfer-line was held at 280°C, the MS source at 230°C, and the MS quad at 150°C. Mass spectra were taken in EI mode (at 70 eV) in the range from 33 m z^{-1} to 350 m z^{-1} with a scanning rate of 4.42 scan s^{-1}. 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±SEM. Statistical significance was assessed with a t-test.

Electrophysiology and odorants

Female transgenic flies were recorded at 5 days after adult eclosion. All other flies were recorded at 5-10 days after adult eclosion. Single sensillum recordings were performed as described[5, 6]. 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 (Ward Hill, MA, USA) and was applied undiluted. Chemical Abstracts Service (CAS) numbers: 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).

30 μl of the desired odour dilution was pipetted onto a filter paper strip (3 × 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. Prior to 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[7].

Data were collected using Autospike (Syntech) and analyzed by custom spike sorting algorithms[5]. 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 analyzed by subtracting average spontaneous activity (expressed as spikes/s) in the 15 s before odour application from activity in the first 600 ms after odour delivery for excitatory odorants or 1 s for inhibitory odorants. This value is referred to as Δ spikes/s, which will typically have a negative value for inhibitory odorants and a positive value for excitatory odorants. The onset of odour-evoked responses varied due 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 for each sensillum recorded based on excitatory responses for each sensillum 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 using OriginPro 8 (OriginLab, Northampton, MA, USA) by a logistic function, except responses to 1-octen-3-ol in Fig. 1d, which used a biphasic function.

Comparisons of paired dose-response curves in Figs. 1, 3, and Supplementary Figs. 1-2, 4 used an F-test to assess 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, 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 fit using a linear regression analysis. The Or59b snake plots in Fig. 4 and Supplementary Fig. 7 were hand-composed based on transmembrane domain predictions generated with the PredictProtein algorithm[8].

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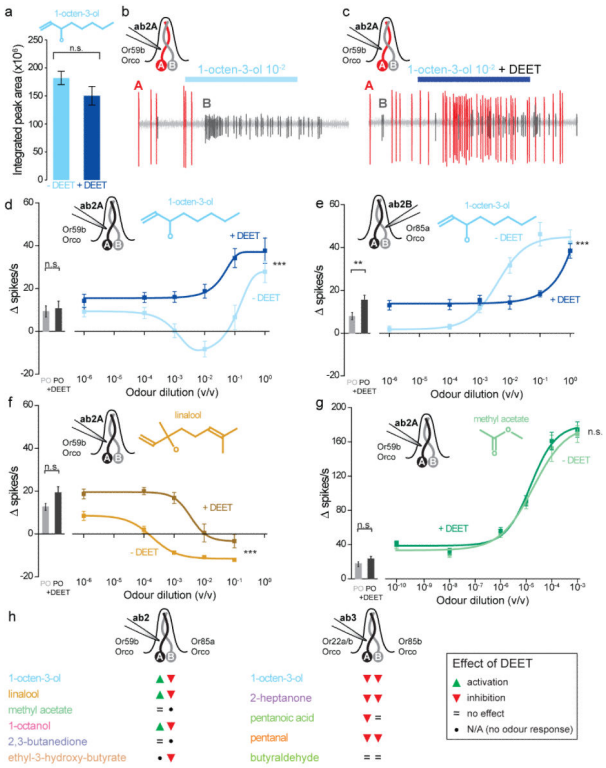

Figure 1. DEET scrambles the *Drosophila* odour code

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 (n.s.=not significant, t-test; mean±SEM, 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 by spike-sorting algorithms.. Bars represent 1s odour stimulus. The delayed odour response onset 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 each dose-response curve represent responses to the solvent paraffin oil in the absence (grey bar) or presence (black bar) of DEET (**p<0.01, ***p<0.001, n.s.=not significant, F-test with Bonferroni correction; mean±SEM, n=8-22). **h**, Summary of effects of DEET on the *Drosophila* ab2 and ab3 odour code derived from dose response curves in Fig. 1 and Supplementary Figs. 1-2. Significance of change in response due to co-application of odorant and DEET was assessed with an F-test.

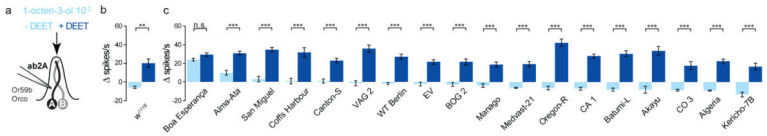

Figure 2. Or59b/Orco sensitivity to DEET varies across wild type *Drosophila melanogaster* strains

a,. Schematic of the screening protocol: 10^{-2} 1-octen-3-ol was delivered in the absence or 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 (***p<0.001, n.s.=not significant, t-test with Bonferroni correction; mean±SEM, n=10-17).

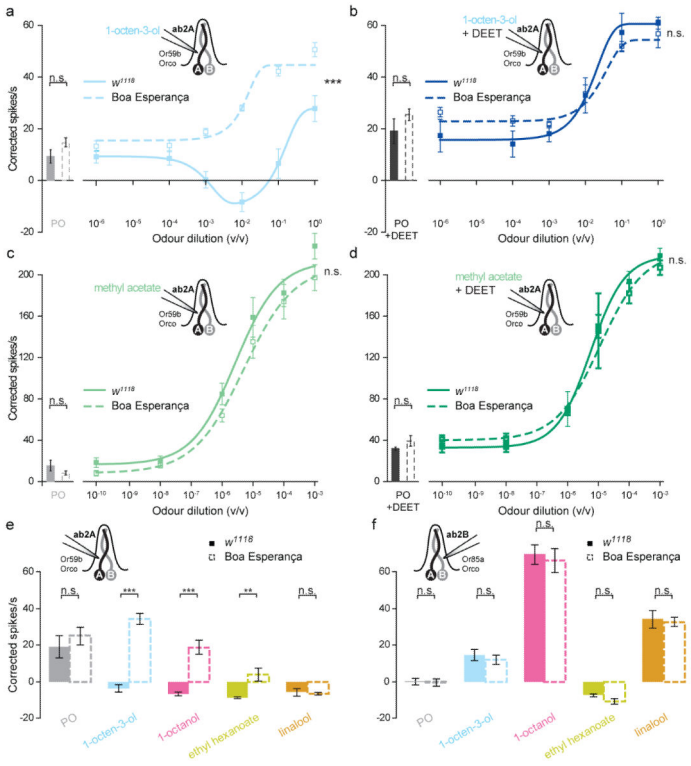

Figure 3. Or59b/Orco neurons in the Boa Esperança strain are insensitive to modulation by DEET

a-d, Dose-response curves of the Or59b/Orco ab2A OSN in wild type w^{1118} (solid line) and Boa Esperança (dotted 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 (***p<0.001, n.s.=not significant, F-test with Bonferroni correction; mean±SEM, n=5-14). The doseresponse curve of w^{1118} to 1-octen-3-ol in (**a**-**b**) is reproduced from Fig. 1d for comparison. Bar plots next to dose-response curves represent responses to the solvent paraffin oil in the absence (grey bar) or presence (black bar) of DEET (n.s.=not significant, F-test with Bonferroni correction; mean±SEM, n=5-11). **e-f**, Bar plots comparing responses of the Or59b/Orco in ab2A (**e**) and Or85a/Orco in ab2B (**f**) in w^{1118} (solid bar) and Boa Esperança (dotted bar) strains to 10^{-2} 1-octen-3-ol, 10^{-1} 1-octanol, 10^{-1} ethyl hexanoate, and 10^{-1} linalool (**p<0.01, ***p<0.001, n.s.=not significant, t-test with Bonferroni correction; mean±SEM, n=9-11).

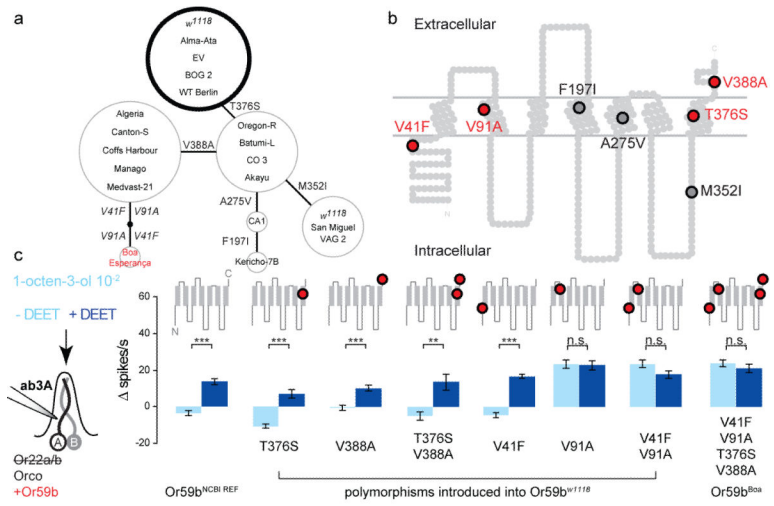

Figure 4. A single natural polymorphism in Or59b confers insensitivity to DEET

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 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/b to 10^{-2} 1-octen-3-ol in the absence (light blue) or presence (dark blue) of DEET. The location of variant amino acids in Or59b is depicted in the cartoon snake plot on top of each set of bar graphs (**p<0.01, ***p< 0.001, n.s.=not significant, t-test with Bonferroni correction; mean±SEM, n=7-11).