Conservation of Olfactory Avoidance in *Drosophila* Species and Identification of Repellents for *Drosophila suzukii*

Christine Krause Pham¹ & Anandasankar Ray¹,²

Flying insects use olfaction to navigate towards fruits in complex odor environments with remarkable accuracy. Some fruits change odor profiles substantially during ripening and related species can prefer different stages. In *Drosophila* species attractive odorants have been studied extensively, but little is understood about the role of avoidance pathways. In order to examine the role of the avoidance cue CO₂ emitted from fruit on behavior of two species with different ripening stage preferences, we investigated the CO₂-detection pathway in *Drosophila melanogaster* and *Drosophila suzukii*, a harmful pest of fruits. Avoidance to CO₂ is not conserved in *D. suzukii* suggesting a behavioral adaptation that could facilitate attraction to younger fruit with higher CO₂ emission levels. We investigated known innate avoidance pathways from five species at different evolutionary distances: *D. melanogaster*, *D. yakuba*, *D. suzukii*, *D. pseudoobscura* and *D. virilis*. Surprisingly, only DEET shows strong repellency across all species, whereas CO₂, citronellal and ethyl 3-hydroxybutyrate show only limited conservation. These findings guide us to test recently discovered safe DEET substitutes, and we identify one that protects fruits from *D. suzukii* thus providing a new behavioral strategy for controlling agricultural pests.

The two related species, *Drosophila melanogaster* (vinegar fly) and *Drosophila suzukii* (spotted wing *Drosophila*) provide an excellent model to study how changes in olfactory behavior are associated with changes in food preference. Drastic changes occur in the composition of volatiles emitted during fruit maturation, ripening and fermentation, potentially providing different cues for different species. *D. melanogaster* primarily feed and lay eggs on overripe and fermenting fruits often fallen from plants and avoid fruit that is still ripening on the plant. During the ripening process, fruits undergo higher rates of respiration and emit a higher level of CO₂, which *D. melanogaster* strongly avoid²⁻⁶. As the fruit over-ripens, levels of CO₂ emission decrease and a concomitant increase in yeast-derived volatiles occur. Volatiles from yeast can attract flies, and there are some (such as hexanol and 2,3-butanedione) that can also inhibit the aversive CO₂ receptor⁷ (Fig. 1A). Conversely, *D. suzukii* prefer to feed on ripening fruits and have evolved a specialized ovipositor that can tear through the skin of ripe berries to lay eggs⁸,⁹ and their larvae emerge inside causing hundreds of millions of dollars worth of agricultural damage worldwide¹⁰,¹¹.

Here we examine the olfactory determinants underlying these behavioral preferences and our findings can serve not only as an important model to understand adaptations in behavior, but can also be exploited to control insects that are harmful to humans. While attractive odor cues emitted from food play an important role in the differential selection process across species¹²,¹³, here we investigate the less studied changes in behavior towards aversive cues.

¹Interdepartmental Neuroscience Program, University of California, Riverside, CA 92521. ²Entomology Department, University of California, Riverside, CA 92521. Correspondence and requests for materials should be addressed to A.R. (email: anand.ray@ucr.edu)
Results

CO2 avoidance behavior is not conserved in all Drosophila species. In order to ask whether the CO2-avoidance pathway has been adapted to suit the D. suzukii change in behavior, we first investigated their ability to detect CO2. The CO2 receptor is comprised of two 7-transmembrane proteins Gr21a and Gr63a, which are housed in the ab1C neuron on the D. melanogaster antenna. We found that the amino acid sequences of both Gr21a and Gr63a are extremely well conserved in the D. suzukii genome (99% and 94%, respectively). In order to test whether the functional expression of the receptors occurs, we used single sensillum electrophysiology on the D. suzukii antenna (N = 5–6). Our results indicate that an ab1C-like neuron in D. suzukii is present and is in fact more sensitive to CO2 than D. melanogaster across different concentrations (Fig. 1B). We next tested D. suzukii preference for CO2 in a T-maze Assay. Surprisingly, D. suzukii did not show avoidance to CO2 at levels that elicit robust avoidance in D. melanogaster (P < 0.001) (Fig. 1C). These results suggest that while D. suzukii can detect CO2, other changes in processing this sensory information have occurred. Since D. suzukii are attracted to ripening fruits which emit CO2, we also tested whether CO2 can enhance attraction in the presence of food odors such as apple cider vinegar (ACV) as has been reported in D. melanogaster4. D. suzukii showed no significant increase in attraction to ACV in the presence of CO2 (P = 0.88) (Fig. 1D). While we do not know the behavioral significance of CO2 detection in this species yet, the ability to sense but not avoid CO2 may offer a distinct evolutionary advantage since D. suzukii feed on ripening fruits that respire and emit CO2.

Avoidance of CO2 is robust in the D. melanogaster lab strain and also in two recently caught wild-type strains tested in the T-maze Assay which suggests that repellency is not due to artificial selection in our lab strains (P = 0.01, P = 0.03) (Supplementary Fig. S1). These results pose an interesting question: how conserved is avoidance of CO2 in other related Drosophila species? In order to answer this question we performed a series of behavioral and electrophysiological experiments using 3 additional Drosophila species (Fig. 2A). Using the T-maze Assay we found that the closely related D. yakuba showed avoidance to carbon dioxide, albeit to a lower degree than D. melanogaster (Fig. 2B). However, like D. suzukii, the more distantly related D. virilis did not avoid carbon dioxide and D. pseudoobscura was only mildly (but not significantly) repelled at the highest concentration tested (Fig. 2B). The Gr21a and Gr63a amino acid sequences are highly conserved across all tested species: D. yakuba (100% and 97%), D. pseudoobscura (97% and 93%) and D. virilis (88% and 85%). We find that a CO2-sensitive ab1C-like neuron is present in each of these species from single sensillum recordings (Fig. 2C). These results suggest that detection of CO2 is conserved; however, the behavioral changes could likely be due to other changes such as processing CO2-detection information in downstream circuitry in the brain.

Since the CO2-pathway is the strongest known repellency pathway for some Drosophila species, we wondered whether other odorants that activate the CO2 receptors would serve as practical repellents that can be applied easily in areas to be protected unlike CO2, which is expensive and impractical for use. In a previous study we had identified pyridine, an animal skin odorant, as one of the strongest activators of the CO2 receptor14. At a 10⁻² concentration, pyridine elicited avoidance behavior in D. melanogaster and D. yakuba as expected from the response to carbon dioxide observed, and also in D. pseudoobscura which avoids CO2 to a lesser degree (Fig. 2D). In D. pseudoobscura, it is conceivable that other olfactory receptors may also contribute to pyridine repellency. Also, as expected the D. suzukii and D. virilis showed...
very little repellency to pyridine. A lower concentration of pyridine (at $10^{-4}$) was also tested; however, none of the species showed behavioral response in the T-maze Assay (data not shown).

The T-maze assays the instantaneous behavioral response of walking flies offered a choice between an odor and control. In order to test behavioral response of flying Drosophila in a long-term behavior we utilized a Two-Choice Trap Assay. Ten male and ten female starved flies are placed in a cylindrical chamber containing two entry traps lured with ACV, one of which also contained the CO$_2$-neuron activator pyridine as a convenient substitute for CO$_2$. Both D. suzukii and D. melanogaster showed no significant avoidance of the pyridine-treated trap (Supplemental Fig. S2) ($P=0.86$). While behavioral responses of free flying Drosophila to CO$_2$ have not been tested, tethered D. melanogaster that can beat their wings do not demonstrate clear anti-tracking behavior to CO$_2$.$^{15}$ This taken together with our results suggest that CO$_2$-receptor activating odorants such as pyridine are unlikely to act as broad-spectrum repellents for Drosophila species, particularly for the agriculturally important pest D. suzukii.

**Conservation of other olfactory avoidance pathways.** These findings prompted a systematic investigation of known repellent olfactory pathways in D. melanogaster and their conservation in related species. A second avoidance pathway in D. melanogaster is mediated by activation of Or85a, a member of another receptor gene family.$^{16}$ The strongest known activator of this receptor (identified by electrophysiology) is the odorant ethyl 3-hydroxybutyrate.$^{17-19}$ We tested two concentrations of ethyl 3-hydroxybutyrate ($10^{-2}$ and $10^{-4}$) in a T-maze Assay. All species showed no preference at the lower concentration. D. melanogaster had some avoidance of ethyl 3-hydroxybutyrate at the higher concentration (Fig. 3A). This response was conserved in D. yakuba. The D. suzukii showed a small repellency; however, the distantly related species D. virilis and D. pseudoobscura showed no behavioral response to ethyl 3-hydroxybutyrate. These behaviors are consistent with the observation that D. pseudoobscura and D. virilis lack a functional copy of the Or85a genes.$^{5,20-22}$

In order to test whether ethyl 3-hydroxybutyrate can reduce attraction towards an attractive odor source and to act over a longer time period, we used a Two-Choice Trap Assay. D. melanogaster avoids the apple cider vinegar trap with 1% ethyl 3-hydroxybutyrate (Fig. 3B). Participation in the Two-Choice Trap Assay for D. suzukii was very low and was not included in this analysis. The odorant was unable to repel the three other species from the lure. These results reinforce our view that for some odorants the avoidance is not conserved, because the receptor gene is not present in the genome. In addition, ethyl 3-hydroxybutyrate may not activate other repellent receptors.

A third known repellent is citronellal, a naturally occurring essential oil found in multiple plant species. Citronellal activates olfactory neurons in the antenna named ab11A and ab12A in D. melanogaster and a Trp channel (TRPA1) is believed to play a role in citronellal’s activation of ab11A but not ab12A.$^{23}$ The odorant receptors in these neurons are unknown. In order to test the conservation in repellency to citronellal we used the previously described Direct Airborne Repellant Assay (DART).$^{23}$ For this non-contact assay, odorant is placed on filter paper at the bottom of a standard 15 ml culture tube, with a mesh screen placed 0.5 ml from the bottom to prevent flies from contacting the filter paper with odorant. Two tubes are placed together to form a long tunnel in which ~100 flies are given 30 minutes...
Safer DEET substitutes repel an agricultural pest, the spotted wing Drosophila. Recently, a number of new naturally occurring repellents were discovered that can substitute for DEET and are strongly repellent to *D. melanogaster* and mosquitoes24. Many of these repellent compounds are present naturally in fruits, have very mild and pleasant odors, are commonly used flavor and fragrance components belonging to a category called generally recognized as safe (GRAS) and are approved for human consumption through addition to food. We tested whether these compounds can be used to repel *D. suzukii*. We measured behavioral responses to three of these DEET-substitute compounds: (1) butyl anthranilate (BA), (2) methyl N,N-dimethylanthranilate (MDA) and (3) ethyl anthranilate (EA) in the previously used Two-Choice Trap Assay in a plate. *D. suzukii* avoided the traps containing 10% of all three compounds (P = 0.926); however, at 1%, ethyl anthranilate did not repel *D. suzukii* (P < 0.05) (Fig. 4A).

We then asked if DEET-like compounds would also act as oviposition deterrents by testing preference for *Drosophila melanogaster* egg-laying using a Two-Choice Oviposition Assay (Fig. 4B). Briefly, 15 male and 25 female flies are released into a 10 gallon closed glass chamber containing two (one with test odorant the other with solvent) Petri dishes with standard grape juice media. After 24 hours, eggs laid on
grape media with test odorant and solvent are counted. D. melanogaster did not oviposit on grape media infused with 0.4% DEET (Fig. 4B). At the lower concentration of 0.2% DEET, there is no avoidance and even initially a preference for ovipositing on the DEET containing media. D. melanogaster avoided ovipositing on MDA, BA or EA at both concentrations tested. Since these DEET-like compounds deter attraction and oviposition, we wondered if they would also deter D. suzukii oviposition on fruit.

In order to test whether BA can protect fruit from D. suzukii we revised the previous assay by replacing the grape juice media with two bowls of fresh, ripe blueberries (a preferred fruit of D. suzukii) and extending the assay time to one week. One bowl of blueberries was painted with BA and the other solvent. This Two-Choice Assay in a glass chamber (Fig. 4C) allowed us to infer egg-laying from a count of eggs, larvae and pupae emerging from each set of fruit. As expected from the time lapsed between the end of the experiment and dissection of exposed blueberries, few eggs were observed, with the exception of one of the six trials of 10% BA where 43 unhatched eggs out of 159 total D. suzukii that were counted. Of the unhatched eggs, 95% were laid on the control blueberries. More importantly, we found a clear dose-dependent decrease in numbers of larvae and pupae emerging from the BA treated blueberries. Remarkably, decreases were observed from the week-long experiment after only a single treatment, with substantial decreases at 2.5% and nearly complete protection at the 10% concentration (Fig. 4D).

This proof of principle experiment indicates that insect repellents with different safety profiles can indeed be useful to reduce fruit damage during ripening.

**Discussion**

Each year D. suzukii damages hundreds of millions of dollars worth of fruits worldwide and there is a great need to find new ways to reduce this loss\(^ {29,30} \). Toxic insecticides are often risky to use directly on fruits, and a safe affordable repellent could provide protection and reduce use of toxic chemicals. Although DEET is repellent to D. suzukii, it is a synthetic chemical that is unlikely to be useful in protecting crops given the human health concerns regarding food supply contamination, as well as the high...
production cost for the large volumes that would be required in agriculture. Other insect repellents we
test here provide an opportunity to develop alternative effective strategies to reduce fruit damage. More
generally, insects destroy a very large fraction of the global agricultural output and necessitate millions
of tons of toxic insecticide use that is environmentally unfriendly and harmful to human populations.
Further analysis of possibly environmentally safer and non-toxic repellents could decrease use of such
insecticides. The analysis of conserved repellent pathways in the insect olfactory system offers an avenue
to design behavioral control strategies of these dangerous pests and ultimately could form a foundation
of novel and safe technologies that can improve both plant and human health.

Materials and Methods

Drosophila stocks. D. yakuba, D. pseudoobscura, D. virilis and D. melanogaster (wild-type lines)
were obtained from the San Diego Stock Center. D. suzukii were a generous gift of R. Stouthammer.
Unless otherwise indicated D. melanogaster were white backcrossed 5X to Canton-S. D. melanogaster
wild-type A1 was caught in La Jolla, California and A2 in Point Loma, California in July 2011. These wild
captured D. melanogaster were tested within five months of being captured. D. melanogaster, D. yakuba and
D. virilis were raised on standard cornmeal in a humidified incubator at 25 degrees Celsius on a 12 hour
light/12 hour dark cycle. D. pseudoobscura were raised in the dark at 18 degrees C. and D. suzukii were
raised on a modified cornmeal diet at room temperature.

Single-sensillum electrophysiology. Recordings were obtained as described previously7.

Short-term assays. Contact and non-contact short-term behavioral assays were conducted to deter-
mine responses to odorants.

The T-maze Assay is ideally suited for highly volatile compounds such as CO₂. Avoidance to carbon
dioxide and pyridine trials were conducted as before7. Briefly, approximately 40 flies are released from
an elevator into the horizontal intersection of a T-shaped apparatus. A test odorant is applied to one
arm of the T-maze and a control odorant to the opposite arm. For trials with other odorants, paraffin oil
was used as the solvent and in the control arm. Flies are given one minute to choose an arm before the
elevator closes. Orientation of arms for test and control were switched between trials. Preference index
was calculated as = (number of flies in test arm-number of flies in control arm)/(number of flies in test
arm + number of flies in control arm).

The Direct Airborne Repellent Test (DART) is suited for volatile compounds that could be confounded
by responses from the taste system. Trials were performed using slight modifications to the previously
reported assay23. Fifty flies were aged per vial and starved in vials with 2 Kimwipes moistened with
3 ml of water. A 6 mm diameter circle of Whatman #1 filter paper was placed in the bottom of a 10 cm
length tube (VWR, #60818-661) to deliver the odor. A brass screen of 8/32 inch diameter was placed
5 mm from the bottom of the tube to gate off the filter paper. Approximately 100 flies were inserted into
the control tube and joined to the tube with test odorant. After 30 minutes exposure in the dark at 25
degrees Celsius, the apparatus was photographed. Flies 5 cm from each screen were counted. Preference
index was calculated.

The Two-Choice Trap Assay in a plate tests less volatile odorants. Trials were performed as described27.
Ten female flies are placed in a Petri dish containing two traps. Traps were made with 1.5 ml micro
centrifuge tubes (USA Scientific) with opening cut in the bottom of the tube. Both traps contain the
fly’s normal laboratory food at the base. The neck of one trap has a filter paper with test odorant, the
other trap has solvent. Five microliters of hexane (control) and five microliters of 10% DEET or test
compounds in hexane were applied to the stem part of filter paper inserted into upper part of pipette tip
near entrance to trap to allow flies to walk over treated surface. Traps were placed in chemical hood for
5 minutes to allow hexane to volatilize before being placed in the 1% agarose treated Petri dish chamber.

Long Term Assays Carriage Return. Two-Choice Trap Assay. Assay was performed to determine24
preference for an attractive food source in the context of a repellent odor. Briefly, ten male and ten
female starved (4–7 day old) flies are placed in a cylindrical chamber containing two traps: (1) with test
odorant and lure, (2) with solvent and lure. Apple cider vinegar (10%) is the lure for all trials except for
D. virilis where liquid malt (25%) was used (D. virilis is not attracted to apple cider vinegar24). To create
a well for separating lure from test odorant, a single cap cut from a BioRad PCR 0.2 ml Tube Flat Cap
strips (#TCS0803) was inserted in a snap top lid of a 3 ml microcentrifuge tube. To run the assay, 35 ul of test
odorant was pipetted into inner well and 90 ul of lure into the outer ring. For all trials, the control trap
had paraffin oil solvent in inner well and lure in outer ring. Flies were given six hours to enter traps.
Preference index was calculated.

Two-Choice Oviposition Assay. Test odorant or solvent was added to warm standard grape juice media
in Petri dishes and set to solidify. Petri dishes were placed at opposite ends of a 10 gallon closed glass
chamber. A 100 ml beaker containing 40 ml of distilled water was placed equidistant between grape plates
to add moisture to the chamber. For each trial, 15 male and 25 female un-starved Canton-S flies were
lightly anesthetized with carbon dioxide and released in the chamber. Assay was run for 24 hours at 25 degrees Celsius on a 12-hour light: 12-hour dark cycle. Preference was determined by counts of eggs on grape plate containing test odorant and control.

**Two-Choice Blueberry Assay in a glass chamber.** Fresh blueberries were obtained from a local grocer and were soaked in distilled water for 30 minutes, rinsed and dried. To prepare the chamber, 31 grams of blueberries were placed in each of 2 plastic bowls. Test compound (0.4 ml) is painted on blueberries in the test bowl and solvent (0.4 ml) on blueberries in control bowl. Bowls are placed at opposite ends of a 10 gallon closed glass chamber. A 100 ml beaker containing 40 ml of distilled water was placed equidistant between fruit to add moisture to the chamber. For each trial, 15 male and 15 female un-starved flies were lightly anesthetized with carbon dioxide and released in the chamber. Assay was run for seven days at 25 degrees Celsius on a 12 hour light: 12 hour dark cycle. After 7 days, each bowl was covered and set aside for an additional six days for eggs and larvae to develop after which the blueberries were dissected under microscope and number of eggs, larvae, pupae and adults were recorded. Preference was determined by inferring egg-laying from a count of eggs, larvae and pupae emerging from each set of fruit.

**References**

1. Faucher, C., Forstreuter, M., Hilker, M. & de Bruyne, M. Behavioral responses of Drosophila to biogenic levels of carbon dioxide depend on life-stage, sex and olfactory context. *The Journal of experimental biology* 209, 2739–2748, doi: 10.1242/jeb.0297 (2006).
2. Suh, G. *et al.* A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila. *Nature* 431, 854–859, doi: 10.1038/nature02980 (2004).
3. Suh, G. *et al.* Light activation of an innate olfactory avoidance response in Drosophila. *Current biology* : CB 17, 905–908, doi: 10.1016/j.cub.2007.04.046 (2007).
4. de Bruyne, M., Foster, K. & Carlson, J. R. Odor coding in the Drosophila antenna. *Neuron* 30, 537–552, doi: 10.1016/S0896-6273(01)00289-6 (2001).
5. Robertson, H. Evolution of the gene lineage encoding the carbon dioxide receptor in insects. *Journal of Insect Science* 9, 19, doi: 10.1673/031.009.1901 (2009).
6. Faucher, C. P., Hilker, M. & de Bruyne, M. Interactions of carbon dioxide and food odours in Drosophila: olfactory hedonics and sensory neuron properties. *PLoS One* 8, e6361, doi: 10.1371/journal.pone.006361 (2013).
7. Turner, S. & Ray, A. Modification of CO2 avoidance behaviour in Drosophila by inhibitory odorants. *Nature* 461, 277–281, doi: 10.1038/nature08295 (2009).
8. Kinjo, H., Kunimi, Y., Ban, T. & Nakai, M. Oviposition efficacy of Drosophila suzukii (Diptera: Drosophilidae) on different aliphatic esters across the Drosophila genus. *J Econ Entomol* 106, 1767–1771 (2013).
9. Aitallah, J., Teixeira, L., Salazar, R., Zaragoza, G. & Kopp, A. The making of a pest: the evolution of a fruit-penetrating ovipositor in Drosophila suzukii and related species. *Proceedings of the Royal Society B: Biological Sciences* 281, 20132840, doi: 0.1098/rspb.2013.2840 (2014).
10. Lee, J. C. *et al.* In Focus: Spotted wing drosophila, Drosophila suzukii, across perspectives. *Pest Manag Sci* 67, 1349–1351, doi: 10.1002/ps.2271 (2011).
11. Goodhue, R. E., Bolda, M., Farnsworth, D., Williams, J. C. & Zalom, F. G. Spotted wing drosophila infestation of California blueberries and raspberries: economic analysis of potential revenue losses and control costs. *Pest Management Science* 67, 1396–1402, doi: 10.1002/ps.2259 (2011).
12. Keesey, I., Knaden, M. & Hansson, B. Olfactory Specialization in Drosophila suzukii Supports an Ecological Shift in Host Preference from Rotten to Fresh Fruit. *Journal of Chemical Ecology*, 1–8, doi: 10.1007/s10886-015-0544-3 (2015).
13. Revadi, S. *et al.* Olfactory responses of Drosophila suzukii females to host plant volatiles. *Physiological Entomology, n/a-n/a*, doi: 10.1111/phen.12088 (2015).
14. Turner, S. *et al.* Ultra-prolonged activation of CO2-sensing neurons disorients mosquitoes. *Nature* 474, 87–91, doi: 10.1038/nature0981 (2011).
15. Wasserman, S., Salomon, A. & Frye, M. A. Drosophila tracks carbon dioxide in flight. *Carr Biol* 23, 301–306, doi: 10.1016/j.cub.2012.09.038 (2013).
16. Semmelhack, J. & Wang, J. Select Drosophila glomeruli mediate innate olfactory attraction and aversion. *Nature* 459, 218–223, doi: 10.1038/nature07983 (2009).
17. Hallem, E. & Carlson, J. Coding of odors by a receptor repertoire. *Cell* 125, 143–160, doi: 10.1016/j.cell.2006.01.050 (2006).
18. Hallem, E. A., Ho, M. G. & Carlson, J. R. The molecular basis of odor coding in the Drosophila antenna. *Cell* 117, 965–979, doi: 10.1016/j.cell.2004.05.012 (2004).
19. Stensmyr, M. C. Novel natural ligands for Drosophila olfactory receptor neurones. *Journal of Experimental Biology* 206, doi: 10.1242/jeb.00143 (2003).
20. McBride, C., Arguello, J. & O’Meara, B. Five Drosophila genomes reveal nonneutral evolution and the signature of host specialization in the chemoreceptor superfamily. *Genetics* 177, 1395–1416, doi: 10.1534/genetics.107.07883 (2007).
21. Guo, S. & Kim, J. Molecular Evolution of Drosophila Odorant Receptor Genes. *Molecular biology and evolution* 24, doi: 10.1093/molbev/msm038 (2007).
22. de Bruyne, M., Smart, R., Zammit, E. & Warr, C. Functional and molecular evolution of olfactory neurones and receptors for aliphatic esters across the Drosophila genus. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology* 196, 97–109, doi: 10.1007/s00359-009-0496-6 (2010).
23. Kwon, Y. *et al.* Drosophila TRPA1 channel is required to avoid the naturally occurring insect repellent citronellal. *Current biology* : CB 20, 1672–1678, doi: 10.1016/j.cub.2010.08.016 (2010).
24. Kain, P. *et al.* Odour receptors and neurones for DEET and new insect repellents. *Nature* 502, 507–512, doi: 10.1038/nature12594 (2013).
25. Lee, Y., Kim, S. & Montell, C. Avoiding DEET through insect gustatory receptors. *Neuron* 67, 555–561, doi: 10.1016/j.neuron.2010.07.006 (2010).
26. Xu, P., Choo, Y. M., De La Rosa, A. & Leal, W. S. Mosquito odorant receptor for DEET and methyl jasmine. *Proc Natl Acad Sci U S A* 111, 16592–16597, doi: 10.1073/pnas.1417244111 (2014).
27. Reeder, N. L., Ganz, P. J., Carlson, J. R. & Saunders, C. W. Isolation of a deet-insensitive mutant of Drosophila melanogaster (Diptera: Drosophilidae). *J Econ Entomol* 94, 1584–1588 (2001).
28. Rytz, R., Croset, V. & Benton, R. Ionotropic receptors (IRs): chemosensory ionotropic glutamate receptors in Drosophila and beyond. *Insect Biochem Mol Biol* 43, 888–897, doi: 10.1016/j.ibmb.2013.02.007 (2013).
29. Calabria, G., Máca, J., Bächli, G., Serra, L. & Pascual, M. First records of the potential pest species Drosophila suzukii (Diptera: Drosophilidae) in Europe. *Journal of Applied Entomology* 136, 139–147, doi: 10.1111/j.1439-0418.2010.01583.x (2012).
30. Cini, A., Ioriatti, C. & Anfora, G. A review of the invasion of Drosophila suzukii in Europe and a draft research agenda for integrated pest management. *Bulletin of Insectology* 65, 149–160 (2012).
31. West, A. Chemical Attractants for Adult Drosophila Species1. *Journal of Economic Entomology* 54, 677–681 (1961).
32. Ometto, L. et al. Linking genomics and ecology to investigate the complex evolution of an invasive Drosophila pest. *Genome biology and evolution* 5, 745–757 (2013).
33. Chiu, J. C. et al. Genome of Drosophila suzukii, the spotted wing drosophila. *G3 (Bethesda)* 3, 2257–2271, doi: 10.1534/g3.113.008185 (2013).

**Acknowledgments**

We thank Sana K. Tharadra for expert technical assistance with electrophysiological recordings, Anupama Dahanukar for valuable discussions and Dyan MacWilliam, Christi Scott, Greg Pask and Pinky Kain for comments on the manuscript. This work was partly funded by University of California Riverside Hatch Funds and a grant 1R01DC014092-01A1 (NIDCD) to A.R. The granting agencies had no role in experimental design or analysis.

**Author Contributions**

C.K.P. performed the experiments and analyzed the data. C.K.P. and A.R. conceived and designed this study and wrote the manuscript. A.R. supervised the project.

**Additional Information**

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: C.K.P. and A.R. are listed as inventors on pending patent applications filed by UC Riverside. A.R. has equity in Olfactor Labs Inc. and is founder of Sensorygen Inc.

**How to cite this article:** Krause Pham, C. and Ray, A. Conservation of Olfactory Avoidance in *Drosophila* Species and Identification of Repellents for *Drosophila suzukii*. *Sci. Rep.* 5, 11527; doi: 10.1038/srep11527 (2015).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/