Journal of Insect Physiology 135 (2021) 104312

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Host plant shift differentially alters olfactory sensitivity in female and male *Drosophila mojavensis*

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ARTICLE INFO

Keywords:
Plant volatiles
Sex-specific
Olfactory receptor neurons
Reproductive isolation

ABSTRACT

Animals may vary in their utilization of plants depending on plant availability, and also on the sex of the animal. Evolutionary adaptations may arise, particularly in specialist animals to the chemistry of the host plants, and these adaptations may differ between the sexes due to differences in their interactions with the plants. *Drosophila mojavensis* uses different host cacti across its range, and volatile chemicals emitted by the host are the primary cue for host plant identification. In this study, we measured responses of individual olfactory sensory neurons to a large suite of odorants across males and females of the two southern *D. mojavensis* populations. We show that a switch in host plant is accompanied by changes in the olfactory system, but the effect of this switch is minor compared to that of sex. That is, we observe differences in olfactory receptor neuron specificity and sensitivity to odorants between sexes, and to a lesser extent between populations. The majority of sensory differences are restricted to only three of the 17 sensory neurons measured. Further, we found numerous differences between sexes that only occur within one population, i.e., sex-by-population interactions.

1. Introduction

Adaptation of animals to their host environment can drive diversification at the genetic and phenotypic levels. Changes in the host, such as a habitat for specialists, can result in alteration of mate choice and may further result in reproductive isolation (Tait et al., 2016). Phytophagous insects vary from being specialists that feed and breed on one or a few plant species, to being generalists which can thrive on many different plants (Bruce et al., 2005). Specialist insects depend on cues emitted by host plants (Bruce et al., 2005; Dekker et al., 2006) and such cues are detected by different sensory modalities, such as olfaction (Matsuo et al., 2007; Linz et al., 2013; Prieto-Godino et al., 2017). In general, insects have evolved peripheral olfactory systems that mediate host identification and localization, social interactions, and predator avoidance. More specifically, many female insects use species-specific and/or species-ratio-specific volatile chemicals emitted by host plants as cues for feeding and ovipositing, whereas males use host plant odors in their search for food and conspecifics for mating (Bruce et al., 2005). Host plant specialization in insects has been shown to alter behavioral responses and odorant receptor gene expression, and olfactory sensory neurons responses, including in flies (Stensmyr et al., 2003; de Bruyne et al., 2010; Date et al., 2013; Ramasamy et al., 2016). For example, behavioral studies have demonstrated that *Rhagoletis pomonella* apple- versus hawthorn-infesting populations differ in their host preference behavior in flight tunnel experiments when presented with their respective host plant odors (Linn et al., 2003). Analysis of the fruit volatile composition revealed differences in the volatile composition of hawthorn and apple (Zhang et al., 1999; Nojima et al., 2003) and changes in olfactory sensory neuron responses were found with a switch in host plant use (Olsson et al., 2006).

Many studies have shown that *Drosophila melanogaster* detects odors in a complex environment using an ensemble of odorant-specific olfactory sensory neurons (OSNs). Two to four OSNs are typically co-localized and housed in a hair-like structure, called a sensillum, on the antennae and maxillary palps. Sensilla are categorized based on their morphology and the combination of functional types of OSNs housed within a sensillum (Shanbhag et al., 1999; de Bruyne et al., 2001; Couto et al., 2005). Two groups of sensilla, the basiconic and trichoid, have OSNs containing seven transmembrane domain olfactory receptors (OR) which detect food odors and pheromones, respectively (Kurtovic et al., 2007; Van der Goes Van Naters and Carlson, 2007). The third group of sensilla, called coeloconic, have OSNs that contain ionotropic receptors

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https://doi.org/10.1016/j.jinsphys.2021.104312
Received 20 May 2021; Received in revised form 26 September 2021; Accepted 27 September 2021
Available online 6 October 2021

(IR) which detect acids and amines in the environment (Yao et al., 2005; Benton et al., 2009; Silbering et al., 2011; Prieto-Godino et al., 2017; Nemeth et al., 2018).

Drosophila mojavensis, a cactophilic fly, is a good model organism for understanding how host preference can alter olfactory peripheral perception and lead to reproductive isolation. *D. mojavensis* is composed of four geographically isolated populations in the southwestern United States and northwestern Mexico, each feeding, breeding, and developing on specific rotting cacti (Heed, 1982; Heed and Mangan, 1986; Ruiz et al., 1990). It is hypothesized that a Mojave/ Sonora-Arizona population diverged from the Baja population 230,000–270,000 years ago, and the Mojave population diverged from Sonora-Arizona 117,000–135,000 years ago (Smith et al., 2012). The host cactus species differ in their volatile composition (Downing, 1985; Date et al., 2013; Wright and Setzer 2014a,b) and these volatile cues are integral to host plant identification (Fogleman and Abril, 1990; Newby and Etges, 1998). *Drosophila mojavensis* feed on yeast that colonizes damaged cactus tissue, and yeast volatiles are important for fly attraction and oviposition (Becher et al., 2012; Date et al., 2017). The colonizing yeast flora varies with cactus substrate, and so the odorant cues emitted during fermentation differ, with a complex interplay between insect, microbial community and plant substrate (Fogleman et al., 1981; Date et al., 2017).

Key volatiles emitted from rotting cactus induce behavioral and neurophysiological responses in *D. mojavensis* (Date et al., 2013, 2017; Crowley-Gall et al., 2016). One behavioral study that prompts the present one was a choice test in which flies chose between different stages of cactus fermentation of their respective host plants. Sex specific behavioral responses were found and females in general were more responsive (Date et al., 2013). Moreover, sex-specific differences were observed to single chemical compounds and compound mixtures (Newby and Etges 1998; Date et al., 2013). At the neuronal response level, electroantennogram and electropalpogram recordings from females of all four populations showed differences in the responses to volatiles present in the rotting cacti, with the Mojave population being distinctly different from the other three according to principal component analysis (Date et al., 2013). Similarly, single sensillum recordings from female Mojave and S. Catalina Island populations revealed differences in OSN spike number in response to the tested odorants (Crowley-Gall et al., 2016). However, the other two populations, Baja and Sonora, which specialize on the agria cactus (*Stenocereus gummosus*), and organ pipe cactus (*S. thurberi*), respectively, remain to be tested for their neuronal responses.

Here we examine the olfactory neurophysiological responses of male and female *D. mojavensis*. We hypothesize that olfactory neuron responses to host plant volatiles in *D. mojavensis* are population, and as such host, and sex specific. We test this hypothesis by recording from the antennal olfactory sensory neurons of males and females of the Sonora and Baja populations.

2. Materials and methods

2.1. Fly lines

D. mojavensis Baja (SQ59a: San Quintin, Baja California, Mexico), and Sonora population (OPNM9: Organ Pipe National Monument, Arizona, USA) stocks were a gift from Dr. William Etges. Flies were reared at 25 °C on a 12 h dark/light cycle on banana cactus medium (563 ml water, 3.75 g agar, 15 g yeast, 0.25 banana, 31.25 ml Karo syrup, 18.75 ml malted barley, 1.5 g Opuntia cactus powder, 3.75 ml propionic acid).

2.2. Odorant stimuli

The most abundant and physiologically relevant compounds from the headspace of rotting cacti, as well as a set of diagnostic odorants used to identify OSNs, gleaned from published *Drosophila* literature (de Bruyne et al., 2001 & 2010; Hallem and Carlson, 2006; Date et al., 2013; Crowley-Gall et al., 2016) were purchased at high purity (see Supplementary Table 1 for chemical details). These odorants were diluted to 10^{-2} in paraffin oil. Twenty microliters of odorant (~200 μg) was loaded on a small piece of filter paper inserted into the large end of a $5^{3/4''}$ Pasteur pipette. Each of these odorant "cartridges" were used a maximum of two times during a recording period.

2.3. Single sensillum recordings (SSR)

Single sensillum recordings were performed on 10–11 days old flies. The fly was restrained in a pipette tip with its antennae exposed (e.g., Pellegrino et al., 2010). Humidified air flowed continuously over the preparation. The tip of a thin glass electrode (Micropipette puller, Sutter Instrument Co., Novato, CA, USA) gently pressed down between second and third segment to prevent torsion of the antenna. Action potentials were recorded with electrolytically (1 M KOH solution) sharpened tungsten electrodes. A reference electrode was inserted into the eye, and a recording electrode into the base of a sensillum with the help of micromanipulator operated by a DC motor controller (Märzhäuser Wetzlar GmbH & Co., Wetzlar, Germany). Odorant stimuli were applied by delivering air through the odor cartridge and into the humidified air stream for 500 ms (Stimulus controller CS55, Syntech Germany), with inter-stimulus intervals of 30 s, or longer if required for the OSN activity return to spontaneous, pre-stimulation level. The signal from the recording electrode was pre-amplified using an IDAC-4 (Syntech), and recorded and analyzed using Autospike software (Autospike v.3.9, Syntech). All of the experimental components were set on an anti-vibration table (Ametek TMC, Berwyn, PA, USA) and shielded by a Faraday cage. The action potentials of different OSNs were distinguished based on their shape and size, aided by Autospike software (Syntech). OSN responses to odorants were calculated by comparing the change in the frequency of action potentials in 0.5 s pre- and post-stimulation periods, and subsequently doubled to obtain the odor-evoked spikes per second. Sensillar types were assigned subtypes by their constituent OSNs identified by their responses to odorants that are diagnostic of known *Drosophila* (*mojavensis*) (de Bruyne et al., 2001 & 2010; Hallem and Carlson, 2006; Date et al., 2013; Crowley-Gall et al., 2016). For each sensillar subtype three to five recordings were made for 57 different odorants.

2.4. Statistical analyses

All responses to a given odorant were included in the statistical analyses for purposes of determining the multiple-test-adjusted critical p-value, but the statistical results from odorants producing negligible responses (-15 to 25 spikes/s) are not indicated or discussed. Three different statistical tests were performed. First, for each of 17 OSNs and each of 57 odorants, we tested for response differences attributable to sex, or to population, by two-way analysis of variance (ANOVA). Second, for each OSN and each odorant, we tested for differences among the four groups Baja female, Sonora female, Baja male and Sonora male, by one-way ANOVA, followed by Tukey-Kramer post hoc tests of the groups' specific statistical relationships and hierarchy. For both of these tests the critical p-value was adjusted for multiple testing by dividing alpha by the number of odorants ($p_{crit} = 0.05/57 = 0.00088$). Third, for each OSN we tested for overall sensitivity differences between females and males, and between Baja and Sonora, by pair-wise subtraction of their responses followed by a student's *t*-test (i.e., is the mean of the 57 differences between female and male responses different from zero?). These tests did not include 'non-responders' with no responses outside the range −15 to 25 spikes/second, and the critical p-value was adjusted for multiple testing by dividing alpha by the number of total *t*-tests ($p_{crit} = 0.05/64 = 0.00078$).

3. Results

We identified and recorded from eight basiconic sensillar subtypes in males and females of the Baja and Sonora populations of *Drosophila mojavensis*. Two additional sensillar subtypes, abX and abZ, were found in these populations, but were encountered too infrequently to draw meaningful conclusions from the data regarding population and/or sex specific differences and were not considered further in this study (data not shown). The ab5, ab8, and ab10 sensillar subtypes have not been reported in *D. mojavensis* (Crowley-Gall et al., 2016). Consistent with previous studies in *Drosophila*, the eight subtypes examined contain between 2 and 4 olfactory sensory neurons (OSNs) designated

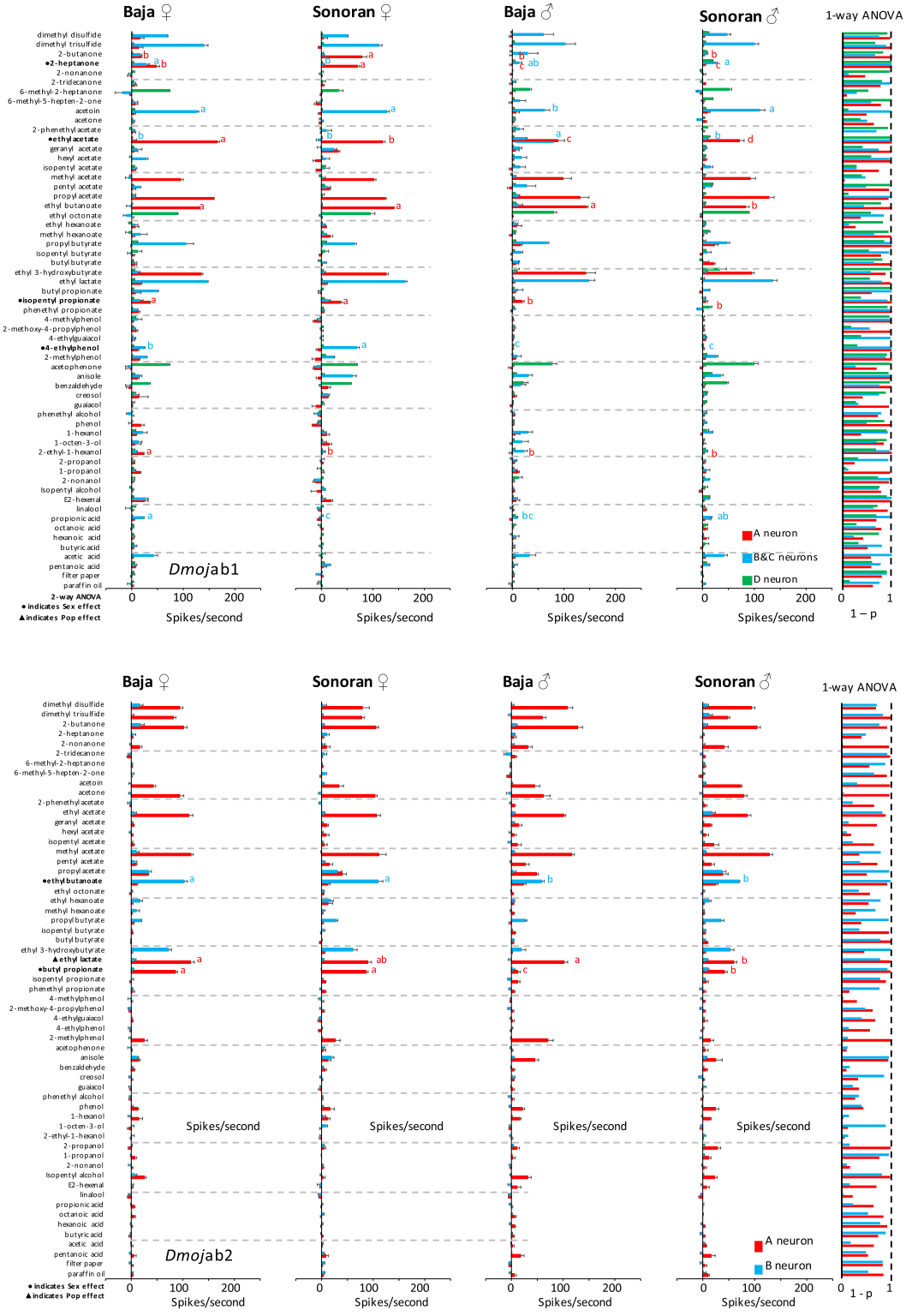

Fig. 1. Single sensillum recordings of olfactory sensory neurons to odorants. The responses (mean ± SEM) of neurons from male and female *Drosophila mojavensis* for the Baja and Sonora populations are shown. Response profiles of each sensillar type are sequentially shown with the statistical results presented in the right hand column where bars indicate 1-p value, i.e., long bars indicate small p-values.

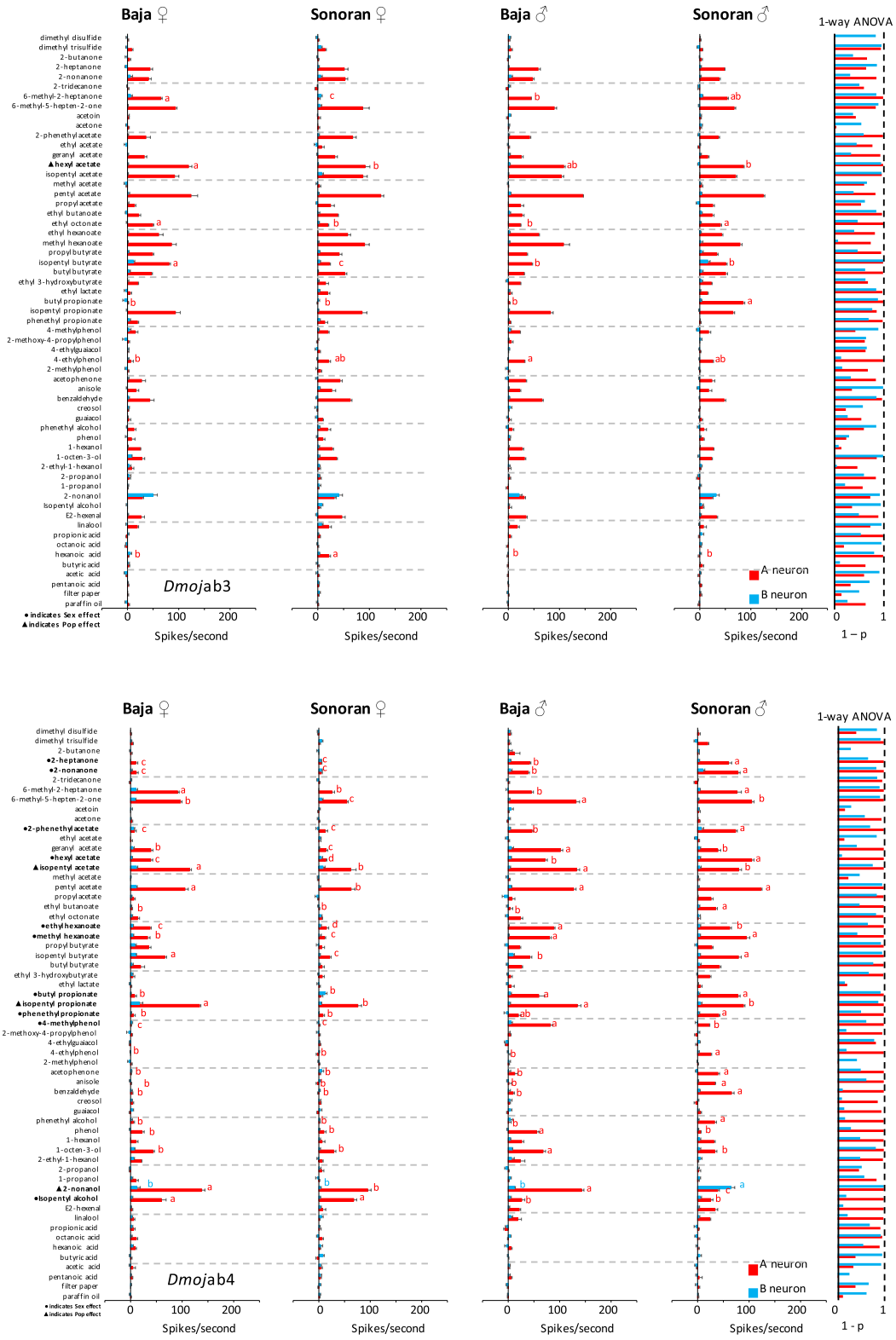

Fig. 1. (*continued*).

alphabetically based on their spike amplitudes, with neuron A having the highest amplitude (de Bruyne et al., 2001). The similarity in spike amplitude of the ab1B and ab1C neurons precluded reliable discrimination of their independent responses and so data were pooled. Homologous neurons were defined by their responses to odorants shown in the previous literature to be diagnostic of each sensillar subtype and by the responses of their paired neuron(s). The response profiles for seven of the sensillar subtypes were classified to the existing *Drosophila* nomenclature given to *Drosophila* subtypes known to exist within and between species, e.g., ab2A (antennal basiconic subtype 2 neuron A; de Bruyne et al., 2001). The remaining abY sensillar subtype contained two neurons with response profiles that defies such classification, and it remains to be determined if this subtype is unique to *D. mojavensis* (Crowley-Gall et al., 2016). The OSNs differed in the breadth and

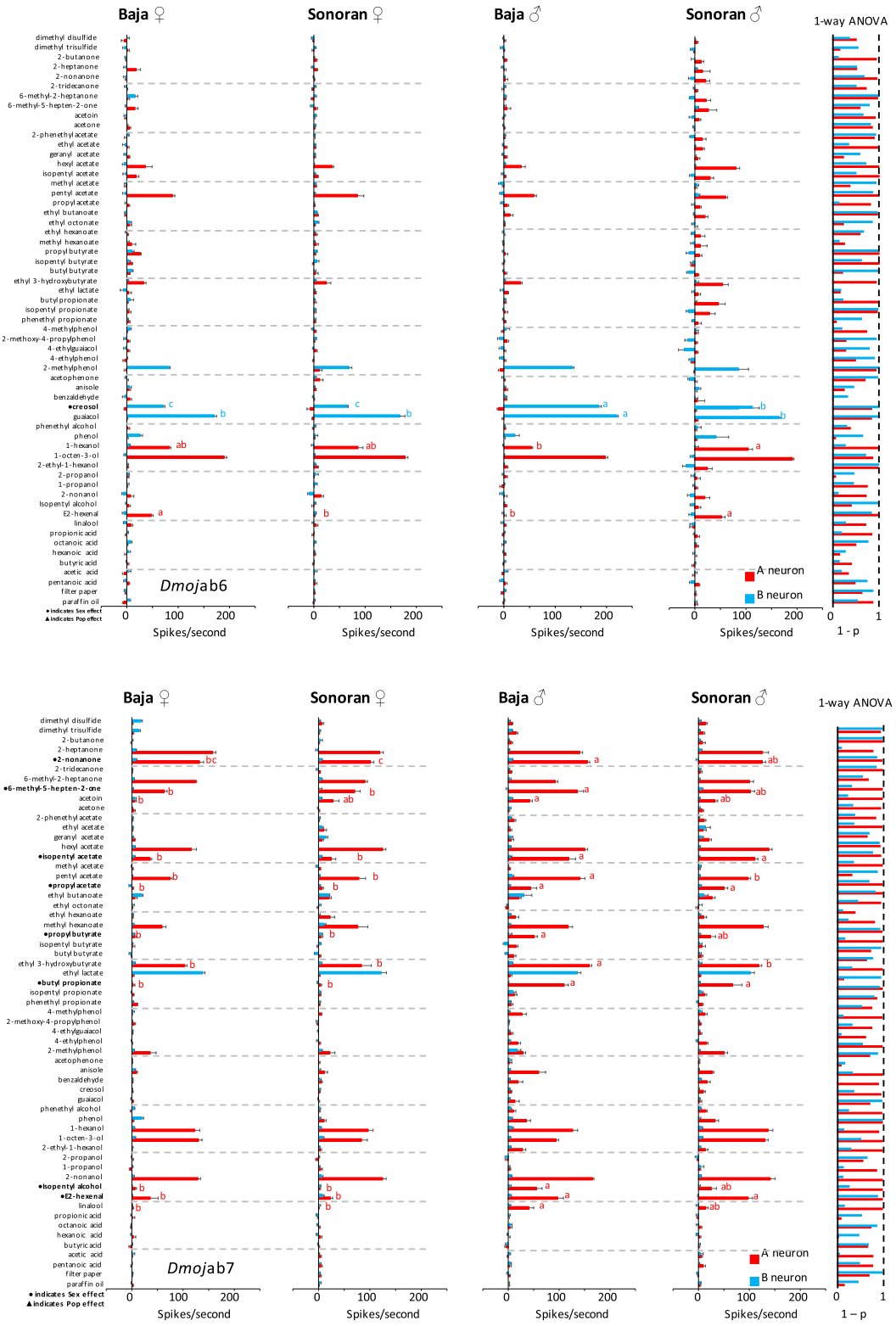

Fig. 1. (*continued*).

magnitude of their response to the tested odorants (Fig. 1). For example, the ab3A and ab7A neurons were broadly responsive, particularly within the esters, and alcohols and esters, respectively, while the ab6B and ab7B neurons were responsive to a handful of aromatics or single odorant, ethyl lactate, respectively. Three additional OSNs showed little to no response to any tested odor stimuli (i.e., ab3B, ab4B, ab9A).

Overall, analyses of 57 odorants presented to 17 OSN types across two populations and two sexes, revealed 41 significant differences in response of some kind. The effect of sex was much more influential than that of population, with 36 instances of a (across-population) sex effect compared to five instances of a (across-sex) population effect, and zero instances of these occurring together (2-way ANOVA, see Fig. 1 where sex and population effects are indicated by● and ▲, respectively, preceding the relevant odorants). Among instances of a sex effect, more are

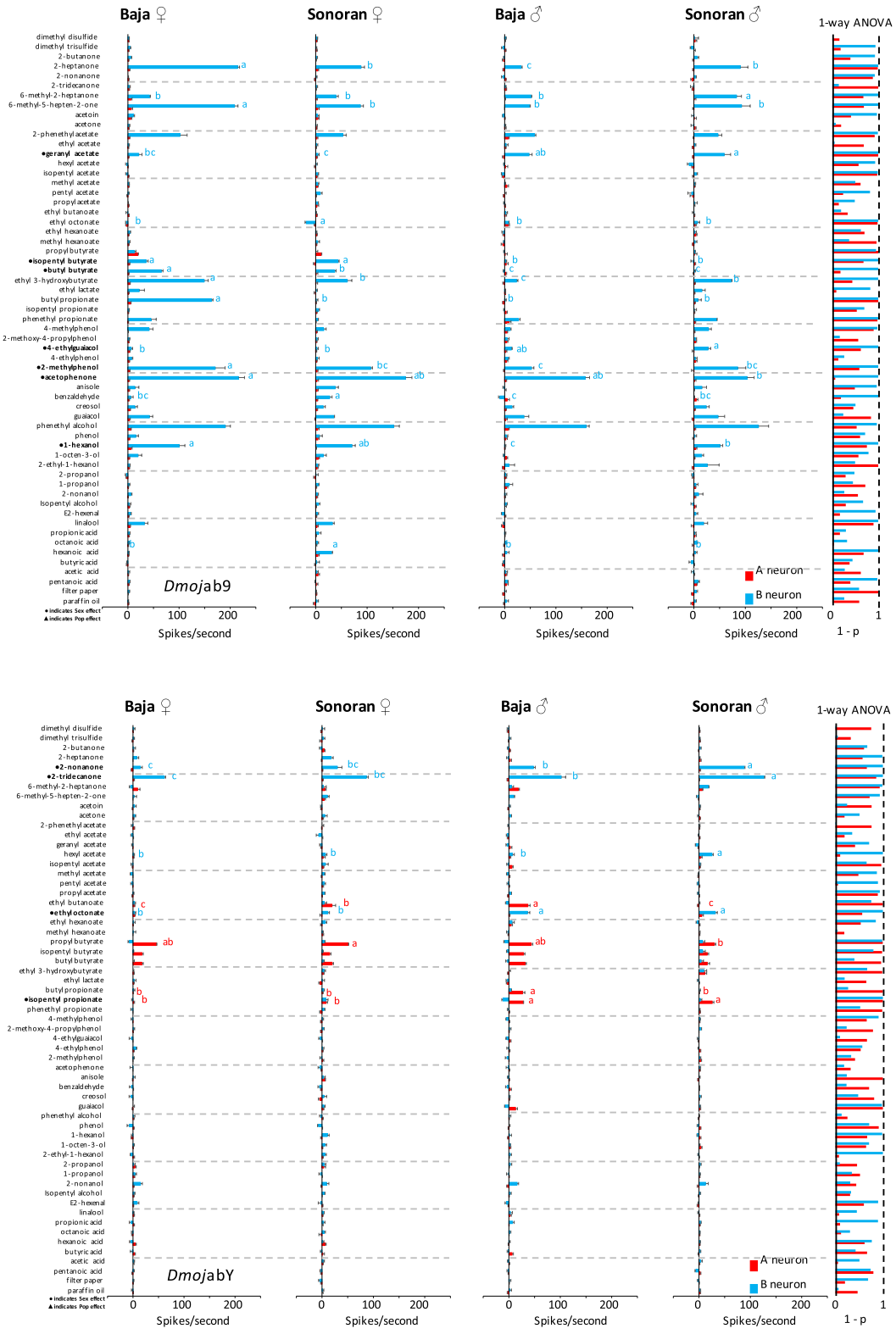

Fig. 1. (*continued*).

caused by males with large responses than are caused by females with large responses, accounting for 24 and 12 significant instances, respectively. Among the five instances of a population effect, all showed the Baja population to be more sensitive (see also Supplementary Table 2).

A similar series of tests was performed for differences among the four sex-population groups within each OSN, followed by post-hoc tests to identify the nature of those differences. Here we are especially interested in significant comparisons that did not exhibit a sex or population effect, and of these there were 46 instances (1-way ANOVA and post-hoc tests, see Fig. 1 where p-values are illustrated in the right hand columns and post-hoc significance hierarchies indicated by alphabetical labels). These consisted of divergent responses (either higher or lower) of either a single sex within a population or, if preferred, a single population within a sex, or both (for example, Baja female and Sonora male ab4A

responses to isopentyl butyrate are significantly greater than the other two groups, but not different from each other; statistically speaking such cases are essentially interactions between sex and population). These divergences in sensitivity to specific odorants are not evenly distributed across the sex-population groups (e.g., male Sonora flies vs any of the other three combinations). The exact numbers of significant instances are difficult to parse out between groups because, as stated, a significant effect can be caused simply by one divergent group, or more subtly by two groups or a hierarchy of responses. Generally, Sonora males account for more high responses than Sonora females, whereas the Baja males and females account for roughly the same number.

Looking at the distribution of these differences across specific OSNs we can add detail to these broader patterns, particularly with regard to sex dependence, the dominant factor in determining differences in sensitivity. First, as mentioned, high sensitivity to many of the test odorants is restricted to a single sex or population, but this often depends on the OSN (see Supplementary Table 2). For instance, the number of significant differences, whether derived from sex- or population-dependence or from an interaction between them, is overrepresented in OSN ab4A: of the 36 total incidents of sex effect, 10 occurred in ab4A (of which nine showed males to be more sensitive), and of the five total incidents of population effect, three were in ab4A (all with Baja more sensitive). Of the 46 significant interactions, 13 were in ab4A. A second tier of OSNs showing high rates of divergence are ab9B and ab7A. All three of these highly variable OSNs correspond well with divergent OSNs identified by Crowley-Gall et al. (2016) in the *D. mojavensis* Mojave and Catalina populations, though that study did not test for any sex differences.

There are significant differences in general sensitivity – that is, across most or all odors tested – between OSNs (see Supplementary Table 3). Within the Baja population there is generally a slight bias toward female sensitivity, though the only statistically significant differences are in ab7A (males over females), and in ab9B (females over males). Within the Sonora population males tend to be more sensitive, and this is significant in ab4A, ab6A and ab7A. OSN sensitivity also varies between populations. Within females the Baja flies tend to be more sensitive and have significantly more sensitive ab4A, though Sonora have more sensitive abYB neurons. Within males, Sonora ab1D and ab6A are significantly more sensitive than in Baja.

4. Discussion

Olfactory sensory neuron response profiles in several *Drosophila* species have been characterized, and in *D. mojavensis* this is the case for two of the primary populations, Mojave and Catalina (Stensmyr et al., 2003; Hallem and Carlson, 2006; Marshall et al., 2010; Mansourian and Stensmyr, 2015; Crowley-Gall et al., 2016). In this study, the odor-evoked response profiles of OSNs were characterized in the other two populations, Baja and Sonora, which are again found to vary in their tuning breadth in ways similar to that found in the other populations and in other drosophilids. For instance, as shown previously in Mojave and Catalina populations the ab3A, ab4A and ab7A neurons are especially broadly responsive, which is also true in *D. melanogaster*. These are some of the neurons broadly tuned to odorants found in feeding substrates (Mansourian and Stensmyr, 2015). On the other hand, the ab2B, ab6B and ab7B neurons are especially narrowly tuned. The ab7B neuron responds to a single odorant, ethyl lactate, which has been suggested to be an indicator of malolactic fermentation and the pH of potential feeding and breeding substrates (Mansourian and Stensmyr, 2015), and the ab6B neuron remains tuned to aromatics (Hallem and Carlson, 2006). There were also three OSNs that showed little or no response to any tested odorants, namely ab3B, ab4B and ab9A. In the case of ab3B, the odorant receptor expressed remains to be determined. The *D. mojavensis* genome has been sequenced and the odorant receptor repertoire has been annotated, several known (from *D. melanogaster*) ORs are not found (Clark et al., 2007; Guo and Kim, 2007), the receptor expressed in the ab3B neuron being among these.

The primary determinant of OSN sensitivity was sex rather than population. Such sex specific differences in the peripheral olfactory system and in the behaviors it mediates are well documented in many taxa. Sexes have been shown to differ in their behavioral preference for and chemosensory perception of food cues, often because of a difference in metabolic and reproductive demands between the sexes (Mowrey and Portman, 2012; Ballhorn et al., 2013). For instance, male fiddler crabs differ from females in their foraging behavior, and also in chemosensory neurons sensitive to food odors (Murai et al., 1983; Weissburg and Derby, 1995; Weissburg, 1999). In *Drosophila*, certain OSNs have been shown to be associated with female specific behaviors. For instance, oviposition preference for citrus in *D. melanogaster* is mediated by *Or19a*-expressing OSNs located within antennal intermediate sensilla tuned to terpene compounds found in citrus fruit (Dweck et al., 2013). In *D. mojavensis*, Date et al. (2013) found differences between the sexes in olfactory behavior in response to host-plant volatiles. Therefore, it is perhaps not unexpected that we have again found sex specific differences at the level of the peripheral olfactory system in individual OSN responses to host plant volatiles.

What appear to be sex specific differences in response to individual odors may not be specific to the odors themselves, but rather may come from a difference in overall sensitivity to odorants. For instance, ab4A and ab7A neurons had many higher responses in males, and ab9B had multiple higher responses in females (Supplementary Table 2). These results may be a reflection of the increased overall sensitivity (within populations, between sexes) of Sonora males in the case of ab4A and Baja females in the case of ab9B, and males of both populations in the case of ab7A (Supplementary Table 3, left two data columns). Indeed, we find that many significant differences in sensitivity to individual odorants are associated with an overall difference in OSN sensitivity, and may not in fact be specific to a particular odorant. In these cases, the differences may be due to factors that are not ligand-specific, such as differences in receptor expression levels within individual OSNs. Still, there are some responses that are not associated with overall OSN sensitivity, i.e., are odorant specific, and these may be caused by differences in ligand binding specificity; examples of this are ab1A, ab1BC, ab2A, ab2B, ab3A, ab6B, abYA. It will be interesting in the future to identify the odorant receptors expressed in these OSNs, and to determine whether sex specific differences lead to divergent behavior through mechanisms governing general sensitivity or through more directly odor-specific means.

Population (host)-specific chemical cues, and subsequent diverging OSN responses to these host-related olfactory cues can arise between populations (Supplementary Table 3, right two data columns). Comparisons of responses between populations within a given sex revealed instances of generalized sensitivity differences. One example is the increased overall sensitivity of ab4A in Baja over Sonora females, which matches the divergent responses found previously in Mojave over Catalina females (Crowley-Gall et al., 2016). Another example is the ab9B neuron, which similarly showed higher sensitivity in Baja than Sonora females (Supplementary Table 3). In *D. melanogaster*, 'olfactogenetic' approaches have identified five OSNs associated with oviposition choice, with activation of *Or67b*-expressing ab9B OSNs resulting in negative oviposition (Chin et al., 2018). Moreover, *Or67b* has been suggested to indicate the presence of favored yeast species (Mansourian and Stensmyr, 2015). However, similar changes in female sensitivity were not observed for the Sonora population. In short, these results might indicate alternative cues in olfactory mediated decision making between populations and sexes.

Finally, it should be noted that all odorants were presented at one concentration, chosen for its tendency to produce a wide range of responses generally, and for comparison with previous work. However, this can affect the ability to detect differences in sensitivity. The chosen concentration has the potential to be at the low or high extreme of an odorant's dose–response curve and thus, in the former case, generated

responses of low signal-to-noise ratio, or, in the latter case, saturated the responses of OSNs with actually divergent sensitivities. Both cases would reduce the ability to statistically detect differences. Future research measuring the dose–response curves for odorants of interest will be able to identify detailed modes of divergence in sensitivity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank members of the Rollmann lab for helpful discussions and/or technical assistance. This work was supported by National Science Foundation grant IOS-1456932 to SMR and JEL.

Author Contributions

BA, SMR, and JEL designed the experiments and wrote the manuscript. BA performed the experiments, collected the data, and made the figure. BA and JEL performed the statistical analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2021.104312.

References

Ballhorn, D.J., Kautz, S., Heil, M., 2013. Distance and sex determine host plant choice by herbivorous beetles. PLoS One 8 (2), e55602. https://doi.org/10.1371/journal.pone.0055602.

Becher, P.G., Flick, G., Rozpedowska, E., Schmidt, A., Hagman, A., Lebreton, S., Larsson, M.C., Hansson, B.S., Piskur, J., Witzgall, P., Bengtsson, M., 2012. Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. Funct. Ecol. 26, 822–828. https://doi.org/10.1111/j.1365-2435.2012.02006.x.

Benton, R., Vannice, K.S., Gomez-Diaz, C., Vosshall, L.B., 2009. Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. Cell 136 (1), 149–162. https://doi.org/10.1016/j.cell.2008.12.001.

Bruce, T.J., Wadhams, L.J., Woodcock, C.M., 2005. Insect host location: a volatile situation. Trends Plant Sci. 10 (6), 269–274. https://doi.org/10.1016/j.tplants.2005.04.003.

Chin, S.G., Maguire, S.E., Huoviala, P., Jefferis, G.S.X.E., Potter, C.J., 2018. Olfactory neurons and brain centers directing oviposition decisions in *Drosophila*. Cell Reports 24 (6), 1667–1678. https://doi.org/10.1016/j.celrep.2018.07.018.

Clark et al. *Drosophila* 12 Genomes Consortium 2007. Evolution of genes and genomes on the *Drosophila* phylogeny. Nature 450 (7167), 203–218. https://doi.org/10.1038/nature06341.

Couto, A., Alenius, M., Dickson, B.J., 2005. Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. Curr. Biol. 15 (17), 1535–1547. https://doi.org/10.1016/j.cub.2005.07.034.

Crowley-Gall, A., Date, P., Han, C., Rhodes, N., Andolfatto, P., Layne, J.E., Rollmann, S. M., 2016. Population differences in olfaction accompany host shift in *Drosophila mojavensis*. Proc. Royal Soc. London B 283, 1–9. https://doi.org/10.1098/rspb.2016.1562.

Date, P., Dweck, H.K.M., Stensmyr, M.C., Shann, J., Hansson, B.S., Rollmann, S.M., 2013. Divergence in olfactory host plant preference in *D. mojavensis* in response to cactus host use. PLoS One 8 (7), e70027. https://doi.org/10.1371/journal.pone.0070027.

Date, P., Crowley-Gall, A., Diefendorf, A.F., Rollmann, S.M., 2017. Population differences in host plant preference and the importance of yeast and plant substrate to volatile composition. Ecol. Evol. 7 (11), 3815–3825. https://doi.org/10.1002/ece3.2017.7.issue-1110.1002/ece3.2993.

de Bruyne, M., Foster, K., Carlson, J.R., 2001. Odor coding in the *Drosophila* antenna. Neuron 30 (2), 537–552. https://doi.org/10.1016/S0896-6273(01)00289-6.

de Bruyne, M., Smart, R., Zammit, E., Warr, C.G., 2010. Functional and molecular evolution of olfactory neurons and receptors for aliphatic esters across the *Drosophila* genus. J. Comp. Physiol. A. 196 (2), 97–109. https://doi.org/10.1007/s00359-009-0496-6.

Dekker, T., Ibba, I., Siju, K.P., Stensmyr, M.C., Hansson, B.S., 2006. Olfactory shifts parallel superspecialism for toxic fruit in *Drosophila melanogaster* sibling, *D. sechellia*. Curr. Biol. 16 (1), 101–109. https://doi.org/10.1016/j.cub.2005.11.075.

Downing, R.J., 1985. The chemical basis for host plant selection in *Drosophila mojavensis*. University of Denver, Denver.

Dweck, H.K.M., Ebrahim, S.A.M., Kromann, S., Brown, D., Hillbur, Y., Sachse, S., Hansson, B.S., Stensmyr, M.C., 2013. Olfactory preference for egg laying on *Citrus* substrates in *Drosophila*. Curr. Biol. 23 (24), 2472–2480. https://doi.org/10.1016/j.cub.2013.10.047.

Fogleman, J.C., Abril, J.R., 1990. Ecological and evolutionary importance of host plant chemistry. In: Baker, J.S.F., Starmer, W.T., MacIntyre, R.J. (Eds.), Ecological and evolutionary genetics of *Drosophila*. Plenum, New York, pp. 121–141.

Fogleman, J.C., Starmer, W.T., Heed, W.B., 1981. Larval selectivity for yeast species by *Drosophila mojavensis* in natural substrates. Proc. National Acad. Sci. USA 78 (7), 4435–4439. https://doi.org/10.1073/pnas.78.7.4435.

Guo, S., Kim, J., 2007. Molecular evolution of *Drosophila* odorant receptor genes. Mol. Biol. Evol. 24, 1198–1207. https://doi.org/10.1093/molbev/msm038.

Hallem, E.A., Carlson, J.R., 2006. Coding of odors by a receptor repertoire. Cell 125 (1), 143–160. https://doi.org/10.1016/j.cell.2006.01.050.

Heed, W.B., 1982. The origin of *Drosophila* in the Sonoran Desert. In: Baker, J.S.F., Starmer, W.T. (Eds.), Ecological genetics and evolution: the cactus-yeast-Drosophila model system. Academic Press, Sydney, pp. 65–80.

Heed, W.B., Mangan, R.L., 1986. Community ecology of the Sonoran desert *Drosophila*. In: Ashburner, M., Carson, H.L., Thompson, J.N. (Eds.), The genetics and biology of *Drosophila*. Academic Press, New York, pp. 311–345.

Kurtovic, A., Widmer, A., Dickson, B.J., 2007. A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone. Nature 446 (7135), 542–546. https://doi.org/10.1038/nature05672.

Linn Jr., C., Feder, J.L., Nojima, S., Dambroski, H.R., Berlocher, S.H., Roelofs, W., 2003. Fruit odor discrimination and sympatric host race formation in *Rhagoletis*. Proc. National Acad. Sci. USA 100 (20), 11490–11493. https://doi.org/10.1073/pnas.1635049100.

Linz, J., Baschwitz, A., Strutz, A., Dweck, H.K.M., Sachse, S., Hansson, B.S., Stensmyr, M. C., 2013. Host plant-driven sensory specialization in *Drosophila erecta*. Proc. Royal Soc. London B 280 (1760), 20130626. https://doi.org/10.1098/rspb.2013.0626.

Mansourian, S., Stensmyr, M.C., 2015. The chemical ecology of the fly. Curr. Opin. Neurobiol. 34, 95–102. https://doi.org/10.1016/j.conb.2015.02.006.

Marshall, B., Warr, C.G., de Bruyne, M., 2010. Detection of volatile indicators of illicit substances by the olfactory receptors of *Drosophila melanogaster*. Chem. Senses 35 (7), 613–625. https://doi.org/10.1093/chemse/bjq050.

Matsuo, T., Sugaya, S., Yasukawa, J., Aigaki, T., Fuyama, Y., 2007. Odorant-binding proteins OBP57d and OBP57e affect taste perception and host-plant preference in *Drosophila sechellia*. PLoS Biol. 5 (5), e118. https://doi.org/10.1371/journal.pbio.0050118.

Mowrey, W.R., Portman, D.S., 2012. Sex differences in behavioral decision-making and the modulation of shared neural circuits. Biol. Sex Differ. 3 (1), 8. https://doi.org/10.1186/2042-6410-3-8.

Murai, M., Goshima, S., Nakasone, Y., 1983. Adaptive droving behavior observed in the fiddler crab *Uca vocans vocans*. Mar. Biol. 76 (2), 159–164. https://doi.org/10.1007/BF00392732.

Nemeth, D.C., Ammagarahalli, B., Layne, J.E., Rollmann, S.M., 2018. Evolution of coeloconic sensilla in the peripheral olfactory system of *Drosophila mojavensis*. J. Insect Physiol. 110, 13–22. https://doi.org/10.1016/j.jinsphys.2018.08.003.

Newby, B.D., Etges, W.J., 1998. Host preference among populations of *Drosophila mojavensis* (Diptera: Drosophilidae) that use different host cacti. J. Insect Behavior. 11, 691–712. https://doi.org/10.1023/A:1022398809881.

Nojima, S., Linn Jr., C., Morris, B., Zhang, A., Roelofs, W., 2003. Identification of host fruit volatiles from hawthorn (*Crataegus* spp.) attractive to hawthorn-origin *Rhagoletis pomonella* flies. J. Chem. Ecol. 29 (2), 321–336. https://doi.org/10.1023/A:1022677827233.

Olsson, S.B., Linn Jr., C.E., Roelofs, W.L., 2006. The chemosensory basis for behavioral divergence involved in sympatric host shifts II: olfactory receptor neuron sensitivity and temporal firing pattern to individual key host volatiles. J. Comp. Physiol. A. 192 (3), 289–300. https://doi.org/10.1007/s00359-005-0066-5.

Pellegrino, M., Nakagawa, T., Vosshall, L.B., 2010. Single sensillum recordings in the insects *Drosophila melanogaster* and *Anopheles gambiae*. J. Vis. Exp. 36, 1–5. https://doi.org/10.3791/1725.

Prieto-Godino, L.L., Rytz, R., Cruchet, S., Bargeton, B., Abuin, L., Silbering, A.F., Ruta, V., Dal Peraro, M., Benton, R., 2017. Evolution of acid-sensing olfactory circuits in drosophilids. Neuron 93 (3), 661–676.e6. https://doi.org/10.1016/j.neuron.2016.12.024.

Ramasamy, S., Ometto, L., Crava, C.M., Revadi, S., Kaur, R., Horner, D.S., Pisani, D., Dekker, T., Anfora, G., Rota-Stabelli, O., 2016. The evolution of olfactory gene families in Drosophila and the genomic basis of chemical-ecological adaptation in *Drosophila suzukii*. Genome Biol. Evol. 8 (8), 2297–2311. https://doi.org/10.1093/gbe/evw160.

Ruiz, A., Heed, W.B., Wasserman, M., 1990. Evolution of the *mojavensis* cluster of cactophilic *Drosophila* with descriptions of two new species. J. Hered. 81, 30–42. https://doi.org/10.1093/oxfordjournals.jhered.a110922.

Shanbhag, S.R., Müller, B., Steinbrecht, R.A., 1999. Atlas of olfactory organs of *Drosophila melanogaster* 1. Types, external organization, innervation and distribution of olfactory sensilla. Int. J. Insect Morphol. Embryol. 28 (4), 377–397. https://doi.org/10.1016/S0020-7322(99)00039-2.

Silbering, A.F., Rytz, R., Grosjean, Y., Abuin, L., Ramdya, P., Jefferis, G.S.X.E., Benton, R., 2011. Complementary function and integrated wiring of the evolutionarily distinct *Drosophila* olfactory subsystems. J. Neurosci. 31 (38), 13357–13375. https://doi.org/10.1523/JNEUROSCI.2360-11.2011.

Smith, G., Lohse, K., Etges, W.J., Ritchie, M.G., 2012. Model-based comparisons of phylogeographic scenarios resolve the intraspecific divergence of cactophilic *Drosophila mojavensis*. Mol. Ecol. 21, 3293–3307. https://doi.org/10.1111/j.1365-294X.2012.05604.x.

Stensmyr, M.C., Dekker, T., Hansson, B.S., 2003. Evolution of the olfactory code in the *Drosophila melanogaster* subgroup. Proc. Royal Soc. London B 270 (1531), 2333–2340. https://doi.org/10.1098/rspb.2003.2512.

Tait, C., Batra, S., Ramaswamy, S.S., Feder, J.L., Olsson, S.B., 2016. Sensory specificity and speciation: a potential neuronal pathway for host fruit odour discrimination in *Rhagoletis pomonella*. Proc. Royal Soc. London B 283 (1845), 20162101. https://doi.org/10.1098/rspb.2016.2101.

Van der Goes van Naters, W., Carlson, J.R., 2007. Receptors and neurons for fly odors in *Drosophila*. Curr. Biol. 17, 606–612. https://doi.org/10.1016/j.cub.2007.02.043.

Weissburg, M.K., 1999. Tuning breadth and sex-specific sensitivity in chemosensory neurons of male and female *Uca pugnax*. J. Comp. Physiol. A. 185 (3), 229–238. https://doi.org/10.1007/s003590050382.

Weissburg, M.J., Derby, C.D., 1995. Regulation of sex-specific feeding behavior in fiddler crabs: physiological properties of chemoreceptor neurons in claws and legs of males and females. J. Comp. Physiol. A. 176, 513–526. https://doi.org/10.1007/BF00196417.

Wright, C.R., Setzer, W.N., 2014a. Characterization of volatiles of necrotic *Stenocereus thurberi* and *Opuntia littoralis* and toxicity and olfactory preference of *Drosophila melanogaster, D. mojavensis wrigleyi* and *D. mojavensis sonorensis* to necrotic cactus volatiles. Nat. Prod. Commun. 9, 1185–1192. https://doi.org/10.1177/2F1934578X1400900833.

Wright, C.R., Setzer, W.N., 2014b. Chemical composition of volatiles from *Opuntia littoralis, Opuntia ficus-indica*, and *Opuntia prolifera* growing on Catalina Island, California. Nat. Prod. Res. 28 (3), 208–211. https://doi.org/10.1080/14786419.2013.867345.

Yao, C.A., Ignell, R., Carlson, J.R., 2005. Chemosensory coding by neurons in the coeloconic sensilla of the *Drosophila* antenna. J. Neurosci. 25, 8359–8367. https://doi.org/10.1523/JNEUROSCI.2432-05.2005.

Zhang, A., Linn Jr., C., Wright, S., Prokopy, R., Reissig, W., Roelofs, W., 1999. Identification of a new blend of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella*. J. Chem. Ecol. 25, 1221–1232. https://doi.org/10.1023/A:1020910305873.