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# Evolution of Multiple Sensory Systems Drives Novel Egg-Laying Behavior in the Fruit Pest *Drosophila suzukii*

## Highlights

- The pest *Drosophila suzukii* prefers to lay eggs on ripening fruit
- Closely related *Drosophila* species prefer to lay eggs on rotten fruit
- Female flies use chemosensation and mechanosensation to choose an oviposition site
- Orco-dependent detection of ripe fruit odors elicits oviposition in *D. suzukii*

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## In Brief

Karageorgi et al. show that the invasive pest *Drosophila suzukii* has evolved a preference to lay its eggs on ripening fruit. The authors dissect the sensory bases of this preference, pointing to a multi-step evolutionary scenario involving the tuning of different sensory modalities.

# Evolution of Multiple Sensory Systems Drives Novel Egg-Laying Behavior in the Fruit Pest *Drosophila suzukii*

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## SUMMARY

The rise of a pest species represents a unique opportunity to address how species evolve new behaviors and adapt to novel ecological niches [1]. We address this question by studying the egg-laying behavior of *Drosophila suzukii*, an invasive agricultural pest species that has spread from Southeast Asia to Europe and North America in the last decade [2]. While most closely related *Drosophila* species lay their eggs on decaying plant substrates, *D. suzukii* oviposits on ripening fruit, thereby causing substantial economic losses to the fruit industry [3–8]. *D. suzukii* has evolved an enlarged, serrated ovipositor that presumably plays a key role by enabling females to pierce the skin of ripe fruit [9]. Here, we explore how *D. suzukii* selects oviposition sites, and how this behavior differs from that of closely related species. We have combined behavioral experiments in multiple species with neurogenetics and mutant analysis in *D. suzukii* to show that this species has evolved a specific preference for oviposition on ripe fruit. Our results also establish that changes in mechanosensation, olfaction, and presumably gustation have contributed to this ecological shift. Our observations support a model in which the emergence of *D. suzukii* as an agricultural pest is the consequence of the progressive modification of several sensory systems, which collectively underlie a radical change in oviposition behavior.

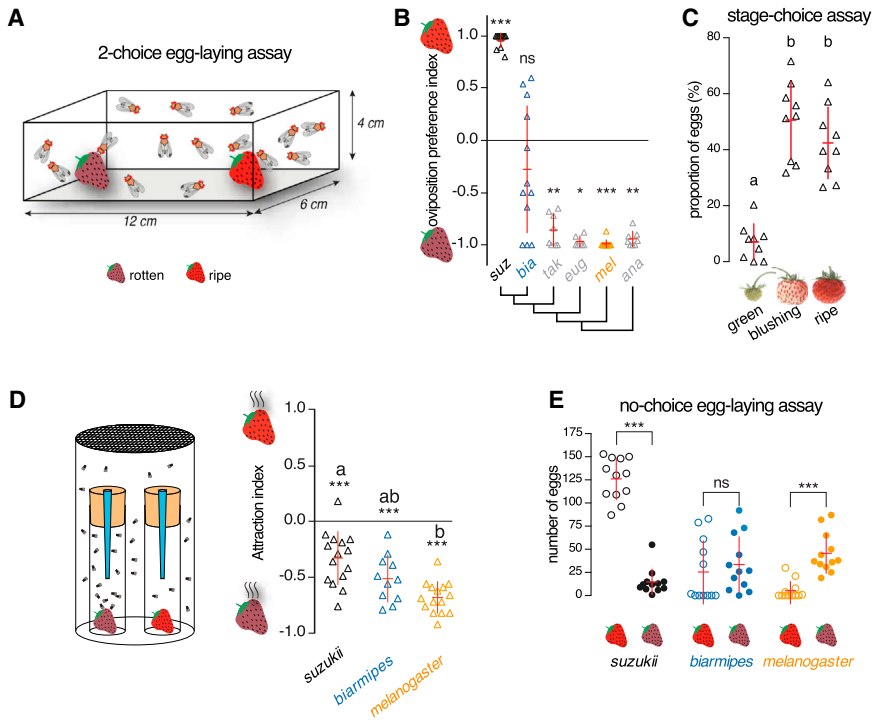
## RESULTS AND DISCUSSION

### *D. suzukii* Females Have Evolved a Preference to Lay Eggs in Ripe Rather Than Rotten Strawberries

We analyzed the oviposition behavior of *D. suzukii* and some close relatives using strawberries (genus *Fragaria*), a main target

of *D. suzukii*, at different stages of maturation [10]. We first compared ripe, pristine strawberries (hereafter referred to as “ripe fruit”), purchased from a local grocery store, to strawberries from a similar batch left to decay for 4 days (hereafter referred to as “rotten fruit”; see [Supplemental Experimental Procedures](#)). We then assessed the egg-laying behavior of *D. suzukii* and five closely related species [11] on ripe and rotten fruit using a two-choice oviposition assay (Figure 1A). We counted the number of eggs laid in each fruit after 19 hr and calculated an oviposition substrate preference index (PI). Using this two-choice assay, we observed a robust oviposition preference for most species and striking differences between *D. suzukii* and the other species (Figures 1B and S1A). *D. suzukii* females laid almost all of their eggs on ripe fruit, whereas *D. ananassae*, *D. melanogaster*, *D. eugracilis*, and *D. takahashii* demonstrated the opposite behavior, targeting rotten fruit almost exclusively. Remarkably, *D. biarmipes* showed an intermediate behavior with no marked preference for either substrate. To test whether the fruit skin is a deterrent barrier resulting in these different behaviors, we repeated the choice assay with ripe fruits sliced in half, to expose their flesh, and rotten fruits. We found that *D. biarmipes* laid approximately equal numbers of eggs on both substrates, while *D. melanogaster* maintained a strong preference for the rotten fruit (Figure S1B). These results establish that the preference for laying eggs on rotten fruit is ancestral to this group of species, and that a preference for oviposition on ripe fruit has evolved in the lineage leading to *D. suzukii*.

To further determine the preferred range of fruit ripening stages [12] targeted by *D. suzukii* for oviposition, we offered *D. suzukii* females the choice between “green,” “early/late blushing,” and “ripe” fruit (Figures S1C and S1D). Although flies managed to lay a few eggs in the green fruit, the vast majority of eggs were laid in fruit of later maturation stages (Figure 1C). We concluded that *D. suzukii* has access to strawberries at the onset of their maturation but strongly prefers blushing and ripe fruit stages. These results are consistent with previous observations that used different strawberry cultivars and other species of berry [10, 13, 14]. Together, they establish that *D. suzukii*, compared to other closely related species, has shifted its



**Figure 1. *D. suzukii* Females Have an Evolutionarily Novel Preference for Laying Eggs in the Early Stages of Strawberry Maturation**

(A) Two-choice oviposition assay on whole fruits, where ten mated *Drosophila* females (mixed with five males) lay their eggs on two different substrates (ripe versus rotten strawberries). Eggs were counted at the end of each assay.

(B) Oviposition preference (two-choice assay as depicted in A) of six closely related species of the *D. melanogaster* species group. Whereas most species show a strong preference to lay their eggs on rotten fruit, *D. suzukii* females have radically shifted their preference to lay eggs on ripe fruit.

Interestingly, *D. biarmipes*, a close relative of *D. suzukii*, displays a mild but not statistically significant preference for rotten fruit. All p values were calculated via Wilcoxon matched-pairs signed-rank test. p values in this and all subsequent figures: \*\*\* $p \leq 0.001$ ; \*\* $p \leq 0.01$ ; \* $p \leq 0.05$ ; ns,  $p > 0.05$ , i.e., non-significant difference. In this and all subsequent figures, the red bars indicate the mean  $\pm$  SD unless otherwise noted. Replicates in preference experiments are represented by triangles ( $n = 6$  to 12 replicates per species).

(C) When given a choice between three stages of the same strawberry cultivar (*Fontaine* strawberries; see [Supplemental Experimental Procedures](#)), *D. suzukii* selects predominantly the

ripening (blushing) or mature (ripe) fruits. Significant differences are denoted by letters (Friedman test;  $\chi^2(2) = 14$ ,  $p < 0.001$ , followed by Conover's test;  $p \leq 0.01$ ).  $n = 9$  replicates per species.

(D) Olfactory attraction assay (trap; design of the assay is shown on the left). All three species (*D. suzukii*, *D. biarmipes*, and *D. melanogaster*) are more attracted to rotten strawberry odors, but *D. suzukii* relatively less than *D. melanogaster*. p values were calculated via one-sample t test for each species; significant differences between species are denoted by letters (ANOVA followed by Tukey's test for multiple comparison;  $p < 0.001$ ).  $n = 11$  or 15 replicates per species.

(E) A no-choice oviposition assay, similar to the two-choice assay in (A), but in which ten females and five males were presented with one type of fruit (ripe or rotten), reveals oviposition on ripe or rotten strawberries for *D. suzukii*, *D. biarmipes*, and *D. melanogaster*. This shows that *D. suzukii* can lay eggs on rotten fruits, although not much, and conversely that *D. melanogaster* can lay eggs on ripe fruits, although not much. *D. biarmipes* appears to lay eggs indifferently on both substrates. All p values were calculated via Mann-Whitney test. In this and all subsequent figures, the number of eggs laid per replicate is presented with open (ripe fruit) or filled (rotten fruit or other) circles.  $n = 12$  replicates per species.

See also [Figure S1](#).

oviposition target from rotten to earlier stages of fruit maturation. We explored this behavioral shift further by focusing on three species: the genetic model *D. melanogaster*; *D. biarmipes*, a close relative of *D. suzukii* not known as a ripe fruit pest; and *D. suzukii* itself.

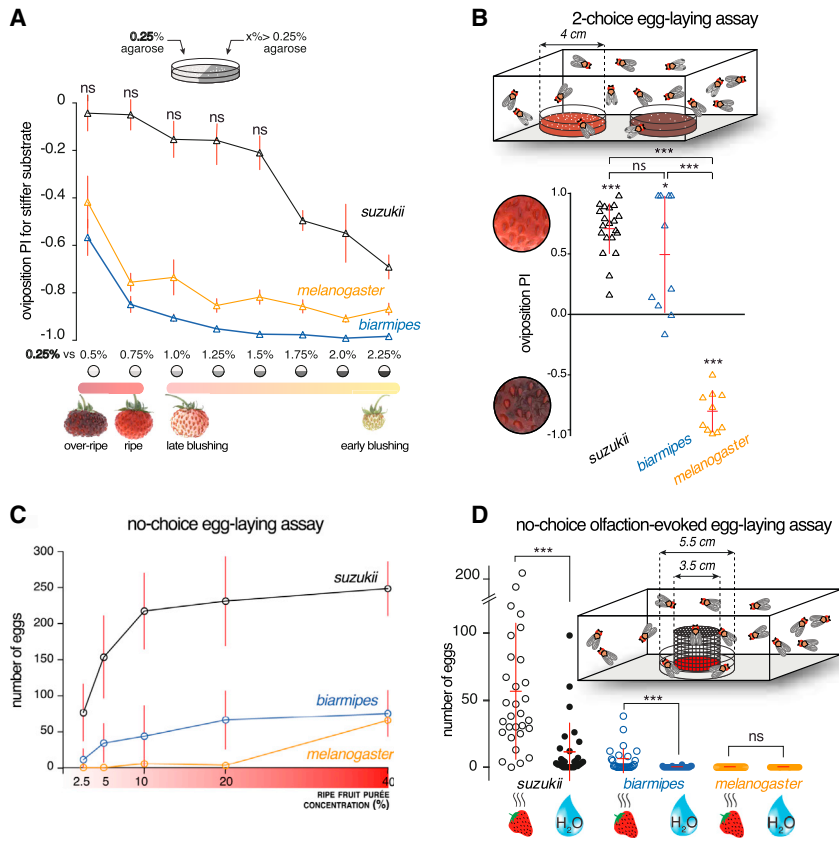
### The Preference of *D. suzukii* for Ripe Fruit Is Specific to Oviposition

We sought to determine whether the preference of *D. suzukii* for ripe fruit is specific to oviposition or a facet of a general ecological shift, as has been observed for other drosophilids [15]. We first found, using an olfactory trap assay, that all three species are more attracted to the odor of rotten fruit, although *D. suzukii* shows a weaker preference ([Figure 1D](#)). Hence, the preference for ripe fruit that *D. suzukii* displays in the context of oviposition seems specific to this behavior. Its attraction to rotten fruit may instead relate to feeding. To examine this possibility, we compared feeding behaviors of all three species with an assay similar to that depicted in [Figure 1A](#), but using either ripe or rotten fruit alone (no-choice assay). While *D. suzukii* fed more on ripe strawberries than *D. melanogaster* (as indicated by the red color of the abdomen), both species show a similarly strong appetite

for rotten fruit ([Figure S1E](#)). We conclude that *D. suzukii* is attracted to rotten fruit mostly for feeding and targets ripe fruit mostly for oviposition, in agreement with published data [16].

We next evaluated the intrinsic capacity of each fruit stage to elicit oviposition using a no-choice oviposition assay. We observed that *D. suzukii* laid more eggs on ripe strawberries alone than on rotten strawberries alone; by contrast, *D. melanogaster* laid more eggs when exposed to rotten fruit, while *D. biarmipes* laid similar numbers of eggs on both fruit stages ([Figure 1E](#)). These results suggest that the relative preferences observed in the two-choice assay result directly from the absolute capacity of each substrate to elicit oviposition for each species. We went on to dissect the properties of the fruit that females select as their preferred egg-laying substrates.

***D. suzukii* Tolerates Stiffer Substrates for Oviposition** The enlarged ovipositor of *D. suzukii* presumably endows the females with the capacity to more easily pierce the stiff skin of a ripe fruit [9]. We wondered whether flies exploit the stiffness of the fruit skin, which decreases with maturation [17], to assess oviposition substrate quality. We exposed females to substrates that differ only in stiffness, in the form of Petri dish halves filled



**Figure 2. Changes in Mechanosensory and Chemosensory Systems Underlie the Evolution of Oviposition Site Preference**

(A) Two-choice oviposition assay for substrate stiffness (schematic depicted above the graph). For each point ( $n = 8$  replicates), a group of females had the choice to lay eggs on 0.25% agarose or an increasingly stiffer substrate from 0.5% to 2.25% agarose in steps of 0.25 percentage point. All substrates were equally supplemented with glucose. The result is expressed as a preference index for the stiffer substrate (red bars indicates here mean  $\pm$  SEM rather than mean  $\pm$  SD, for the sake of figure clarity). Whereas *D. melanogaster* and *D. biarmipes* showed a consistent strong preference for the softer substrate—even when the alternative was only marginally stiffer (0.25% versus 0.5%)—*D. suzukii* was relatively indifferent to the substrate stiffness until the concentration difference was higher than 1.5 percentage points. The turning point in the preference of each species matched the stiffness of the fruit stage in which they preferred to lay eggs in choice assays (Figures 1B, 1C, and S1A). All p values were calculated via Wilcoxon tests for each point with comparison to a theoretical value of 0 (no preference).  $p \leq 0.01$  for all samples, except where “ns” is indicated.

(B) Two-choice oviposition assay for substrates of different chemical composition. Ten *Drosophila* females (and five males) had the choice between two substrates of equal stiffness (1% agar) but different chemical composition. One egg-laying plate contained 35% w/v ripe strawberry purée, while the other plate contained 35% w/v rotten

strawberry purée. The results recapitulate the preference of *D. melanogaster* and *D. suzukii* observed in a similar two-choice assay using whole fruits (Figure 1A), while *D. biarmipes* shows in this assay a mild and variable preference for ripe fruit purée. p values were calculated via Wilcoxon tests with comparison to a theoretical value of 0 (no preference) for each species, and via Kruskal-Wallis test followed by Dunn’s test ( $p < 0.001$ ) for interspecies comparisons.  $n = 10$ –19 replicates per species.

(C) Oviposition in *D. suzukii* increases in response to increasing concentrations of ripe strawberry purée. *D. melanogaster* hardly responds to this substrate, while *D. biarmipes* shows a moderate response.  $\log EC_{50}$  of *D. suzukii* (5.7%) and *D. biarmipes* (8.6%) are significantly different (measured via a non-linear regression analysis of dose response,  $p < 0.001$ ); the  $\log EC_{50}$  of *D. melanogaster* (23.9%) is not statistically comparable to the other species due to a poor fit of the data.  $n = 16$  replicates per species. Each replicate includes ten females.

(D) Oviposition response elicited by the odor of ripe fruit or water in *D. suzukii*, *D. biarmipes*, and *D. melanogaster*. In this no-choice egg-laying assay, females were placed in a chamber similar to the one depicted in Figure 1A and were offered an agar plate for egg laying. A source of odor placed at the center of the plate was covered by a mesh and therefore could not be directly contacted by the flies. *D. suzukii* laid abundantly on the agar when exposed to the odor of ripe strawberry purée but laid significantly less when exposed to water. By contrast, strawberry odors hardly elicited egg laying in *D. biarmipes* and did not elicit egg laying at all in *D. melanogaster*. p values were calculated via Mann-Whitney test.  $n = 28$ –30 replicates per condition (15 females and 0 males per replicate).

See also Figures S1 and S2.

with agarose at different concentrations and equally sweetened with glucose to stimulate ovipositioning (Figure 2A, top). We then measured and correlated the stiffness of agarose at different concentrations to that of strawberries at different stages (Figure S2A). Exposed to a choice between agarose at 0.25% (stiffness of a rotten fruit) and any stiffer substrate, up to 2.25% agarose (earlier fruit maturation stages), *D. melanogaster* and *D. biarmipes* always strongly preferred softer substrates. By contrast, *D. suzukii* displayed hardly any preference for softer substrates until the stiffer substrate reached 1.75% agarose (Figure 2A). We concluded that all three species exploit substrate stiffness for oviposition site selection, but in different ranges. The relaxation of the stiffness threshold observed in *D. suzukii* implies functional changes in the mechanosensory system of this species.

### Chemical Cues Drive Species-Specific Oviposition Substrate Preferences

Chemical composition also changes with fruit maturation [12, 18]. To test its influence on oviposition site selection, we used substrates of fixed stiffness (1% agarose) containing either ripe or rotten strawberry purée (see Supplemental Experimental Procedures). We then tested oviposition preference in a two-choice assay (Figure 2B, top) in darkness (to circumvent slight color differences). The chemical stimuli were sufficient to recapitulate the egg-laying preference of *D. melanogaster* and *D. suzukii* on whole fruits (compare Figures 1B and 2B), while *D. biarmipes* showed an intermediate behavior (Figure 2B). We concluded that the chemical composition of the substrate is a primary determinant guiding oviposition site selection for *D. suzukii* and *D. melanogaster*. For *D. biarmipes*, the contrasting results



obtained with whole fruits (Figure 1B) or agar-based substrates (Figure 2B) suggest that this species has evolved a mild preference for the chemicals of ripe fruit that is balanced with a strong preference for soft substrates. The previous results suggest that chemical cues from ripe fruit elicit variable oviposition responses in different species. To compare the quantitative response to these cues, we exposed *D. suzukii*, *D. biarmipes*, and *D. melanogaster* females to a dilution series of ripe strawberry purée plates. We observed that the egg laying increased with the concentration of ripe fruit purée (Figure 2C). Although the dose response was shared by all species, *D. suzukii* responded much more, and at lower concentrations of ripe fruit purée, than *D. melanogaster* or, to some degree, *D. biarmipes*. These results show that the oviposition site preference of *D. suzukii* for ripe fruit is mediated at least in part by chemical cues. They also suggest that the chemosensory system involved in oviposition has changed in *D. suzukii* compared to *D. biarmipes* and *D. melanogaster*.

### **Strawberry Odors Are Sufficient to Evoke Oviposition in *D. suzukii***

We then set out to determine how *D. suzukii* females perceive the chemical cues that elicit their oviposition on ripening fruit. We first tested the sufficiency of olfaction to respond to these cues and elicit oviposition. Specifically, we asked whether the odor of ripe strawberries alone could evoke egg laying in *D. suzukii*, *D. biarmipes*, and *D. melanogaster*. We placed flies in a chamber containing agar plates with, at the center, a cup filled with ripe strawberry purée or water; the cup was covered with a metallic mesh allowing the flies to smell but not to contact its content (Figure 2D). The odor of ripe strawberries alone elicited oviposition by *D. suzukii* on plain agar, and to a lesser extent by *D. biarmipes* as well (Figure 2D). By contrast, it did not elicit *D. melanogaster* to lay eggs at all (Figure 2D). To eliminate the possibility that *D. melanogaster* did not lay eggs simply because females disliked plain agar, we created conditions for an oviposition baseline by supplementing the agar with 5% fructose. We then surveyed oviposition enhancement from this baseline upon exposure to fruit odor and found that the odor of ripe fruit enhanced oviposition in *D. suzukii*, but not in *D. melanogaster* (Figure S2B). Finally, we demonstrated that the oviposition enhancement in *D. suzukii* is not the indirect result of a stronger attraction to the odor source. First, replacing strawberry odors by acetoin, a potent attractant of *D. melanogaster* [19] and *D. suzukii* (Figure S2C), did not enhance *D. suzukii* oviposition (Figure S2B). Second, *D. suzukii* and *D. melanogaster* were equally attracted to ripe strawberry volatiles (Figure S2D). Together, these results reveal that ripe strawberry odors are sufficient to elicit oviposition in *D. suzukii*.

**OR-Mediated Olfaction Elicits Oviposition in *D. suzukii*** To measure the contribution of olfaction to the selection of an oviposition site, we first ablated the antennae (the main olfactory organs) of female *D. suzukii*. In a two-choice assay with fruit purée plates, the ablated flies displayed a reduced preference for ripe fruit compared to the non-ablated control flies (Figure S2E), mostly due to a reduction of egg laying on ripe fruit substrate (Figure S2F). We concluded that olfaction from antennae is partially necessary for selecting between ripe and rotten fruits in

*D. suzukii*. This also indicates that the maxillary palps (the other olfactory organs) or the perception of chemosensory cues by direct contact can partly compensate for the absence of antennae. We further analyzed the role of olfaction in egg laying in *D. suzukii* by impairing olfaction genetically. We focused on the odorant receptor (OR) system, one of the two olfactory receptor families expressed in antennae chemosensory neurons [20, 21]. We targeted the obligate co-receptor *Orco*, or the neurons that express it, to interfere with OR-mediated olfaction [19, 22].

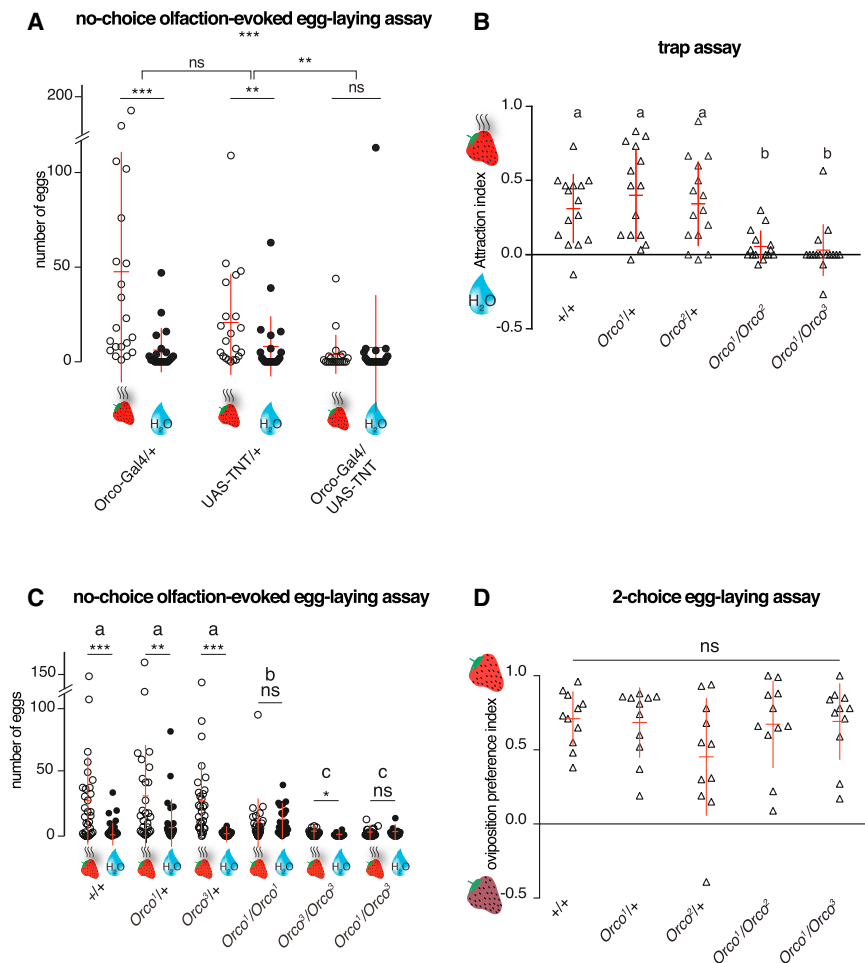
We first generated a *D. suzukii Orco*-Gal4 line [19] to target *Orco*-positive sensory neurons (Figure S3A) and a UAS-CD4-tdTomato line [23] to visualize the projections of *Orco*-Gal4-positive cells. In *D. suzukii*, the *Orco*-Gal4 and UAS-CD4-tdTomato combination labels neurons projecting to the antennal lobe (Figures S3B<sub>4</sub> and B<sub>5</sub>), consistent with what has been described in *D. melanogaster* (Figures S3C<sub>4</sub> and C<sub>5</sub>). These neurons also express endogenous *Orco*, detected by an antibody in the antenna (Figures S3B<sub>1</sub>-B<sub>3</sub> and C<sub>1</sub>-C<sub>3</sub>), showing that the *Orco*-Gal4 construct targets *Orco*-positive cells.

To block synaptic transmission and impair *Orco* neuron-mediated olfaction, we created a *D. suzukii* UAS-TNT transgenic line [24] and crossed it to our *Orco*-Gal4 line. In a trap assay with acetoin, an odor perceived by *Orco*-expressing neurons [19], we observed a severe reduction of attraction to acetoin (Figure S3D) in *Orco*-Gal4, UAS-TNT flies but normal locomotion (Figure S3E), confirming that these constructs impair OR-mediated olfaction. We then subjected *Orco*-Gal4, UAS-TNT *D. suzukii* females to the olfaction-evoked oviposition assay (Figure 2D) with ripe strawberry odors. We found that olfaction-evoked oviposition was almost abolished compared to the control genotypes (Figure 3A), showing that *Orco*-positive neurons are involved in the oviposition elicited by ripe fruit odors in *D. suzukii*.

We also generated *D. suzukii Orco* mutants using the CRISPR-Cas9 system [25]. We obtained three alleles, named *Dsuz\Orco*<sup>1</sup>, *Dsuz\Orco*<sup>2</sup>, and *Dsuz\Orco*<sup>3</sup> (Figure S4A). These mutants are protein null (Figure S4B). While wild-type *D. suzukii* antennae respond to ripe strawberry odor and to the control odor acetoin in electroantennograms (EAGs), mutant antennae did not respond to either smell (Figure S4E). Consistent with this data, the attraction of *Dsuz\Orco* mutants to acetoin (Figure S4C) or ripe strawberry odors (Figure 3B) was severely impaired, although their locomotion was unaffected (Figure S4D), revealing that they are loss-of-function alleles.

We then exposed *Dsuz\Orco* mutants to the olfaction-evoked oviposition assay (as in Figure 2D). The mutant females were not stimulated, or were significantly less stimulated, to lay eggs in response to ripe strawberry odors compared with the control genotypes (Figure 3C), similar to what we observed upon silencing the *Orco*-expressing neurons (Figure 3A). Altogether, these results reveal that the OR subsystem is essential in *D. suzukii* for the perception of ripe fruit volatiles and the oviposition elicited by these odors.

Finally, we tested the oviposition preference of the *Dsuz\Orco* mutants in a two-choice assay with whole ripe and rotten strawberries (Figure 1A). We found no difference in oviposition preference between the *Dsuz\Orco* mutants and the wild-type controls (Figure 3D), revealing that OR-mediated olfaction becomes redundant for egg-laying site selection when other



**Figure 3. Orco and Orco-Expressing Neurons Mediate Oviposition Enhancement in Response to the Odor of Ripe Strawberries in *D. suzukii***

(A) In a no-choice olfaction-evoked egg-laying assay (as depicted in Figure 2D), the enhanced egg laying elicited by the odor of ripe fruits in *D. suzukii* was abolished when neurotransmission was specifically blocked in Orco-positive neurons.  $n = 21$  replicates per condition (15 females and 0 males per replicate).  $p$  values were calculated via Mann-Whitney test for each genotype, using a negative binomial generalized linear model followed by a general linear hypothesis test for multiple comparisons with a false discovery rate (FDR) correction method.  $n = 21$  replicates per condition (15 females and 0 males per replicate).

(B) The loss of Orco function in *D. suzukii* prevents attraction to the odors of ripe strawberries in a trap assay against water. Significant differences are denoted by letters (ANOVA followed by Tukey's test for multiple comparison;  $p < 0.001$ ).  $n = 10$ –13 replicates per condition (15 males and 15 females per replicate).

(C) The enhanced egg laying elicited by the odor of ripe fruits in a no-choice olfaction-evoked egg-laying assay was also significantly diminished or abolished in *D. suzukii* when the function of Orco is lost.  $p$  values were calculated via Mann-Whitney test for each genotype, using a negative binomial generalized linear model followed by a general linear hypothesis test for multiple comparisons with a FDR correction method to compare the genotypes.  $n = 26$ –30 replicates per condition (10 females and 0 males per replicate).

(D) In a two-choice assay with whole strawberries, in which the flies could contact and presumably taste the fruits, *D. suzukii* Orco mutants preferred

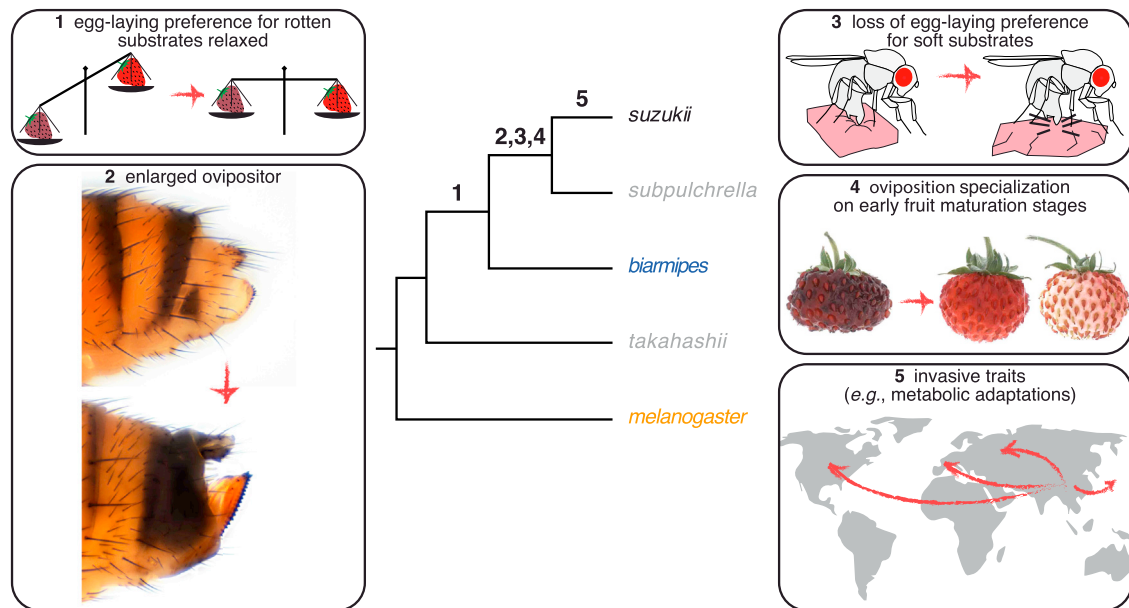
to lay eggs on ripe fruits as much as wild-type females did.  $n = 11$  replicates per condition (15 females and 0 males per replicate) for multiple comparison;  $p < 0.001$ . See also Figures S3 and S4.

sensory stimuli are available. Consistent with this, when females in which Orco neuron output is silenced could contact strawberry purée, and presumably taste it, they laid eggs at levels comparable to wild-type flies (Figure S3F). These results suggest that contact chemosensation, in conjunction with olfaction, also contributes to the oviposition behavior of *D. suzukii* on ripe fruit.

### A Multi-step Evolutionary Scenario for the Making of a Pest Species

The evolution of *D. suzukii* as a pest species could be regarded as the result of a single key innovation: its enlarged, serrated ovipositor that enables females to pierce the skin of many ripe fruits. We have found that the egg-laying substrate preference of *D. suzukii* has evolved in concert with its morphology and was instrumental in the shift to a new reproductive niche. Our work shows that the divergence in oviposition behavior is associated with the modification of multiple sensory modalities, namely mechanosensation and chemosensation, that determine differences in the egg-laying site choice between *D. melanogaster*, *D. biarmipes*, and *D. suzukii*.

The comparison of *D. suzukii* with multiple closely related species, in particular *D. biarmipes*, suggests a possible scenario for the evolution of *D. suzukii* as a pest species (Figure 4). In this scenario, oviposition in species like *D. melanogaster* is strongly elicited by rotten fruit and is inhibited by stiff substrates. In the lineage leading to *D. suzukii*, the oviposition response to ripe fruit has progressively increased, as in *D. biarmipes*, whose behavior is intermediate between that of *D. melanogaster* and *D. suzukii*. Such species, however, can only exploit ripe or slightly damaged fruit of sufficient softness. Presumably, the small ovipositor in these species prevented the full exploitation of the ripe fruit niche. Only in *D. suzukii*, and its close relative *D. subpulchrella* [9], did chemosensory specialization for ripe fruit cues, broadening of substrate stiffness preference, and evolution of an enlarged ovipositor come together and endow the flies with the capacity to fully use ripe fruit as oviposition substrates. Finally, additional physiological adaptations (e.g., metabolic changes [27]) may have provided *D. suzukii* with the potential to adapt to different environments and invade new geographical areas. In this stepwise scenario, the evolution of the ovipositor of *D. suzukii* was certainly a key acquisition, but it was secondary



**Figure 4. A Possible Evolutionary Scenario for the Shift in *D. suzukii* Oviposition Behavior**

The emergence of *D. suzukii* as a pest species is associated with multiple evolutionary changes, either identified or suspected. These comprise (1) a broadening of the range of possible oviposition sites to include earlier fruit maturation stages, (2) the morphological expansion and strengthening of the ovipositor, (3) the tuning of mechanosensory preferences to accept stiffer substrates, (4) the tuning of chemosensory modalities to earlier stages of fruit maturation in the context of egg laying, and (5) physiological and metabolic changes enabling the fly to spread across a broad geographic range. The order of these changes is speculative, but the intermediate state of *D. biarmipes* for some of these traits suggests the genetic potentiation of the clade containing these species to gain such traits [26].

to the behavioral changes that endowed some ancestors with an opportunistic egg-laying behavior toward ripe fruits.

We propose that the evolutionary origin of *D. suzukii* as a pest species was therefore made possible by the progressive tuning of multiple sensory systems, which might be mirrored in changes in its sensory receptor genes [28, 29] or the determinants of neuronal connectivity. Our results suggest that these traits may have emerged in a clade predisposed [26] for this behavioral shift.

#### SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and Supplemental Experimental Procedures and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2017.01.055>.

#### AUTHOR CONTRIBUTIONS

B.P. and N.G. conceived the project. B.P., N.G., M.K., L.B.B., and S.L. designed the experiments. M.K., L.B.B., and S.L. carried out the behavioral experiments and the statistical analyses. C.M. and M.K. built the constructs used in Figure 3 and S3 (Orco-Gal4 [C.M.]; UAS-CD4-tdTomato and UAS-TNT [M.K.]). M.C. characterized the expression of Orco and reporter genes (Figures S3 and 4). B.P., C.M., and M.K. generated all transgenic and CRISPR mutant lines. N.G. documented the strawberry stages. I.C.G.K. and K.P.S. designed and analyzed the electrophysiology experiments (Figure S4E), which K.P.S. carried out. B.P. and N.G. wrote the manuscript with the help of all authors.

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