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Report

Olfactory Shifts Parallel Superspecialism for Toxic Fruit in *Drosophila melanogaster* Sibling, *D. sechellia*

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Summary

Olfaction in the fruit fly *Drosophila melanogaster* is increasingly understood, from ligand-receptor-neuron combinations [1–4] to their axonal projection patterns into the antennal lobe [5, 6]. *Drosophila* thus offers an excellent opportunity to study the evolutionary and ecological dynamics of olfactory systems. We compared the structure and function of the generalist *D. melanogaster* with that of specialist *D. sechellia*, which oviposits exclusively on morinda fruit [7]. Our analyses show that whereas the fruit's headspace was dominated by acids, antennae responded most strongly to hexanoates. *D. sechellia* exhibited an extraordinarily strong response to methyl hexanoate (MeHex). Behaviorally, *D. sechellia* was much more attracted to these morinda fruit volatiles than was *D. melanogaster*. The high sensitivity to MeHex was paralleled by a 2.5×–3× overrepresentation of MeHex neurons on the antenna and a concordant 2.9× increase in volume of the corresponding glomerulus as compared to *D. melanogaster*. In addition, the MeHex neuron exhibited an extreme sensitivity down to femtograms of its ligand. In contrast, no peripherally mediated shift was found paralleling *D. sechellia*'s increased attraction to acids. These findings are a demonstration of evolution acting at several levels in the olfactory circuitry in mediating a fruit fly's unique preference for fruit toxic to its sibling species [8–10].

Results and Discussion

Morinda Fruit Volatiles, Detection, and Behavior

The *Drosophila melanogaster* species complex, consisting of nine sibling species, originated in subsaharan Africa. Whereas most species today are generalists, several have evolved some level of specialization [7, 11]. *D. sechellia* is by far the most stringent specialist, ovipositing on the fruit of *Morinda citrifolia* (morinda fruit). To other members of the *melanogaster* subgroup, this fruit is toxic and avoided. It is generally thought that morinda fruit acids, particularly octanoic acid, are key cues for host-searching *D. sechellia* [8, 9], a preconception probably caused by the focus on octanoic acid as the compound causing morinda fruit toxicity, *D. sechellia*'s oligogenic adaptations to cope with octanoic acid [9, 12, 13], and the fact that it stimulates oviposition in *D. sechellia* [10, 12]. We verified the fly's detection of ripe morinda fruit (Figure 1B) volatiles by using gas chromatography coupled with electro-antennographic detection (GC-EAD; Figure 1A) and mass spectrometry (GC-MS, see Supplemental Data available with this article online).

GC-EAD and GC-MS

Morinda fruit headspace is dominated by medium-chain aliphatic acids and esters, which give the fruit a characteristic smell, reminiscent of pineapple and blue cheese (Figure 1C). Fly antennae responded by far most strongly to the hexanoate esters (Figure 1C), whereas acids elicited only very weak responses. In addition, *D. sechellia* antennae showed a strongly increased response to MeHex (MeHex) compared to *D. melanogaster*. We verified the importance of these major morinda fruit volatiles, acids and hexanoates, in behavioral assays.

Behavior to Acids

In agreement with the GC-EAD recordings, both *D. sechellia* and *D. melanogaster* showed a low behavioral sensitivity to acids; no attraction was observed below 1000-fold dilution of pure compound (Figures 2A–2D). Compared to *D. melanogaster*, *D. sechellia* was more attracted to all acids, even when presented pure, and more strongly preferred hexanoic acid over octanoic and ethanoic acid (Figures 2A–2D).

Behavior to Hexanoates

MeHex is attractive to both species at midrange concentrations (Figure 2F). In contrast to *D. melanogaster*, however, *D. sechellia* showed no tapering in the attraction to MeHex at high concentrations, and attraction was supported down to at least 10× lower concentrations. The lower-concentration tests may even underestimate the actual response thresholds, because of the nonlinear evaporation of the minute amounts of MeHex and the slow trap-entry responses of flies.

The results show that *D. sechellia*'s behavior is tuned to the major volatiles of its sole host, morinda. In addition, *D. sechellia* is behaviorally much more sensitive to hexanoates than to hexanoic acid. Although the precise head-space concentrations are not known, the 10^6 difference in dilution supports several orders of magnitude difference in headspace concentration. This supports the notion that hexanoates are much more important in fruit-searching *D. sechellia* than acids. We infer that acids are detected only very close to the host and mediate behaviors such as oviposition [12].

Peripheral Changes of *D. sechellia*'s Olfactory Appendages

We investigated in detail whether any possible peripheral changes could account for *D. sechellia*'s enhanced antennal response to MeHex, as well as the increased behavioral preference for MeHex and acids. In a previous study, we found that the olfactory code among eight sibling species of *D. melanogaster* was highly conserved,

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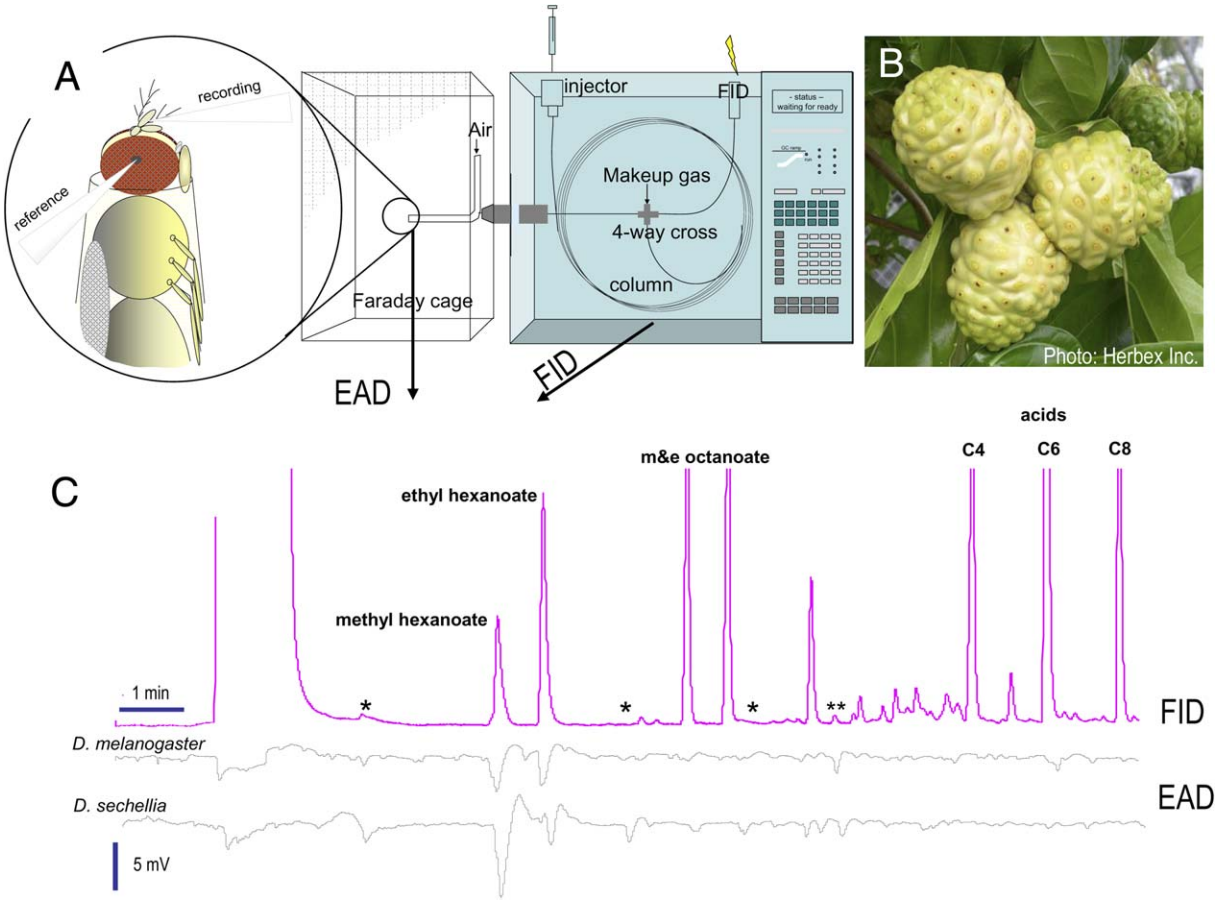

Figure 1. GC-EAD Analysis of Morinda Fruit

(A) The setup for gas chromatography combined with flame ionization and electroantennographic detection (GC-EAD; odor collection, GC-MS see [1]). FID, flame ionization detector. *, response not repeatable; **, unidentified peak.

(B) Fresh ripe morinda fruits on a tree.

(C) GC-EAD traces of *D. melanogaster* and *D. sechellia* to headspace collections of ripe morinda fruit. Note the strongly increased response of *D. sechellia* to methyl hexanoate compared to its sibling.

with only in *D. mauritiana* and *D. sechellia* deviating from the ancestral physiological characteristics [14].

Morphology

D. sechellia antennae were morphologically distinct from *D. melanogaster*, with trichoid (T) sensilla ~60% shorter than those of *D. melanogaster* (Figure 3A). The T1 sensillum responded in both species to Z11-vaccenyl acetate, a male-produced pheromone of the *Drosophila melanogaster* subgroup [15, 16]. Several, but not all, small basiconic sensilla on *D. sechellia* antennae also appeared smaller than in its sibling species. Other sensilla appeared morphologically similar across the sibling species. Interestingly, the abundance of hair-like structures also seem to be reduced in larval *D. sechellia* [13], although the ecological significance is not clear.

ab3 Overexpression

Ethyl hexanoate (EtHex) and MeHex have been described previously as key ligands of the ab3A neuron and its olfactory receptor (OR), Or22a [1, 14, 16]. We verified the response patterns from more than 2000 large basiconic sensilla from 98 *D. sechellia* females obtained from different lines. Concomitant with the increased EAD response to MeHex, all *D. sechellia* lines overexpressed ab3 at the cost of ab2 and ab1 sensilla in comparison to *D. melanogaster* (see also [14]). In several individuals, we verified the overexpression through recordings from up to 80% of the large basiconic sensilla (Figure 3B). Small clusters of ab1 sensilla were found distally and medially of the sacculus (7–15, depending on the line; Figure 3B), and sparsely medially, whereas no (DsJ, Ds0248.7, Ds0248.8) or up to 2 (Ds0248.1) ab1 sensilla were found on the anterior side of the antenna. In total, *D. sechellia* had 10–18 ab1 sensilla (with some variation between strains), a reduction of ~60%–80% compared to *D. melanogaster*. We found 0–2 ab2 sensilla (0 ab2 in Ds0248.1 and Ds0.248.8, 1–3 ab2 in DsJ and Ds0248.7), a reduction of 93%–100%. The total number of ab3 on *D. sechellia* antennae was 80–93, a 2.5- to 3-fold increase compared to *D. melanogaster*. Visualization of the higher abundance of ab3 sensilla was performed with cryosections of hybrid male *D. sechellia* × female *D. melanogaster* Or22A-nsyb-GFP. Hybrids had an intermediate number of ab3 sensilla, ascertained through counts of anti-GFP-stained cell bodies (Figure 3C).

We do not currently know whether the ORs that are expressed in ab2 neurons in *D. melanogaster*, Or59b

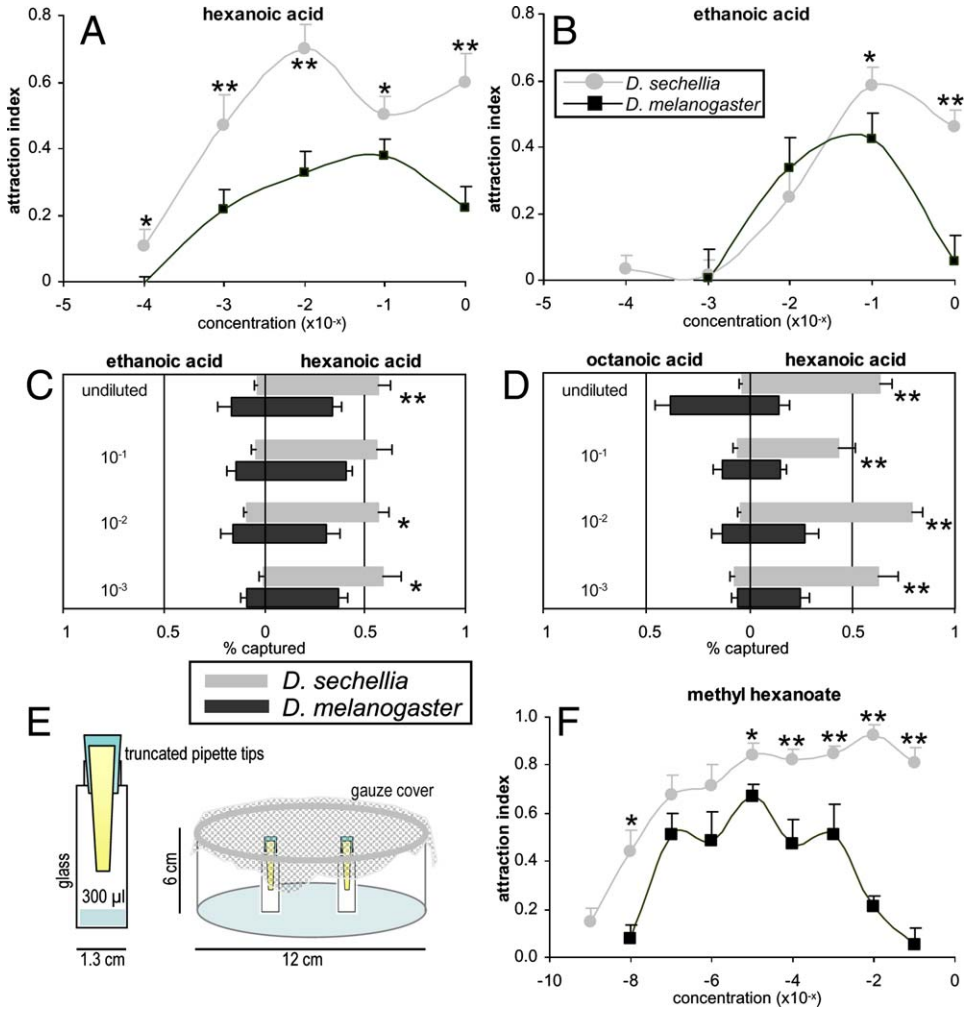

Figure 2. Two-Choice Behavioral Bioassays

(A–D) Responses to acids (±SE).

(E) Two-choice bioassays.

(F) Responses to methyl hexanoate (±SE). For explanation, see text. Attraction index in (A) and (B): (T − C)/(T + C + NR − D), in which T is the number of flies in the treatment, C number in the control, NR number remaining in the arena, and D the number of dead flies in the arena. Assays were performed under L:D 12:12 and lasted 18 hr. Odors (>98% purity, Sigma-Aldrich) were diluted in water + 0.2% Triton-X. *$p < 0.05$, **$p < 0.005$ (t test).

and Or85a [5, 6], are not expressed in *D. sechellia* antennae at all or may have shifted location. However, both ORs mediate responses to volatiles not present in morinda fruit, indicating that, because of *D. sechellia*'s specialization, the putative loss of ORs may not have been countered by selection.

The overexpression of a sensillar subtype resembles at a finer scale the effect of transcription factors *atonal*, which determines the expression of coeloconic versus basiconic and trichoid sensilla [17], and *amos*, which regulates in a dose-dependent manner basiconic to trichoid fate [18, 19]. In another study, a single mutation at the *ovo/shaven-baby* locus caused the loss of cuticular bristles in *D. sechellia*, underlining the potentially strong effects of single genes on patterning of hair-like structures. Further research is required to determine how many genes are involved in the ab3 overexpression in *D. sechellia*.

Hypersensitivity to MeHex

In addition to the higher abundance, *D. sechellia* ab3A neurons responded more strongly to MeHex than EtHex ($10^2\times - 10^3\times$; Figure 3D). *D. melanogaster* ab3A neurons, on the other hand, were slightly more sensitive to EtHex than MeHex (around 10×; Figure 3D). *D. sechellia* ab3A neurons generally displayed also a higher sensitivity to hexanoates (>10 times compared to *D. melanogaster* ab3A), responding down to 5 fg MeHex on a filterpaper (Figure 2D), the highest sensitivity ever reported for an insect ORN. The increased sensitivity could possibly be caused by upregulation of ORs. Similarly, but on a different timescale, it has been found that insect ORN sensitivity and OR expression may fluctuate with, e.g., feeding status [20] and circadian rhythms [21, 22]. In mosquitoes, several but not all Ors are downregulated within 12 hr following a blood meal [20]. The OR downregulation correlated with a 5×–10× lower ORN

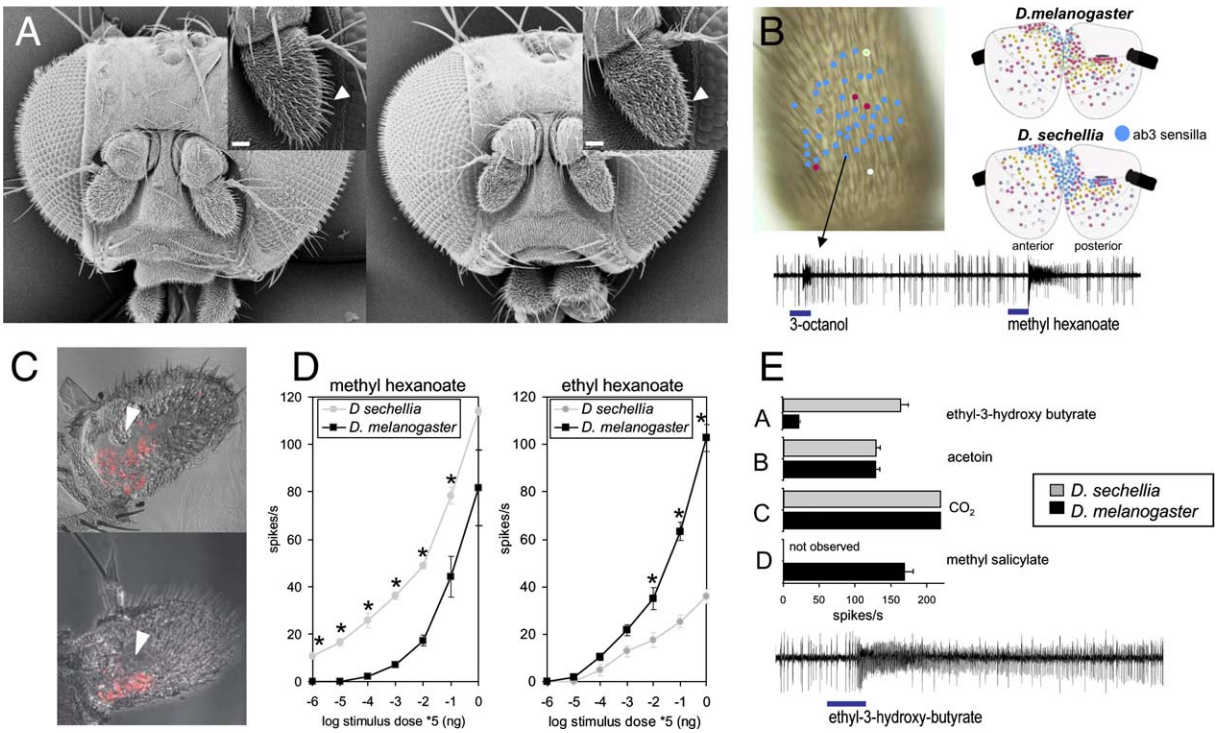

Figure 3. Morphology and Physiology of *Drosophila* Antennae

(A) Scanning electron micrographs of *D. melanogaster* (left) and *D. sechellia* (right) antennae. Insets: arrowhead indicates the short trichoid sensilla on *D. sechellia* antennae, compared to *D. melanogaster*. Scale bar equals 20 μm.

(B) As many recordings from large basiconics per individual as possible were performed, mapped on a micrograph (left), and analyzed (red circle, ab1; green circle, ab2; blue circle, ab3; open circle, other, recording procedures [1, 14]). Trace shows a typical response of ab3 neurons to a 200 ms stimulation with 3-octanol (B cell, cell with smaller spiking amplitude) and MeHex (A cell, cell with larger spiking amplitude). Right: comparison of antennal sensillar map of *D. melanogaster* (original map from [4], permission kindly granted by J.R. Carlson) and *D. sechellia* (blue circle, ab3).

(C) Cryosections of Or22a-4x-GFP flies expressing GFP mostly in the cell bodies (courtesy Dr. B. Dickson) and their hybrids with *D. sechellia.* Posterior view of the antennae. Red, anti-GFP labeling. In total, 48 GFP-positive cells were found on the hybrid, whereas between 18 and 30 were found in *D. melanogaster*. Top, hybrid female *D. sechellia* × *D. melanogaster*; bottom, *D. melanogaster*. Arrowhead indicates sacculus. Note the presence of anti-GFP labeling (indicating Or22a-expressing neurons) in the hybrid distal from the sacculus, but not in *D. melanogaster* (see also sensillar map in [B]).

(D) ab3A neuron responses of *D. sechellia* and *D. melanogaster* to hexanoates (±SE). *$p < 0.05$, **$p < 0.005$ (one-way ANOVA).

(E) Deviant response spectra of ab1 sensilla from *D. sechellia* in comparison to its sibling species (±SE). The ab1A cell responded strongly to 50 ng/filterpaper of ethyl-3-hydroxy-butyrate (same response found in ab2B in *D. melanogaster*). Trace represents a recording from an ab1 sensillum. Stimulation bar: 500 ms 50 ng/filterpaper ethyl-3-hydroxy-butyrate stimulation with a strong response from the ab1A cell ($p < 0.0001$, one-way ANOVA). The ab1D cell was not observed in *D. sechellia*. CO_2 responses (breath puff) were not quantified. Odors were diluted in hexane. Hexane was evaporated before stimulation. Blank responses were subtracted.

sensitivity to L-lactic acid [23], an important host odor for *An. gambiae s.s.* [24], and coincided with a reduced behavioral response to host odors [25]. We suggest that the increased sensitivity of the ab3A neuron to MeHex serves *D. sechellia* to better detect and respond to morinda fruit.

The increased sensitivity in *D. sechellia* to MeHex compared to EtHex may also be caused by changes in the Or22a homolog in *D. sechellia*. By searching the draft genomes of *D. sechellia*, *D. simulans*, *D. yakuba*, and *D. erecta*, we identified or22a homologs in *D. sechellia*, *D. simulans*, *D. yakuba*, and *D. erecta* (Figure 4A). Or22a is highly conserved throughout, with only nine amino acids different between *D. melanogaster*, *D. yakuba*, and *D. erecta* on the one hand and *D. sechellia* and *D. simulans* on the other hand. ab3A neurons (expressing Or22a) in *D. simulans* respond more strongly to MeHex than EtHex, similar to *D. sechellia* [14]. Conversely, the ab3a neurons in *D. yakuba* and *D. erecta* respond more strongly to EtHex than MeHex, similar to *D. melanogaster* ab3A. The minor shift in affinity is likely caused by the aforementioned difference in amino acid sequence.

A bootstrap analysis of the sequences shows the close link between the or22a orthologs of *D. simulans* and *D. sechellia* (Figure 4B). The same tree also displays Or22b orthologs. This olfactory receptor gene is coexpressed in ab3A neurons but does not seem to have any functional significance in *D. melanogaster* [16]. In *D. sechellia*, Or22a is a pseudogene and similarly nonfunctional, with a highly interrupted coding sequence. Exhaustive searches did not reveal Or22b orthologs in the more distantly related species *D. yakuba* and *D. erecta*, which suggests that Or22b is the result of a melanogaster clade-specific duplication of Or22a. More research is needed to verify if any of the

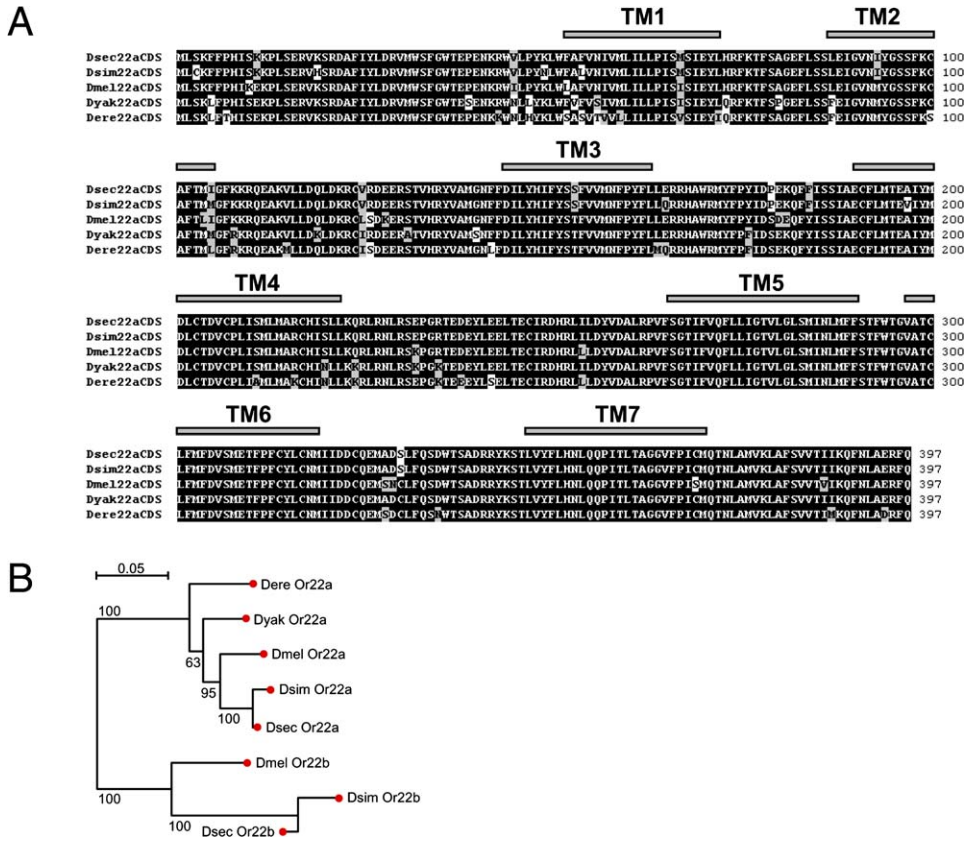

Figure 4. Sequences of OR22a and b Orthologs in *D. melanogaster* Siblings

(A) Multiple alignments of Or22a orthologs from *D. melanogaster*, *D. simulans, D. sechellia*, as well as two outgroup species, *D. yakuba* and *D. erecta*. TM1-7 indicate the putative transmembrane domains.

(B) Consensus neighbor-joining tree showing the relationship of Or22a and Or22b orthologs of *D. melanogaster*, *D. simulans*, and *D. sechellia*. Numerical values refer to bootstrap support.

abovementioned factors contributed to the shift in affinity and sensitivity reported here.

The extreme sensitivity is unexpected for a "general odor" OR. The general tenet is that pheromone responses are mediated by highly sensitive and specifically tuned ORNs, whereas general odor ORs would be more broadly tuned [2, 26], although exceptions have been reported before [27]. Our results demonstrate that ORs tuned to general odors can actually be both very sensitive as well as specific, i.e., only at $10^2\times-10^3\times$ higher concentrations did the second best ligand EtHex induce responses from the ab3A cell in *D. sechellia*. Perhaps the "Or22a-hexanoate receptor-ligand" interactions are an unusually close match, whereas in most cases general odor responses, even those of ecological direct significance, rely more on the "promiscuity" of the OR. As concentrations of general odors generally exceed pheromone concentrations manifold, loose fits are evolutionarily permissible or perhaps even favorable to increase potential flexibility in odor coding space.

Mismatch of ORN Pairing Rules

In addition to overexpression and hypersensitivity, we found that *D. sechellia* large basiconic sensilla were more variable in the precise response spectra than *D. melanogaster*. Although *D. melanogaster* large basiconic sensilla infrequently also did not "obey" the coding rules described by De Bruyne et al. [4], such occurrences were much less frequent. We found reshuffling among spiking classes within subtypes, altered sensitivities, nonresponding and nonclassifiable large basiconic sensilla, as well as putatively misexpressed ORs. Of particular interest is the fact that ab1A cells of *D. sechellia* responded strongly to ethyl-3-hydroxy-butyrate (key ligand of ab2B in *D. melanogaster*; Figure 3E), reminiscent of a shift of Or85a expression [2] from the "lost" ab2B neuron to ab1A. The higher variation in large basiconic coding may be a pleiotropic effect of the mutation(s) that caused the overexpression of ab3 sensilla. As the ORNs of ab1 and ab2 sensilla do not detect morinda fruit volatiles, the "error" may be of little consequence to the fitness of the fly and permissible in the eye of evolution.

Acids

To verify whether *D. sechellia*'s increased attraction to acids, particularly hexanoic acid, could be traced back to changes in the peripheral olfactory organs, we did both extensive EAG and single sensillum recordings. No apparent differences between *D. sechellia* and *D. melanogaster* were found (see Supplemental Data). By using single sensillum recordings across a large subset of antennal sensilla, we noted mostly unspecific responses to acids from several sensilla, i.e., acid-responding cells responding much stronger to other,

key ligands. More specific responses (but with low sensitivity) were found in coeloconic sensilla, including responses to propionic and valeric acid. As the ORNs inhabiting the large basiconics did not respond to acids, the behavioral shift can not be accounted for by the shift found in these sensilla. No apparent differences in ORN responses to acids were found between the two species (data not shown).

Palpae

The palpae of both species were morphologically similar. By using a panel of 14 odors, we found that the response patterns of the ORNs inhabiting the three types of pb sensilla of *D. sechellia* were identical to *D. melanogaster* ([3], data not shown). Acids did not elicit any significant responses from the palpal ORNs. Iso-amyl acetate, and to a lesser extent EtHex and MeHex, elicited low responses in pb1B and pb3B in both species. No differences between the two species were found in the number of pb sensilla.

In summary, the substantial peripheral shifts in the large basiconic sensilla clearly parallel the large behavioral shift to MeHex. In contrast, no peripheral shifts were found accounting for the observed increase in acid attraction. Did we miss some fine-scale alterations in sensitivities or number of neurons? In other insects, lack of clear peripheral changes that match shifts in host preferences have been reported. In a recent niche differentiation and radiation event, some populations of *Rhagoletis pomonella* flies adopted new hosts, including apple and dogwood, leading to a premating barrier based primarily on odors [28]. However, no differences were detected between the *R. pomonella* races in the response of antennal ORNs to odors used in host discrimination [29]. Similarly, ORNs of *Anopheles gambiae s.l.* showed similar response profiles to various host odors despite clear differences in host preference [30, 31]. These studies as well as the present one indicate that evolution may act at different levels of integration in modulating the final behavioral output.

Rewiring in the Antennal Lobe

As ORs are not directly involved in axonal guidance in *Drosophila* [17, 32], we asked whether the axons of ORNs in the "new" ab3 sensilla (i.e., "replaced" ab1 and ab2 sensilla) still project to their "cognate" glomeruli or rewire to arborize in "ab3 glomeruli." ab3A neurons of *D. melanogaster* project to a dorsomedially located glomerulus, DM2 (Figure 5A; [33]). By using nc82 overview staining, we identified two glomeruli in the same dorsomedial region that were clearly enlarged in *D. sechellia*, suggestive of increased axonal input (Figure 5B). A microelectrode filled with neurobiotin was placed on *D. sechellia* antennae in an area inhabited by only ab3 sensilla. The obtained backfills were found projecting to those two enlarged glomeruli (Figure 5C). By using hybrid male *D. sechellia* × female *D. melanogaster* Or22a-nsyb-GFP, which were mosaic with respect to the DM2 glomerulus size, we identified which of the two glomeruli received input from the ab3A neurons (Figure 5D). The *D. sechellia* DM2 glomerulus was 2.9× more voluminous than the corresponding DM2 glomerulus in *D. melanogaster* (see insets in Figures 5C and 5D, the bright green glomerulus receives input from the ab3A neurons).

Apparently, the synaptic input from more than 80% of the large basiconic sensilla converges onto two greatly enlarged glomeruli, one of which channels information from morinda fruit volatile MeHex. The volume of glomeruli depends not only on the number of ORNs projecting to it, but also on the number and extent of interneuronal arborizations [34]. Moreover, the volume can be modulated over adult lifespan and in response to odor exposure [35, 36]. In our study, the 2.9× volume increase of *D. sechellia* DM2 closely approximates the numerical increase in ORNs synapting in DM2. We infer that the enlarged DM2 is due to the increased number of synapting ORNs expressing the MeHex-sensitive OR.

The observed changes in the ORN projection patterns are behaviorally and ecologically highly relevant. However, the behavioral data suggest not a simple enhanced sensitivity, as the curve of *D. sechellia*'s behavioral response to MeHex is not merely shifted to the right. The curve is also higher and shows no tapering at high concentrations, casting the question of whether there are additional changes. Projection neurons (PNs) in *D. melanogaster* are devoted to a particular glomerulus and have more or less stereotypical arborization patterns in the mushroom bodies and the lateral horn [37]. What has changed in the wiring in the *D. sechellia* DM2 glomerulus? Several scenarios can be envisioned. Is the ratio of ORN-PN increased, such that a similar number of PNs receives a 3× higher synaptic input in *D. sechellia*? Or are the PNs that originally arborized in one of the neighboring ab2 glomeruli [5, 6] through fusion of glomeruli now arborizing in the enlarged DM2 glomerulus? How is the signal modulated through local interneuronal connectivity? We are currently investigating how downstream signaling is precisely affected by the increase in volume of the DM2 glomerulus.

How do the reported peripheral and central and behavioral shifts fit with what is known of *Drosophila*'s ecology and evolution, particularly of *D. sechellia*?

D. sechellia's Ecology and the Value of Olfactory Specialization

Morinda fruit ripens throughout the year and trees are patchily distributed. Each tree normally bears some ripening fruit. Once ripe, the fruit drops off in a few days and deteriorates. It is unknown whether *D. sechellia* oviposits on fresh fallen fruit or only on fruit still in the tree. However, the fruit most likely offers *D. sechellia* only a small window of opportunity for oviposition, as ripe fruit quickly disintegrates, and the stronger competitor *D. simulans* can sometimes be found breeding on the less toxic, rotten fruit [38]. Emerging adult *D. sechellia* therefore need to search for new fresh fruits in the right stage of ripeness. The shifts in the olfactory circuitry reported here indicate a very important role of MeHex in this searching process.

The Evolution of *D. sechellia* and the Origin of Its Olfactory Specialization

It is uncertain whether *D. sechellia*'s present preference mirrors that of the past. Although *D. sechellia* diverged from *D. simulans* several hundred thousand years ago [39, 40], its morinda specialization may have been a much more recent event [41]. Today, *M. citrifolia* is found across the Indian and Pacific Ocean regions,

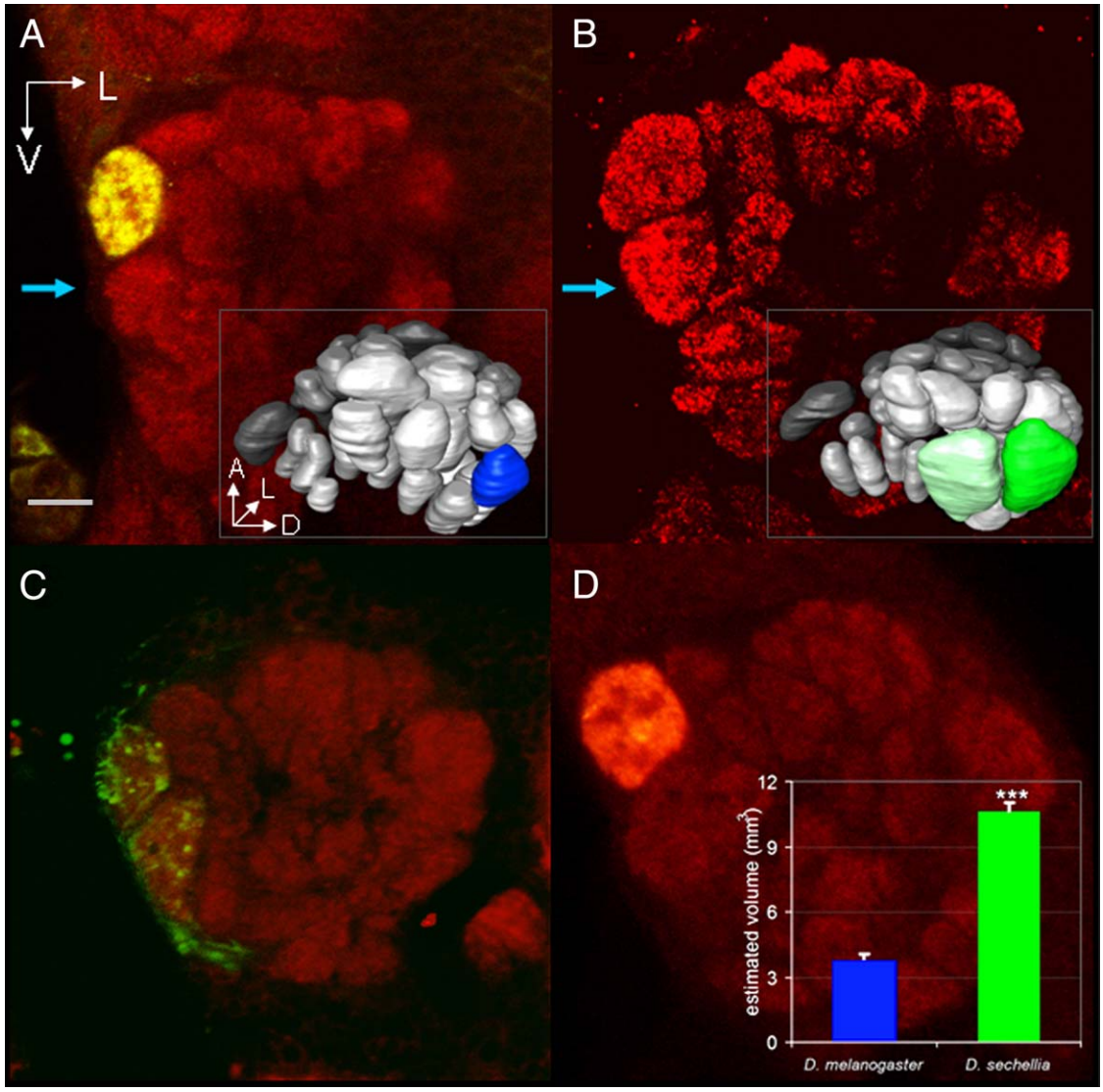

Figure 5. Wiring of ab3 ORNs into Antennal Lobe Glomeruli

(A) Female *D. melanogaster* expressing nsyb-GFP under Or22a promoter control (LV38.3). Axons target the DM2 glomerulus (yellow, anti-GFP antibody, background staining with α-synapsin [42]). Arrow, the medial-lateral direction of view for the inset. Inset: reconstructed antennal lobe with DM2 highlighted. A, anterior; D, dorsal; L, lateral; V, ventral.

(B) NC82 overview staining of a female *D. sechellia* with two enlarged glomeruli in the area of the DM2 glomerulus. Inset: reconstructed antennal lobe of *D. sechellia*. The enlarged glomeruli are targeted by neurons inhabiting the ab3A and B neurons (bright and faint green, respectively, see [C] and [D]).

(C) Backfill with neurobiotin (green, 025 M KCl + 2% neurobiotin, Molecular Probes, Carlsbad, CA) from ab3 sensilla (2 hr) in *D. sechellia* on an α-synapsin background staining. Axons target the two enlarged glomeruli.

(D) Hybrid female *D. melanogaster* × *D. sechellia* expressing Or22a-GFP (see text). The upper enlarged glomerulus is targeted by ab3A neurons, expressing Or22a. Inset: graphic display of *D. sechellia* DM2 volume increase compared to *D. melanogaster* (±SE; n = 6 and 7, respectively, one-way ANOVA, $p < 0.00001$). For confocal microscopy details, see [34].

spread primarily through human transportation. It may have been introduced by humans to the Seychelles only a few hundred years ago, casting doubt on the antiquity of the morinda-sechellia association [40]. The proposed more recent shift of *D. sechellia* to morinda is thought to be driven primarily to avoid competition from *D. simulans* [41].

The question arises whether the genetic adaptations thought typical for its morinda fruit adaptation are likely to take place in such a short time. However, its oligogenic resistance to the toxic morinda fruit acids [9] may have evolved on another host plant with similarly high acid levels [41]. In contrast, the MeHex-related mutation(s) in the olfactory circuitry should have occurred after *D. sechellia*'s shift to morinda, as there exist no other hexanoate-rich fruit species on the Seychelles on which *D. sechellia* could have preadapted. Interestingly, rapid adaptations in the olfactory circuitry have been inferred for, e.g., *Rhagoletis* flies (see above), where major shifts in odor-based host preference evolved over a period of less than 150 years [28]. We found no clear differences between *D. sechellia* lines originating from Cousin

(most lines) or Praslin island (0248.8). However, the current populations across the Seychelles could have originated from a single morinda-adapted ancestor population and recently been transported by humans across the archipelago.

In summary, we have started to unravel how *D. sechellia*'s unique taste for toxic morinda fruit has affected its sense of smell. Our results clearly demonstrate how evolution has "synced" *D. sechellia*'s olfactory circuitry to the odor of its sole host. In short, *D. sechellia*'s olfactory circuitry has undergone several shifts at peripheral and central levels, culminating in increased sensitivity and attraction to morinda fruit volatiles. These findings are important for our understanding of the host odor-driven evolution of the olfactory code and its structural basis in *Drosophila*.

Supplemental Data

Supplemental Data include one figure and Supplemental Experimental Procedures and can be found with this article online at http://www.current-biology.com/cgi/content/full/16/1/101/DC1/.

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