Evolution of taste processing shifts dietary preference

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Summary

Food choice is an important driver of speciation and invasion of novel ecological niches. However, we know little about the mechanisms leading to changes in dietary preference. Here, we use the three closely related species *Drosophila sechellia*, *D. simulans* and *D. melanogaster* to study taste circuit[1] and food choice evolution. *D. sechellia,* a host specialist, feeds exclusively on a single fruit (*Morinda citrifolia*, noni) - the latter two are generalists living on diverse diets[2]. Using quantitative feeding assays, we recapitulate the preference for noni in *D. sechellia* and detect conserved sweet but altered bitter sensitivity via calcium imaging in peripheral taste neurons. Noni surprisingly activates bitter sensing neurons more strongly in *D. sechellia* due to a small deletion in one single gustatory receptor. Using volumetric calcium imaging in the ventral brain[3], we show that instead of peripheral physiology, species-specific processing of noni and sucrose signals in sensorimotor circuits recapitulates differences in dietary preference. Our data support that peripheral receptor changes alone cannot explain altered food choice but rather modifications in how sensory information is transformed into feeding motor commands.

Introduction

The decision to accept or reject a food source is critical for survival. While some animals consume a broad range of substrates, others are specialised feeders reliant on a single nutrient source. Such dietary shifts, even among closely related species, are often linked to changes in taste perception – the primary driver of food intake and a key determinant of acceptance or avoidance across animals[1,4]. Taste cells express diverse, rapidly evolving chemosensory receptors[5], with gene gains and losses often correlating with species-specific feeding habits. For example, the giant panda lost its umami taste receptor and feeds solely on bamboo[6], cats, as carnivores, lost sweet perception through sweet receptor pseudogenisation[7], and hummingbirds, after ancestral loss of sweet receptors, re-purposed their umami receptor to detect nectar[8]. In insects, dietary shifts often reflect novel preferences for host-specific metabolites, such as unripe citrus volatiles or glucosinolates detected by specifically tuned taste receptors in the Chinese citrus fly[9] or *Pieris rapae*[10], respectively. While these studies link receptor evolution to peripheral taste physiology, how sensory signals are processed centrally to influence food choice remains poorly understood.

Drosophilids provide a powerful model for studying feeding evolution, thanks to genetic tools developed in *Drosophila melanogaster* (*Dmel*) and now applied to related species with distinct diets. In *Drosophila*, gustatory sensory neurons (GSNs), located on the labellum of the proboscis, legs, wings, and pharynx[1] detect multiple taste modalities[1], promoting food acceptance or rejection[11,12]. Peripheral proboscis and a subset of leg GSNs project to the SubEsophageal Zone (SEZ) of the brain[1] where innervations are spatially separated by modality[13]. Here, they connect to local inter- and second-order neurons transmitting taste information within the SEZ or to higher brain areas[14-16]. The separation of taste modalities at the periphery converges, for sugar and water taste already at second order, or in higher processing layers[14-18] which activate motor neurons driving behaviours like proboscis extension or food ingestion[16,17]. This organisation mirrors that of mammals, yet evolutionary studies have largely focused on the sensory periphery.

Several drosophilid species have evolved specialised feeding habits. The agricultural pest *D. suzukii* shifted preference from fermented to ripe fruits[19], *Scaptomyza flava* adopted herbivory[20], and *D. sechellia* (*Dsec*) specialised on

Morinda citrifolia ("noni")[2]. Electrophysiological recordings revealed altered GSNs responses compared to *Dmel* in these species, linked to their host environment. In *D. suzukii,* reduced sugar sensitivity coincides with fewer sweet-sensing GSNs despite its oviposition preference for sweet substrates[19,21]. Bitter sensing neurons in all three species exhibit narrowed response profiles, probably reflecting reduced exposure to environmental bitter compounds[20,22,23]. In *Dsec,* bitter neurons also respond less to sugar and fatty acid mixtures (the latter being abundant in noni)[24]. These changes likely result from gene loss or down-regulation of chemosensory genes for bitter[22,23,25] and sweet detection[21], including olfactory binding proteins[26], gustatory[24,27] or ionotropic receptors[24]. However, most evidence remains correlative, inferred from *Dmel* loss-of-function studies without functional validation in the respective species.

While these examples highlight the link of peripheral receptors and dietary preferences, how central taste circuits adapt remains unexplored. Here, we investigate peripheral sensing and central taste processing in the specialist *Dsec* to uncover how neural circuits evolve to shape feeding preferences in an ecologically relevant paradigm.

Results

Dsec shows noni feeding preference

To replicate *Dsec*'s host preference in the lab, we exposed groups of flies to four fruits (banana, apple, grape, and noni) and tracked their spatial distribution over 24 hours. As expected, *Dsec* strongly preferred noni, while the generalists *D. simulans* (*Dsim*) and *Dmel,* which diverged from *Dsec* 0.1-0.24 and 3-5 million years ago[2], respectively, preferred other fruits (**Fig. 1a, Extended Data Fig. 1a**). This preference persisted in near-anosmic flies (mutants for the odorant and ionotropic co-receptors Orco and Ir8a, **Fig. 1a, Extended Data Fig. 1a**), which lack olfactory noni attraction in trap assays[28], suggesting that non-olfactory cues contribute to *Dsec's* noni choice.

We next analysed taste-driven noni preference in three binary choice assays: a 72-well plate[12], a petri-dish group assay[29], and the flyPAD (on single flies)[30] comparing grape and noni juice. The latter mimics noni fruit chemistry[28] and elicits similar behaviours[28,31]. In all assays, *Dmel* and *Dsim* preferred grape juice, while *Dsec* consumed more noni (**Fig. 1b**), with no sex differences and comparable feeding rates across species (**Extended Data Fig. 1b-d**). Temporal analysis of flyPAD data revealed rapid substrate selection: *Dsec* chose noni, whereas *Dmel* and *Dsim* preferred grape juice (**Fig. 1c**), suggesting that species-specific food choice is driven by pre-digestive sensory cues rather than post-ingestive feedback or learning.

To test olfactory contributions to feeding preference, we generated an *orco* allele in *Dsim* (matching *DsecOrco*^{1} of *Dsec*[28], **Extended Data Fig. 2a**) and removed antennae in Orco mutants of all three species, eliminating Or-mediated olfactory input (maxillary palp OSNs all depend on Orco[32]). *Dsim* and *Dmel* still preferred grape, while *Dsec* showed a neutral preference index in the petri dish assay, consistent with results from flies lacking maxillary palps and antennae (=MPAR) or olfactory receptor mutants in the flyPAD (**Fig. 1d, Extended Data Fig. 2b-d**). Principal component analysis of flyPAD data (incorporating feeding microstructure, **Extended Data Fig. 2e-g**) clearly separated *Dsec* from its relatives, independent of olfaction (**Fig. 1e**). These findings suggest that, while olfaction contributes to noni attraction, taste differences likely participate in food preferences: *Dmel* and *Dsim* show strong grape preference, which is lost in *Dsec*.

Grape, banana, and apple are rich in sugars compared to noni[33] (**Extended Data Fig. 2h**). While *Dmel* and *Dsim* thrive on carbohydrate-rich diets, *Dsec* shows developmental delays and reduced lifespan when fed sugars, indicating a shift in nutritional requirements[33-35]. To test sugar's role in feeding preference, we offered sucrose versus noni juice in petri dish and flyPAD assays (**Fig. 1f, g**). *Dmel* preferred sucrose, *Dsec* fed almost exclusively on noni, and *Dsim* displayed sugar preference (petri dish, **Fig. 1f**) or an intermediate phenotype (flyPAD, **Fig. 1g**, **Extended Data Fig. 2i-j**).

Together, these results indicate a dietary shift from sugar-rich fruits like grape in *Dmel* and *Dsim* to noni in *Dsec*, driven by olfactory preference and lower sugar attraction of the latter.

Bitter/sweet taste impact noni feeding

Adult flies evaluate food using GSNs located on the labellum and legs (**Fig. 1h**). Activation of sweet sensing neurons in these organs reduces locomotion[36] and promotes feeding[1,36] while bitter neuron activation triggers aversion[1]. Labellar taste peg neurons modulate feeding duration on yeast[37]. Additionally, internal GSNs in three pharyngeal organs (labral, ventral cibarial, and dorsal cibarial) assess ingested food and influence consumption[38,39].

To explore the role of GSNs in species-specific food choice, we screened *Dmel* GSN populations using the flyPAD and genetic tools. First, *Pox neuro* (*Poxn*) mutants, which lack external taste bristles[39], shifted their preference from grape to noni (**Fig. 1i**, **Extended Data Fig. 3a**), suggesting that external taste and pre-digestive signals influence food choice.

Next, we silenced specific GSNs in the labellum, legs, and pharynx (**Fig. 1h**) using Gal4 drivers and *UAS-Kir2.1*. Inactivation of sugar-sensing neurons (*DmelGr64f-Gal4*) reduced grape but not noni juice intake, resulting in a positive noni preference index (**Fig. 1i, Extended Data Fig. 3a**), supporting the role of sugar in promoting grape juice intake. Silencing bitter-sensing neurons (*DmelGr66a-Gal4*) increased noni feeding and abolished food preference (**Fig. 1i, Extended Data Fig. 3a, b** for sucrose/noni comparison), likely due to reduced aversive signalling.

Silencing ppk23 or ppk28 neurons, which detect pheromone/high salt and water, respectively[1], had no effect on preference, though ppk23 manipulation mildly increased grape intake (**Fig. 1i, Extended Data Fig. 3a**). Inactivation of Ir94e, involved in salt and amino acid sensing[40], did not alter intake nor preference. Notably, simultaneous silencing of sweet ($Gr64f^+$) and bitter ($Gr66a^+$) sensing neurons induced a strong noni preference in *Dmel*, resembling *Dsec*'s phenotype (**Fig. 1b, i, Extended Data Fig. 3a**). These results support a role of peripheral sweet and bitter bristle GSNs in shaping food choice between grape and noni juice.

To investigate multisensory integration, we removed antennae and maxillary palps from *Poxn* mutants and flies with silenced sweet and/or bitter neurons. Loss of olfactory input in *Poxn* mutants reduced noni intake and abolished noni preference in *Dmel* (**Fig. 1j, Extended Data Fig. 3c**). Similarly, anosmic flies with silent $Gr64f^+$, $Gr66a^+$, or both neurons lost substrate preference (or remained neutral, $Gr66a^+$) (**Fig. 1j, Extended Data Fig. 3c**).

These results suggest that while noni is olfactorily attractive to *Dmel*[28,41], activation of pre-ingestive sweet and bitter sensing neurons can override this attraction – promoting grape juice intake and aversion to noni. In *Dsec*, both mechanisms appear absent: smell-blind flies lose olfactory attraction to noni but remain neutral in food choice.

Comparison of sweet sensing circuits

Given the role of sweet and bitter sensing neurons in food choice, we investigated these populations in *Dsec* to understand its reduced sugar attraction and lack of noni aversion. Using CRISPR, we inserted Gal4 cassettes into early exons of *Ir25a* and *Gr64f,* generating reporters under native *cis*-regulatory control (**Extended Data Fig. 4a**).

Similar to *Dmel*, *DsecIr25a*^{Gal4} showed broad expression across the taste system, including leg neurons, labellar bristles and pegs, pharynx, and olfactory neurons[32,42] (**Fig. 2a, Extended Data Fig. 4b**). *DsecGr64f*^{Gal4} expression was more restricted to sweet-sensing neurons in the legs, labellum, pharynx, and a few olfactory populations[11] (**Fig. 2b, Extended Data Fig. 4b, c**). To enable cross-species anatomical comparisons with minimal transgenic artefacts, we generated analogous *Gr64f*^{Gal4}-knock-ins in *Dsim* and *Dmel*. Quantification of *Gr64f*-expressing cells in the labellum, legs, and labral sense organ revealed no major species differences (**Fig. 2c, Extended Data Fig. 4d, e, Sup. Table 1**), unlike *D. suzukii*, which shows reduced sweet sensitivity and a one-third reduction in labellar sweet-sensing neurons[19]. This suggests that reduced sugar appetite in *Dsec* is unlikely due to loss of peripheral sweet-sensing neurons.

We next probed pre-ingestive taste responses in peg and sweet-sensing taste bristle neurons via calcium imaging in *Dsec* and *Dmel.* Flies were stimulated on the labellum or foreleg with noni or grape juice, selected noni acids[43] or sweet agonists, while recording contact-evoked SEZ responses (**Fig. 2d,** the oesophagus was transected to prevent ingestion).

Taste peg neurons, inaccessible to electrophysiology and uncharacterized in *Dsec*, respond to carbonation[42], hexanoic acid[44], and yeast, and prolong yeast feeding in *Dmel*[37]. Using *Ir25a*^{Gal4}, we detected robust responses to hexanoic acid and stronger activation by noni than grape juice in *Dsec* (**Fig. 2d, Extended Data Fig. 4f**, normalised data). However, overall activation patterns and dynamics were similar in both species (**Fig. 2e**). Likewise, foreleg stimulation evoked conserved SEZ responses, except for a significantly heightened hexanoic acid sensitivity in *Dmel* (**Extended Data Fig. 5a, b**), suggesting that peg and leg neuron physiology does probably not explain *Dsec*'s altered noni consumption.

We then imaged calcium responses in Gr64f^{+} labellar bristle neurons, which respond strongly to sucrose (**Fig. 2f, Extended Data Fig. 5c,** normalised data) and drive feeding initiation[1]. Despite *Dmel*'s sucrose preference, its sugar sensitivity was not higher than *Dsec*'s. *Dsec* showed significantly stronger responses to hexanoic acid, yet Gr64f neurons responded equally to grape and noni juice, with grape sensitivity even exceeding *Dmel*'s (**Fig. 2f**). Noni responses in *Dsec* were independent of Gr64f function (**Extended Data Fig. 5d**), suggesting involvement of other receptors. Overall, labellar sweet neuron responses were temporally similar across species (**Fig. 2g**) and did not correlate with *Dmel's* sugar preference or *Dsec's* noni attraction.

Consistent with these findings, when offered increasing concentrations of sucrose, fructose, or glucose, all three species discriminated low from high sugar levels, but *Dsec* consumed significantly less, indicating reduced sugar attractiveness and phagostimulatory effect (**Fig. 2h, Extended Data Fig. 5e**). Adding acids enhanced sucrose intake in *Dsec*[24], while *Dmel* and *Dsim* were repelled by high acid concentrations – likely due to bitter pathway activation[24] (**Fig. 2h, Extended Data Fig. 5e**).

In sum, peripheral sweet sensing neuron responses alone do not explain feeding patterns. While all species can discriminate sugar levels, differences likely arise from how sensory input is translated into sustained feeding.

Altered bitter sensing in *Dsec*

Previous studies and our neuron silencing experiments (**Fig. 1i, j**) suggest that altered bitter sensing contributes to noni feeding in *Dmel/Dsim/Dsec*[23,24,27]. This is supported by accelerated loss of bitter gustatory receptors in *Dsec*[25], the presence of bitter compounds like coumarin derivatives in noni[45], and its high acid content, which may activate bitter-sensing neurons[46]. To monitor activity in these circuits, we generated *Gr66a*^{Gal4} reporter alleles in all three species. As in *Dmel*[12] and *Dsim*, the *Dsec* transgene labelled leg, labellar bristles, and pharyngeal neurons projecting to the SEZ (**Fig. 3a, b, Extended Data Fig. 6a, b**).

We quantified Gr66a$^+$ neurons in the legs, labellum, and labral sense organ. Compared to earlier *promoter-Gal4* studies[12], our knock-in allele revealed more labellar Gr66a$^+$ cells in *Dmel*, consistent with electrophysiogical studies[12,23]. In *Dsec*, bitter neuron numbers were largely conserved, with a slight reduction in males (**Fig. 3b, Extended Data Fig. 6a, b**), suggesting that reduced cell counts are unlikely to explain *Dsec*'s diminished aversion - unlike in *D. suzukii*[22].

We next examined Gr66a circuit physiology in *Dmel* and *Dsec* (**Fig. 3c, Extended Data Fig. 6c**, normalised data). Foreleg responses were similar across species (**Extended Data Fig. 6d**), but *Dsec* showed markedly reduced caffeine sensitivity, consistent with prior electrophysiological data[23] (and independent of our knock-in strategy, **Extended Data Fig. 6e**). Responses to denatonium, hexanoic and octanoic acid were conserved (**Fig. 3c, Extended Data Fig. 6c, f**, *Dsim* and normalised data) and we detected off-responses[47] upon tastant removal across stimuli (**Fig. 3d**) without species differences. Unexpectedly, *Dsec* bitter-sensing neurons were highly sensitive to coumarin and noni juice, showing stronger activation than *Dmel* (**Fig. 3c**), challenging the hypothesis that reduced bitter sensitivity underlies *Dsec*'s noni specialisation. Loss of Gr66a abolished denatonium responses[23] (and others, **Extended Data Fig. 6g**) but not noni sensitivity, indicating a Gr66a-independent mechanism for noni detection. Together, these findings suggest that peripheral bitter sensing neuron physiology does not explain – with *Dsec* responding even stronger to noni – *Dsec*'s reduced noni aversion.

Molecular components of bitter responses

The gain of coumarin and noni sensitivity, alongside reduced caffeine responses in *Dsec* bitter-sensing neurons, prompted investigation into the genetic basis of this divergence. *Dsec* shows accelerated loss of bitter receptors[25], and several - Gr33a, Gr39a, Gr66a, Gr93a – are implicated in caffeine sensing in *Dmel*[23]. Among these, the *Gr39a* locus stands out for its broad expression (**Fig. 4a**) and four splice isoforms (*Gr39a.a-a.d*). One isoform, *Gr39a.b* (following FlyBase naming, in some studies *Gr39a.a*[48]) is pseudogenised in *Dsec*[25] due to an 8bp deletion (**Fig. 4b**). Although this locus is evolutionary dynamic[48] and may influence taste physiology in herbivorous drosophilids[20], expressing *DmelGr39a.b* in *Dsec* Gr66a$^+$ neurons did not restore caffeine sensitivity (**Extended Data Fig. 7a**), arguing against a direct role of *Gr39a.b* loss in this phenotype.

Focusing on other isoforms, sequence analysis revealed a *Dsec*-specific three-amino acid deletion (Δ202-204) in Gr39a.a (**Fig. 4b**) in the intracellular anchor domain, implicated in receptor tetramer assembly (based on AlphaFold[49] and recent Gr

structures[50], **Fig. 4c**). To test its function, we expressed *Dmel*Gr39a.a or *Dsec*Gr39a.a in Gr39a.a^{+} neurons of *Gr39a*$^{-/-}$ mutants in *Dmel*. While *Dmel*Gr39a.a. restored caffeine responses (**Fig. 4d**)[23], *Dsec*Gr39a.a did not to the same level. Deleting the same three residues in *Dmel*Gr39a.a (=*Dmel*Gr39a.a$^{\Delta 3}$) reproduced similar phenotypes as the *Dsec* orthologue, confirming that this deletion impairs caffeine detection (**Fig. 4d**).

To assess whether heightened coumarin and noni sensitivity in *Dsec* Gr66a^{+} neurons is influenced by the *Gr39a* locus, we imaged SEZ activity in the same *Dmel* transgenics exposed to coumarin and noni juice (**Fig. 4e**). While wild-type *Dmel* showed minimal responses, *Gr39a* deletion enhanced coumarin sensitivity but not significantly for noni. Re-expression of *Dmel*Gr39a.a restored a wild-type-like response, whereas *Dsec*Gr39a.a and *Dmel*Gr39a.a$^{\Delta 3}$ failed to do so, mimicking the *Gr39a*$^{-/-}$ phenotype (**Fig. 4e**). These findings suggest that both caffeine sensitivity loss and coumarin gain stem from the same genetic alteration.

To test whether *Dmel*Gr39a.a affects neuron physiology in *Dsec*, we expressed *Dmel*Gr39a.a in endogenous *Dsec* Gr66a^{+} neurons. Wild-type *Dsec* showed minimal caffeine responses; these “rescue” animals, however, gained caffeine sensitivity (**Fig. 4f**). Expression of *Dmel*Gr39a.a also reduced coumarin and noni responses, indicating a shift in tuning (**Fig. 4f**). Thus, sequence changes in Gr39a.a likely explain interspecies differences: in *Dmel*, a functional Gr39a.a receptor mediates caffeine sensitivity and dampens coumarin responses; in *Dsec*, a three-amino acid deletion likely renders *Dsec*Gr39a.a non-functional, reversing this profile.

Given that Gr66a neuron activity predicts behavioural aversion[4,12], we tested how Gr39a.a isoform expression affects feeding in *Dmel*. In a two-choice petri dish assay, wild-type flies avoided caffeine-laced sucrose, while *Gr39a*$^{-/-}$ mutants showed neutral preference, rescued to some extent upon re-expression of *Dmel*Gr39a.a (**Fig. 4g, i**). In contrast, *Dsec*Gr39a.a and *Dmel*Gr39a.a$^{\Delta 3}$ failed to restore aversion to wild-type levels (**Fig. 4g**), linking peripheral Gr66a neuron responses to feeding decisions in *Dmel*. *Dsec* wild-type flies, lacking peripheral caffeine responses, showed increased consumption of caffeine-laced food compared to *Dmel* (**Fig. 4h, i**)[23,27]. However, despite restored Gr66a caffeine sensitivity via re-expression of *Dmel*Gr39a.a, *Dsec* flies did not exhibit strong aversion (compare **Fig. 4f** with **Fig. 4h**) suggesting that peripheral bitter information is processed differently in *Dsec*.

To test whether these effects are caffeine-specific, we assessed feeding behaviour in wild-type flies using a two-choice petri dish assay with increasing concentrations of caffeine and coumarin. *Dsec* showed reduced caffeine aversion, consistent with Gr66a neuron physiology. In contrast, despite strong neuronal activation by coumarin, *Dsec* did not exhibit the same robust aversion seen in *Dmel* and *Dsim*[27] (**Fig. 4i**). However, bitter aversion is not generally impaired in *Dsec*, as denatonium avoidance is conserved (**Extended Data Fig. 7b**). Thus, while bitter neuron activity predicts aversion for some compounds (e.g. caffeine, denatonium), others (e.g. coumarin, noni) elicit divergent behavioural outcomes in *Dsec*, consistent with species-specific noni preference.

To directly assess the role of bitter and sweet neurons in *Dsec* noni feeding, we generated and validated a *Dsec UAS-eGFP-Kir2.1* transgene (**Extended Data Fig. 7c, d**). Silencing individual or both populations (and *Gr66a* and *Gr64f* mutations, **Extended Data Fig. 7e**) maintained robust noni preference in the flyPAD, indicating involvement of multiple sensory modalities (**Extended Data Fig. 7f**). Noni does not act as an aversive stimulus in *Dsec*, as bitter neurons silencing did not enhance intake.

These findings suggest that, similar to sugar, processing of peripheral information may be altered in *Dsec* compared to *Dmel*.

Altered sensorimotor processing of taste

Taste signals from the labellum are relayed via sensory neurons through interneurons to motor neurons (MNs)[16,17] that initiate feeding via proboscis extension (MN9) and maintain ingestion through pharynx pumping (MNs 10-13) (**Fig. 5a**)[51,52]. To examine how evolution may have shaped this sensory-motor transformation, we performed volumetric calcium imaging across the entire SEZ[3].

We expressed GCaMP6s pan-neuronally[28] and imaged SEZ activity in response to noni, grape juice, denatonium, sucrose, and acids. For each species, we generated average brain responses and segmented regions using shared anatomical and functional landmarks. We annotated two sensory input zones: a central region enriched for bitter neuron axons ("sensory central") and a lateral region enriched for sweet and water-sensing input[14-16] ("sensory lateral"), both also containing inter- and projection neuron processes. Adjacent to these, we identified two putative interneuron zones ("interneuron region 1" and "interneuron region 2"), likely housing SEZ local inter- and projections neurons[14-16], and one region likely enriched for motor neuron outputs[52] ("motor region", **Fig. 5b, Extended Data Fig. 8, Suppl. Video 1**).

In the central sensory region, *Dmel* and *Dsec* showed strong responses to denatonium, with noni signals significantly stronger than grape in *Dsec* (**Fig. 5c**). In the lateral sensory regions, all species responded robustly to water, sucrose and noni. Notably, noni responses exceeded grape in *Dsec*, possibly due to heterogeneous innervation (**Extended Data Fig. 9a**) or differences in postsynaptic processing. In the two putative interneuron regions[14-16], *Dmel* showed stronger response to grape than noni, while *Dsec* showed the opposite pattern, with noni evoking stronger activity than grape and sucrose in interneuron region 1. This supports the idea that sugar-driven downstream activity[17] is diminished in *Dsec*. *Dsim* showed no differential activity across stimuli (**Extended Data Fig. 9b**), mirroring its flyPAD behaviour (**Fig. 1**).

Across species, grape consistently evoked stronger signals in *Dmel* interneuron regions. In the motor neuron arborization region of MNs10-13 (motor region), *Dsec* showed weak responses to sucrose and grape, but elevated noni activity (**Fig. 5c, d**), though not significantly different from *Dmel*. Acids did not elicit differential responses across species (**Extended Data Fig. 9b**).

These findings suggest species differ in higher-order sensorimotor activity. *Dmel* (and *Dsim* to a lesser degree) maintains strong grape/sucrose but not noni responses across the SEZ, while *Dsec* shows sustained noni responses. These SEZ activity patterns across species align with their feeding preference in two-choice assays (**Fig. 1**).

To directly link peripheral taste sensation to motor output, we measured the proboscis extension response (PER) – a motor programme necessary to initiate feeding – upon labellar stimulation with sucrose, grape or noni juice (**Fig. 5e)**. Reflecting weak motor region activity, *Dsec* showed reduced PER to sucrose and grape compared to noni, supporting diminished sugar-driven feeding. Surprisingly, noni provoked strong PER not only in *Dsec* but also *Dsim* (and less so in *Dmel*) indicating that PER alone does not fully reflect food consumption (**Fig. 5e**). To assess feeding vigor, we quantified proboscis extension duration upon tastant presentation as a proxy for ingestion rate (**Fig. 5f**)[53]. *Dmel* and *Dsim* showed longer proboscis extension times to sucrose and grape than noni while *Dsec* showed uniformly low ones, with slightly longer responses to noni than to grape (**Fig. 5f**).

In sum, these results align with our imaging data suggesting that, despite similar peripheral taste neuron responses, sugar substrates lead to heightened activity in central brain areas and subsequently feeding in *Dmel* compared to the noni specialist *Dsec* which lost sugar appetite.

Discussion

We used *Dsec*'s noni specialisation to study taste and feeding evolution in insects (**Fig. 5g**). Although *Dsec* is strongly attracted to noni via olfaction, experiments in smell-blind flies[24,27] show that olfaction alone does not explain species-specific preference across the *Dmel*/*Dsim*/*Dsec* trio. *Dmel* lacking peripheral taste input is attracted to noni via olfaction, but sweet-driven sugar preference and bitter-mediated noni aversion shifts its preference towards sugary fruits. While multimodal integration likely occurs in the central brain[54], our physiological data from flies lacking antenna and maxillary palps reveals species differences in taste-related signals in the SEZ and beyond.

In species like *D. suzukii*, reduced sweet responses stem from fewer sweet sensing neurons or lower receptor expression[19,21]. *Dsec* instead appears to rely on altered downstream processing, as suggested by our volumetric imaging. While sugar-sensing downstream circuits have been started to be mapped in *Dmel*[14-17], our data indicate that *Dsec* has lost the high efficiency by which sugar signals recruit feeding motor neurons along this sensorimotor processing loop in *Dmel* (and probably *Dsim*). This resembles *D. suzukii's* oviposition preference for sugar, which also cannot be explained by peripheral sensitivity[19,21] and may involve central circuitry changes. Consistent with previous work, *Dsec* and *Dmel* exhibit similar physiological responses towards the noni-enriched octanoic and hexanoic acids[24]. Although sweet-acid integration may differ between species at periphery[24], this variation does not significantly affect noni sensing, as peripheral responses to noni are not elevated in *Dsec*.

In bitter sensing circuits, our calcium imaging captures population-level activity across all neurons targeted by our Gal4 drivers. Supporting prior electrophysiological data, we confirm the loss of caffeine and bitter responses in *Dsec*[23]. The resulting absence of behavioural caffeine aversion aligns with studies showing that diminished aversive signals in peripheral neurons can facilitate novel food exploitation[19,20,23,24,27]. However, unlike *D. suzukii* or *S. flava*, *Dsec*'s reduced aversion to noni is not due to fewer bitter neurons (as in *D. suzukii*) or reduced bitter sensitivity (both species)[20,22] but likely reflects altered signal processing.

Some changes in *Dsec* bitter neuron responses to caffeine and coumarin can be recapitulated in *Dmel* and traced to a short deletion in *Gr39a.a*. Our data suggest this *Dsec*-specific deletion – not pseudogenisation of *Gr39a.b* – disrupts receptor function, likely by impairing receptor complex assembly. The resulting altered physiology support the idea that Grs, which likely form heteromeric tetramers, engage in complex, potentially inhibitory interactions[12,23]. Beyond ligand binding sites mutations - evolutionary hotspots in olfactory receptor genes[28,55] - Gr receptor-receptor interaction domains may offer an alternative evolutionary route to modulate gustatory responses.

The increased bitter neuron sensitivity to noni in *Dsec,* without corresponding behavioural aversion, suggests altered signal integration in interneurons[14-17]. In *Dmel*, downstream bitter circuits inhibit sugar-evoked behaviours via inhibitory connections[36], and such circuits[14] may distribute information differently across

species. Evolutionary changes in these or modulatory systems[56,57] may have re-shaped bitter-sweet integration in *Dsec*, dampening aversive responses to noni.

The reduced feeding rate and lack of motor region activation by sucrose in *Dsec* align with possible metabolic changes in this species. It's inability to metabolise high-sugar diets and the negative effects of a carbohydrate rich environment[33-35] limit sweet food intake. Future comparisons with *Dmel* may reveal how carbohydrate-related signalling in the brain[11], gut-brain axis[58], and reproductive system[59] evolved to maintain nutrient homeostasis under distinct feeding regimes. Moreover, field validation will be essential to understand feeding circuit function in natural environments.

References

1 Scott, K. Gustatory processing in *Drosophila melanogaster*. *Annu Rev Entomol* **63**, 15-30 (2018).
2 Auer, T. O., Shahandeh, M. P. & Benton, R. *Drosophila sechellia*: A genetic model for behavioral evolution and neuroecology. *Annu Rev Genet* (2021).
3 Munch, D., Goldschmidt, D. & Ribeiro, C. The neuronal logic of how internal states control food choice. *Nature* **607**, 747-755 (2022).
4 Yarmolinsky, D. A., Zuker, C. S. & Ryba, N. J. Common sense about taste: from mammals to insects. *Cell* **139**, 234-244 (2009).
5 Policarpo, M., Baldwin, M. W., Casane, D. & Salzburger, W. Diversity and evolution of the vertebrate chemoreceptor gene repertoire. *Nat Commun* **15**, 1421 (2024).
6 Li, R. *et al.* The sequence and *de novo* assembly of the giant panda genome. *Nature* **463**, 311-317 (2010).
7 Li, X. *et al.* Pseudogenization of a sweet-receptor gene accounts for cats' indifference toward sugar. *PLoS Genet* **1**, 27-35 (2005).
8 Toda, Y. *et al.* Early origin of sweet perception in the songbird radiation. *Science* **373**, 226-231 (2021).
9 Zhang, G. *et al.* Identification and expression profiles of gustatory receptor genes in *Bactrocera minax* larvae (*Diptera: Tephritidae*): Role of BminGR59f in larval growth. *Insect Sci* **29**, 1240-1250 (2022).
10 Yang, J. *et al.* Identification of a gustatory receptor tuned to sinigrin in the cabbage butterfly *Pieris rapae*. *PLoS Genet* **17**, e1009527 (2021).
11 Fujii, S. *et al. Drosophila* sugar receptors in sweet taste perception, olfaction, and internal nutrient sensing. *Curr Biol* **25**, 621-627 (2015).
12 Weiss, L. A., Dahanukar, A., Kwon, J. Y., Banerjee, D. & Carlson, J. R. The molecular and cellular basis of bitter taste in *Drosophila*. *Neuron* **69**, 258-272 (2011).
13 Harris, D. T., Kallman, B. R., Mullaney, B. C. & Scott, K. Representations of Taste Modality in the Drosophila Brain. *Neuron* **86**, 1449-1460 (2015).
14 Li, J., Dhaliwal, R., Stanley, M., Junca, P. & Gordon, M. D. Functional imaging and connectome analyses reveal organizing principles of taste circuits in *Drosophila*. *Curr Biol* (2025).
15 Walker, S. R., Pena-Garcia, M. & Devineni, A. V. Connectomic analysis of taste circuits in *Drosophila*. *Sci Rep* **15**, 5278 (2025).
16 Tastekin, I. *et al.* From sensory detection to motor action: the comprehensive *Drosophila* taste-feeding connectome. *bioRxiv*, 2025.2008.2025.671814 (2025).
17 Shiu, P. K., Sterne, G. R., Engert, S., Dickson, B. J. & Scott, K. Taste quality and hunger interactions in a feeding sensorimotor circuit. *Elife* **11** (2022).
18 Snell, N. J. *et al.* Complex representation of taste quality by second-order gustatory neurons in *Drosophila*. *Curr Biol* **32**, 3758-3772 e3754 (2022).
19 Cavey, M. *et al.* Increased sugar valuation contributes to the evolutionary shift in egg-laying behavior of the fruit pest *Drosophila suzukii*. *Plos Biol* **21**, e3002432 (2023).
20 Pelaez, J. N. *et al.* Taste evolution in an herbivorous drosophilid. *bioRxiv* (2024).
21 Wang, W. *et al.* Sugar sensation and mechanosensation in the egg-laying preference shift of *Drosophila suzukii*. *Elife* **11** (2022).
22 Dweck, H. K., Talross, G. J., Wang, W. & Carlson, J. R. Evolutionary shifts in taste coding in the fruit pest *Drosophila suzukii*. *Elife* **10** (2021).
23 Dweck, H. K. M. & Carlson, J. R. Molecular logic and evolution of bitter taste in *Drosophila*. *Curr Biol* **30**, 17-30.e13 (2020).
24 Dey, M., Brown, E., Charlu, S., Keene, A. & Dahanukar, A. Evolution of fatty acid taste in drosophilids. *Cell Rep* **42**, 113297 (2023).
25 McBride, C. S. Rapid evolution of smell and taste receptor genes during host specialization in *Drosophila sechellia*. *Proc Natl Acad Sci U S A* **104**, 4996-5001 (2007).

26 Matsuo, T., Sugaya, S., Yasukawa, J., Aigaki, T. & Fuyama, Y. Odorant-binding proteins OBP57d and OBP57e affect taste perception and host-plant preference in *Drosophila sechellia*. *Plos Biol* **5**, 985-996 (2007).

27 Reisenman, C. E., Wong, J., Vedagarbha, N., Livelo, C. & Scott, K. Taste adaptations associated with host specialization in the specialist *Drosophila sechellia*. *J Exp Biol* **226** (2023).

28 Auer, T. O. *et al.* Olfactory receptor and circuit evolution promote host specialization. *Nature* **579**, 402-408 (2020).

29 Charlu, S., Wisotsky, Z., Medina, A. & Dahanukar, A. Acid sensing by sweet and bitter taste neurons in *Drosophila melanogaster*. *Nat Commun* **4**, 2042 (2013).

30 Itskov, P. M. *et al.* Automated monitoring and quantitative analysis of feeding behaviour in *Drosophila*. *Nat Commun* **5**, 4560 (2014).

31 Alvarez-Ocana, R. *et al.* Odor-regulated oviposition behavior in an ecological specialist. *Nat Commun* **14**, 3041 (2023).

32 Task, D. *et al.* Chemoreceptor co-expression in *Drosophila melanogaster* olfactory neurons. *Elife* **11** (2022).

33 Watanabe, K. *et al.* Interspecies comparative analyses reveal distinct carbohydrate-responsive systems among *Drosophila* species. *Cell Rep* **28**, 2594-2607 e2597 (2019).

34 Abe, M. *et al.* Shortened lifespan induced by a high-glucose diet is associated with intestinal immune dysfunction in Drosophila sechellia. *J Exp Biol* **225** (2022).

35 Melvin, R. G. *et al.* Natural variation in sugar tolerance associates with changes in signaling and mitochondrial ribosome biogenesis. *Elife* **7** (2018).

36 Jacobs, R. V. *et al.* Overlap and divergence of neural circuits mediating distinct behavioral responses to sugar. *Cell Rep* **43**, 114782 (2024).

37 Steck, K. *et al.* Internal amino acid state modulates yeast taste neurons to support protein homeostasis in *Drosophila*. *Elife* **7** (2018).

38 LeDue, E. E., Chen, Y. C., Jung, A. Y., Dahanukar, A. & Gordon, M. D. Pharyngeal sense organs drive robust sugar consumption in Drosophila. *Nat Commun* **6**, 6667 (2015).

39 Chen, Y. D., Park, S. J., Joseph, R. M., Ja, W. W. & Dahanukar, A. A. Combinatorial pharyngeal taste coding for feeding avoidance in adult *Drosophila*. *Cell Rep* **29**, 961-973 e964 (2019).

40 Guillemin, J. *et al.* Taste cells expressing Ionotropic Receptor 94e reciprocally impact feeding and egg laying in Drosophila. *Cell Rep* **43**, 114625 (2024).

41 Takagi, S. *et al.* Olfactory sensory neuron population expansions influence projection neuron adaptation and enhance odour tracking. *Nat Commun* **15**, 7041 (2024).

42 Sanchez-Alcaniz, J. A. *et al.* An expression atlas of variant ionotropic glutamate receptors identifies a molecular basis of carbonation sensing. *Nat Commun* **9**, 4252 (2018).

43 Lima, D. B. M., Dos Santos, A. L., Cardoso, C. A. L., Krause, L. C. & Caramao, E. B. Studies related to the chemical composition, biological activities and toxicity of methanolic extracts of noni (*Morinda citrifolia*) fruits and leaves. *Nat Prod Res* **36**, 5868-5871 (2022).

44 Tauber, J. M. *et al.* A subset of sweet-sensing neurons identified by IR56d are necessary and sufficient for fatty acid taste. *PLoS Genetics* **13**, 1-18 (2017).

45 Ikeda, R., Wada, M., Nishigaki, T. & Nakashima, K. Quantification of coumarin derivatives in noni (*Morinda citrifolia*) and their contribution of quenching effect on reactive oxygen species. *Food Chemistry* **113** (2009).

46 Ahn, J. E., Chen, Y. & Amrein, H. Molecular basis of fatty acid taste in *Drosophila*. *Elife* **6** (2017).

47 Devineni, A. V., Deere, J. U., Sun, B. & Axel, R. Individual bitter-sensing neurons in *Drosophila* exhibit both ON and OFF responses that influence synaptic plasticity. *Curr Biol* **31**, 5533-5546 e5537 (2021).

48 Gardiner, A., Barker, D., Butlin, R. K., Jordan, W. C. & Ritchie, M. G. Evolution of a complex locus: exon gain, loss and divergence at the *Gr39a* locus in *Drosophila*. *PLoS One* **3**, e1513 (2008).

49 Jumper, J. *et al.* Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583-589 (2021).

50 Ma, D. *et al.* Structural basis for sugar perception by *Drosophila* gustatory receptors. *Science* **383**, eadj2609 (2024).

51 Shiu, P. K. *et al.* A Drosophila computational brain model reveals sensorimotor processing. *Nature* **634**, 210-219 (2024).

52 McKellar, C. E., Siwanowicz, I., Dickson, B. J. & Simpson, J. H. Controlling motor neurons of every muscle for fly proboscis reaching. *Elife* **9** (2020).

53 Gonzalez Segarra, A. J., Pontes, G., Jourjine, N., Del Toro, A. & Scott, K. Hunger- and thirst-sensing neurons modulate a neuroendocrine network to coordinate sugar and water ingestion. *Elife* **12** (2023).

54 Schlegel, P. *et al.* Information flow, cell types and stereotypy in a full olfactory connectome. *Elife* **10** (2021).

55 Prieto-Godino, L. L., Schmidt, H. R. & Benton, R. Molecular reconstruction of recurrent evolutionary switching in olfactory receptor specificity. *Elife* **10** (2021).

56 Chu, B., Chui, V., Mann, K. & Gordon, M. D. Presynaptic gain control drives sweet and bitter taste integration in *Drosophila*. *Current Biology* **24**, 1978-1984 (2014).

57 Inagaki, H. K., Panse, K. M. & Anderson, D. J. Independent, reciprocal neuromodulatory control of sweet and bitter taste sensitivity during starvation in *Drosophila*. *Neuron* **84**, 806-820 (2014).

58 Cui, X., Meiselman, M. R., Thornton, S. N. & Yapici, N. A gut-brain-gut interoceptive circuit loop gates sugar ingestion in *Drosophila*. *bioRxiv*, 2024.2009.2002.610892 (2024).

59 Carvalho-Santos, Z. *et al.* Cellular metabolic reprogramming controls sugar appetite in *Drosophila*. *Nat Metab* **2**, 958-973 (2020).
60 Ling, F., Dahanukar, A., Weiss, L. A., Kwon, J. Y. & Carlson, J. R. The molecular and cellular basis of taste coding in the legs of *Drosophila*. *J Neurosci* **34**, 7148-7164 (2014).
61 Li, H. *et al.* Fly Cell Atlas: A single-nucleus transcriptomic atlas of the adult fruit fly. *Science* **375**, eabk2432 (2022).

Figure Legends

Figure 1. *Dsec* shifts preference from sucrose to noni

a, Four-choice assay testing preference in wild-type and anosmic (*DsecIr8a*$^{-/-}$/*Orco*$^{1-/-}$)[28] flies. Percentage of flies per quadrant; phylogeny above. Significant differences to equal distribution at 24 h indicated. One-sample Student's *t*-test (H_0 = 25%, two-sided). **b**, Preference index (PI) for noni or grape juice in a 72-well (left), petri dish (middle) or flyPAD (right) two-choice assay. Kruskal-Wallis test (72-well: $H_{(5)}$=73.394, P=2.012e^{-14}; petri dish: $H_{(5)}$=64.468, P=1.445e^{-12}; flyPAD: $H_{(5)}$= 270.48, P=2.2e^{-16}), followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). Wilcoxon test (two-sided): asterisks = significant deviation from 0 (neutrality) (same in **d, f, g, i**). **c**, Temporal profile of cumulative PI. **d**, Left: PI of *Orco*-mutants after antennae removal (AR) ($H_{(2)}$=19.227, P=6.681e^{-05}). Right: PI of flies after maxillary palps and antennae removal (MPAR) ($H_{(2)}$=19.227, P=6.681e^{-05}). **e**, Principal component analysis of flyPAD data (**b**, **d**, **Extended Data Fig. 2e**). **f**-**g**, PI for feeding on noni juice vs. 200 mM sucrose in (**f**) petri dish ($H_{(5)}$=28.526, P=6.393e^{-07}) and (**g**) flyPAD ($H_{(5)}$=93.908, P=2.2e^{-16}) assays. **h**, Left: labellum schematic with taste pegs, bristles and labral sense organ. Right: (fore)leg highlighting sweet- and bitter-sensing taste bristles neurons[60]. Below: markers for labellar neuron types. **i**, Left: PI (noni/grape) in controls and *Poxn* mutants ($H_{(5)}$=55.484, P=2.572e^{-11}). Right: Control (*UAS-Kir2.1*) and experimental flies with silenced neurons. **j**, PI (noni/grape) in *Poxn* mutants and with neurons silenced +/- MPAR. Wilcoxon test (two-sided). In **b**-**j**: Box plots show median (centre line), interquartile range (IQR) (box), 1.5× IQR (whiskers); (***P < 0.001; **P < 0.01; *P < 0.05; NS, not significant: P > 0.05). In **c**: line = mean; shaded area = ±SEM. Here and other figures: Tests and *p*-values in **Sup. Table 5**, *n* = independent biological replicates. Schematics indicate assay type; fly/noni schematics adapted from ref.[2].

Figure 2. Taste peg and labellar sweet neuron characterisation

a, Left: Fly brain with SEZ. AL = antennal lobe. Middle: immunofluorescence of a *DsecIr25a*^{Gal4}*>GCaMP6s* brain (anti-GFP, green; nc82, magenta) and labellum (anti-GFP). Scale bars: 25 µm, inlet highlights taste pegs (scale bar: 5 µm). Right: axonal SEZ innervations; taste bristle neurons target PMS4, taste pegs AMS1. Scale bar: 10 µm. **b**, Anti-GFP/nc82 brain (left) and labellum (right) staining in *DsecGr64f*^{Gal4}*>GCaMP6s*. Scale bars: 25 µm. **c**, Quantification of Gr64f^{+} neurons in labellum and legs (tarsal segments t5–t2) (scale bars: 25 µm; male and pharyngeal counts: **Extended Data Fig. 4d, e, Sup. Table 1**). Kruskal-Wallis test followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). **d**, Left: calcium imaging setup. Middle: raw fluorescence (top) and 0.5% hexanoic acid-evoked responses (bottom) in peg neurons (scale bar: 10 µm). ROIs outlined. Right: SEZ response quantification. **e**, Temporal fluorescence in peg neuron projections (from **d**). **f**, Left: Raw fluorescence (top) and noni- or sucrose-evoked responses (middle, bottom) in *DsecGr64f*^{Gal4}*>GCaMP6s* taste bristle projections

(scale bar: 10 µm). ROIs outlined. Right: SEZ response quantification. **g**, Temporal fluorescence (from panel **f**). **h**, Top: dose-dependent PI for sucrose, glucose, and sucrose + hexanoic acid. *Dmel*/*Dsim* die at highest acid concentration. Bottom: percentage feeding. Both: Kruskal-Wallis test (***P < 0.001; **P < 0.01; *P < 0.05; NS: P > 0.05) followed by Dunn's test (two-sided, Bonferroni). In **c**,**h**: mean ± SEM. In **e**,**g**,**h**: line = mean; shaded area = ±SEM. Black bar = tastant application. In **d**,**f**: box plots show median (centre line), IQR (box), 1.5× IQR (whiskers). Asterisks next to box plots indicate significant responses compared to water (***P < 0.001; **P < 0.01; *P < 0.05; NS: P > 0.05). Wilcoxon test (two-sided, Bonferroni); same test for *Dmel/Dsec* comparison.

Figure 3. Bitter neuron numbers and physiology

a, Immunofluorescence in the SEZ and labellum of *DsecGr66a*^{Gal4}*>UAS-GCaMP6s* flies. AL = antennal lobe. Scale bars: 25 µm. **b**, Quantification of Gr66a^{+} neurons in labellum (top) and legs (bottom). Scale bars: 25 µm; male and pharyngeal cell numbers: **Extended Data Fig. 6a, b, Sup. Table 1**. Kruskal-Wallis test (labellum: $H_{(2)}$=13.614, P=0.001106; foreleg: $H_{(2)}$=4.8756, P=0.08735; midleg: $H_{(2)}$=3.0446, P=0.2182; hindleg: $H_{(2)}$=3.4719, P=0.1762) followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). **c**, Left: raw fluorescence (top) and noni-evoked responses (bottom) in *DsecGr66a*^{Gal4}*>GCaMP6s* bristle neurons (scale bar: 10 µm). ROIs outlined. Right: SEZ response quantification. Asterisks next to box plots indicate significant responses compared to water. Wilcoxon test (two-sided, Bonferroni). Wilcoxon test between species (two-sided), (***P < 0.001; **P < 0.01; *P < 0.05; NS: P > 0.05; **Extended Data Fig. 6f** for *Dsim* data). **d**, Temporal fluorescence in bitter taste bristle neuron projections (from panel **c**). In **b**: bars = mean ± SEM. In **c**: box plots show median (centre line), IQR (box), 1.5× IQR (whiskers). In **d**: line = mean; shaded area = ±SEM. Black bar = tastant application.

Figure 4: **A deletion in Gr39a.a recapitulates *Dsec* GSN physiology**

a, *Gr39a* locus with four splice isoforms sharing the last three exons (red). Below: Anti-GFP/nc82 immunofluorescence in SEZ, labellum, and foreleg of *DmelGr39a.a-Gal4>UAS-GCaMP6s* flies (scale bars: 25 µm). Right: *Gr39a* expression (red) in proboscis and maxillary palp (FlyCellAtlas[61]). **b**, An 8bp deletion in *Gr39a.b* (FlyBase nomenclature; in some studies *Gr39a.a*[48]) causes a frameshift/premature stop codon after 33 residues in *Dsec* (left, nucleotide alignment). Right: *Dsec*-specific 3-aa deletion in Gr39a.a (residues 202–204; blue = divergent sites). **c**, Predicted *Dmel* Gr39a.a monomer structure. Ligand-binding near pore domain (PD); the intracellular anchor domain mediates tetramer assembly. The *Dsec* deletion is nearby. TMD = transmembrane domain (domains per ref.[50]). **d**-**e**, Quantification of calcium responses in *Gr39a.a*$^{+}$ bristle projections of *Dmel* wild-type, *Gr39a*$^{-/-}$ mutants, and rescue lines (*DmelGr39a.a*, *DsecGr39a.a*, *DmelGr39a.a*$^{\Delta 3}$). Top: caffeine, middle: coumarin, bottom: noni juice. Kruskal–Wallis test followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). **f**, Quantification of water-, caffeine-, coumarin-, and noni-evoked calcium responses in *Dsec Gr66a*$^{+}$ bristle projections of wild-type and *DmelGr39a.a*-expressing flies. Wilcoxon test (two-sided, **P < 0.01; NS: P > 0.05). **g**-**h**, PI for caffeine (10 mM) in 5 mM vs. 2 mM sucrose. Top: *Dmel*, bottom: *Dsec*, for the wild-type and rescue lines. Kruskal–Wallis test (*Dmel* $H_{(4)}$=31.118, P=2.896e^{-06}; *Dsec* $H_{(2)}$=0.26381, P=0.8764) followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). **i**, Top: dose-dependent feeding aversion for caffeine and coumarin. Bottom: percentage feeding.

Both: Kruskal-Wallis test (***P < 0.001; **P < 0.01; *P < 0.05; NS: P > 0.05) followed by Dunn's test (two-sided, Bonferroni). In **d**-**h**: box plots show median (centre line), IQR (box), 1.5× IQR (whiskers). In **i**: line = mean; shaded area = ±SEM; bars = mean ± SEM.

Figure 5. Volumetric brain imaging uncovers processing differences
a, Left: simplified SEZ circuitry showing peripheral, inter-, and motor neuron (MN) connections. Right: SEZ arborisation of MN 10–13. Below: pharynx with muscle insertions (white arrowheads)[52]. **b**, *Dsec* SEZ imaging volume (anterior view, inverted signal; A=anterior, D=dorsal) with segmentation of central (purple) and lateral (green) sensory projections, interneuron regions 1–2 (grey), and motor neuron arborisations (blue). Bottom: lateral view (z-stack in **Sup. Video 1**). **c**, Left: Volume segmentation. Middle: Maximum tastant-evoked responses in *Dmel*/*Dsec*. Extreme outliers excluded from plots/included in stats. Wilcoxon test (two-sided), (***P < 0.001; **P < 0.01; *P < 0.05; NS: P > 0.05); letters = significant differences between species. Right: Temporal fluorescence changes (line = mean; shaded area = ±SEM. Black bars = tastant applications). **Extended Data Fig. 9** = dataset including *Dsim*. **d**, Maximum intensity projections of motor region fluorescence to sucrose or noni juice application in *Dmel* (top), *Dsec* (bottom). ΔF/F volumes averaged across animals; time-dimension projections show peak responses per voxel. **e**, Probability of PER to sucrose, grape and noni juice. Kruskal-Wallis test (sucrose: $H_{(2)}$=39.073, P=3.276e^{-09}; grape juice: $H_{(2)}$=25.82, P=2.474e^{-06}; noni juice: $H_{(2)}$=6.8942, P=0.03184) followed by Dunn's test (two-sided, Bonferroni correction); same letter = no significant difference (P > 0.025) (same in **f**). **f**, Proboscis extension time for sucrose, grape or noni juice (sucrose: $H_{(2)}$=11.173, P=0.003748; grape juice: $H_{(2)}$=28.491, P=6.504e^{-07}; noni juice: $H_{(2)}$=9.0659, P=0.01075). In **c**,**f**: box plots show median (center line), IQR (box), 1.5× IQR (whiskers). In **e**: bars = mean ± SEM. **g**, Schematics of taste processing in *Dmel*/*Dsec*. Both are attracted to noni via olfaction (*Dsec*>*Dmel*). Peripheral signals are transmitted to the central brain. Sugars activate feeding motor neurons in *Dmel*. In *Dsec*, probably altered transformation of peripheral sugar and noni signals results in reduced sugar attraction/lost noni aversion.

Online-only Methods

Data reporting
Preliminary experiments were used to assess variance and determine adequate sample sizes in advance of acquisition of the reported data. Several experiments were carried out repeatedly because they served as controls for different genetic manipulations. For calcium imaging, data were collected from multiple flies on multiple days in randomised order. Within datasets the same tastant dilutions were used for acquisition of the dataset. The experimenter was not blinded to the genotype of flies in physiological experiments.

Drosophila husbandry
Drosophila stocks in the Auer lab were maintained on standard wheat flour/yeast/fruit juice medium under a 12 h light:12 h dark cycle at 22-25°C. For all *Dsec* strains, a few g of Formula 4-24® Instant *Drosophila* Medium, Blue (Carolina Biological Supply Company) soaked in noni juice (Raab Vitalfood) were added on top of the standard food. Flies used for volumetric imaging were reared on a yeast-based medium (containing per litre of water: 8 g agar (NZYTech), 80 g barley malt syrup (Prövida),

22 g sugar beet syrup (Grafschafter), 80 g cornflour (Próvida), 10 g soya flour (A. Centazi), 18 g instant yeast (Saf-instant, Lesaffre), 8 ml propionic acid (Argos), and 12 ml nipagin (Tegospet, Dutscher) (15% in 96% ethanol)). Food was supplemented with instant yeast granules on the surface (Saf-instant, Lesaffre) and stocks were kept at 25°C, 70% relative humidity in a 12 h light:12 h dark cycle. Wild-type, published and newly created transgenic lines used in this study are listed in **Sup. Table 2**.

Drosophila microinjections

Dsec transgenesis was performed in-house as described[28]. For CRISPR-Cas9-mediated homologous recombination, a mix of a sgRNA-encoding construct (150 ng μl^{-1}) and donor vector (400 ng μl^{-1}) was injected into *Dsec nanos-Cas9*[28]. Site-directed integration into attP sites was achieved by co-injection of pBS130 (400 ng μl^{-1}) (encoding *PhiC31 integrase* under control of a heat shock promoter (Addgene #26290)[62]) and an *attB*-containing vector (400 ng μl^{-1}). For *piggyBac* transgenesis, a *piggyBac* vector (300 ng μl^{-1}) and a *piggyBac*[63] helper plasmid (300 ng μl^{-1}) were co-injected. All concentrations are given as final values in the injection mix.

CRISPR/Cas9-mediated genome engineering and molecular cloning

sgRNA expression vectors: For expression of single sgRNAs, oligonucleotide pairs (**Sup. Table 3**) were annealed and cloned into *BbsI*-digested *pCFD3-dU6-3gRNA* (Addgene #49410)[64]. To express multiple sgRNAs from the same vector backbone, oligonucleotide pairs (**Sup. Table 4**) were used for PCR and inserted into *pCFD5* (Addgene #73914)[65] via Gibson Assembly.

Donor vectors for homologous recombination: To target the *orco* locus of *Dsim* a similar strategy as for the *orco*^{1} allele in *Dsec* was applied[28]. Homology arms were amplified from *Dsim* genomic DNA and inserted via restriction cloning into *pHD-3P3-DsRed*. To generate Gal4 reporter alleles, a 2A-Gal4 expression vector (GeneScript) was synthesised and either *Dsec*, *Dsim* or *Dmel* specific homology arms generated via gene synthesis (GeneScript) were introduced via seamless cloning flanking the expression cassette. Sequence details can be obtained from the corresponding author upon request.

UAS-expression vectors: cDNA vectors for *Dmel Gr39a.a*, *Gr39a.b* and *Dsec Gr39a.a* were generated via gene synthesis (GeneScript). cDNA was amplified via PCR and inserted into *pUAST-attB*[66] via restriction cloning resulting in *pUAS-DmelGr39a.a-attB, pUAS-DmelGr39a.b-attB*, and *pUAS-DsecGr39a.a-attB.*

Site directed mutagenesis: A three amino acid deletion was introduced into *Dmel pUAS-DmelGr39a.a-attB* resulting in *pUAS-DmelGr39a.a*$^{\Delta 3}$*-attB* via PCR mediated site directed mutagenesis (sequences of oligonucleotides are listed in **Sup. Table 4**).

Immunohistochemistry

Immunofluorescence on adult brains, proboscis, and legs was performed as previously described[67] using mouse monoclonal antibody nc82 1:10 (Developmental Studies Hybridoma Bank, Cat Nr. nc82; RRID:AB_2314866), anti-Ir25a (guinea pig, gift from R. Benton), and rabbit anti-GFP 1:500 (Invitrogen #A-11122). Alexa488-, Cy3- and Cy5-conjugated goat anti-guinea pig, goat anti-mouse, and goat anti-rabbit IgG secondary antibodies (Molecular Probes, Jackson Immunoresearch, 111-545-003, 115-165-003, 112-175-143) were used at 1:500.

Image acquisition and processing

Confocal images of brains, legs and proboscis were acquired on an inverted confocal microscope (Zeiss LSM 710) equipped with an oil immersion 40Å~ objective (Plan Neofluar 40Å~ oil immersion DIC objective; 1.3 NA) and images were processed in Fiji[68]. *Dsec* brains were registered to a *Dsec* reference brain[28] using the Fiji CMTK plugin (https://github.com/jefferis/fiji-cmtk-gui), as previously described[69].

Behavioural assays

4-choice arena: A group of 20 male and 50 female flies was anaesthetised 5 days after eclosion on ice and transferred to an arena (20x20x5 cm) containing four different fruits (pieces of apple, banana, grapes, and noni) placed on a small petri dish (6 cm diameter). The arena was covered with a mesh, placed under a camera and snapshots were take every 10 min for 24 h. Apples, banana and grape were purchased freshly from a local supermarket; noni at the ripe stage was harvested from the greenhouse at the University of Lausanne. Assays were run at red light under temperature (25°C) and humidity (60%) controlled conditions starting at 11 am for each replicate. The position of fruits was randomised for each experiment. At every timepoint, the number of flies per quadrant was counted; data is represented as percentage of flies per quadrant.

72-well plate feeding assay: The 72-well plate feeding assay was adapted from previous studies[67,12]. Briefly, agarose (1%) was dissolved in noni or grape juice, heated in a microwave and mixed with food colorants (Food Red No. 106; 500 µg ml^{-1}; Food Blue No. 1, 125 µg ml^{-1}; Tokyo Chemical Industry Co., Tokyo, Japan). 10 µl of each solution were pipetted into alternating wells of 72-well microtiter plates (Sigma M5812). Groups of 80 male or female 3-7-day old, mated flies were wet-starved for 22 h at 22°C, anaesthetised on ice for 1 min and transferred into the plates where they were allowed to feed for 1.5 h in darkness at 25°C, 60% relative humidity. Flies' feeding was terminated by plate flash freezing and food ingestion was scored by two independent operators. Juice-to-colourant association was swapped at every replicate. The percentage of feeding was calculated by scoring flies without colourant in the abdomen and comparison to flies with food consumption. The preference index was calculated with the following formula: (number of flies feeding on A - number of flies feeding on B)/total number of flies feeding.

Petri dish feeding assay: The petri dish feeding assay was adapted from previous reports[29,70]. Briefly, groups of 20 male or female 3-7 day old mated flies were wet-starved for 22 h at 22°C, anaesthetized on ice for 1 min and transferred into small Petri dishes (60x15 mm, Sarstedt 82.1194.500) where food substrates in 1.3% moderate EEO-agarose (SeaKem ME agarose, CatNo 50010) labelled with blue or red colourants (as above) were pipetted as 18 (9x2) semisolid drops (10 µl) on the dish surface. A template was used for reproducible and equally spaced spot distribution on the petri dish surface. For experiments with antennectomised flies, surgery was performed on freshly eclosed flies followed by 3-5 days of recovery before starvation. The preference index was calculated as for the 72-well plate feeding assay.

FlyPAD: The flyPAD (fly Proboscis and Activity Detector) rig was purchased from EasyBehaviour and used in a two-choice configuration where food substrates were pipetted into the sensor wells as 1.3% moderate EEO-agarose solutions (as for the petri dish assay). Starved flies (22 h wet starvation at 22°C) from different genetic backgrounds were loaded in the arena using a mouth aspirator in random order and allowed to feed under dim red light for 1 h at 25°C, 60% relative humidity. The flyPAD capacitance signal was analysed as previously reported[30] and multiple feeding parameters were extracted. We only considered flies that performed at least 10 sips

on both substrates over 1 h recording. In the case of *Dsec* Kir2.1 experiments, we lowered this threshold to 1 sip on both substrates. The preference index was calculated as (number of sips on food A - number of sips on food B)/total number of sips in 1 h. We used Bonsai (v2.0.2) and Analyze_flyPAD_data.exe (EasyBehaviour) for data acquisition. Principal component analysis was performed including all flyPAD extracted parameters using the *prcomp()* function in R.

Proboscis extension response (PER): PER was assessed on starved female flies (22 h wet starvation at 22°C) mounted inside a pipette tip so that only the head was exposed for labellar stimulation. After mounting, flies were allowed to recover in a humidified chamber for 1 h and water was provided until satiation before testing. Liquid tastant solutions were applied to the labellum with a blunted needle syringe and stimulation was repeated for three times with a 1 min interval between stimulations. PER was scored when a fly showed full proboscis extension, and the experimenter was blind to the genotype of tested flies.

Temporal consumption assay: The temporal consumption/proboscis extension time of flies was quantified as previously described[53] with slight modifications: 3-5 day old females flies were wet-starved at 22°C for 22 h, anesthetized using CO_2 and fixed to a glass slide with glue (Pritt). Flies recovered for 1-2 h in a humidified box and were given water until satiation before testing. In testing, flies were presented with one tastant (200 mM sucrose, grape juice or noni juice) 10 times, flies were able to touch tastants with their leg and labellum, and consumption/proboscis extension time was manually recorded for each fly and summed up. Stimuli order was randomized. If flies did not respond to a tastant they were tested for response to any of the other two and only included if they responded to at least one of the stimuli. All experiments were done in light, at constant temperature (25°C) and humidity, and the experimenter was blind to the genotype of tested flies.

Calcium imaging

Widefield imaging: SEZ imaging was performed as previously described[42]. For labellum stimulation experiments, 3-7 day old non-starved female flies raised in groups at 22-25°C were used. These were anaesthetised on ice, mounted into self-built imaging blocks with extended proboscis and fixed with glue (UV glue, Tetric EvoFlow, A1, Ivoclar Vivadent). For tarsal stimulation experiments, older flies were used (10-20 days old), forelegs were immobilised with UV glue under a CO_2 flow and legs were positioned so that tarsi were accessible for stimulation. The proboscis was extended and, in both preparations, sealed from the head capsule to prevent buffer leakage.
The head was surgically opened frontally by removing the antennae and the SEZ was exposed to the microscope objective in AHL buffer (Adult Haemolymph-Like saline buffer: 108 mM NaCl, 5 mM KCl, 2 mM CaCl2, 8.2 mM MgCl2, 4 mM NaHCO3, 1 mM NaH2 PO4, 5 mM trehalose, 10 mM sucrose, 5 mM HEPES, pH 7.5) after removal of the oesophagus.
A CCD camera (CoolSNAP-HQ2 Digital CameraSystem) mounted on a fluorescence microscope (upright fixed stage Carl Zeiss Axio Examiner D1) equipped with a 40x water-immersion objective (W Plan-Apochromat 40Å~ /1,0 VIS-IR DIC) was used for GFP signal acquisition. The excitation light (470 nm) was produced with an LED light (Cool LED pE-100, VisiChrome). Tastants were delivered manually using a micromanipulator (Narishige) equipped with a blunted needle syringe containing the liquid tastant solution. Stimulation was initiated 10 s after the LED was turned on and maintained for 10 s, after which contact was terminated while recording continued for an additional 10 s. The order of different tastant application was partly randomized:

water was always applied first followed by other tastants. Water was applied also in between stimulation to wash away residues from previous applications on the labellum, though these water applications were not quantified. Juice solutions were used fresh from frozen aliquots. The other tastants were re-used for a maximum of 3 months. Time course images (16-bit) were acquired using Metafluor software (Visitron) at a rate of 3 Hz with a 200 ms exposure time per frame. For experiments in Extended Data figures 5b, 5d, 6d, 6e, and 6g, image acquisition was performed using a Hamamatsu ORCO-Flash4.0 CCD camera with LED illumination from a ZEISS Colibri 5 system, controlled by ZEN 2 software (v3.9.3). All other parameters were identical to those described above. The signal time course was quantified using Fiji[68] by extracting the maximum pixel value over time from manually selected ROIs. ROIs were drawn around SEZ projections and four background areas and the average background intensity was subtracted from SEZ projections intensity at each time point. Basal fluorescence (F) was determined by averaging the intensity of 10 timepoints prior to the onset of the stimulation, while peak fluorescence was identified as the maximum intensity within the 10 timepoints following the onset. The relative changes in fluorescence ($\Delta F/F$) were then calculated. To account for species-specific differences in GCaMP levels and to enable inter-species comparisons, we normalised the relative changes in fluorescence for each tastant application by the average response within the same preparation (normalised data). We excluded samples from our analysis that did not respond to initial tastant applications or after they stopped responding to diagnostic tastants after a series of stimulations.

Volumetric imaging: Pan-neuronal volumetric imaging in the SEZ was performed as previously described[3]. 1-15 d old female flies were cold anaesthetised on ice and fixed to a custom-made imaging block using fast UV curing glue (Bondic). The proboscis was fixed in an extended position and the front legs were removed to prevent the flies from touching the food stimulus. The anterior part of the head was isolated from the rest of the body through a hole in a weigh ship and covered with *Drosophila* AHL (103 mM NaCl, 3 mM KCl, 5 mM TES, 10 mM trehalose dihydrate, 10 mM glucose, 2 mM sucrose, 26 mM NaHCO3, 2 mM CaCl2 dihydrate, 4 mM MgCl 2 hexahydrate, 1 mM NaH2PO4, pH 7.3). A window was cut between the eyes and the ocelli, thereby removing the antennae, the oesophagus was transected to allow for unoccluded visual access to the SEZ.

A resonant-scanning two-photon microscope (Scientifica) equipped with a 20×NA 1.0 water immersion objective (Olympus) and a piezo-electric z-focus drive, was used to allow for fast volumetric scans. SEZ volumes in the three different species were acquired for 60 s at a 1 Hz volume rate covering 512 × 256 × 60 voxels at voxel dimensions of ~0.5 × 0.5 × 3.6 µm using SciScan (Scientifica). A Chameleon Ultra II Ti:Sapphire laser (Coherent) was used to excite GCaMP6s at 920 nm. During imaging, the brain was constantly perfused with AHL bubbled with carbogen (95% O_2, 5% CO_2). Manual gustatory stimulations of the proboscis were performed using glass capillaries filled with taste solution, mounted on a micromanipulator (Sensapex).

Species-specific brain templates were generated using the Advanced Normalisation Toolkit (ANTs) script antsMultivariateTemplateConstruction2.sh. We corrected for movement artifacts and shifts between recordings within an animal using ANTs. Recordings were then pre-aligned to the respective species template by using manual landmarks that were defined in ITK-SNAP[71]. Finally, ANTs was used for automated fine registration of each recording to the respective template.

We created average brain volumes for each stimulus-species combination and performed a maximum response projection. Based on these volumes, we defined non-

overlapping 3D ROIs in ITK-SNAP, which were then used for time course extraction from each recording. ROIs were segmented based on a combination of anatomical landmarks and stimulus-evoked activity patterns to ensure consistent identification across species. The anterior motor region, spanning both hemispheres, resembles the arborization patterns of motor neurons. Sensory projection regions were defined based on location (central vs. lateral) and the response patterns induced by sucrose and denatonium stimulations and resemble bristle sensory projections (PMS2/3 and PMS4). Two additional broad interneuron regions were defined: 'Interneurons region-1', located laterally to the sensory projections, was selected based on functional responsiveness; 'Interneurons region-2' was positioned posterior to the sensory regions. All ROIs were deliberately defined broadly and conservatively to allow reproducible segmentation and cross-species comparison. Data quantification was performed as explained above.

AlphaFold structural prediction

Dsec Gr39a.a peptide sequence was translated from aligned nucleotide sequences with *Dmel*, protein structure predicted using ColabFold[72] and plotted using PyMOL (Version 1.8 Schrödinger, LLC).

Fly Cell Atlas analysis

Single nuclear transcriptomic data were obtained from the Fly Cell Atlas[61] datasets (s_fca_biohub_proboscis_and_maxillary_palps_10x, s_fca_biohub_antenna_10x) and transcript co-expression was determined for transcripts with n>1 transcript per cell.

Statistics and reproducibility

Data were analysed and plotted using Microsoft Excel (version 16.89.1), R (v4.4.1; R Core Team 2024) and the tidyverse suite[73]. All statistical details are listed in the figures and figure legends and exact *p*-values are listed in **Sup. Table 5**. Micrographs shown in the manuscript are representative pictures taken from at least three independently performed biological replicates.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All relevant data supporting the findings of this study, and all unique biological materials generated in this study (e.g., mutants, plasmids and transgenic fly strains) are available at https://github.com/AuerTomLab/Bertolini2025 or from the corresponding author upon request.

Code availability

All code used to analyse and present data in this study is available at https://github.com/AuerTomLab/Bertolini2025 or on request from the corresponding author.

Methods References

62 Gohl, D. M. *et al.* A versatile *in vivo* system for directed dissection of gene expression patterns. *Nat Methods* **8**, 231-237 (2011).
63 Arnoult, L. *et al.* Emergence and diversification of fly pigmentation through evolution of a gene regulatory module. *Science* **339**, 1423-1426 (2013).
64 Gratz, S. J. *et al.* Highly specific and efficient CRISPR/Cas9-catalyzed homology-directed repair in *Drosophila*. *Genetics* **196**, 961-971 (2014).
65 Port, F., Chen, H.-M., Lee, T. & Bullock, S. L. Optimized CRISPR/Cas tools for efficient germline and somatic genome engineering in *Drosophila*. *Proc Natl Acad Sci U S A* **111**, E2967-2976 (2014).
66 Bischof, J., Maeda, R. K., Hediger, M., Karch, F. & Basler, K. An optimized transgenesis system for *Drosophila* using germ-line-specific phiC31 integrases. *Proc Natl Acad Sci U S A* **104**, 3312-3317 (2007).
67 Sanchez-Alcaniz, J. A., Zappia, G., Marion-Poll, F. & Benton, R. A mechanosensory receptor required for food texture detection in *Drosophila*. *Nat Commun* **8**, 14192 (2017).
68 Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nat Methods* **9**, 676-682 (2012).
69 Cachero, S., Ostrovsky, A. D., Yu, J. Y., Dickson, B. J. & Jefferis, G. S. Sexual dimorphism in the fly brain. *Curr Biol* **20**, 1589-1601 (2010).
70 Ribeiro, C. & Dickson, B. J. Sex peptide receptor and neuronal TOR/S6K signaling modulate nutrient balancing in *Drosophila*. *Curr Biol* **20**, 1000-1005 (2010).
71 Yushkevich, P. A. *et al.* User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage* **31**, 1116-1128 (2006).
72 Mirdita, M. *et al.* ColabFold: making protein folding accessible to all. *Nat Methods* **19**, 679-682 (2022).
73 Wickham Hadley *et al.* Welcome to the tidyverse. *The Journal of Open Source Software* **4**, 1686 (2019).

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Author contribution

T.O.A. and E.B. conceived the project. All authors contributed to experimental design, analysis and interpretation of results. Specific experimental contributions were as follows: E.B. performed molecular cloning, behavioural assays, immunostainings, and physiological experiments. D.M. performed volumetric calcium imaging. J.P. performed molecular cloning, behavioural assays, and immunostainings. N.S. and M.B. performed behavioural assays. C.R. provided guidance to D.M. T.O.A. performed molecular cloning, immunostainings, preliminary physiological experiments, and generated transgenic lines. T.O.A. and E.B. wrote the paper with input from all authors. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional Information

Supplementary Information is available for this paper.

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Extended Data Figure Legends

Extended Data Figure 1: Feeding preference is not sexually dimorphic

a, Distribution of flies per quadrant in the 4-choice assay from 10 min to 24 h after assay start, separated by species. Significant differences at each time point to an equal distribution across fruits are indicated (One-sample Student's t-test, two-sided, H_0 = 25%; ***P < 0.001; **P < 0.01; *P < 0.05). For *Dsec*, only the smallest *p*-value for both genotypes is indicated. **b**, Feeding preference for female (left) and male (right) flies in the 72-well, petri dish and flyPAD assay. Data re-plotted from **Fig. 1**. Kruskal-Wallis test (72-well, females: $H_{(5)}$=60.22, P=1.095e^{-11}; 72-well, males: $H_{(5)}$=29.109, P=2.207e^{-05}; petri dish, females: $H_{(5)}$=35.773, P=1.055e^{-06}; petri dish, males: $H_{(5)}$=33.573, P=2.895e^{-06}; flyPAD, females: $H_{(5)}$=270.48, P=2.2e^{-16}; flyPAD, males: $H_{(5)}$=31.49, P=7.496e^{-06}) followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). Wilcoxon test (two-sided): asterisks = significant deviation from 0 (neutrality, ***P < 0.001; **P < 0.01; *P < 0.05; NS: P > 0.05). **c**, Percentage of female (left) and male (right) flies feeding on either noni juice, grape juice or both in the 72-well (two panels to the left) and petri dish assay (two panels to the right). **d**, Number of sips in one hour after assay start on noni ($H_{(5)}$=147.64, P=2.2e^{-16}) or grape juice ($H_{(5)}$=110.73, P=2.2e^{-16}) in the flyPAD assay. Kruskal-Wallis test followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). The same data was used to calculate the preference index shown in **Fig. 1b**. In **a**: line = mean; shaded area = ±SEM; points overlaid. In **b**,**d**: box plots show median (center line), inter-quartile-range (IQR) (box), 1.5× IQR (whiskers), datapoints overlaid. In **c**: mean ± SEM; datapoints overlaid. Here and other figures: Statistical tests and exact *p*-values are provided in **Sup. Table 5**, sample sizes indicating independent biological replicates are indicated.

Extended Data Figure 2: *DsimOrco$^{1-/-}$* mutants and behavioural analysis

a, Left: schematic of the *Dsim* orco locus. The *3P3-DsRed* marker was inserted into the first exon to match the *DsecOrco^{1}* allele[28]. In red: epitope recognised by Orco antibody. This allele is a strong hypomorph but does not fully disrupt Orco function[28]. Right: Anti-Orco immunofluorescence in wild-type (DSSC 14021-0251.04) and *DsimOrco$^{1-/-}$* antennae. Orco signal is strongly reduced but not absent. Scale bar = 20 µm. **b**, Left: percentage of flies feeding on noni, grape or both for wild-type, AR and *orco*-mutant flies. Right: number of sips per substrate in wild-type and MPAR flies measured by flyPAD (Kruskal-Wallis test: noni $H_{(5)}$=70.918, P=6.6e^{-14}; grape $H_{(5)}$=96.801, P=2.2e^{-16}; followed by Dunn's test (two-sided, Bonferroni); ***P < 0.001; **P < 0.01; *P < 0.025; NS: P > 0.025); data from **Fig. 1d c**, Temporal profile of PI for wild-type strains +/- MPAR (from **Fig. 1b**). **d**, PI for noni vs. grape juice in flyPAD for olfactory mutants (*Dmel Ir8a$^{-/-}$/Orco$^{-/-}$/Ir25a$^{-/-}$/Gr63a$^{-/-}$*, *Dsim Orco$^{1-/-}$*, *Dsec Ir8a$^{-/-}$/Orco$^{1-/-}$*). Kruskal-Wallis test ($H_{(2)}$=16.537, P=0.0002564) followed by Dunn's test (two-sided,

Bonferroni). Same letters = no significant difference (P > 0.025). Wilcoxon test (two-sided): asterisks = significant deviation from 0 (***P < 0.001; *P < 0.05, same in **e**). **e**, PI for female flies lacking eye pigmentation ($H_{(2)}$=77.005, P=2.2e^{-16}). **f**, Schematic of flyPAD feeding parameters (adapted from ref.[30]). **g**, Loadings of principal component 1 and 2 from **Fig. 1e. h**, Sugar content (g/100 ml for juices/sucrose and g/100 g for fruits) of apple (12,2), banana (15,8), grape (16,1), grape juice (18,0), noni juice (2,5), noni fruit (2,2) and of 200mM sucrose (6,85). Data was taken from the U.S. Department of Agriculture, Agricultural Research Service FoodData Central Database (https://fdc.nal.usda.gov/, identifiers: banana (9040), apple (9050), grapes (100280)) or the Malaysian Food Composition Database (https://myfcd.moh.gov.my/, identifier: *Morinda citrifolia* (105056)). **i**, Percentage of flies feeding on noni, sucrose or both (from **Fig. 1f**). **j**, Temporal profile of PI for wild-type strains from **Fig. 1g. b**–**e**: Box plots show median, IQR, 1.5× IQR, datapoints overlaid. In **b**,**i**: mean ± SEM; datapoints overlaid. In **b**,**d**: box plots show median (center line), IQR (box), 1.5× IQR (whiskers), datapoints overlaid. In **c**,**j**: line = mean; shaded area = ±SEM; datapoints overlaid.

Extended Data Figure 3: Sip counts in flies with impaired GSNs

a, Number of sips per substrate as measured in the flyPAD assay in *Poxn* alleles (left) and after silencing of defined taste neuron populations (right). The same data was used to calculate the preference index shown in **Fig. 1i**. Kruskal-Wallis test followed by Dunn's test (two-sided, Benjamini-Hochberg) results are shown in the figure. Same letters = no significant difference (P > 0.025). (***P < 0.001; *P < 0.025; NS: P > 0.025), exact *p*-values in **Sup. Table 5**. **b**, Left: Feeding preference of female control flies and flies with silenced bitter and sweet sensing neurons for noni or 200mM sucrose in the flyPAD assay. Kruskal-Wallis test ($H_{(2)}$=43.573, P=3.454e^{-10}) followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). Right: Number of sips per substrate. Kruskal-Wallis test (noni juice ($H_{(2)}$=83.671, P=2.2e^{-16}), grape juice ($H_{(2)}$=54.424, P=2.506e^{-12}) followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). **c**, Number of sips per substrate as measured in the flyPAD in *Poxn* alleles and after silencing of defined taste neuron populations and without or with maxillary palp and antennal removal (MPAR). The same data was used to calculate the preference index shown in **Fig. 1j**. Wilcoxon test (two-sided) (*Poxn*: noni juice (W=2026.5, p=3.985e^{-09}), grape juice (W=1423, p=0.1112). Kruskal-Wallis test taste neuron silencing (noni juice ($H_{(5)}$=138.29, P=2.2e^{-16}), grape juice ($H_{(5)}$=146.95, P=2.2e^{-16}) followed by Dunn's test (two-sided, Bonferroni). (***P < 0.001; **P < 0.01; *P < 0.025; NS: P > 0.025), exact *p*-values in **Sup. Table 5**. In **a**-**c**: box plots show median (centre line), IQR (box), 1.5× IQR (whiskers), datapoints overlaid.

Extended Data Figure 4: Sensory neuron population analysis

a, Schematic illustrating the CRISPR/Cas9-mediated knock-in strategy to generate reporter *Gal4*-alleles at receptor loci. **b**, Expression of *Ir25a*, *Gr64f*, and *Gr66a* in individual neurons of the fly proboscis and maxillary palp (left, top: *Gr64f* and *Ir25a*, bottom: *Gr66a* and *Ir25a*) or fly antenna (right, *Gr64f* and *Ir25a*). Data from the FlyCellAtlas[61]. **c**, Immunofluorescence in the labellum of *DsecGr64f^{Gal4}>UAS-GCaMP6s* transgenic flies with an anti-GFP (detecting GCaMP6s expression) and anti-Ir25a antibody. Gr64f expressing cells represent a subpopulation of Ir25a expressing neurons. Scale bar: 20 µm. **d**, Number of Gr64f^{+} neurons in the labellum and legs of male *Dmel*, *Dsim* and *Dsec* based on transgenic labelling. Kruskal-Wallis test (labellum: $H_{(2)}$=14.05, P=0.0008894; foreleg: $H_{(2)}$=17.631, P=0.0001484; midleg:

$H_{(2)}$=6.0282, P=0.04909; hindleg: $H_{(2)}$=1.3862, P=0.5) followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). **e**, Left: representative picture of GFP expressing neurons in the proboscis of a female of the *DsecGr64fGal4>UAS-unc84:GFP* transgenic line. Arrows point towards neurons in the labral sense organ (LSO). Scale bar: 25 µm. Right: Number of Gr64f$^+$ neurons in the LSO of female (left) or male (right) *Dmel*, *Dsim* and *Dsec* based on transgenic labelling. Kruskal-Wallis test (females: $H_{(2)}$=1.4, P=0.4966) followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). **f**, Normalised calcium imaging data in taste pegs (same as **Fig. 2d**). Asterisks next to single box plots indicate significant responses compared to water. Wilcoxon test (two-sided, Bonferroni). Wilcoxon test between species (two-sided); (***P < 0.001; **P < 0.01; *P < 0.05; NS: P > 0.05). In **d,e**: mean ± SEM; points overlaid. In **f**: box plots show median (centre line), IQR (box), 1.5× IQR (whiskers), datapoints overlaid.

Extended Data Figure 5: Calcium imaging in forelegs and sweet neuron characterisation

a, Left: representative pictures of GFP expressing Ir25a$^+$ neurons in *Dsec* (top) and *Dmel* (bottom) forelegs (scale bars: 25 µm). Middle: Schematic of the calcium imaging set-up to characterise physiological responses in the SEZ upon tastant presentation to the foreleg. Right: Example image of raw fluorescence (top) and tastant-evoked (noni juice) calcium responses (bottom) in *Dsec* tarsal bristle neurons (labelled by *Ir25a^{Gal4}* driven GCaMP6s expression, scale bar = 25 µm). The colour scale depicts relative fluorescent changes (∆F/F). Regions of Interest (ROIs) used for signal quantification are indicated with dashed lines (dashed black lines: signal quantification, white dashed lines: background signal). Note that only one foreleg was stimulated resulting in lateralised, asymmetric responses. **b**, Quantification of tastant-evoked calcium responses in Ir25a$^+$ foreleg neuron projections in the SEZ. Data is reported as GCaMP6s fluorescence changes. **c**, Normalised calcium imaging data in taste bristles (same as **Fig. 2f**). **d**, Quantification of tastant-evoked calcium responses in Gr64f$^+$ bristle neuron projections of the fly labellum in the SEZ of *DsecGr64f^{Gal4}>UAS-GCaMP6s* (wild-type) and Gr64f mutant flies (*Gr64f$^{-/-}$*) being homozygous for the *DsecGr64f^{Gal4}* allele. Data is reported as GCaMP6s fluorescence changes. **e**, Top of each panel: dose-dependent feeding preference for fructose (top) or sucrose containing increasing concentrations of octanoic acid (bottom) across species in the petri dish assay, 18-20 females per datapoint; sample size for each condition is indicated below. Bottom of each panel: percentage of flies feeding in the petri dish assay. Both: Kruskal-Wallis test (**P < 0.01; *P < 0.05; NS: P > 0.05) followed by Dunn's test (two-sided, Bonferroni), exact *p*-values in **Sup. Table 5**. In **b**,**c**,**d**: box plots show median (centre line), IQR (box), 1.5× IQR (whiskers), datapoints overlaid. Asterisks next to single box plots indicate significant responses compared to water. Wilcoxon test (two-sided, Bonferroni). Wilcoxon test between species/genotypes (two-sided); (***P < 0.001; **P < 0.01; *P < 0.05; NS: P > 0.05). In **e**: line = mean; shaded area = ±SEM; datapoints overlaid. Bars: mean ± SEM; datapoints overlaid.

Extended Data Figure 6: Bitter sensory neuron characterisation

a, Number of Gr66a$^+$ neurons in the labellum and legs of *Dmel*, *Dsim* and *Dsec* males. For cell counts, bars = mean ± SEM; datapoints overlaid. Kruskal-Wallis test (labellum: $H_{(2)}$=14.05, P=0.0008894; foreleg: $H_{(2)}$=17.631, P=0.0001484; midleg: $H_{(2)}$=6.0282, P=0.04909; hindleg: $H_{(2)}$=1.3862, P=0.5) followed by Dunn's test (two-

sided, Bonferroni). Same letters = no significant difference (P > 0.025). **b**, Left: GFP-expressing neurons in the proboscis of *DsimGr66aGal4>UAS-unc84:GFP* males. Arrows = neurons in the labral sense organ (LSO). Scale bar: 25 µm. Right: Number of Gr66a^{+} neurons in the LSO of females (left) or males (right). Kruskal-Wallis test followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). **c**, Normalised calcium imaging data in labellar bristles (same as **Fig. 3c**). **d**, Top: Example image of raw (top) and noni-evoked (bottom) calcium responses in *Dsec* tarsal taste neurons (*Gr66a^{Gal4}*>GCaMP6s). Scale bar: 25 µm. ROIs indicated. Only one foreleg was stimulated, resulting in lateralised responses. Bottom: Quantification of foreleg Gr66a^{+} projections in the SEZ. **e**, Quantification of labellar bristle responses in *Dmel* comparing a *Gr66a promoter-Gal4* vs. *Gr66a^{Gal4}* knock-in allele. **f**, Quantification of labellar Gr66a^{+} bristle responses to water, sucrose, caffeine, denatonium. **g**, Quantification of labellar Gr66a^{+} bristle responses in *DsecGr66a^{Gal4}>UAS-GCaMP6s* (wild-type) and *Gr66a$^{-/-}$* mutants. In **a**,**b**: mean ± SEM; datapoints overlaid. In **c**-**g**: box plots show median (centre line), IQR (box), 1.5× IQR (whiskers), datapoints overlaid. Asterisks next to single box plots indicate significant responses compared to water. Wilcoxon test (two-sided, Bonferroni). Wilcoxon test between species/genotypes (two-sided); (***P < 0.001; **P < 0.01; *P < 0.05; NS: P > 0.05).

Extended Data Figure 7: Physiology of *DmelGr39a.b* rescue and testing of a *Dsec UAS-Kir2.1-eGFP* line

a, Quantification of tastant-evoked calcium responses in Gr66a^{+} bristles neuron projections in the SEZ of *Dsec* expressing *DmelGr39a.b*. Wilcoxon test (two-sided). No significant differences to wild-type (NS: P > 0.05). **b**, PI for denatonium (10 mM) in 50 mM sucrose vs. 5 mM sucrose feeding in the petri dish assay for all three species. The variance of the data = 0. **c**, Number of progenies from crossing males carrying a pan-neuronal *nsyb-Gal4* transgene (located on the X chromosome, marked with *miniW*) with heterozygote *UAS-eGFP-Kir2.1* (marked with *3P3-DsRed*) females. No adult flies carrying both transgenes emerge. **d**, Anti-GFP immunofluorescence in the labellum of *DsecGr64fa^{Gal4}>UAS-eGFP-Kir2.1* (left) and *DsecGr66a^{Gal4}>UAS-eGFP-Kir2.1* (right) transgenic flies. Scale bar: 25 µm. **e**, PI and sip counts for heterozygous control and experimental (*Gr64$^{f-/-}$* and *Gr66a$^{-/-}$* mutant) flies in *Dsec* in the flyPAD assay when presented with noni vs. grape juice. Kruskal-Wallis test (PI ($H_{(3)}$=11.318, P=0.01013); sips on noni ($H_{(3)}$=27.103, P=5.603e^{-06}); sips on grape ($H_{(3)}$=14.156, P=0.002701)) followed by Dunn's test (two-sided, Bonferroni); ***P < 0.001; NS: P > 0.025. **f**, PI and sip counts for control (wildtype *Dsec.30*, *UAS-Kir2.1*) and experimental flies after silencing of selected taste cell populations in *Dsec* in the flyPAD assay when presented with noni vs. grape juice. Kruskal-Wallis test (PI ($H_{(3)}$=38.121, P=2.664e^{-08}); sips on noni ($H_{(3)}$=45.999, P=5.673e^{-10}); sips on grape ($H_{(3)}$=39.071, P=1.676e^{-08})) followed by Dunn's test (two-sided, Bonferroni). Same letters = no significant difference (P > 0.025). In **a**-**f**: box plots show median (centre line), IQR (box), 1.5× IQR (whiskers), datapoints overlaid.

Extended Data Figure 8: Segmentation of responsive regions in the SEZ

Anatomy- and activity-based manual segmentation exemplified on the *D. melanogaster* brain. Top: segmented areas with relative depth within the stack, middle: activity map upon sucrose (200 mM) stimulation of the proboscis; bottom: activity map upon denatonium benzoate (10 mM) stimulation of the proboscis. See **Suppl. Video 1** for a detailed Z-stack with annotated segmentations.

Extended Data Figure 9: Volumetric imaging across all three species

a, Temporal fluorescence changes in lateral sensory axonal innervations in *Dmel* and *Dsec* upon water and denatonium benzoate (10mM) presentation. The black bars indicate the interval of tastant application. **b**, Quantification of tastant-evoked neuronal activity (maximum responses) in the segmented areas shown in **Fig. 5b** in all three species. For visualisation purposes, several outliers are excluded from the display but included in the statistical analysis. Wilcoxon test (two-sided, Bonferroni). Asterisks for significantly different intraspecific comparisons (***P < 0.001; **P < 0.01; *P < 0.05; NS: P > 0.05). Significant differences to *Dmel* responses are shown in the figure with letters. In **a**: line = mean; shaded area = ±SEM; datapoints overlaid. Bars: mean ± SEM; points overlaid. In **b**: box plots show median (centre line), IQR (box), 1.5× IQR (whiskers), datapoints overlaid.