nature communications

Article

https://doi.org/10.1038/s41467-025-56354-3

Yeast-derived volatiles orchestrate an insect-yeast mutualism with oriental armyworm moths

Received: 28 April 2024

Accepted: 8 January 2025

Published online: 10 February 2025

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Interactions among insects, plants, and microorganisms are fundamental to ecosystem dynamics, with floral nectar and pollen serving as key resources for various organisms. Yeasts, such as *Metschnikowia reukaufii*, commonly found in nectar, influence nectarial attraction through volatile compounds (VOCs), yet the underlying biological mechanisms remain elusive. Here, we show that isoamyl alcohol, a prominent yeast VOC, attracts oriental armyworm moths (*Mythimna separata)* to pollen-rich, yeast-fermented nectar. In a series of electrophysiological and behavioral assays, we show that isoamyl alcohol activates a single class of highly specific olfactory sensory neurons expressing the olfactory receptor MsepOR8. In the moth antennal lobe, these neurons target the AM2 glomerulus, which responds to isoamyl alcohol. Genetic disruption of MsepOR8 leads to complete abolition of both physiological and behavioral responses to isoamyl alcohol, resulting in an impaired ability to locate nectar sources. Moreover, we show that isoamyl alcohol-induced foraging behavior fosters a mutualistic relationship between yeast and moths to some extent, enhancing yeast dispersal and increasing moth reproductive success. Our results unveil a highly specific mechanism by which a yeast-derived VOC facilitates insect-yeast mutualism, providing insights into insect-microbe interactions within pollination ecosystems.

The intricate interdependence between insects and angiosperms forms a captivating symbiotic relationship crucial to both parties. Within this alliance, insects play the role of dedicated carriers, facilitating the essential transfer of pollen grains among flowering plants. This process initiates the vital cycle of fertilization, leading to the production of seeds. For the majority of angiosperms relying on insect pollination, investing in elaborate floral manifestations and nutrient-rich rewards to attract their insect counterparts is essential[1,2].

At the heart of this alliance lies the pivotal role of reproduction, with pollen serving as the currency for plant propagation. While pollen

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is a vital food resource for many flower-visiting insects, its collection and consumption exact a toll on plants, depleting their energy and reproductive potential with each harvested grain[3–5]. In contrast, floral nectar is a more convenient and appealing reward for both parties, providing sugars (mainly sucrose, glucose, and fructose) for the pollinators[6,7]. However, floral nectar is susceptible to microbial colonization, primarily by yeasts and bacteria, which can alter its characteristics[8–13]. The proliferation of floral microorganisms is influenced by the presence of pollen in nectar, which are ubiquitous among field floral nectar[14,15], and pollen's nutrient substances promote microbial growth[16–18]. These microorganisms, with their strong metabolism, affect the pH, content, composition, and temperature of the nectar, potentially influencing the flower-visiting behavior of insects[19–23].

To optimize pollination efficiency, flowering plants have evolved multiple strategies to attract and engage pollinators[24–27]. Beyond visual cues, many plant species emit complex blends of VOCs, serving as potent olfactory signals to attract both pollinators and seed dispersers, thereby enhancing reproductive success[28,29]. Insects detect these VOCs using specialized sensilla on their antennae[30]. In moth species, among these sensilla are trichodea, basiconica, and coeloconica types, with sensilla containing different types of olfactory sensory neurons that play crucial roles in detecting these signals[31–34]. The precise composition, quantity, and context of these VOCs intricately shape the behavioral responses of pollinators and are tailored to attract specific partners and ensure effective pollination[35]. In addition, floral microorganisms emerge as significant modulators within this intricate ecosystem, influencing plant and floral odors by contributing their own emissions or by modifying plant volatiles[36]. For instance, previous studies have shown that VOCs produced by floral microorganisms can significantly impact plant–pollinator interactions[19,37,38]. However, the underlying biological mechanisms remain largely unexplored.

Among the microorganisms commonly found in floral nectar, yeasts, especially *Metschnikowia reukaufii*, are prevalent[15,39]. These yeasts exhibit robust growth in pollen-rich nectar and rapidly release a multitude of VOCs[16,17]. Interestingly, yeast VOCs share similarities with typical floral volatile signals[40]. Some of the yeast VOCs are also known to attract common Lepidoptera, such as the oriental armyworm moth, *Mythimna separata*[40,41]. *M. separata* moths are documented as pollen carriers for over 60 plant species, with the highest concentrations of pollen found on their proboscises[42–44]. As long-distance migratory insects, *M. separata* moths can transport pollen over extended distances. Although their effectiveness as pollinators may vary depending on the plant species and environmental conditions, their role as significant pollen carriers is well-established[42].

In this work, we identify isoamyl alcohol as a significant, yeast-produced scent molecule that attracts moths to pollen-rich, yeast-fermented nectar. We pinpoint a specific type of olfactory sensory neuron expressing the olfactory receptor MsepOR8, which responds specifically to isoamyl alcohol. Genetic disruption of MsepOR8 disrupts moth reaction to isoamyl alcohol, confirming its vital role in attracting these moths to yeast-fermented nectar. Additionally, we find that isoamyl alcohol increases yeast dispersal. The presence of yeast also boosts moth reproduction by encouraging the moths to seek out pollen-rich nectar, which in turn boosts their fecundity. These results underscore how microorganisms like yeasts fostering mutualistic relationships between yeast and insects. Understanding how yeast scents aid in the mutualistic relationship between yeast and insects broadens our understanding of ecosystem dynamics and emphasizes the importance of considering microbial partners in insect-microbe interactions.

Results

The role of yeast in enhancing moth attraction to pollen-rich nectar

To test the contribution of yeast-associated odors in aiding moths to efficiently locate pollen-rich nectar, we conducted experiments in a wind tunnel (Fig. 1a), observing the behavioral responses of female and male oriental armyworm moths to various synthetic nectar solutions, including nectar (NEC), yeast-fermented nectar (NEC + Y), pollen-rich nectar (NEC + P), and pollen-rich, yeast-fermented nectar (NEC + Y + P). The synthetic nectar used in our experiments was formulated according to the method described by ref. 45, which mimics the main components of natural floral nectars, predominantly consisting of sugars such as sucrose, fructose, and glucose[6]. This composition was designed to closely replicate the key ingredients found in natural nectar.

To validate the feasibility of our synthetic nectar, we analyzed the volatile organic compounds (VOCs) in both natural nectar (from Chinese rose, a known flower visited by *M. separata*) and synthetic nectar. The VOC profiles of the two nectars were nearly identical (Fig. S1), supporting the use of synthetic nectar as an effective substitute for natural floral nectar in our studies. Our results revealed that both female and male moths displayed a clear increase in attraction when navigating toward nectar solutions enriched with a combination of pollen and yeast. Conversely, nectar solutions lacking yeast failed to elicit any significant attraction, irrespective of pollen being present or not (Fig. 1b, c). Subsequently, we explored whether the attractiveness of pollen-rich, yeast-fermented nectar was influenced by the type of pollen present in the nectar. Comparing rape, sunflower, and maize pollen, we could show that the presence of yeast significantly enhanced the moths' ability to locate pollen-rich nectar, regardless of pollen type (Fig. 1b, c). These findings suggest a potential interaction between yeast and pollen-enriched nectar, where yeast-derived compounds within the nectar facilitate the moths' detection of pollen-enriched nectar-containing yeast. Next, we wanted to identify the chemical cues involved in this interaction.

Identification of attractive odors in pollen-rich yeast-fermented nectar

To identify odors within pollen-rich yeast-fermented nectar that may attract moths, we conducted a chemical analysis of VOCs emitted from various nectar solutions (NEC, NEC + Y, NEC + P, NEC + Y + P). We found that pollen-rich nectar-containing yeast emits two main additional compounds, ethanol (EtOH) and isoamyl alcohol, and one minor additional compound, 2-methyl-1-propanol (Fig. 2b, c), compared to nectar devoid of yeast and/or pollen. To assess the electrophysiological effects of these compounds, we performed gas chromatography-coupled electroantennographic (GC-EAG) analysis on moth antennae. Isoamyl alcohol elicited significant responses, whereas EtOH produced only minimal responses, and the 2-methyl-1-propanol did not provoke any notable activity (Fig. 2c). This led us to focus further on isoamyl alcohol and EtOH. Next, we investigated the effects of varying initial yeast densities, pollen concentrations, different pollen types and fermentation times and temperature on the emission of EtOH and isoamyl alcohol. Our findings indicate the emission of EtOH and isoamyl alcohol from pollen-rich nectar-containing yeast gradually increased with fermentation time and pollen concentration, while it remained unaffected by the initial density of yeast, pollen type or fermentation temperature (Fig. S2). We then quantified the total concentration of isoamyl alcohol and EtOH in pollen-rich nectar with yeast. Considering the significant emission of VOCs within a 24-h timeframe, we selected NEC + Y+Rape P (nectar + yeast 100/μL + pollen 2 μg/μL) fermented for one day for the analysis. We determined the concentrations of isoamyl alcohol and EtOH using standard curves of concentration and volatility (Fig. S3a, b), mimicking the natural release from NEC + Y + P to conduct further assays.

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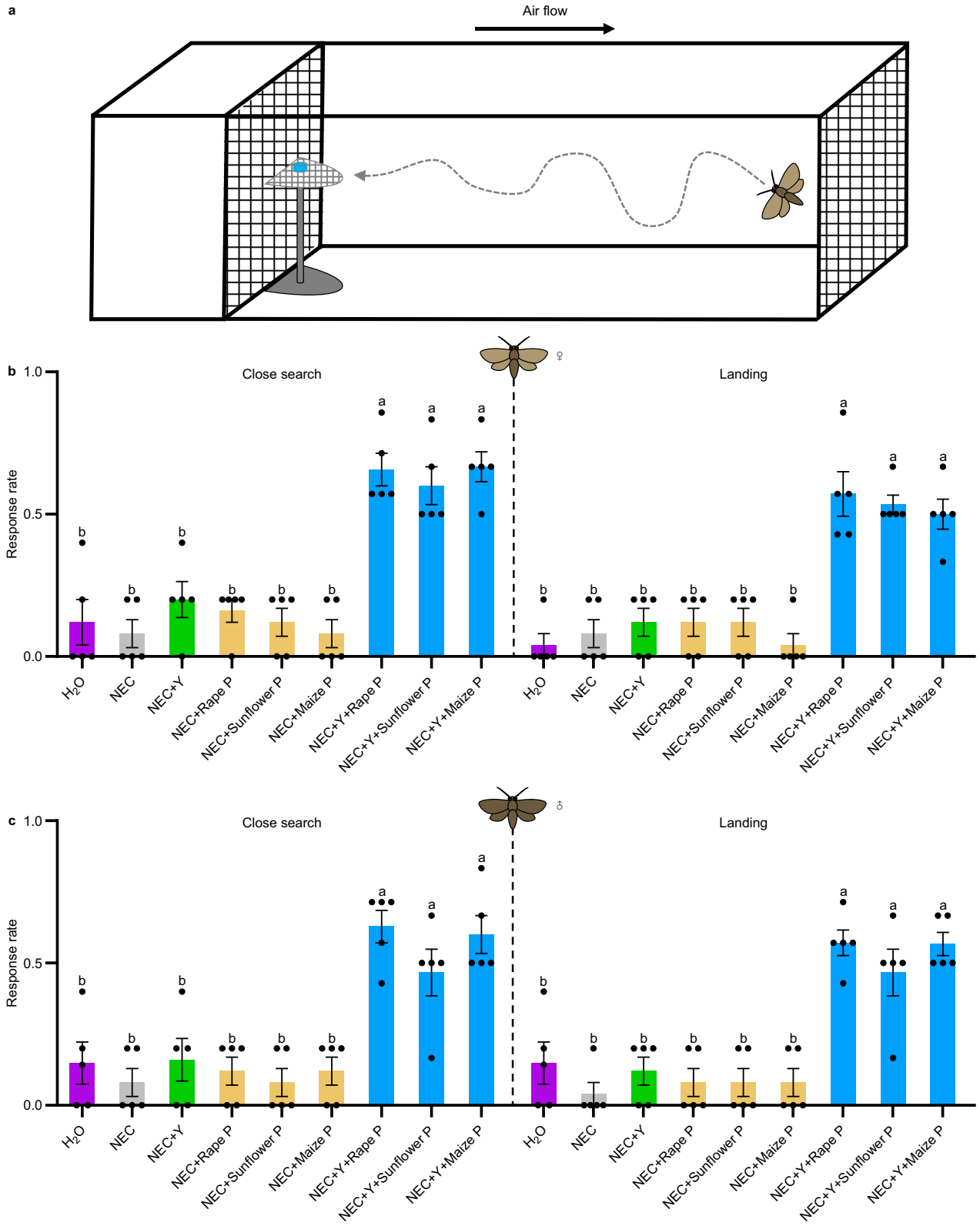

Fig. 1 | Attraction of oriental armyworm moths to pollen-rich, yeast-fermented nectar. a Schematic drawing of the wind tunnel. **b** Female oriental armyworm moth behavioral responses to various stimuli: water (H_2O), pure nectar (NEC), yeast-fermented nectar (NEC + Y), pollen-rich nectar (NEC+Rape P, NEC+Sunflower P, NEC+Maize P), and pollen-rich yeast-fermented nectar (NEC + Y+Rape P, NEC + Y+Sunflower P, NEC + Y+Maize P). $n = 5$ replicates, each replicate consisted of 5–8 individuals. $p = 2.825 \times 10^{-11}$ in close search and $p = 3.369 \times 10^{-11}$ in landing, one-way ANOVA followed by Tukey's test. Error bars represent the standard error of the mean (SEM). **c** Male oriental armyworm moth behavioral responses to the same stimuli as described in (**b**). $n = 5$ replicates, each replicate consisted of 5–7 individuals. $p = 8.776 \times 10^{-9}$ in close search and $p = 4.294 \times 10^{-10}$ in landing, statistical analysis and error bars were performed as mentioned. Source data are provided as a Source Data file.

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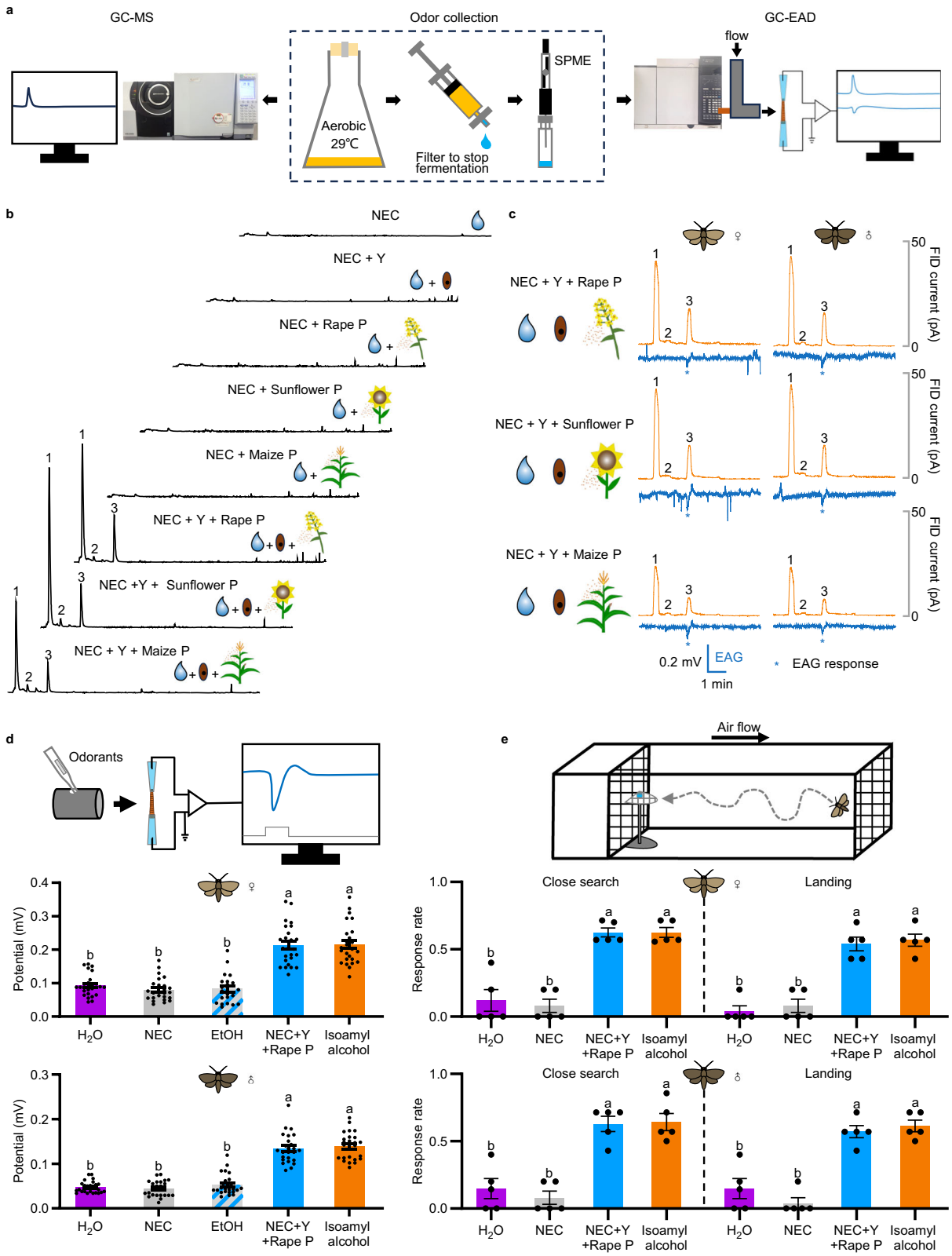

Having access to the chemical information, we proceeded to investigate the electrophysiological and behavioral effects of isoamyl alcohol and EtOH compounds. By measuring the electroantennographic (EAG) responses from moth antennae, we observed that only isoamyl alcohol elicited significant and dose-dependent responses, while stimulation with EtOH elicited no activity (Fig. 2d and Fig. S3c, d). To study potential behavioral activity, we conducted wind tunnel experiments, where we found that isoamyl alcohol attracted moths as strongly as NEC + Y+Rape P (Fig. 2e). Additionally, we explored whether isoamyl alcohol could enhance moth attraction within natural floral volatile bouquets, we conducted additional behavioral experiments using the floral volatile component, phenylacetaldehyde (PAA)[46], which is known to attract numerous species of Lepidoptera. Our results demonstrate that isoamyl alcohol significantly enhances

https://doi.org/10.1038/s41467-025-56354-3

Fig. 2 | Isoamyl alcohol is the principal attractant for oriental armyworm moth in pollen-rich, yeast-fermented nectar. a Schematic drawing of odor collection by solid-phase microextraction (SPME) for gas chromatography–mass spectrometry (GC–MS) and gas chromatography-electroantennographic detection (GC-EAD). **b** Gas chromatograms showing the composition of volatiles emanating from different nectar sources: pure nectar (NEC), yeast-fermented nectar (NEC + Y), pollen-rich nectar (NEC+Rape P, NEC+Sunflower P, NEC+Maize P), and pollen-rich yeast-fermented nectar (NEC + Y+Rape P, NEC + Y+Sunflower P, NEC + Y+Maize P). **c** Electroantennographic responses of female and male oriental armyworm moth antennae to different pollen-rich, yeast-fermented nectars, including NEC + Y+Rape P, NEC + Y+Sunflower P, NEC + Y+Maize P, linked with gas chromatography. Flame Ionization Detector (FID). **d** Electroantennogram responses to isoamyl alcohol and volatile organic compounds (VOCs) from NEC + Y+Rape P. $n = 25$ antennae (one antenna was used from each moth). $p = 1.121 \times 10^{-26}$ in females and $p = 8.683 \times 10^{-37}$ in males, one-way ANOVA followed by Tukey's test. Error bars represent the standard error of the mean (SEM). **e** Behavioral responses of oriental armyworm moths to different stimulus in a wind tunnel including H_2O, NEC, isoamyl alcohol and VOCs from NEC + Y+Rape P. $n = 5$ replicates, each replicate consisted of 5–8 individuals, $p = 4.537 \times 10^{-7}$ in close search and $p = 1.713 \times 10^{-7}$ in landing in females, $p = 4.0 \times 10^{-6}$ in close search and $p = 6.688 \times 10^{-7}$ in landing in males, statistical analysis and error bars were performed as mentioned. In all experiments, EtOH (ethanol) and isoamyl alcohol were used at concentrations consistent with those found in NEC + Y+Rape P, with 1.1% (v/v) and 0.00044% (v/v), respectively. Numbers at the peaks in panels **b** and **c** denote (1) Ethanol (EtOH); (2) 2-methyl-1-propanol; (3) isoamyl alcohol. Source data are provided as a Source Data file.

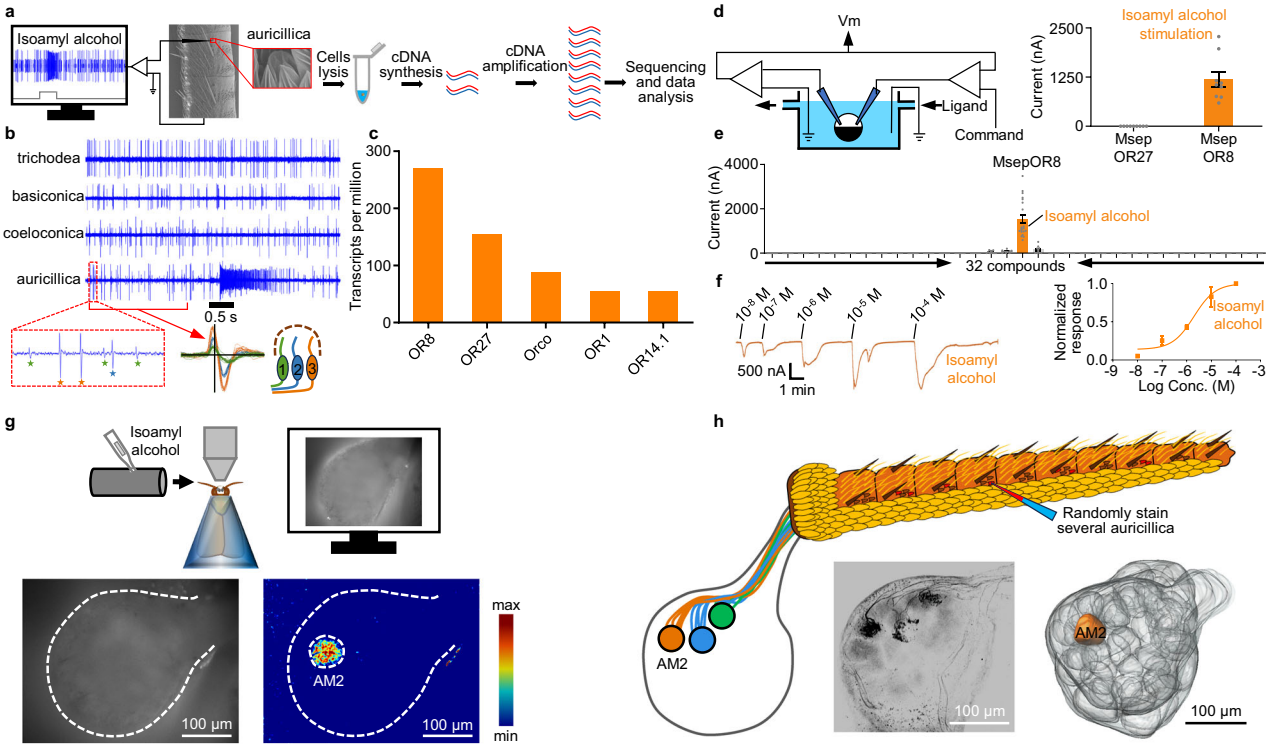

Fig. 3 | Isoamyl alcohol activates a single type of olfactory sensory neuron expressing MsepOR8. a Schematic drawing of single-sensillum recording and sequencing. **b** Single-sensillum recordings showing three neurons housed in sensilla auricillica, with the 3rd neuron (characterized by the largest spikes) responding to isoamyl alcohol. **c** Single-sensillum sequencing reveals the expression of four MsepORs plus Orco in cells under auricillica sensilla. **d** Electrophysiological responses of oocytes expressing MsepOR8/MsepOrco and MsepOR27/MsepOrco to a 10^{-4} M concentration of isoamyl alcohol revealing MsepOR8 as the isoamyl alcohol-detecting receptor. **e** Electrophysiological responses of oocytes expressing MsepOR8/MsepOrco to 32 odorants at a 10^{-4} M concentration showing the high specificity of the interaction between MsepOR8 and isoamyl alcohol. **f** Dose response of MsepOR8/MsepOrco-expressing oocytes to isoamyl alcohol. EC50 = 1.74×10^{-6} M. **g** Calcium imaging recordings from the antennal lobe showing specific isoamyl alcohol-induced calcium responses in the AM2 glomerulus. The white and black figure is the transmission image of the same field, and the white dashed line in the image represents the recorded field of the antennal lobe. $n = 4$. **h** Neural tracing reveals three innervated glomeruli out of which one is the AM2 glomerulus. $n = 3$. In **d**–**f**, replicate numbers are indicated ($n = 9$ for (**d**), $n = 7$–20 for (**e**), $n = 3$–6 for (**f**)), and error bars represent the standard error of the mean (SEM). Source data are provided as a Source Data file.

the attraction of oriental armyworm moths when combined with PAA compared to PAA alone (Fig. S4). This suggests that isoamyl alcohol acts synergistically with other floral volatiles, further enhancing moth attraction to natural floral scents. Collectively, these results confirm that isoamyl alcohol is a key attractant in pollen-rich yeast-fermented nectar and likely plays a central role in moth foraging behavior in natural settings.

Identification of olfactory sensory neurons responding to isoamyl alcohol

Olfactory sensory neurons (OSNs) expressing distinct olfactory receptors (ORs) are housed within specialized olfactory sensilla on the antenna of the moth. To determine the sensillum type(s) housing neurons detecting isoamyl alcohol, we conducted single-sensillum recordings (SSRs) from four types of moth's sensilla (trichodea, basiconica, coeloconica, and auricillica), while stimulating with isoamyl alcohol. Only OSNs present in sensilla auricillica, discreetly positioned at the border of the sensillum and scale regions of each segment, exhibited robust responses to isoamyl alcohol stimulation (Fig. 3a, b and Fig. S5a–c). These sensilla contained three OSNs, distinguished by their spike amplitudes, with the neuron displaying the largest spike amplitude being responsive to isoamyl alcohol (Fig. 3b). Using precise dissection techniques, we isolated sensilla auricillica and collected cells around the base region, thereby capturing the cell bodies of the OSNs present in sensillum. Transcriptome sequencing using Smart-seq2 revealed distinct expression of four OR and one olfactory

receptor co-receptor (Orco) transcripts, with OR8 and OR27 exhibiting the highest expression levels (Fig. 3c), leading us to focus on MsepOR8 and MsepOR27. Functional validation through heterologous expression together with the moth Orco in the *Xenopus* oocyte system demonstrated that the expression of MsepOR8/MsepOrco resulted in dose-dependent and sensitive responses to isoamyl alcohol, while expression of MsepOR27/MsepOrco did not (Fig. 3d, f). Additional screening of 31 volatiles (Supplementary Data 1), well-known as various plant flower-derived odors and isoamyl alcohol analogs[46–48] revealed a high degree of specificity of MsepOR8/MsepOrco to isoamyl alcohol, with no other odors eliciting a response (Fig. 3e).

Next, we wanted to establish the response pattern to isoamyl alcohol among the olfactory glomeruli present in the moth primary olfactory center, the antennal lobe. To achieve this goal, we employed functional imaging of calcium dynamics. After stimulation of the antenna with isoamyl alcohol we could observe one single and specifically responding glomerulus, denoted as the AM2 glomerulus, displaying a robust increase in calcium concentration (Fig. 3g and Fig. S5d). This observation was further supported by the backfilling of OSNs present in eight single sensilla auricillica using micro-ruby to trace their innervation patterns into the antennal lobe followed by three-dimensional reconstruction (Fig. 3h). In all eight auricillica, three OSNs were stained and were shown to target three separate glomeruli in the antennal lobe, out of which one was the AM2 glomerulus. In summary, our experiments strongly indicate that isoamyl alcohol activates a single class of OSNs present in sensilla auricillica, expressing the olfactory receptor MsepOR8 and innervating the AM2 glomerulus.

Validation of MsepOR8 as the key odorant receptor detecting isoamyl alcohol

To verify the pivotal role of MsepOR8 as the primary odorant receptor governing the response to isoamyl alcohol, we employed CRISPR-Cas9 genome editing to disrupt the gene encoding MsepOR8. Utilizing a single-guide RNA (sgRNA) targeting the first exon of the MsepOR8 gene, we induced a 364-base pair (bp) deletion, resulting in a truncated MsepOR8 protein (Fig. S6). Subsequent examinations of the electrophysiological and behavioral responses of oriental armyworm moths to isoamyl alcohol validated the significance of MsepOR8 in mediating these responses. Electroantennographic (EAG) recordings revealed a marked reduction in the response to isoamyl alcohol in the MsepOR8$^{-/-}$ line compared to the wildtype (Fig. 4a and Fig. S7a–c). Importantly, when stimulated with the sex pheromone Z11-16: Ald, which is not a ligand of MsepOR8, the MsepOR8$^{-/-}$ line still exhibited a strong response to Z11-16: Ald as that of the wildtype, indicating the absence of any phenotypical off-target effects of the CRISPR-Cas9 manipulations (Fig. S7d). Additionally, a search for potential off-target binding sites using bioinformatics tools revealed no such sites at other gene regions, demonstrating the high specificity of this sgRNA. Subsequent single-sensillum recordings (SSR) and functional imaging tests again affirmed the complete loss of response to isoamyl alcohol or VOCs released from pollen-rich, yeast-fermented nectar in the mutant line relative to the wildtype (Fig. 4b, c and Fig. S7e–h). Finally, wind tunnel experiments also revealed a complete loss of attraction to isoamyl alcohol and to VOCs emanating from pollen-rich, yeast-fermented nectar in the MsepOR8$^{-/-}$ line compared to the wildtype (Fig. 4d and Fig. S8). Collectively, our results from the CRISPR-Cas9-based experiments demonstrate that MsepOR8 is an indispensable and specific receptor governing the attractive response to isoamyl alcohol in oriental armyworm moths.

Impact of isoamyl alcohol-induced foraging behavior on yeast dispersal and reproductive success in oriental armyworm moths

The behavior of insects visiting flowers for foraging inevitably leads to the dissemination of pollen. The visitation rate of insects, to a large extent, reflects the efficiency of pollen dissemination[1,49]. To assess this, we conducted an I_2-KI stain assay (Fig. S9a–c) to evaluate the viability of pollen on the proboscis of moths. Our results indicate that some of the pollen adhering to the moths' proboscis remained viable. Additionally, we observed that the presence of yeast did not induce pollen rupture (Fig. S9d), consistent with findings from Rachel Vanette's team[18]. These results suggest that while yeast-moth interactions do not directly enhance pollination, the viability of pollen on the moths' proboscises implies a potential, albeit indirect, role in pollination. Next, we wanted to investigate the role of isoamyl alcohol in moth foraging behavior. We provided wild-type and MsepOR8$^{-/-}$ moths with meals containing pollen-rich, yeast-fermented nectar, mimicking the natural flower-visiting system. We observed that wild-type moths exhibited a significantly higher visitation rate to the meals compared to the MsepOR8$^{-/-}$ line (Fig. 5a and Fig. S10a), suggesting that isoamyl alcohol-induced foraging behavior may enhance pollen uptake in moths.

We then wanted to know whether isoamyl alcohol-induced pollination behavior in moths could facilitate yeast dispersal. By comparing the efficiency of yeast dispersal between moths initially feeding on pure nectar and those on pollen-rich, yeast-fermented nectar, we observed that moths feeding on pollen-rich, yeast-fermented nectar could carry yeasts from the yeast-containing nectar and transport them to other nectar sources, even after long flight periods (Fig. S10b, c). In the next step, we fed wild-type and MsepOR8$^{-/-}$ moths with pollen-rich, yeast-fermented nectar and compared their transmission abilities. Our results showed that wild-type moths exhibited a greater extent of yeast dispersal compared to the MsepOR8$^{-/-}$ line (Fig. 5b and Fig. S10d), suggesting that isoamyl alcohol-induced moth foraging behavior facilitates yeast dispersal.

Considering the nutritional benefits added by pollen to nectar sources and the positive impact of pollen on insect fertility[4,17,50–55], we evaluated the effects of different nectar formulations (NEC, NEC + Y, NEC + P, NEC + Y + P) on the fecundity of mature moth. Through close monitoring of oviposition patterns among cohorts provided with these varying nectar compositions (Fig. 5c), we observed a significant increase in egg deposition in moths consuming nectar enriched with pollen compared to those fed pure nectar (Fig. 5c). To rule out the possibility that the positive impact on insect fertility was due to isoamyl alcohol enhancing nectar consumption, we conducted a direct comparison experiment, where wild-type moths were provided with either nectar (NEC) alone or isoamyl alcohol-supplemented nectar. The results showed no significant increase in nectar intake with the addition of isoamyl alcohol (Fig. S11), suggesting that isoamyl alcohol primarily aids in locating nectar sources rather than directly enhancing consumption. These findings highlight the crucial role of pollen as a nutritional determinant in the reproductive processes of nectar-feeding moths, while at the same showing that yeast supplementation is not necessary for reproduction overall.

Discussion

The intricate relationship between insects and flowering plants is pivotal for the reproduction of plants and for providing energy and nutrients to insects. This interaction relies heavily on sensory mechanisms that facilitate the interplay between the two parties. Floral nectar, a crucial reward for visiting insects, is often subject to microbial colonization, with yeasts notably impacting its composition and characteristics[36]. However, the underlying molecular mechanisms of the influence of yeast-derived VOCs on insect behavior has remained elusive.

Here, we provide compelling evidence that isoamyl alcohol, a prominent VOC produced by yeasts, plays a significant role in attracting oriental armyworm moths to pollen-rich, yeast-fermented nectar. Moreover, we reveal the molecular mechanisms underlying this phenomenon, identifying a specific olfactory receptor, MsepOR8,

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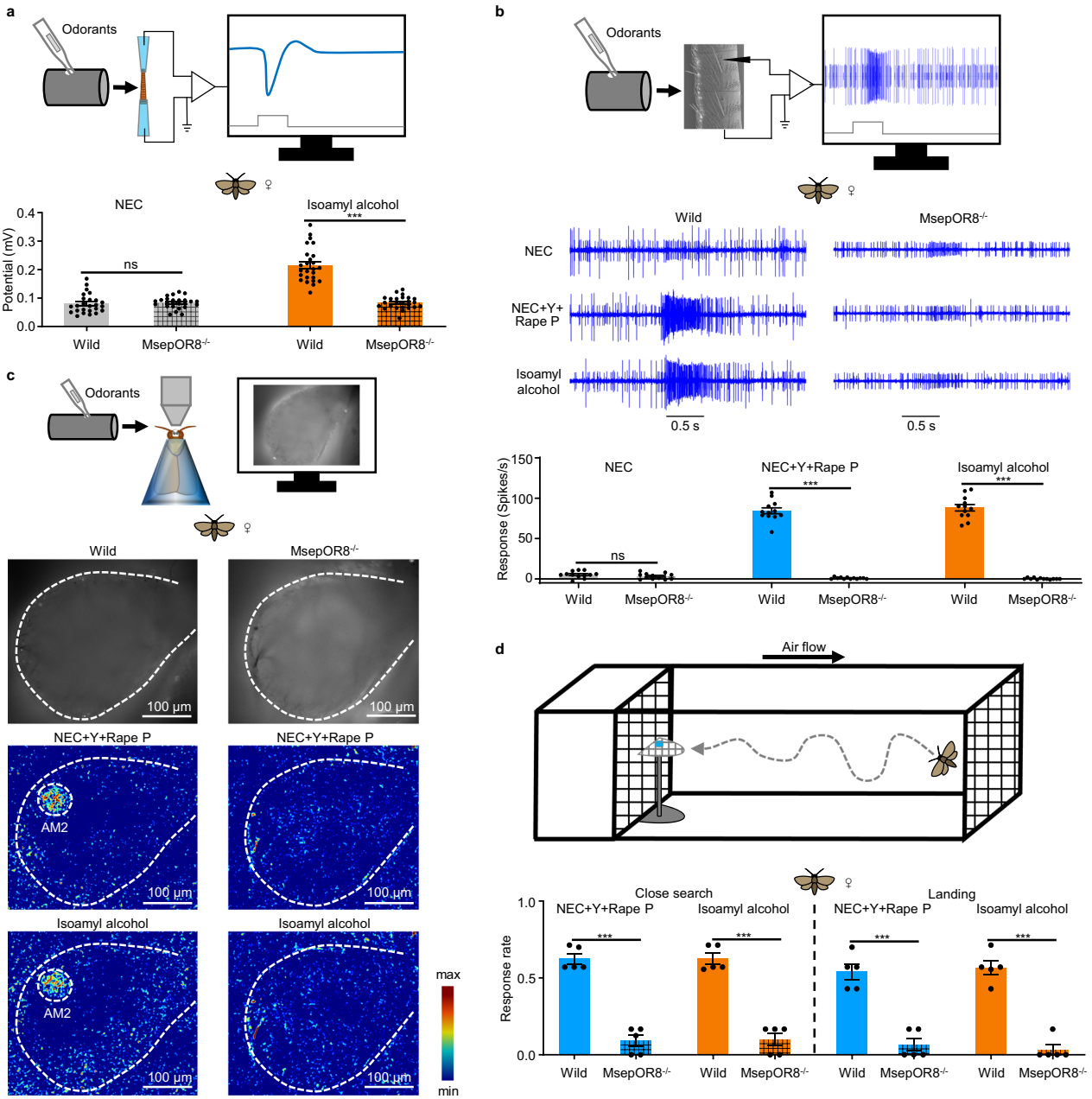

Fig. 4 | MsepOR8 is essential for the response of oriental armyworm moth to isoamyl alcohol. a Electroantennographic (EAG) response comparison between wild-type (WT) and MsepOR8 knockout (MsepOR8$^{-/-}$) oriental armyworm moths to isoamyl alcohol. $n = 25$, $p = 0.684$ in NEC (pure nectar) stimuli and $p = 2.662 \times 10^{-11}$ in isoamyl alcohol stimuli. **b** Single-sensillum recording (SSR) responses from sensilla auricillica in WT and MsepOR8$^{-/-}$ oriental armyworm moths to NEC + Y+Rape P (rape pollen-rich yeast-fermented nectar) and isoamyl alcohol. $n = 12$, $p = 0.225$ in NEC stimuli, $p = 1.292 \times 10^{-10}$ in NEC + Y+Rape P stimuli and $p = 2.040 \times 10^{-10}$ in isoamyl alcohol stimuli. **c** Calcium responses among antennal lobe glomeruli of WT and MsepOR8$^{-/-}$ oriental armyworm moths to NEC + Y+Rape P and isoamyl alcohol. The white and black figure is the transmission image of the same field, and the white dashed line in the image represents the recorded field of the antennal lobe. $n = 3$. **d** Behavioral response comparison between WT and MsepOR8$^{-/-}$ oriental armyworm moths to NEC + Y+Rape P and isoamyl alcohol. $n = 5$ replicates, each containing 5–8 individuals, in close search, $p = 6.0 \times 10^{-6}$ in NEC + Y+Rape P stimuli and $p = 1.2 \times 10^{-5}$ in isoamyl alcohol stimuli, in landing, $p = 9.0 \times 10^{-5}$ in NEC + Y+Rape P stimuli and $p = 2.1 \times 10^{-5}$ in isoamyl alcohol stimuli. Statistical analyses in panels **a**, **b**, and **d** were performed using two-sided *t*-tests. Error bars represent the standard error of the mean (SEM). Source data are provided as a Source Data file.

responsible for the moth's response to isoamyl alcohol. Genetic disruption of MsepOR8 resulted in a complete loss of physiological and behavioral responses to isoamyl alcohol, confirming its indispensable role in the moth's ability to locate pollen-rich, yeast-fermented nectar sources. Previous studies have indicated that isoamyl alcohol is a common compound attracting many Lepidoptera species in the field[40], especially the noctuidae species[56]. This fact raises the question if the dedicated olfactory pathway is conserved in other nectar-feeding moths. Our phylogenetic analysis of MsepOR8 among related lepidopterans shows that MsepOR8 is primarily conserved among nectar-feeding moths (Fig. S12), suggesting that this receptor may play a specialized role in nectar foraging. However, further functional studies are required to test this hypothesis and enhance our understanding of the evolutionary mechanisms shaping such systems in moths. In other insects, this kind of "ecologically labeled lines" through the olfactory system are mainly known from sex pheromone communication systems in different insects, where the male typically possesses extremely specific and sensitive olfactory sensory neurons

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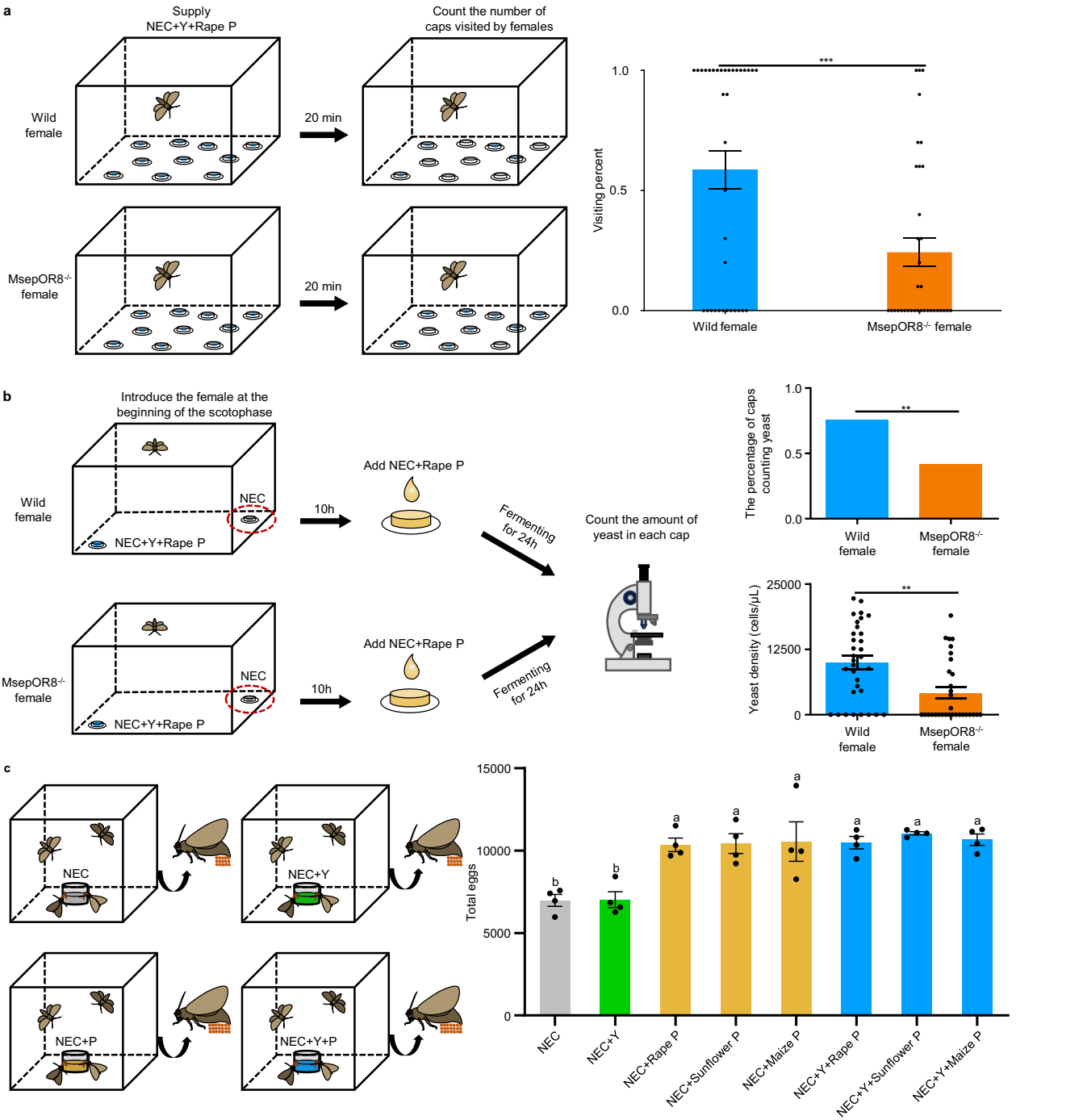

Fig. 5 | Isoamyl alcohol-mediated foraging behavior promotes the dispersal of yeast and augments the reproduction of oriental armyworm moth. a Female MsepOR8$^{-/-}$ oriental armyworm moth exhibit a significantly reduced visiting rate to rape pollen-rich yeast-fermented nectar (NEC + Y+Rape P) compared to wild-type individuals. $n = 35$ replicates in both wild-type group and MsepOR8$^{-/-}$ group, $p = 9.76 \times 10^{-4}$, statistical analyses were performed using a two-sided t-test. Error bars represent the standard error of the mean (SEM). **b** Female MsepOR8$^{-/-}$ oriental armyworm moth exhibit significantly reduced capacity to disseminate yeasts from NEC + Y+Rape P compared to wild-type individuals. $n = 33$ replicates in the wild-type group and $n = 31$ replicates in the MsepOR8$^{-/-}$ group, $p = 0.0025$ and analyzed using the Chi-square test in the above column chart, $p = 0.00105$ and analyzed using the two-sided t-test in the below column chart. Error bars represent the standard error of the mean (SEM). **c** Pollen significantly augments the fecundity of female oriental armyworm moth. $n = 4$ replicates per treatment, and each replicate comprised a cohort of newly emerged moths consisting of 10 females and 12 males. $p = 2.8 \times 10^{-5}$, one-way ANOVA followed by Tukey's test. Error bars represent the standard error of the mean (SEM). Source data are provided as a Source Data file.

(OSNs) to detect the female pheromone[57,58]. However, also in other parts of the olfactory system similar lines have been identified for other, ecologically highly important odors. In *Drosophila melanogaster*, information about geosmin, an odor signifying the presence of harmful microbes, is detected and processed in such a labeled line[59]. The same goes for a system detecting the pheromone of an enemy parasitoid[60]. Food odors are usually mixtures, and insects sense some of them to assess the fitness of food, like the fruit ripeness[61]. Food odors are typically detected by less specific OSNs, allowing ample cross-fiber coding in the system[62–64]. In the oriental armyworm moth, and likely in many other moths, evolution has, however, equipped these insects with an ecologically labeled line detecting and processing

information regarding isoamyl alcohol, which points to its very high importance in the life history of these moths. The function of isoamyl alcohol as a general, but highly important attractant also opens possibilities for its use in moth management strategies. In consideration of isoamyl alcohol attracting both sexes of oriental armyworm moths, it may be a potential mate-finding cue for insects, like the plant volatiles[65].

An interesting aspect of the isoamyl alcohol detection mechanism is that OSNs responsible for detecting the compound are located in sensilla auricillica. These sensilla are, in general, more scarce than other sensillum types on the moth antenna and have, in general, been less investigated. In the herald moth (*Scoliopteryx libatrix*), SSR recordings revealed responses to a number of plant-related compounds[66]. While in the codling moth (*Cydia pomonella*), one of the auricillica-associated OSNs was clearly tuned to the female sex pheromone, the other OSNs responded to plant-associated volatiles[67]. Isoamyl alcohol was, however, not included among the stimuli tested in any of these studies.

Flower nectar is primarily composed of sugars such as sucrose, fructose, and glucose, creating a highly imbalanced nutritional solution with a high osmotic pressure and carbon-to-nitrogen ratio, resembling a carbohydrate-based aqueous solution[66]. However, the presence of pollen and microorganisms can significantly alter its composition, thus balancing the nutritional content of nectar and providing a more optimal food source for visiting insects[17,19]. Our study reveals that the consumption of pollen-rich nectar notably augments moth fecundity compared to reproductive levels sustained by pure nectar lacking pollen. This fact highlights that pollen has a vital, though not necessary role in moth reproduction.

The proliferation of floral yeast within the nectar depends heavily on the presence of pollen. Pollen releases amino acids into the solution, stimulating yeast growth[16,17]. However, when yeast growth saturates, chemicals detrimental to floral ecosystems might be produced. Additionally, the yeast itself may struggle to derive sufficient nutrition from the nectar system[68]. In light of this ecological context, the moth foraging behavior elicited by the odor of yeast, isoamyl alcohol, potentially offers a solution by facilitating the transmission of yeast to new, unexploited nectar sources. The moth thus provides the yeast with mobility. These findings emphasize the intricate interplay between microbes and insects, and exemplify the importance of deeper understanding when striving to preserve ecosystem resilience and biodiversity.

In our foraging experiments, we show that isoamyl alcohol-induced foraging behavior may enhance pollen uptake in moths. However, we currently lack direct evidence to show that yeast-derived volatiles benefit plants by facilitating pollination. Due to current limitations, we are unable to conduct semi-field or field pollination experiments, which might be an interesting direction for future work.

The ability of insects to detect and respond to specific, yeast-derived compounds like isoamyl alcohol thus suggests a fascinating co-evolutionary process between plants, yeasts, and insects[40,69]. This interplay may have been instrumental in shaping pollination strategies and foraging behaviors over evolutionary timescales. Plants, in association with yeasts, have evolved to produce compounds that attract specific pollinators, potentially optimizing their reproductive success[37,49]. Concurrently, insects may have developed sensory mechanisms to efficiently locate these yeast-modified floral resources, enhancing their foraging efficiency and ensuring access to vital nutrients[70]. This co-evolutionary relationship likely represents a finely tuned adaptation, showcasing the intricate interdependence of different organisms within ecosystems. Therefore, our study also paves the way for future investigations into several intriguing aspects of insect-plant interactions and the role of yeast-derived compounds. For example, exploring other yeast-derived compounds beyond isoamyl alcohol could provide a more comprehensive understanding of the chemical language involved in guiding insect behavior. Further research could also delve into the broader spectrum of insect-plant-microbe interactions affected by VOCs, considering different plant, microbe, and insect species.

Methods

Insects

Mythimna separata larvae were collected from corn-cultivated fields in Xinxiang, Henan Province, China. The larvae were reared on an artificial diet under controlled conditions: a 14-h light and 10-h dark photoperiod, temperature maintained at 25 ± 1 °C, and relative humidity at 50 ± 10%. Upon pupation in soil, larvae were left undisturbed until pupa emergence. The moths were provided with synthetic nectar containing 3% sucrose, 6% glucose, and 6% fructose (w/v). The synthetic nectar was formulated according to the method described by Rering et al.[45], replicating the primary sugar composition found in natural floral nectar[6]. This synthetic nectar was also used in our experiments.

Yeast fermentation assays

Metschnikowia reukaufii yeasts were obtained from the China Center of Industrial Culture Collection (Beijing, China), while pollen (Rape, Sunflower, and Maize) were purchased from Xinzhou Wutaishan Bee Industry Co., Ltd. (Shanxi Province, China). Pollen were sterilized with ultraviolet light for 30 min in a super clean bench, and synthetic nectar was filtered through a 0.22-μm filter and exposed to ultraviolet light for 1 h. All vessels underwent autoclaving before utilization. Fermented solutions were prepared in conical flasks equipped with sand core silicone foam stoppers to ensure sufficient oxygen availability for the yeasts. These solutions were composed of various initial densities of yeasts (10, 100, and 1000 cells/μL) and pollen (0.00, 0.02, 0.20, and 2.00 μg/μL) in synthetic nectar. The solutions were incubated at 29 °C for 12, 24, 48, 72, and 96 h. After incubation, the solutions were sterilized by filtration through a 0.22 μm filter, exposed to ultraviolet light for 1 h, and then stored at −20 °C. For the experiments, a yeast density of 100/μL and a pollen density of 2 μg/μL, fermented for 24 h, were selected. Additionally, we also prepared yeast-fermented nectar with rape pollen (yeast density 100 cells/μL, rape pollen density 2.00 μg/μL) in different temperatures (20, 25, 29 °C) to compare their volatile profiles.

Wind tunnel assays

The experiments were conducted in a wind tunnel device (160 cm × 60 cm × 60 cm), maintaining a constant airspeed of ~30 cm/s, with room conditions set at 25 ± 1 °C and 40–50% relative humidity. To minimize liquid splatter resulting from the fluttering wings of moths, odor sources were enclosed within 15 mL centrifuge tube covers, which were relatively deep. Each cover was filled with 500 μL of liquid to cover the bottom and ensure consistent volatilization across different treatments. Freshly emerged moths were collected at the beginning of the photophase and were maintained without food until the completion of the wind tunnel experiments in the subsequent scotophase. At the start of scotophase, we allowed the moths approximately half an hour to adapt to the diffusive red light (0.5 lux). Subsequently, we conducted the experiments, which were completed within about 4 h. The behavioral responses of moths to lures were classified and recorded according to the following typical categories: Flight—moths took off from the release cage; Upwind—moths flew in the upwind direction and over the halfway line; Close search—moths continued flying upwind and reached about 10 cm from the odor source; Landing—moths landed on the lure to suck the liquid. During the experiments, we observed that oriental armyworm moths were highly active, and there were no differences among different liquids in the categories of "Flight" and "Upwind". Therefore, only the categories "Close search" and

"Landing" were retained for analysis. Each moth was observed only once for 4 min or until landing, and the lure was changed after each moth was tested.

Chemical analysis

VOCs were collected using solid-phase microextraction (SPME) at 25 °C. To compare the volatile profiles of the solutions used in the wind tunnel experiments, 1 mL of each liquid was added to a 20 mL headspace bottle and allowed to equilibrate for over 2 h to ensure saturation of the liquid odor volatilization. Subsequently, an SPME fiber (50/30 µm DVB/CAR/PDMS) was introduced into the bottle through the cap to collect odorants for 30 min. Following collection, the SPME fiber was retracted and immediately inserted into the inlet (250 °C) of a gas chromatography–mass spectrometry (GC–MS) system equipped with a DB-5 column. The column temperature was initially held at 40 °C for 1 min, then increased to 100 °C at a rate of 5 °C/min and maintained for 1 min, followed by a subsequent increase to 260 °C at a rate of 50 °C/min with a final stage held for 3 min at 260 °C. Chemical identification was performed by comparing mass spectra against synthetic standards and NIST 2.0 library matches. We also collected the fresh Chinese rose nectar and detected its VOCs by the same method above to compare it with the VOCs of pure nectar.

Electroantennogram (EAG) experiments

The antenna was prepared by cutting off both ends and immediately mounted with two glass capillary Ag/AgCl electrodes containing 0.1 M KCl. One capillary was connected to a grounding electrode, while the other was connected to a 10 × universal AC/DC probe (SYNTECH; Netherlands), and the signal was then transmitted to an analog/digital converter (IDAC-4, SYNTECH, Netherlands) and transferred to a computer. Odor stimuli were delivered from a glass Pasteur pipette (1 µL solution was added on a 4 mm × 30 mm filter paper) via a pulse of air (100 ml/min) into the main humidified air stream (1500 ml/min) through a 7 mm inner diameter stainless steel tube ending ~1 cm from the antenna. Each pipette was used for stimulation no more than five times. The response was recorded for 10 s, starting 1 s before the stimulation period of 0.1 s. Data were analyzed using EAGPro software, version 2.0 (SYNTECH).

Gas chromatography-electroantennographic detection (GC-EAD) experiments

The GC-EAD experiments procedures mainly merged from the above GC–MS (odor sample introduction) and EAG (antenna connection) procedures. The data was collected and analysed by GcEad 2014 v1.2.5 (SYNTECH).

Single-sensillum recording (SSR)

A moth was immobilized within a 1 ml blue pipette, with its head securely held in place using dental wax. The base of the antenna was then fixed, and the scales on the antenna were carefully removed. Next, the antenna was affixed to a wall of dental wax to aid in the insertion of a recording electrode. Extracellular signals originating from the olfactory sensory neurons (OSNs) were recorded by inserting a tungsten wire electrode connected to a 10 × universal AC/DC probe (SYNTECH; Netherlands) into the base of a sensillum, with a reference electrode inserted into the compound eye. The electrical signal was transmitted to an analog/digital converter (IDAC-4, SYNTECH, Netherlands) and transferred to a computer, where it was sampled at a rate of 10,666 samples/s and filtered (300–2000 Hz with 50/60-Hz suppression). Odor stimuli were prepared using a glass Pasteur pipette (1 µL solution was added on a 4 mm × 30 mm filter paper) to deliver a pulse of air (100 ml/min) into the main humidified air stream (1500 ml/min) via a 7 mm inner diameter stainless steel tube, positioned ~1 cm from the antenna. Each pipette was used for stimulation no more than five times. The response was recorded for a duration of 10 s, starting 3 s before the onset of the 0.5 s stimulation period. The data were analyzed and saved using AutoSpike software, version 3.9 (SYNTECH). Response values for odor stimuli were calculated as the difference between the spike number observed 1 s before the stimulus delivery point and 1 s after stimulus delivery.

Two-choice behavioral assays

Two-choice behavioral assays were conducted using a Y-tube olfactometer (glass, 20 cm stem length, 4 cm diameter, with two 25 cm long, 4 cm diameter arms positioned at a 60° angle). Clean, humidified air was delivered to the olfactometer through activated charcoal and water at a flow rate of 1.0 L/min to each arm. For each trial, 10 µL of odor solution was applied to a 10 mm × 15 mm filter paper and placed at the entrance of each arm. Each filter paper was used only once per moth. To initiate a trial, a single moth was placed at the base of the olfactometer stem, and its behavior was recorded for 3 min. Moths typically respond quickly to the odor source. Each moth was tested only once. To minimize potential contamination, the olfactometer was cleaned after every five trials or whenever excreta were observed. The positions of the odor sources were alternated every five trials to account for potential positional effects. The odor solutions used were: pure nectar (NEC), phenylacetaldehyde (PAA), and isoamyl alcohol. Freshly emerged *M. separata* moths were collected at the start of the photophase and starved for 14 h (one photophase) in glass vials. At the onset of scotophase, moths were allowed to acclimate under red light (0.5 lux) for 30 min before experiments commenced.

Single-sensillum sequencing

Antennal scales and sensilla surrounding the auricillica were initially removed. A 2-day waiting period was observed to allow for the degeneration of sensory neurons around the sensilla auricillica. Subsequently, targeted sensilla auricillica were carefully dissected using a specialized micro-scalpel, and a small cluster of cells around their base was collected. Samples were then processed using Smart-seq2 and subjected to transcriptome sequencing analyses

Receptor expression in oocytes and electrophysiological recordings

The open reading frames (ORFs) encoding MsepOR8, MsepOR27, and MsepOrco were amplified and cloned into the pT7Ts vector. Subsequently, cRNAs were synthesized using the mMESSAGE mMACHINE T7 kit (Ambion, Austin, TX). Electrophysiological recordings were conducted following previously reported protocols[71,72]. Mature healthy oocytes (stage V–VII) (Nasco, Salida, California) were treated with collagenase I (GIBCO, Carlsbad, CA) in washing buffer (96 mM NaCl, 2 mM KCl, 5 mM $MgCl_2$, and 5 mM HEPES [pH = 7.6]) for ~1 h at room temperature. After overnight incubation at 18 °C, oocytes were microinjected with 27.6 ng ORs cRNA and 27.6 ng Orco cRNA. Post-injection, oocytes were further incubated for 4–7 days at 18 °C in 1X Ringer's solution (96 mM NaCl, 2 mM KCl, 5 mM $MgCl_2$, 0.8 mM $CaCl_2$, and 5 mM HEPES [pH = 7.6]) supplemented with 5% dialyzed horse serum, 50 mg/ml tetracycline, 100 mg/ml streptomycin, and 550 mg/ml sodium pyruvate. Whole-cell currents were recorded from the injected *Xenopus* oocytes using a two-electrode voltage clamp. Odorant-induced currents were measured with an OC-725C oocyte clamp (Warner Instruments, Hamden, CT) at a holding potential of −80 mV. Data acquisition and analyses were conducted using Digidata 1440 A and pCLAMP 10.2 software (Axon Instruments Inc., Union City, CA). The tested compounds were dissolved in dimethyl sulfoxide (DMSO) to 1 M stock solutions and stored at −20 °C. Prior to testing, the stock solutions were diluted with 1 X Ringer's buffer (96 mM NaCl, 2 mM KCl, 5 mM $MgCl_2$, 0.8 mM $CaCl_2$, and 5 mM HEPES [pH = 7.6]).

Neural tracing of olfactory sensory neurons housing in the sensilla auricillica

To trace the olfactory sensory neurons housed in auricillica sensilla, the moth was carefully immobilized in a 1 ml blue pipette, with its head secured using dental wax. The base of the antenna was then fixed, and the antenna scales were removed. A wet towel was placed around the insect to maintain a high-humidity micro-environment, crucial for the staining experiment. Tungsten electrodes were used to puncture auricillica sensilla several times to facilitate the subsequent insertion of a glass pipette. A micro-ruby solution was injected into the glass pipette and inserted into each sensilla auricillica, with a staining duration of 10 min per auricillica. Following staining, the antenna was sealed with vaseline, and the insect was placed in a high-moisture dark box overnight at 4 °C. Subsequently, the brain was dissected in Ringer's saline and fixed in a freshly prepared 4% paraformaldehyde solution in phosphate-buffered saline (PBS) for 2 h at room temperature. The brains were then rinsed four times for 15 min each in PBST, dehydrated using an ascending ethanol series (50, 70, 90, 95, 100% × 3, 5 min each), cleared in xylene, and mounted in ZEISS Immersion Oil (ne = 1.518). The samples were imaged using a laser scanning confocal microscope (LSM 980 META Zeiss, Jena, Germany) equipped with a Plan-Apochromat 40×/1.2 w objective. A HeNe1 laser with a 546-nm line was used to excite the Micro-Ruby, while a 488-nm line of an argon laser was used to excite brain autofluorescence. Images were obtained with a resolution of 1024 × 1024 pixels in the xy-plane and an interslice distance of 2 μm. To identify the glomeruli responding to isoamyl alcohol, a 3D atlas of the antennal lobe glomeruli was created using Amira 5.3 (Visage Imaging, Fürth, Germany). This atlas was compared with the system nomenclature of *M. separata* antennal lobe glomeruli established in previous work, along with the results of antennal lobe calcium imaging to confirm the glomeruli names.

In vivo optical imaging of moth antennal lobes

The moth was carefully placed in a plastic tube and secured with dental wax. Subsequently, the tube was affixed in a custom-made chamber. After removing the head scales and mouthparts, a window was created in the head between the two compound eyes, the muscles connecting the antennae were dissected, and the neck muscles were also carefully excised to prevent brain movement during the procedure. Throughout the preparation process, the brain was kept in Ringer's solution. Before staining, the dye was dissolved in 20% Pluronic-127 in dimethyl sulfoxide and then diluted in Ringer's solution to achieve a final concentration of 30 mmol/L. The brain was then stained with 20 μL of Calcium Green-1 (Molecular Probes; Invitrogen) for ~1 h at 4 °C. Following staining, the brain was rinsed three times with Ringer's solution and prepared for imaging. An upright microscope (OLYMPUS BX51WI, Tokyo, Japan) equipped with a 20 × water-immersion objective (NA 0.50, OLYMPUS) was utilized to observe and record the antennal lobe. Imaging data were captured using a digital camera (ORCA-03G, HAMAMATSU, Japan), along with a camera adapter (A13206-01, HAMAMATSU, Japan), Polychrome V, and CCM2 (FEI, USA). Data acquisition and analysis of in vivo optical imaging were performed using Live Acquisition (Till Photonics, Gräfelfing, Germany), Offline Analysis (FEI, USA), and ImageJ (NIH, USA). The stimulus delivery setup is referred to as the single-sensillum recording (SSR) setup. For each stimulus, 30 continuous frames were acquired at a sampling rate of 4 Hz, with a 0.5 s pulse stimulation taking place at frame 7. Data analysis procedures followed those outlined by ref. 73 and were conducted using ImageJ software. Raw fluorescent intensities were normalized into relative changes (ΔF/F), with F representing the average of frames 1–6 before stimulus onset. The response to the solvent (pure nectar) was subtracted from all stimulus-induced signals.

Establishment of the MsepOR8 mutant line using CRISPR-Cas9

The establishment of MsepOR8 mutant moths using CRISPR-Cas9 followed a previously described protocol[71,74]. Two target sequences (Supplementary Data 2) were selected, and the corresponding single-guide RNAs (sgRNAs) were synthesized using the GeneArtTM Precision sgRNA Synthesis Kit (Thermo Fisher Scientific, USA). After DNA removal by DNase I digestion, the synthesized sgRNAs were purified using the sgRNA Clean Up Kit (Thermo Fisher Scientific, Pittsburgh, PA, USA). Subsequently, a mixture of the two target sgRNAs and Cas9 protein (GeneArt™ Platinum™ Cas9 Nuclease, Thermo Fisher Scientific, Pittsburgh, PA, USA) was injected into each freshly laid egg using a FemtoJet and InjectMan NI 2 microinjection system (Eppendorf, Hamburg, Germany). The Cas9/gRNAs mixture comprised 150 ng/μL of each sgRNA and 150 ng/μL of Cas9 protein. Following injection, the eggs were incubated at 25 ± 1 °C and 50 ± 10% relative humidity for 3–4 days until hatching. The individual genotypes of the G0 generation were examined by performing genomic fragment amplification from the genomic DNA using specific primers (refer to Supplementary Data 2) and subsequent Sanger sequencing of PCR amplicons. Heterozygous mutant moths with the same genotype were then hybridized to generate the F1 generation. Upon eclosion, the F1 homozygous mutants were screened out using the same procedure for further analysis.

Yeasts transmission bioassay

To investigate the potential impact of foraging behavior influenced by sensing isoamyl alcohol on yeast transportation, we conducted two behavioral experiments. In the first experiment, we want to know whether moths transmit yeasts from nectar sources. Freshly emerged moths were captured at the onset of the photophase and divided into two groups randomly. At the beginning of the subsequent scotophase, one group was supplied with sufficient NEC (nectar), while the other received NEC + Y + P (Rape pollen). After four hours, the food containers were removed to halt feeding. During the following photophase, each individual was transferred to a new clean cage. At the beginning of the subsequent scotophase, a sterile cap containing 200 μL of sterile NEC was provided during the scotophase, after 10 h, the empty caps were collected, 200 μL of sterile NEC + P (Rape pollen) was added into each empty cap, and then incubated in a sterile, high-humidity, aerobic environment (29 °C) for one day. Subsequently, the yeast density associated with each moth was quantified using a blood-counting chamber under a microscope. In the second experiment, we investigated whether foraging behavior mediated by sensing isoamyl alcohol promotes yeast dispersal. Two caps, one containing 50 μL of NEC and the other containing 50 μL of NEC + Y + P (Rape pollen), were positioned diagonally in a cage. A single freshly emerged moth was introduced into the cage at the beginning of the scotophase. At the start of the subsequent photophase, the empty NEC caps were collected, 200 μL of sterile NEC + P (Rape pollen) solution were added into each empty cap+, and then incubated in a sterile, high-humidity, aerobic environment (29 °C) for one day. The number of yeasts in each cap was quantified using a blood-counting chamber under a microscope. Both experiments utilized sterile caps, NEC, NEC + P (Rape pollen), petri dishes, pipettes, cages et al. Prior to the experiments, the room environment underwent sterilization using a UV lamp for two hours, followed by ventilation to eliminate any residual ozone produced by the UV radiation using an exhaust fan, because the ozone could affect insect behavior[75]. Additionally, a dense fiber filter was placed on the air inlet to filter the incoming air, ensuring a sterile experimental environment.

Moth foraging behavior assay

Freshly emerged moths (*M. separata*) were collected at the onset of the photophase, starved individually in glass vials for 14 h (one photophase), and used for experiments during the subsequent scotophase.

To assess feeding behavior, we set up a cage (60 cm × 40 cm × 40 cm) with ten feeding caps, each containing 10 μL of rape pollen-rich, yeast-fermented nectar (NEC + Y+Rape P). The caps were randomly arranged on the bottom of the cage. Moths were allowed to feed for 20 min. After the feeding period, all caps were removed and inspected to record the number of caps with consumed nectar. Observations during the assay indicated that moths spent the majority of their time searching for nectar caps. Upon locating a cap, the moths consumed the nectar rapidly before moving on to search for additional sources. Each cap was either completely emptied or left untouched (we checked the caps in a stereo microscope and found there was no nectar in the empty caps, but unreduced nectar in the untouched caps). At the end of the feeding period, the number of empty caps in each cage was counted as "*n*", and *n*/10 as the visiting percent to nectar sources. The assay was repeated for 35 replicates in both the wild-type and MsepOR8$^{-/-}$ groups.

Pollen viability assay

Sunflower plants were grown, and pollen was collected at the time of bloom. The pollen was then divided into three equal portions: the first was stored as a dry powder (control), the second was placed in pure nectar, and the third was mixed with nectar-containing yeasts. All samples were incubated for 24 h at 29 °C in an aerobic environment. After incubation, a drop of each sample was transferred to a conical flask, and a moth was allowed to feed on the nectar, thereby transferring pollen to its proboscis. Subsequently, a drop of synthetic nectar was provided on a microscope slide, and pollen was released onto the slide as the moth fed. Pollen viability was assessed by staining with I2-KI solution and examined under a microscope. Viable pollen was stained in blue.

Moth oviposition assay

Freshly emerged oriental armyworm moths, collected at the onset of the photophase, were utilized for the experiment. Each experimental group comprised 10 females and 12 males, considering their natural tendency to congregate. An ample quantity of food was provided, replenished daily at the beginning of each scotophase to ensure freshness. Folded papers were supplied as egg-laying sites starting from the second day until the natural demise of all moths. Each morning, the folded papers were gathered, and the eggs were enumerated using a stereo microscope.

Nectar consumption assay

A small hole was drilled in a 2 mL plastic centrifuge tube to allow the moth to feed while minimizing nectar volatilization. The tube was filled with either pure nectar (NEC) or NEC with isoamyl alcohol ($44/10^{-7}$ (v/v)). The tube was weighed before and after the moth consumed the nectar during the scotophase (night phase). The difference in weight was used to calculate the mass of nectar consumed, and divided by nectar density to get the consumed volume.

Phylogenetic analysis

The phylogenetic analysis was performed using 659 ORs from 13 lepidopteran species, including *Agrotis segetum*, *Agrotis ipsilon*, *Athetis lepigone*, *M. separata*, *Mythimna lorgi*, *Spodoptera frugiperda*, *Spodoptera exigua*, *Spodoptera litura*, *Helicoverpa assulta*, *Helicoverpa armigera*, *Bombyx mori*, *Ostrinia furnacalis* and *Chilo suppressalis*. The tree was built using the Fast Tree program with default parameters. and displayed using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/Figtree/).

Statistics and figure preparation

All graphs were generated using Prism 6 software (GraphPad Software, La Jolla, CA), while all statistical analyses were performed using SPSS Statistics 25 (IBM). The statistical tests employed included the Chi-square test, two-sided *t*-tests, or one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) test for multiple comparisons of mean values.

Reporting summary

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

Data availability

The raw data used to generate all figures in this study are available in the Supplementary Information/Source Data file. Transcriptomics data have been deposited in the SRA database under accession code PRJNA1110715. Source data are provided as a Source Data file. Source data are provided with this paper.

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Acknowledgements

We thank Mrs. Liyan Yang (our lab) for rearing insects and Dr. Yanbing Gong (Wuhan University) for providing yeast. This work was funded by the National Natural Science Foundation of China (32130089 to G.W.), Shenzhen Science and Technology Program (Grant No. KQTD20180411143628272 to G.W.), special funds for science technology innovation and industrial development of Shenzhen Dapeng New District (Grant No. PT202101-02 to G.W.) and Innovation Program of Chinese Academy of Agricultural Sciences (CAAS-CSCB-202302 to H.C.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article.

Author contributions

B.M., H.C., B.S.H., and G.W. designed the research; B.M., H.C., M.G., D.A., J.W., R.C., and X.L. performed the experiments; B.M., H.C., B.S.H., and G.W. analyzed the data; All authors discussed the results; B.M., H.C., and G.W. wrote the paper with contributions from B.S.H. and B.R. All authors approved the final paper.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-025-56354-3.

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Peer review information *Nature Communications* thanks Monica Barman, who co-reviewed with Nicole van Dam, and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. A peer review file is available.