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Comparison of functions of pheromone receptor repertoires in *Helicoverpa armigera* and *Helicoverpa assulta* using a *Drosophila* expression system

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ABSTRACT

Helicoverpa armigera and *H. assulta* are sympatric closely related species sharing two sex pheromone components, (*Z*)-11-hexadecenal (Z11-16:Ald) and (*Z*)-9-hexadecenal (Z9-16:Ald) but in opposite ratios, 97:3 and 3:97 respectively. This feature makes them a feasible model for studying the evolution of pheromone coding mechanisms of lepidopteran insects. Despite a decade-long study to deorphanize the pheromone receptor (PR) repertoires of the two species, the comparison of the function of all PR orthologs between the two species is incomplete. Moreover, the ligands of OR14 and OR15 have so far not been found, likely due to the missing of the active ligand(s) in the compound panel and/or incompatibility of heterologous expression systems used. In the present study, we expressed the PR repertoires of both *Helicoverpa* species in *Drosophila* T1 neurons to comparatively study the function of PRs. Among those PRs, OR13, OR6, and OR14 of both species are functionally conserved and narrowly tuned, and the T1 neurons expressing each of them respond to Z11-16:Ald, (*Z*)-9-hexadecenol (Z9-16:OH), and (*Z*)-11-hexadecenyl acetate (Z11-16:Ac), respectively. While HarmOR16-expressing neurons respond strongly to (*Z*)-9-tetradecenal (Z9-14:Ald) and (*Z*)-11-hexadecenol (Z11-16:OH), the neurons expressing HassOR16 mainly respond to Z9-14:Ald and also weakly respond to (*Z*)-9-tetradecenol (Z9-14:OH). Moreover, HarmOR14b-expressing neurons are activated by Z9-14:Ald, whereas HassOR14b-expressing neurons are sensitive to Z9-16:Ald, Z9-14:Ald, and (*Z*)-9-hexadecenol (Z9-16:OH). In addition, HarmOR15-expressing neurons are selectively responsive to Z9-14:Ald. However, the *Drosophila* T1 neurons expressing either HarmOR11 or HassOR11 are silent to all of the compounds tested. In summary, except for OR11, we have deorphanized all the PRs of these two *Helicoverpa* species using a *Drosophila* expression system and a large panel of pheromone compounds, thereby providing a valuable reference for parsing the code of peripheral coding of pheromones.

1. Introduction

Insect sex pheromones are constituted by a blend of multiple long-chain unsaturated hydrocarbon derivatives that are produced by one individual and arouse members of the opposite sex of the same species (Rizvi et al., 2021; Zhang et al., 2012). The insect uses odorant receptors (ORs) that contain seven transmembrane domains to detect the ever-changing chemical world (Hallem and Carlson, 2006; Leal, 2013). Pheromone receptors (PRs) are a member of ORs and many specialize in pheromone detection (Krieger et al., 2004; Sakurai et al., 2004; Yang and Wang, 2021). Insect ORs are ligand-gated non-selective cation channels that consist of two tuning ORs and two highly conserved odorant receptors co-receptors (ORco) (Butterwick et al., 2018; Mármol et al., 2021; Sato et al., 2008; Wicher et al., 2008). As a central molecular element operating in the peripheral process of insect olfaction, ORs determine the response profiles of olfactory receptor neurons (ORNs), thereby gating the input of peripheral olfactory information into the central olfactory neurons in the brain (Dobritsa et al., 2003; Nakagawa et al., 2005). Therefore, deorphanization of PRs is the key to understanding the molecular basis of pheromone detection.

Helicoverpa armigera and *Helicoverpa assulta* are closely related species that occur in sympatry, sharing the same two sex pheromone components, (*Z*)-11-hexadecenal (Z11-16:Ald) and (*Z*)-9-hexadecenal (Z9-16:Ald), but in opposite ratios, 97:3 in *H. armigera* and 7:93 in

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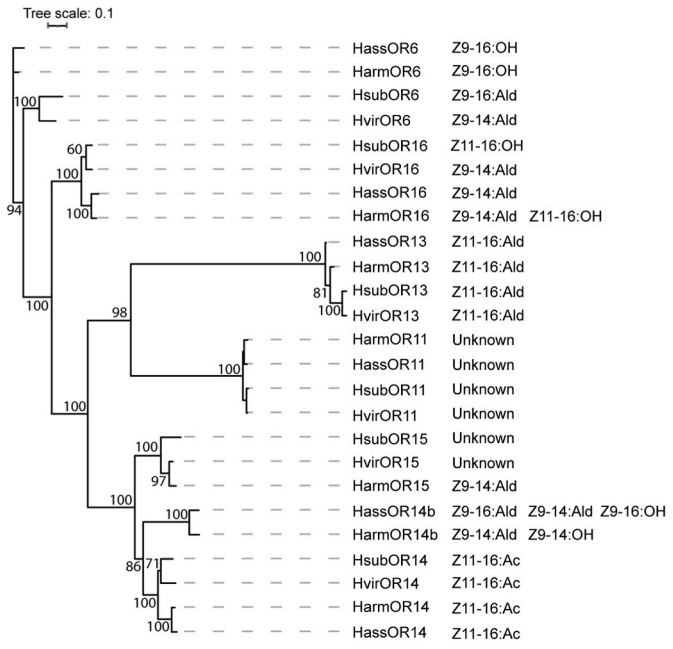

Fig. 7. Phylogenic analysis and functional annotation of PRs from representative heliothine species. The maximum likelihood tree was constructed from the amino acid sequences of four representative heliothine species. The most effective ligands of HarmORs and HassORs listed were reported by this study. The ligands of HvirORs from *H. virescens* were reported by Große-Wilde et al. (2007) and Wang et al. (2011), and HsubORs from *H. subflexa* by Cao et al. (2021). The numbers on clade branches indicate the bootstrap values ranging from 50 to 100.

Z9-14:Ald and Z11-16:OH (Fig. 7). The functions of OR6 vary between *Helicoverpa* species and *H. virescens* (Fig. 7). Both HarmOR6 and HassOR6 are mainly responding to Z9-16:OH, whereas HvirOR6 and HsubOR6 are tuned to Z9-14:Ald (Fig. 7). Interestingly, so far, OR14b clade has only been found in the two *Helicoverpa* species and is functionally divergent (Yang et al., 2017). In this study, we validate that HarmOR14b strongly responds to Z9-14:Ald and Z9-14:OH, whereas HassOR14b responds to Z9-16:Ald, and to a less extent, to Z9-14:Ald and Z9-16:OH (Figs. 3 and 7). HarmOR15 is the only member of OR15 clade in heliothine that has been deorphanized, which precludes us from an analysis of the functional conservation of OR15 clade (Fig. 7).

In conclusion, our results not only further validate the response spectra of OR13, OR6, OR16, and OR14b in the *Drosophila* T1 neurons, but also deorphanize OR14 and OR15. We compile the hitherto identified ligands of HarmPRs and HassPRs into Table 1, providing a holistic view of the tuning profiles of the PR repertoire of the two *Helicoverpa* species.

Availability of data and material

The raw data and materials including transgenic flies are available from the corresponding author upon request.

Author contributions

HG and CZW designed the experiments; HG, LQH, and XLG performed the experiments; HG and CZW analyzed the data; CZW conceived the project; HG and CZW wrote the manuscript.

Declaration of competing interest

The authors declare no competing financial interests and the funding agencies were not involved in the experimental design and data collection.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibmb.2021.103702.

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