Candidate pheromone receptors of coding moth *Cydia pomonella* respond to pheromones and kairomones

**Cydia pomonella**.

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**Background**

Olfaction plays a dominant role in the mate-finding and host selection behaviors of the coding moth (*Cydia pomonella*), an important pest of apple, pear and walnut orchards. Antennal transcriptome analysis (Bengtsson et al., 2012; Walker et al., 2016) revealed a number of abundantly expressed genes related to the moth olfactory system, including those encoding the olfactory receptors (ORs) CpomOR1, CpomOR2 and CpomOR5, which belong to the putative pheromone receptor (PFR) lineage, and the cop-carboxyesterase (CpomCE) family. Using heterologous expression, in both human embryonic kidney (HEK293T) cells and in *Drosophila* olfactory sensory neurons, coupled with calcium imaging and electrophysiological recording, respectively, we characterized the basic physiological and pharmacological properties of these receptors and demonstrate that they form functional ionotropic receptor channels.

**Functional expression of CpomORs in Human Embryonic Kidney (HEK293T) cells**

Functional expression of homomeric CpomORs were verified by Calcium imaging and single patch-clamp recording experiments (n = 200 μM VUAA, scale bar = 20 μm).

**Activation of homomeric CpomORs and heteromeric CpomOR/OR complexes by VUAA agonists.** Different OR complexes showed different sensitivity to VUAA1 and VUAA2 agonists and demonstrated different activities in electrophysiological recordings.

**Functional characterization of CpomORs.** Left: comparison of CpomOR/OR complexes of the Calcium response (mean of the mean response ± SEM) of OR complexes (n = 30) (VUAA1 = 29.23 ± 27.28 μM; VUAA2 = 135.73 ± 48.46 μM) to 250 μM (E,Z)-6-decadienol (neutral odorant; (E,Z)-2,6-dimethyl-4-heptanol (moderate odorant; (E,Z)-2,6-dimethyl-4-heptanol (moderate odorant; (E,Z)-2,6-dimethyl-4-heptanol (moderate odorant). Black bar stimulus. Right: normalized dose response of peak current (n = 10) and mean amplitudes (n = 10) of VUAA1 and VUAA2. **Functional characterization of CpomORs vs. Orco.** Left: comparison of CpomOR/OR complexes of the Calcium response (mean of the mean response ± SEM) of OR complexes (n = 30) (VUAA1 = 29.23 ± 27.28 μM; VUAA2 = 135.73 ± 48.46 μM) to 250 μM (E,Z)-6-decadienol (neutral odorant; (E,Z)-2,6-dimethyl-4-heptanol (moderate odorant; (E,Z)-2,6-dimethyl-4-heptanol (moderate odorant). Black bar stimulus. Right: normalized dose response of peak current (n = 10) and mean amplitudes (n = 10) of VUAA1 and VUAA2.

**Conclusion**

We characterized recombinant Coding Moth ORs transiently expressed in HEK293T cells.

- Using Calcium imaging and whole-cell outside-out patch-clamp recordings, we demonstrated that both the homomeric CpomOR channel forming submit and heteromeric CpomOR complexes have intrinsic properties that are affected by the OR agonist VUAA1 and VUAA2 and they are also susceptible to inhibition by amidinated and amidine agonists.
- Whole-cell outside-out patch-clamp recordings demonstrated CpomOR complexes forming functional ionotropic receptor channels.
- Electrophysiological expression of CpomORs confirmed sensitivity to planar lipid bilayer (Bengtsson et al., 2016) and in the pharmacological assay.

**Functional expression of CpomORs demonstrated sensitivity to (E,Z)-6-decadienol agonists.**

- Heterologous expression of Coding Moth ORs demonstrated activation to the same ligands and identified potential agonists for CpomORs.

**Functional expression of CpomORs therefore represents a valuable tool that can be utilized to further investigate mechanisms of insect OR functions and develop novel means to intervene and control this pest’s behavior (Cattaneo 2019).**

**Acknowledgements**


The chemosensory receptors of *Cydia pomonella* demonstrated sensitivity to (E,Z)-6-decadienol agonists. *Chem. Senses* 38, 362-373.