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RAPID COMMUNICATION

Odorant Receptor from the Southern House Mosquito Narrowly Tuned to the Oviposition Attractant Skatole

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Abstract Oviposition attractants are environmental cues that allow *Culex* gravid female mosquitoes to locate suitable sites for egg-laying and, therefore, may be exploited for environmentally friendly strategies for controlling mosquito populations. Naturally occurring skatole has been identified as an oviposition attractant for the Southern House mosquito, *Culex quinquefasciatus.* Previously, we identified in *Cx. quinquefasciatus* female antennae an olfactory receptor neuron (ORN) highly sensitive to skatole and an odorant-binding protein involved in the detection of this semiochemical. Here, we describe the characterization of an odorant receptor (OR), CquiOR10, which is narrowly tuned to skatole when expressed in the *Xenopus* oocyte system. Odorant-induced response profiles generated by heterologously expressed CquiOR10 suggest that this OR is expressed in the mosquito ORN sensitive to skatole. However, geranylacetone, which stimulates the antennal ORN, was not detected by CquiOR10-expressing oocytes, thus raising interesting questions about reception of oviposition attractants in mosquitoes.

Key Words Odorant receptor · CquiOR10 · *Culex quinquefasciatus* · *Xenopus* oocyte expression system · 3-Methylindole · 2-Methylphenol

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Introduction

Culex mosquitoes are vectors of pathogens including the human filarial nematode, *Wuchereria bancrofti*, and encephalitis-causing viruses, such as St. Louis, Japanese, Venezuela equine, Western equine encephalitis, and West Nile virus (Nasci and Miller, 1996).[1] Given the resistance of *Culex* populations to modern insecticides, alternative methods of controls are sorely needed. Larval development is a particularly vulnerable phase in the life cycle of *Culex* mosquitoes, as eggs are laid in rafts from which hundreds of larvae emerge in confined areas—thus facilitating management. Gravid females rely on environmental oviposition attractants to locate oviposition sites. Skatole, a natural product found in animal excreta and also a product of fermentation of organic material, has been identified as an oviposition attractant for the Southern House mosquito, *Culex quinquefasciatus* (Millar et al., 1992). Field studies have demonstrated that traps baited with optimal doses of skatole collected significantly more eggs (Mboera et al., 2000) and gravid females (Leal et al., 2008) than control traps, thus suggesting that in combination with a biological agent, *Bacillus thuringiensis* var. *israelensis* (Barbosa et al., 2010) oviposition attractants may be used in "attract-and-kill" strategies. Chemical ecology and olfaction are the pillars of these semiochemical-based, environmentally friendly strategies. Therefore, identification of olfactory proteins involved in the reception of these semiochemicals may open the door for development of better oviposition attractants. Recently, we demonstrated by RNA interference that an odorant-binding protein (OBP) from *Cx. quinquefasciatus*, CquiOBP1, is involved directly in the

[1] We apologize for not being able to cite all the relevant literature due to reference limitations of rapid communication format.

reception of skatole and other oviposition attractants (Pelletier et al., 2010a). We also have characterized an odorant receptor (OR) from this mosquito species, CquiOR2, which is highly sensitive to indole and moderately sensitive to skatole (Pelletier et al., 2010b). Here, we characterize CquiOR10 and show this OR to be highly sensitive and narrowly tuned to skatole.

Methods and Materials

Expression of CquiOR10 in the Xenopus Oocyte System Oocytes were prepared as previously described (Pelletier et al., 2010b). CquiOR10 and CquiOR7, initially cloned into pBlueScript (Pelletier et al., 2010b), were transferred to pGEMHE for use as templates for synthesis of capped cRNA by using mMessage mMachine kits (Ambion). Twenty-five ng of cRNA encoding each OR subunit were injected into Stage V–VI Xenopus oocytes. Oocytes were incubated at 18°C in Barth's saline (in mM: 88 NaCl, 1 KCl, 2.4 NaHCO3, 0.3 CaNO3, 0.41 CaCl2, 0.82 MgSO4, 15 HEPES, pH 7.6, and 100 μg/ml amikacin) for 2–5 d prior to electrophysiological recording.

Electrophysiology and Data Analysis Odorant-induced currents were recorded under two-electrode voltage clamp from oocytes expressing ORs, by using an automated parallel electrophysiology system (OpusXpress 6000A; Molecular Devices). Oocytes were perfused with ND96 (in mM: 96 NaCl, 2 KCl, 1 CaCl2, 1 MgCl2, 5 HEPES, pH 7.5). Odorants were diluted in ND96 and applied for 20 sec at a flow rate of 1.65 ml/min with extensive washing in ND96 (5–20 min at 4.6 ml/min) between applications. Current responses approached a plateau during the 20 sec application (Pelletier et al., 2010b). Micropipettes were filled with 3 M KCl and had resistances of 0.2–2.0 MΩ. The holding potential was −70 mV. Current responses were filtered (4-pole, Bessel, low pass) at 20 Hz (−3 db), sampled at 100 Hz, and were captured and stored with OpusXpress 1.1 software (Molecular Devices). Initial analysis of electrophysiological data was done with Clampfit 9.1 software (Molecular Devices). Curve fitting of concentration-response data was done with Prism 4 (Graphpad).

Results and Discussion

In our search for molecular targets that may be used in a reverse chemical ecology approach for the development of better oviposition attractants (Leal et al., 2008), we recently have mined the genome of *Cx. quinquefasciatus* and identified an OR sensitive to indole, CquiOR2, which also responded to methylindoles, including skatole (IUPAC name, 3-methylindole). By mapping the antennae of female *Cx. quinquefasciatus*, we previously observed that a skatole-detecting ORN also responded to geranylacetone and ethyl hexanoate, but not indole (Syed and Leal, 2009). These observations prompted us to examine the odorant response profile of CquiOR10, an OR closely related to CquiOR2 (Pelletier et al., 2010b). Full-length coding sequence of CquiOR10 and the obligatory co-receptor CquiOR7 (Pelletier et al., 2010b) were cloned in pGEMHE for heterologous expression in *Xenopus* oocytes.

To identify the best ligand for this receptor, oocytes expressing CquiOR10 + CquiOR7 were screened first with a panel of odorants (Fig. 1a), each applied for 20 sec at a concentration of 10 μM with extensive washing between applications. Skatole (3-methylindole) elicited the largest current responses, but the receptor also responded with lower sensitivity to indole, other methylindoles, and 2-methylphenol. Interestingly, CquiOR10 was unresponsive to many compounds in the test panel of 23 odorants, including other oviposition attractants such as trimethylamine, nonanal, and the mosquito oviposition pheromone (MOP) (Leal et al., 2008).

Next, we performed concentration-response analyses for skatole and two other ligands, indole and 2-methylphenol, which were identified as the best ligands among the indoles and phenols, respectively, for the related receptor CquiOR2 (Pelletier et al., 2010b). Skatole was the most potent of these compounds, activating the CquiOR10 + CquiOR7 receptor with an EC_{50} of 90 nM. Indole and 2-methylphenol were less potent, activating CquiOR10 + CquiOR7 with EC_{50} values of 2.4 μM and 41 μM, respectively. Interestingly, indole and 2-methylphenol also displayed lower efficacy (maximal response) than skatole (40±2% and 69±3% of skatole, respectively). In addition, the response threshold for skatole was two to three orders of magnitude higher than that observed for indole and 2-methylphenol (Fig. 1b, c). Thus, we found that heterologously expressed CquiOR10 is highly sensitive and narrowly tuned to the oviposition attractant skatole.

In female antennae of the Southern House mosquito, skatole is detected by a small-spike-amplitude ORN housed in A1 sensilla (Syed and Leal, 2009), which is also sensitive to a lower degree to geranylacetone and ethyl hexanoate, but does not respond to indole or 2-methylphenol [see Figs. S5, S6, supporting information in (Syed and Leal, 2009)]. In the *Xenopus* oocyte system, CquiOR10 was unresponsive to geranylacetone. Although expression of ORs in heterologous systems, such as the *Xenopus* oocyte system, is an invaluable tool for de-orphanizing and characterizing receptors, it does not completely mimic the insect olfactory system. Typically, these systems are devoid of OBPs, odorant-degrading enzymes, sensory neuron membrane proteins, and other

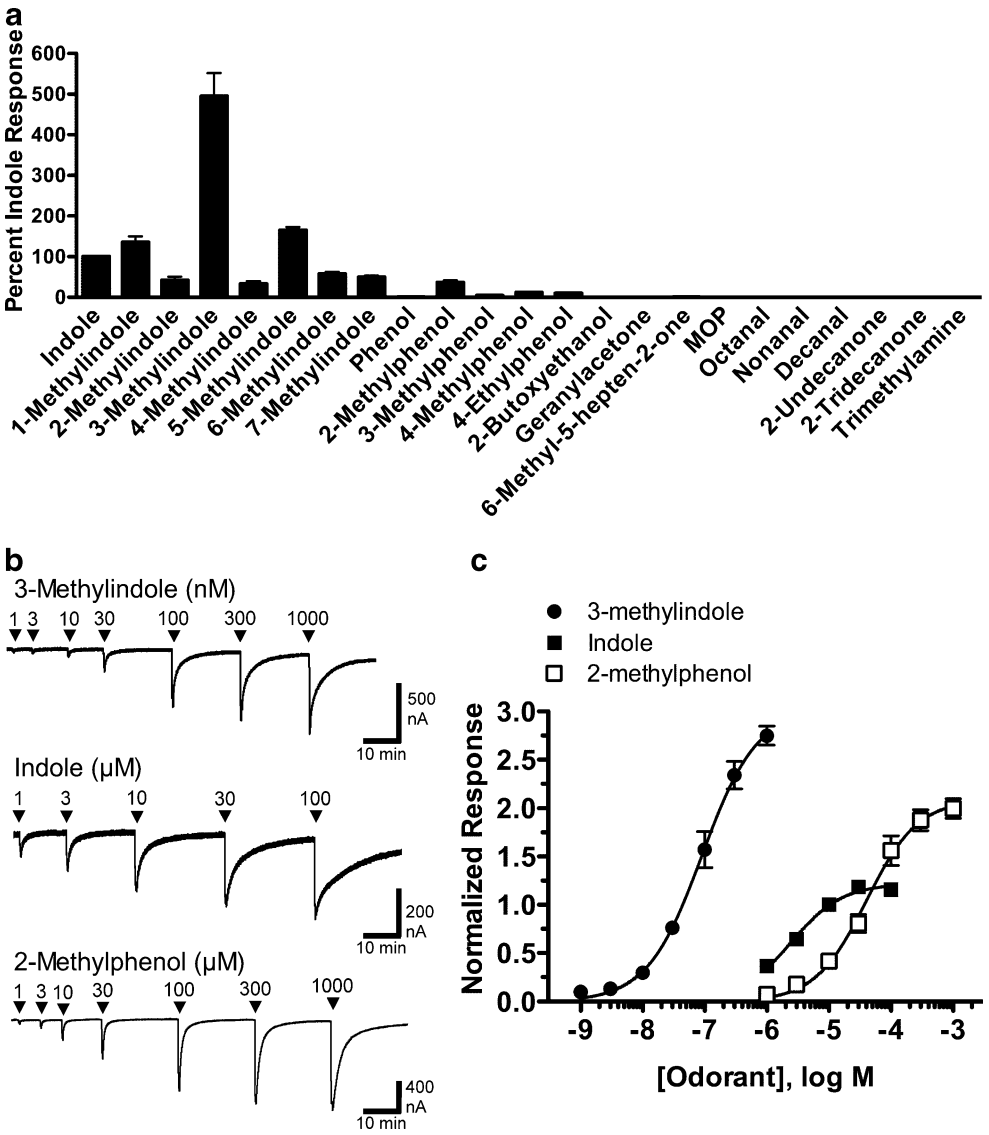

Fig. 1 Odorant receptor CquiOR10 is highly sensitive to skatole (3-methylindole). **a** Oocytes expressing CquiOR10 + CquiOR7 were challenged with a panel of odorant compounds. Each odorant was applied at a concentration of 10 μM for 20 sec with 10 min washes between applications. All responses are normalized to the response of the same oocyte to 10 μM indole (mean ± SEM, N=4–5). **b** Oocytes expressing CquiOR10 + CquiOR7 were challenged with a range of concentrations of 3-methylindole (*top trace*), indole (*middle trace*) or 2-methylphenol (*bottom trace*). Each odorant was applied for 20 sec with 5–20 min washes between applications. Note different scales: from top to bottom 500, 200 and 400 nA. **c** Oocytes expressing CquiOR10 + CquiOR7 were challenged with a range of concentrations of 3-methylindole, indole, and 2-methylphenol. Responses were normalized to the response of each oocyte to 10 μM indole and are presented as mean ± sem (N=3–5 for each odorant tested). Data were fit to the equation: $I = I_{max}/(1 + (EC_{50}/X)^n)$ where I represents the current response at a given concentration of odorant (X), I_{max} is the maximal response, EC_{50} is the concentration of odorant yielding a half maximal response, and n is the apparent Hill coefficient. Derived values are: 3-methylindole, EC_{50}=90±17 nM, N=1.0±0.1; indole, EC_{50}=2.4±0.3 μM, N=1.1±0.2; 2-methylphenol, EC_{50}=41±7 μM, N=1.0±0.1

olfactory proteins that may play a part in the selectivity and sensitivity of the olfactory system. Thus, it is conceivable that heterologously expressed CquiOR10 and the receptor in its native environment differ in the detection of geranylacetone because the former is devoid of OBPs. However, one cannot rule out the possibility that a separate ORN responding to geranylacetone has the same spike amplitude as the skatole-detecting ORN (Syed and Leal, 2009), thus rendering them indistinguishable by single unit recordings. Alternatively, the same small-spike neuron sensitive to skatole may express another OR along with CquiOR10. In marked contrast to the mammalian olfactory system, co-expression of ORs has been documented in *Drosophila melanogaster*. Co-expression of CquiOR10 and another OR would not be entirely surprising given the number of putative odorant receptors in the Southern House

mosquito genome (Pelletier et al., 2010b) and the number of ORNs in their sensory system (Syed and Leal, 2007, 2008). Future research aimed at testing these three hypotheses might lead to deeper understanding of odorant reception in mosquitoes.

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