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Molecular bases of sensory processes in kissing bugs, vectors of Chagas disease

Short title: Kissing bug sensory protein coding genes

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Abstract

Sensory processes represent an information gathering interface between animals and their surrounding world. Therefore, they serve to scan the environment for resources and threats. The behavior of kissing bugs has been studied to aid their control because they transmit Chagas disease to humans. Besides, a few triatomines represent important insect models since Wigglesworth times. These hematophagous insects rely on different sensory systems to scan their environment for blood-sources, mating partners and hiding places. The study of the molecular bases of sensory processes has undergone a dramatic progress due the advent of new technologies allowing mass-sequencing of genes. Here we focus on reviewing the fundamental knowledge gathered to date about the molecular bases of kissing bug sensory processes.

Highlights

- Kissing bugs are hematophagous insects that act as vectors of human disease;
- The detection of multimodal sensory inputs related to host, shelter and sexual partner relies on receptor proteins belonging to diverse gene families;
- Genome sequences have been characterized and sensory gene families described;
- Targeted genes code mostly for olfactory, taste, thermo and hygro-receptors.

Introduction

Sensory processes and their molecular bases in model insects.

An impressive progress in our understanding of the molecular bases of sensory processes has been promoted by the advent of next-generation sequencing (NGS) technologies. This is especially true for insects because of ease of access and straightforward experimental manipulation. In particular, *Drosophila melanogaster* has become the topic's flagship due to its well annotated genome and the enormous wealth of genetic tools available for this species [1, 2]. Genome projects and transcriptomic studies have allowed sequencing and identifying genes underlying sensory processes for many insect species. The study of these sequences has allowed characterizing the evolution of the different gene families involved in stimulus detection by insects [3-5]. For instance, this has allowed determining that a relatively large gene family exclusive of insects, the odorant receptors, has been subjected to fast rates of change indicating that selective pressures promoted diversification through tandem duplication, elimination and pseudogenization events [5-7]. Besides, the identification of gene coding sequences has also allowed comparing their relative expression between different tissues or physiological conditions. Additionally, characterizing their specific functions in olfaction, taste, hygro and thermoreception became feasible [8, 9]. These functional aspects represent a new frontier for the generation of tools required for the manipulation of insect behavior through the development of better ligand-based baits or the *in silico* design of receptor-blocking agents [10]. Alternatively, other new concepts oriented to the development of control tools propose spreading RNAi vectoring vehicles to target the expression of genes impacting insect fitness [11].

Kissing bugs and their potential as models for the study of the molecular bases of sensory processes

Kissing bugs are hemipterans that belong to the subfamily Triatominae. These insects can transmit *Trypanosoma cruzi*, the causative agent of Chagas disease. Currently, the absence of vaccines points to the necessity of better vector control tools. Kissing bugs are nocturnal insects that search for hosts, including humans, exploiting multimodal sensory cues emitted by

them [12]. Furthermore, other relevant sensory processes are those related to the detection of triatomine aggregation signals, sexual and alarm pheromones, stridulation signals mediating communication between sexes and microclimates (i.e., both relative humidity and temperature) of hiding places [12]. These features of bug biology represent opportunities for behavioral manipulation to promote their control. Therefore, the molecular machinery involved in bug sensory processes represents a diverse array of new targets that require functional characterization before proceeding to the design of next-generation control tools.

Molecular bases of sensory processes in kissing bugs

1 - Chemoreception

Olfaction - Two main families of receptors have been shown to mediate insect olfaction. Odorant receptors (ORs) are a group of proteins characteristically showing seven transmembrane domains and a high rate of diversification [5-7]. On the other hand, the more recently identified ionotropic receptors (IRs) mediate the detection of volatile compounds such as aliphatic amines and short-chain fatty acids. A subset of them called antennal IRs seems to present a high degree of sequence and functional conservation [3, 13]. Two families of soluble proteins are responsible for transporting odorant molecules to the receptors located at the dendritic membranes of olfactory sensory neurons (OSNs): odorant binding proteins (OBPs) and chemosensory proteins (CSPs) [14].

The sequencing of the *Rhodnius prolixus* genome, the only available for a triatomine to date, has allowed the characterization of bug chemoreceptor families [15]. This species has a rather large OR family with 116 members organized in small subfamilies generated by older and recent gene expansion events. Regarding IRs, a total of 33 genes were identified in the genome, including the co-receptor genes (Ir25a, Ir8 and Ir76b), as well as the orthologs of several antennal IRs. Interestingly, *R. prolixus* has a great expansion of Ir75 subfamily with 16 genes [15]. An antennal transcriptome study reported that *R. prolixus* adult bugs present a leap in the abundance of antennal transcripts for several ORs, suggesting that these receptors

may have adult-specific roles [16]. Likewise, several ionotropic receptors have shown increased expression in the antennae of adult bugs, a few also presenting differential expression between males and females. Specifically, Ir8a and some members of the Ir75 subfamily presented increased expression in adult male antennae [16]. Furthermore, the expression of olfactory co-receptors, both OrCo and IR co-receptors, seems to depend on kissing bug age and nutritional status [17]. Interestingly, many kissing bug ORs and IRs have been shown to be expressed in the rostrum, tarsi and genitalia, suggesting chemoreceptive roles for these structures [18].

Transcriptome and functional genetics studies focused on the sensory machinery of kissing bugs are still scarce. Latorre-Estivalis and collaborators [16] have characterized antennal expression changes through the imaginal molt and proposed several gene candidates potentially involved in adult-specific behavior. Moreover, a study based on the same dataset has reported the antennal expression of modulatory factors including neuropeptides (NPs) and NP receptors, which suggests that insect antennae have several local systems dedicated to the modulation of sensory function [19]. Marchant and collaborators [20] performed chemosensory transcriptomes with *Triatoma brasiliensis* from different ecotopes and reported lower expression of OBPs and CSPs in insects collected at human dwellings. Besides, recent studies reported the use of RNAi to knock-down the expression of *OrCo* [21]; *Obp27* [22]; and *Or80* [23] in *R. prolixus*, showing that this methodology is feasible to study the sensory biology of triatomines. Finally, an heterologous expression system based on the use of *Xenopus* oocytes allowed deorphanizing four *R. prolixus* odorant receptors [23]. Among them, *RproOr80* has been suggested to respond to DEET, the main known insect repellent.

Taste - Insect gustatory receptors (GRs) are a family of proteins also having seven transmembrane domains and said to be basal to the ORs [6]. To date, they have been better functionally characterized for *D. melanogaster*, having been related to the detection of food during foraging, the recognition of cuticular sexual signals and the sensing of CO₂ [24]. Kissing

bugs rely on taste sensing for the recognition of hosts and sexual partners and a total of 28 GRs have been described in the genome of *R. prolixus* [12, 15, 25]. Nevertheless, no bug GR has so far been functionally characterized. The *R. prolixus* GRs include a single receptor (*RproGr1*) showing homology with *DmelGr43a*, which has been shown to function as a fructose receptor [26]. Two GRs mediate the detection of CO₂ in *D. melanogaster* [27], while three have been reported to be required for this task in mosquitoes [28]. Curiously, no orthologues have been found for these genes in the genome of *R. prolixus* [15], even though kissing bugs are well-known for their ability of exploiting this host cue during foraging [12]. Therefore, it is clear that a different mechanism should mediate this sensory competence in kissing bugs, as it is the case also for honeybees, ants and other insects lacking this GR lineage [29].

As already mentioned, behavioral experiments have shown that kissing bug males detect cuticular signals mediating female recognition [25]. This is expected to be either mediated by GRs or *pickpocket* (PPK) receptor genes, the latter being a conserved group of degenerin epithelial Na⁺ channels (DEG/ENaC) reported to mediate several taste-related functions in *D. melanogaster* [24]. A transcriptome study has reported GR genes with increased expression in male bug antennae, making them candidates for the detection of contact sexual signals [16].

Host skin recognition by taste receptors, as well as that of blood taste properties, has been shown to be relevant for skin piercing and feeding in these bugs [30, 31]. The identification of the receptors underlying the molecular bases of NaCl, KCl and ATP recognition, three of the main signals mediating the evaluation of skin and blood quality features, is yet to be revealed.

As well, kissing bugs have been reported to detect bitter substances that induce aversive responses [32], but the molecular mechanisms allowing them to recognize these compounds have not been studied. The PPK family of *R. prolixus* may include orthologues of *D. melanogaster* receptor genes involved in salt (*Rproppk19*), water (*Rproppk28*) and pheromone detection (*Rproppk23*). Whether these receptors are also responsible for the detection of kissing bug host cues and sexual signals deserving testing.

2 - Thermo and hygrosensation

Due to their limited control of temperature and water balance, insects rely on the detection of heat and relative humidity (RH) for homeostatic tasks. As insects are fundamentally ectothermic animals, environmental temperature is a critical factor affecting their locomotion. Therefore, foraging intensity and the speed of predator-avoidance responses depend on prevailing temperatures. Therefore, insects rely on a complex set of receptors mediating the recognition of different temperatures and allowing adequate environmental choices. Most of these belong to a gene family named transient receptor potential receptors (TRPs), channels dedicated to hygro and chemosensation, as well as mechanoreception or phototransduction [33]. As with previous cases, the molecular bases of heat detection have been mostly shown for *D. melanogaster*, but a few examples have reported genes involved in heat detection in mosquitoes [34], cockroaches [35], and kissing bugs [36]. Recently, Ir93a, Ir25a, Ir40a and Ir21a have also been reported to be involved in thermo and hygrosensation in *D. melanogaster* [37]. In fact, a transcriptome study showed that these IRs and some members of the TRPA subfamily presented high expression in the antennae of *R. prolixus* [16]. Whether they share these sensory properties also for kissing bugs deserves experimental testing.

As already mentioned, kissing bug heat reception is fundamental for choosing proper hiding-places based on temperature. Even most importantly, bugs recognize their hosts by the infrared radiation emitted by their bodies, as well as by the humidity expelled by hosts [12]. It is also known that kissing bugs choose their shelters depending on RH. Furthermore, engorged bugs prefer drier refuges to promote the passive loss of excess water [38, 39]. The mentioned IR and/or TRP receptors *nanchung* and *waterwitch* probably mediate this sensory ability in kissing bugs, but functional studies have yet to be delivered. The high antennal expression reported for *waterwitch* by Latorre-Estivalis and collaborators [16] makes this gene an interesting candidate for air humidity detection in bugs.

3 - Mechanoreception

Mechanoreception mediates proprioceptive, nociceptive and vibratory/auditory sensing in insects. Diverse mechanoreceptor genes have been functionally characterized in *D. melanogaster*, indicating that most mechanoreceptors, but not all, are related to the TRP gene family. Among them, the genes named *painless* and *NompC* have been shown to mediate mechanoreceptive processes in *D. melanogaster* [33]. Other genes coding for mechanoreceptive proteins present diverse sequence identity, suggesting that this sensory modality relies in proteins that have undergone different evolutionary histories. These include the genes called *piezo*, *narrowabdomen*, *mrityu*, *Chloride channel-b* and *NMDA receptor 1* and *2* [9]. Kissing bugs have been reported to rely on mechanoreception for skin texture probing, thigmotactic responses mediating their semi-social aggregation processes and communication through stridulation in their sexual context [40]. Nevertheless, to date no specific genes have been suggested to be potentially involved in mediating these sensory abilities. The antennal expression observed for *painless* and *piezo* [16] suggests them as main mechanoreceptor candidates in kissing bugs.

4 - Vision

Sensory processes mediating insect vision depend on the production of proteins called opsins to mediate the transduction of light into electrical impulses. These proteins have specific properties that allow them to react to light in a wavelength-specific manner. Therefore, this allows spectral discrimination and, in some insects presenting different opsins, color vision [41].

Triatomines are nocturnal insects and their visual system has been elegantly shown to be finely adapted to adjust to daily changes in light intensity. These insects show an intense negative phototaxis that promotes the search for dark shelters during daylight hours. Starved kissing bugs are known to forage at night, searching for vertebrate hosts from which they obtain blood meals [12]. Even though vision could aid this search and host recognition, it has not yet been related to this context. Interestingly, starved adults frequently engage in dispersal

through flight and orientation during flight seems to be mediated by light [42]. Four opsins were found in the genome of *R. prolixus*: ciliary opsin; r-opsin 7; ultraviolet sensitive opsin; and long wavelength sensitive 1 [15]. The presence of the last two opsins suggests kissing bugs could detect green and ultraviolet light. Nevertheless, no efforts have been made to date in order to confirm these suggestions and their putative functional roles remain unknown.

Perspectives

The chemical blends that compose sexual and aggregation pheromones have been characterized for several triatomine species [43-45]. As well, relevant host-emitted odorants have been identified [46]. However, the large number of olfactory receptor candidates identified in the *R. prolixus* genome represents a complex target for the selection of candidates for gene silencing or heterologous expression studies. The sensory organs of triatomines are expected to present molecular changes under different developmental and physiological conditions (e.g. after blood feeding). Therefore, transcriptomic data are necessary to allow understanding odor-receptor-behavior relationships by means of functional genetics studies in sensory organs. Finally, the virtually finished characterization of the genome sequence of *Triatoma infestans* will allow its comparison to the *R. prolixus* genome, bringing light about unknown tendencies of expansion/elimination of relevant gene lineages related to the sensory physiology of kissing bugs.

Declarations of interest: none

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