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An inside look at the sensory biology of triatomines

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Abstract

Although kissing bugs (Triatominae: Reduviidae) are perhaps best known as vectors of Chagas disease, they are important experimental models in studies of insect sensory physiology, pioneered by the seminal studies of Wigglesworth and Gillet more than eighty years ago. Since then, many investigations have revealed that the thermal, hygric, visual and olfactory senses play critical roles in the orientation of these blood-sucking insects towards hosts. Here we review the current knowledge about the role of these sensory systems, focussing on relevant stimuli, sensory structures, receptor physiology and the molecular players involved in the complex and cryptic behavioural repertoire of these nocturnal insects. Odours are particularly relevant, as they are involved in host search and are used for sexual, aggregation and alarm communication. Tastants are critical for a proper recognition of hosts, food and conspecifics. Heat and relative humidity mediate orientation towards hosts and are also important for the selection of resting places. Vision, which mediates negative phototaxis and flight dispersion, is also critical for modulating shelter use and mediating escape responses. The molecular bases underlying the detection of sensory stimuli started to be uncovered by means of functional genetics due to both the recent publication of the genome sequence of *Rhodnius prolixus* and the availability of modern genome editing techniques.

Introduction

The sensory systems of triatomines have captured the interest of scientists since the early 30s, when Wigglesworth and Gillett (1934) started investigations on the role of thermal, hygric, visual and olfactory information in the orientation of these insects towards their hosts. Since then, numerous behavioural, anatomical and physiological studies have considerably increased our knowledge about the sensory capabilities of these epidemiologically important insects. Because these insects are vectors of Chagas disease, knowledge about their sensory systems provides an opportunity to develop sustainable methods to reduce and/or prevent vector-host contacts and disease transmission.

Natural habitats are complex environments that insects need to navigate and exploit in order to survive. The sensory systems of triatomines, as those of many other insects, mediate behaviours such as food, mate and shelter search, habitat selection, avoidance of environmental risks, escape from predators, and communication with conspecifics. As a consequence, the sensory structures of insects are specialized in the detection of a great variety of stimuli, such as light intensity and colour, visual target movement, conductive or convective heat, infrared radiation, sound frequency or syllable pattern, air or mechanical pressure, and a plethora of volatile and contact chemicals. Visual, thermal/hygric, mechanosensory, olfactory and taste stimuli are first detected at the peripheral level and then information is relayed to downstream brain centres for further processing, including integration across modalities, and modulated by the internal (e.g. starvation, endogenous clocks and mating status) and the external (e.g. light intensity, temperature) state, to finally produce an appropriate behavioural response.

In this review, we summarize the current knowledge about the olfactory, taste, thermal and visual sensory systems of triatomines with particular emphasis on their morphological, physiological and molecular aspects. Lesser studied aspects, such as mechano and hygroreception, are also briefly presented. Additionally, we discuss how the recent annotation of the genome of *Rhodnius prolixus* opens new possibilities to apply modern functional genetics strategies to study and further comprehend triatomine sensory biology.

1. The olfactory sense

In triatomines, like in other insects, the olfactory system mediates important behaviours, such as host- and mate-seeking, oviposition, and alarm responses (e.g., Manrique et al., 2006; Guerenstein and Lazzari, 2009; Manrique and Lorenzo, 2012; Guidobaldi and Guerenstein, 2015), some of which are modulated by both internal and external factors (e.g. Bodin et al., 2009a, b; Reisenman et al., 2013). The olfactory-driven behaviour of triatomines has been recently reviewed (Lazzari et al., 2013) and therefore, it will be described here only briefly. While several studies at the peripheral level have started to provide significant knowledge about the function of the triatomine olfactory system in a natural context (see below), we still lack an understanding of how downstream neurons process olfactory information in these insects.

1.1. Olfactory sensory structures

In all animals, volatile chemical signals are detected by olfactory sensory neurons (OSNs), which in insects and other arthropods are housed inside multiporous olfactory sensilla. Triatomine olfactory sensilla are present on the surface of the antenna, which is composed of three segments, the scape, the pedicel and two flagellomeres (Figure 1A). Olfactory sensilla are only found on the distal and proximal flagellomeres and, depending on the species, also on the pedicel. For example, adults of species within the Tribe Triatomini (including *Triatoma infestans*) but not of species within the Rhodniini (including *R. prolixus*) have olfactory sensilla on the pedicel (Chaika 1980; Catalá and Schofield, 1994; Catalá, 1997; Gracco and Catalá, 2000; Catalá and Dujardin, 2001). Three types of olfactory sensilla have been described on the flagellum of a number of triatomine species: the basiconic (also called thin-walled wall-pore trichoid) (Figure 1B, C), the trichoid (also called thick-walled wall-pore trichoid) (Wigglesworth and Gillet, 1934; Catalá and Schofield, 1994; Guerenstein and Guerin, 2001; May-Concha et al., 2016) and the grooved-peg (also named double-walled wall-pore sensilla) (Figure 1B, D, E). Notably, the number and the diversity of these olfactory sensilla vary between species of triatomines that inhabit different biogeographical regions (Carbajal de la Fuente and Catalá, 2002). Thus, it was suggested that those species living in less stable habitats have higher number of olfactory sensilla (Carbajal de la Fuente and Catalá, 2002).

Ultrastructural studies in *T. infestans* showed that basiconic sensilla are non-articulated, 25-30 μm long, with a high density of wall pores linked to pore tubules (Bernard 1974). In *R. prolixus* these sensilla have many OSNs (ca. 15, Wigglesworth and Gillet, 1934), but more recent reports in *T. infestans* suggested that this type of sensilla houses about 21-41 OSNs (Guerenstein and Guerin, 2001) and about eighty dendritic arborisations (Bernard 1974). Trichoid sensilla are non-articulated, 35 μm long, with a lower density of wall pores than basiconic sensilla. They house five OSNs in *R. prolixus* and one or two *T. infestans* (Wigglesworth and Gillet, 1934; Bernard, 1974). Grooved pegs are non-articulated, longitudinally grooved, 8–18 μm long, with wall pores that communicate via spoke channels crossing the double cuticular wall to the central lumen of the sensillum (Bernard, 1974). These sensilla house fewer OSNs (about five) in both species mentioned above (Wigglesworth and Gillet, 1934; Bernard 1974; Guerenstein and Guerin, 2001). Regarding their sensory function, Wigglesworth and Gillet (1934) proposed olfactory and thermal functions for the basiconic and the trichoid sensilla, respectively. Nowadays, all three types are thought to be olfactory, although no odour stimulus tested so far was able to evoke responses in trichoid sensilla OSNs (reviewed in Guerenstein and Lazzari, 2010).

1.2. Sensory aspects of olfaction

Once inside the sensillum cavity, volatiles reach the dendrites of the OSNs. Many components involved in the signalling process, such as odour binding proteins (OBPs), chemosensory proteins (CSPs), odour degrading enzymes (ODEs), olfactory receptors (ORs), ionotropic receptors (IRs) and sensory neuron membrane proteins (SNMPs) have been identified in the genome of *R. prolixus* (Mesquita et al. 2015; see section 6).

Electrophysiological techniques such as electroantennography (EAG, Figure 1F) and single sensillum recordings (SSRs) have been useful to uncover the diversity of odours detected by triatomine OSNs, their sensitivity, and the existence of functional classes of sensilla. We next summarize findings from such studies in the two most studied triatomines: *R. prolixus* and *T. infestans*.

1.2.1. Host-related odour detection

A pioneer study using SSRs reported the first responses to biologically relevant odours such as breath in *T. infestans* (Mayer, 1968). Later studies reported responses of basiconic sensilla and grooved pegs to the host-derived odorants DL-lactic acid and pyruvic acid, while grooved pegs additionally responded to ammonia and butyric acid (Bernard 1974). Further electrophysiological investigations characterized odour responses to a larger spectrum of odorants, and the coupling of SSR to gas-chromatographic analysis (GC-SSR) allowed for the identification of bio-active sheep odour components (Guerenstein and Guerin, 2001). In *T. infestans*, OSNs within basiconic sensilla responded to aldehydes such as heptanal, octanal, nonanal and a number of terpenes, including (+)-pinene and (–)-limonene (Guerenstein, 1999; Guerenstein and Guerin, 2001). In *R. prolixus* and *Dipetalogaster maxima* basiconic sensilla also responded to nonanal (Guerenstein, 1999). In contrast, in *T. infestans*, grooved peg OSNs responded to different aliphatic amines such as methyl-, dimethyl-ethyl-amine, isobutylamine and short-chain carboxylic acids like isobutyric, isovaleric and 2-methylbutyric acids (Taneja and Guerin, 1997; Guerenstein and Guerin, 2001; Diehl et al., 2003; Guerenstein, 1999).

In general, the responses of most OSNs are excitatory and phasic-tonic in *T. infestans* (Guerenstein and Guerin, 2001; Diehl et al., 2003). Responses of grooved peg OSNs to different odours vary between sensilla, consequently at least three different functional sensillum types, named GP1-3, have been proposed in *T. infestans* (Diehl et al., 2003). All the three types have an OSN which is excited by aliphatic amines, while GP1 and GP2 also contain a cell that is activated by ammonia, and only GP2 is excited by short-chain carboxylic acids. To date, the odour tuning of at least nine OSN classes has been characterized in *T. infestans* (Guerenstein and Lazzari, 2009; 2010).

While most studies have examined responses at the peripheral level, not much is known about how odours are processed in downstream olfactory centres. As in other insects, the axons of OSNs project to the primary processing centre of olfactory information in the brain, the paired antennal lobes (ALs) (Figure 1G, H). In *R. prolixus*, each AL is composed of twenty-two spheroid structures called glomeruli, without obvious sexual dimorphism (Barrozo et al., 2009).

1.2.2. Host-associated behavioural responses

In their pioneering behavioural work, Wigglesworth and Gillet (1934) showed that *R. prolixus* orient to host (mouse) odours and moreover, found that adding a thermal cue enhanced the probing of an odour-heat source. The responses of triatomines to natural vertebrate odours were later confirmed in many studies. *T. infestans* follow airstreams carrying host odours using positive anemotaxis, a directional response to wind direction modulated by odour (hereafter called attraction for simplicity), in which both mechanical

and chemical cues are relevant (Barrozo et al., 2003; Barrozo and Lazzari, 2004a,b; 2006).

As for virtually all hematophagous insects, CO₂ is a host odour constituent that plays an important role in triatomine activation and host attraction (Núñez, 1982; 1987; Taneja and Guerin, 1995; Barrozo and Lazzari, 2004a; 2006). When tested alone, in *T. infestans* the behavioural response threshold lies between 300 and 400 ppm above ambient level (Barrozo and Lazzari, 2004a). *T. infestans* and *R. prolixus* are most attracted towards CO₂ at the beginning of the scotophase, when they search for food (Barrozo et al., 2004; Barrozo and Lazzari, 2004a; Bodin et al 2008). Moreover, the response to CO₂ is state-dependent, being modulated by an endogenous circadian rhythm (Barrozo et al., 2004; Bodin et al 2008), the insect nutritional status (Bodin et al., 2009a), and the post-ecdysis time (Bodin et al., 2009b). As in the case of CO₂, the behavioural attraction of *R. prolixus* to other host odorants, such as (-)-limonene, is modulated by their feeding status (Reisenman et al., 2013). This may be at least partially explained by the higher EAG responses observed in starved insects with respect to those of fed insects to the host odour ammonia (Reisenman, 2014). Moreover, this modulation of antennal responses by the feeding status only happens during the night, when insects are active (Figure 4; Reisenman, 2014).

Other constituents of host odours that activate and attract bugs include nonanal (Guerenstein and Guerin, 2001) isobutyric acid (Guerenstein and Guerin, 2001) and 1-octen-3-ol (Barrozo and Lazzari, 2004a). These odours have been tested at different concentrations, likely including those presented in nature. It remains to be tested if all those odours have the same behavioural role across different species of triatomines. Ammonia has additionally been shown to contribute to attraction within odour mixtures of pentanoic acid and lactic acid in *R. prolixus* and *T. infestans* (Guidobaldi and Guerenstein, 2013; 2016) as it also occurs in mosquitoes (Kröckel et al., 2006; Smallegange et al., 2005), highlighting possible convergent evolution.

In nature, insects encounter odour mixtures, often presented in an irrelevant odorous background, rather than single compounds. In fact, natural odour mixtures are more efficient in attracting triatomines than single odorants (e.g., Núñez, 1987; Barrozo and Lazzari, 2004a,b; Ortiz and Molina, 2010). For example, L-lactic acid *per se* does not evoke orientation, but when combined with a sub-threshold amount of CO₂ produces oriented responses in *T. infestans* (Barrozo and Lazzari, 2004a). More complex mixtures having human skin odour constituents are indeed particularly attractive (Barrozo and Lazzari, 2004b; Guidobaldi and Guerenstein, 2013, 2016). In particular, because L-(+)-lactic acid evokes consistent EAG responses (Figure 1F, Barrozo, 2003), it would be informative to further characterize the physiological responses of the corresponding OSNs.

Most odorants that are detected by triatomine OSNs (section 1.2.1.) also evoke behavioural responses (Guerenstein and Guerin, 2001; Reisenman et al., 2013, Taneja and Guerin, 1997; Otálora-Luna and Guerin, 2014; Barrozo and Lazzari, 2004b). However, although much is known about the behavioural responses of triatomines towards CO₂, the olfactory sensilla and OSNs involved in CO₂ detection have not yet been identified. Virtually all host odorants reported to be detected by *T. infestans* and *R. prolixus* are also detected by other blood-sucking insects, notably mosquitoes (Braks et al. 2001; Ghaninia et al. 2008; Syed and Leal, 2009; Harraca et al., 2012; see

also Guerenstein and Lazzari 2009 and references therein). This, along with the limited range of volatiles used by most blood feeders during host seeking (Syed, 2015), suggests that different blood-sucking insects use the same host odours. Convergent evolutionary pressures thus appeared to act on diverse blood-sucking arthropods conferring them the capacity of detecting such conspicuous signatures of host presence. Gustatory receptor proteins mediate CO₂ detection in Diptera (Jones et al., 2007). Interestingly, even though lice, bees and hemipterans are capable of CO₂ detection, they lack orthologs of these gustatory receptors, indicating that CO₂ receptors have evolved independently at least twice in insects (Robertson and Kent 2009). Blood-feeding is thought to have evolved independently at least six times across distant arthropod groups (Ribeiro, 1995; Yao et al., 2014). In any case, the origin of blood-feeding dates back to at least the early cretaceous (Yao et al., 2014), indicating an ancient (and likely not unique) origin for sensory mechanisms underlying host detection in blood-sucking arthropods and convergent evolution.

Apart from host-seeking, host odours have other behavioural effects. Thus, it has been reported that natural host odours (e.g., those released by chicken feathers) stimulate oviposition in *R. prolixus* (Schilman et al., 1996; Guidobaldi and Guerenstein, 2015), likely serving to guide the search of oviposition sites (Guidobaldi and Guerenstein, 2015).

1.2.3. Detection of pheromones

1.2.3.1. Alarm pheromones

Immediately after mechanical disturbance, adult triatomines release a pungent odour that functions as an alarm pheromone (Barrett, 1976; Kälin and Barret, 1975; Ward, 1981; Manrique et al., 2006). This odour is a secretion produced from the paired Brindley's glands (Pattenden and Staddon, 1972), which are exclusively present in adult insects. The alarm pheromone is a complex mixture of volatiles that varies according to the species (Guerenstein and Guerin, 2004), without sexual differences in either gland contents or mixtures emitted (e.g., Cruz-López et al., 1995; Rojas et al., 2002; Guerenstein and Guerin, 2004; Manrique et al., 2006).

Isobutyric acid is the main component of the alarm pheromone in all studied species having functional Brindley's glands, although other short-chain carboxylic acids such as butyric and 2-methyl-butyric acid are normally present. These types of compounds are detected by OSNs in antennal grooved pegs (Guerenstein and Guerin, 2001; Diehl et al., 2003). Detection of alarm signals elicits avoidance behaviour (Barrett, 1976; Kälin and Barret, 1975; Schofield, 1979a; Rojas et al., 2002; Manrique, 2006; May-Concha et al., 2015). However, attraction to low concentrations of isobutyric acid (a compound also found in vertebrate odour, e.g., Guerenstein and Guerin, 2001) as well as to other short-chain carboxylic acids has also been reported (Schofield, 1979b; Guerenstein and Guerin, 2001; Rojas et al., 2002). Thus, this single compound can evoke different behaviours depending on its concentration. How this information is encoded and processed along the olfactory pathway according to the behavioural context needs and merits further investigation.

1.2.3.2. Sex pheromones

In *R. prolixus*, the number of basiconic and trichoid sensilla increases after the moult to the adult stage (Catalá and Schofield, 1994), which suggests that these sensilla play a role in the detection of chemical signals relevant to adults, such as sex pheromone components.

In triatomines, evidence for the use of chemical substances mediating sexual communication includes ambiguous references in the literature (reviewed in Manrique and Lorenzo, 2012 and refs. therein). *R. prolixus* and *T. infestans* males aggregate around mating pairs, and this behaviour was shown to be mediated by chemical signals (Baldwin et al., 1971; Manrique and Lazzari, 1995; Pontes and Lorenzo, 2012). However, male aggregation might not be consistent across all triatomines (Pires et al. 2004). In *T. infestans*, EAG responses to odours emitted by mating couples pointed towards the presence of a detection system for sexual signals (de Brito Sánchez et al., 1995). Adult triatomines have a pair of metasternal glands (MGs) for which no clear role was known until ten years ago (Cruz-López et al., 1995; Rossiter and Staddon, 1983; Schofield and Upton, 1978). The main secretion of the MGs of *T. infestans* was identified as 3-pentanone, which was also detected during mating (Manrique et al., 2006). Mostly short chain aliphatic ketones and alcohols were identified in the MGs of *R. prolixus* and other species within the subfamily (Manrique and Lorenzo, 2012). The female MG secretion was necessary and sufficient to increase male take-off frequency and to promote male orientation and mating also within other triatomines (Crespo and Manrique, 2007; Pontes et al., 2008; Vitta et al., 2009; Zacharias et al., 2010; May-Concha et al. 2013). Furthermore, Vitta et al. (2009) showed that several compounds emitted by the MGs of female *T. brasiliensis* were electrophysiologically active. Besides, dioxolanes have been reported to be only produced by the MGs of adults belonging to the genus *Triatoma* (Unelius et al 2010; Bohman et al., 2011). Ongoing SSR studies suggest that OSNs housed in male basiconic sensilla are sensitive to several compounds produced by the MGs of *R. prolixus* (Björn Bohman, pers. com.). Thus, further investigations are needed to uncover sexually active compounds and their cognate OSNs. While the chemical identity of all triatomine sex pheromones is not completely elucidated, it is likely that different species are attracted to blends that differ in either chemical composition or proportion, particularly in the case of species which are sympatric or co-domiciliated.

The recent publication of the genome of this species (Mesquita et al., 2015) reported the identification of 111 ORs and 33 IRs that include the probable molecular bases of triatomine olfaction (see section 6). Therefore, a broad repertoire of triatomine receptors may be potentially involved in the detection of these sexually-related gland secretions mixtures.

1.2.3.3. Aggregation pheromones

Triatomines spend most of their life hidden inside dark and narrow shelters. Bug aggregations inside those micro-environments are induced by signals emitted by their faeces and by cuticular components left behind on the shelter substrate. The use of aggregation signals by triatomines has been thoroughly proven for many species in several genera (Cruz-López et al., 1993; Lorenzo Figueiras and Lazzari, 1998a, 2002;

Mota et al., 2014). Moreover, inter-specific responses induced by faeces from different species are well documented within the subfamily (Lorenzo Figueiras and Lazzari, 1998b, 2002; Pires et al., 2002).

While most aggregation components reported are polar, their chemical identity and behavioural activity often differed across species. The faeces of *T. infestans* emit low quantities of quinazolines, acetophenone and other components, although no aggregation effects were observed when these compounds were used in behavioural assays (Cruz-López et al., 1995). Ammonia has also been reported in triatomine faeces and it induced positive anemotaxis (Taneja and Guerin, 1997), but this emission was likely an artefact produced by the humidification of collected material. Volatile compounds emitted by faeces of several triatomine species (*T. infestans*, *T. brasiliensis* and *Panstrongylus megistus*) are able to recruit bugs into shelters, mimicking aggregation effect of faeces (Mota et al., 2014). These compounds include highly volatile substances which are common by-products of microbial metabolism, such as short-chain fatty acids, a diol, and an amide. As mentioned above, *T. infestans* grooved peg OSNs can detect short chain fatty acids (Diehl et al., 2003). It is thus possible that these sensilla play a role in the detection of aggregation signals in this species.

2. The taste sense

The taste sense (or contact chemoreception) is involved in the detection of non-volatile chemicals mediating important processes such as food ingestion. Feeding decisions often occur after a brief gustatory evaluation of a potential food source. The gustatory system of many animals is able to detect the presence of phagostimulant substances that signal nutritious food and promote feeding, while some bitter substances, such as toxins and poisonous compounds, elicit rejection or aversive behaviours (Chapman, 2003; Dethier, 1976). Besides its relevance for recognizing appropriate food sources, taste also plays an important role in mediating insect communication. Mate recognition, aggression and assembling are often mediated, at least in part, by contact with non-volatile compounds present in the cuticle of insects or in the surrounding substrate.

2.1. Contact chemosensory structures

Gustatory sensory neurons (GSNs) can detect solid or liquid stimuli. GSNs are housed in contact chemosensory sensilla, which depending on the insect species, can be found on the antenna, mouthparts, pharynx, legs, wings, and sometimes on the female ovipositor (Liman et al., 2014). The taste sensilla or so-called gustatory sensilla, are different from olfactory sensilla in that they bear a single apical pore through which stimuli penetrate.

In triatomines, the presence of uniporous sensilla in the antenna of *R. prolixus* and *T. infestans* was reported more than 40 years ago (Bernard, 1974; Gracco and Catalá, 2000; Insausti et al., 1999). Scanning electron microscopy examination of the antennae of *R. prolixus* allowed differentiating four 70-80 µm long uniporous sensilla (called chaetic sensilla) at the tip of the distal flagellomere (Figure 2A).

The tarsi and tibia of *R. prolixus*, as those of other insects, also have uniporous sensilla (Figure 2E, F, G). Differently from antennal sensilla, these have a flexible socket at the

base and are about 20 μm long (Figure 2F). Up to date there is no evidence of the presence of gustatory sensilla in the maxillae and mandibles of triatomines, as only mechanoreceptive structures have been reported in these mouthparts (Bernard, 1974; Pinet, 1968).

Uniporous sensilla are also found in internal structures, such as the walls of the food canal (Figure 2B). In *R. prolixus* and *T. infestans* a group of 8-11 uniporous sensilla were described in the anterior region of the epipharynx (Bernard, 1974; Kraus, 1957; Pontes et al., 2014), but their shape is quite different in both species (Figure 2C, D). *R. prolixus* epipharyngeal sensilla (Figure 2C) are 2 μm pegs inside a 2 μm -diameter pit and have a circular apical pore. In *T. infestans*, however, the pegs are 1.5 μm -diameter semicircular domes, 1.2 μm in height, with a single narrow opening that extends along the dome (Figure 2D).

2.2. Sensory aspects of taste

In general, each GSN responds to a single taste quality (e.g. bitter, sweet, water, salts) (Liman et al., 2014). Discrimination between taste qualities requires specific sets of GSNs expressing different proteins such as gustatory receptors (GRs), ionotropic receptors (IRs) and OBPs (see section 6.1) (Freeman and Dahanukar, 2015). Binding of chemicals to one or several of these proteins elicits an electrophysiological response in GSNs (Figure 2H). The axons of GSNs terminate in the brain primary gustatory centre (the suboesophageal ganglion, Figure 1G) and the ventral nerve cord (Isono and Morita, 2010).

In triatomines, behavioural and electrophysiological studies revealed that antennal taste sensilla are involved in host recognition and participate in intraspecific communication, whereas epipharyngeal sensilla inform the brain about the quality of ingested food (Bernard, 1974; Lorenzo Figueiras and Lazzari, 1998b; Pontes et al., 2014).

2.2.1. Substrate quality recognition

After finding a host, the insect walks over the host skin and decides whether to pierce it or not. The role of antennal taste receptors in feeding initiation was recently studied in experiments which offered an appetitive solution through a latex membrane impregnated with bitter compounds such quinine and caffeine (Pontes et al., 2014). Such compounds are known to inhibit feeding in other insects, via specialized GSNs (e.g. Chapman, 2003; Glendinning et al., 1999; Meunier et al., 2003; Schoonhoven and Van Loon, 2002; Weiss et al., 2011). Contact with membranes embedded with quinine or caffeine inhibited feeding, while the presence of phagoestimulants in the membrane was not necessary for feeding initiation (Pontes et al., 2014). Feeding inhibition was not observed when only the distal flagellomere of each antenna was ablated, indicating that these bitter compounds are detected by gustatory sensilla within this antennal segment (Pontes et al., 2014). Corresponding electrophysiological recordings showed that the four chaetic hairs located in the distal flagellomere (Figure 2A) respond to quinine and caffeine (Pontes et al., 2014). *R. prolixus* GSNs within these sensilla likely detect salts (Figure 2H), as stimulation with NaCl, KCl, and LiCl elicits dose-dependent

spiking activity (Barrozo, pers. comm.). Further studies are needed to determine the number of GSNs inside these sensilla and to investigate the spectrum of taste modalities triatomines can detect.

2.2.2. Food quality recognition

When triatomines bite they take a sip of blood which bathes epipharyngeal sensilla, which further evaluate food quality (Bernard, 1974). If the sampled fluid fulfils quality requirements the insect feeds a much larger volume but if not, it leaves the host and starts a new search (Smith and Friend, 1970).

The hedonic value of different tastants has been studied in triatomines using artificial feeders which allow manipulating the composition of the feeding solution (Friend, 1965; Guerenstein and Nuñez, 1994; Núñez and Lazzari, 1990; Pontes et al., 2014). The identification of phagostimulants, molecules that trigger gorging, has been reported for different blood-feeding insects (see Friend and Smith, 1977). In most cases the presence of substances such as purinergic nucleotides, NaCl, KCl, NaHCO₃, albumin, reduced glutathione, glucose and sucrose seemed to be critical for food acceptance. Adenosine triphosphate (ATP) is the main phagostimulant for *R. prolixus* (Friend, 1965); other nucleoside phosphates (i.e. ADP, CTP, GTP, CDP, ITP, 3'5'-cyclic AMP, IDP, GDP, and AMP) can elicit gorging in *R. prolixus* but with a considerably lower potency (Friend, 1965; Friend and Smith, 1971). In *R. prolixus* the absence of ATP in the food prevents engorgement (Friend and Smith, 1971). However, when insects are severely starved, they can feed on saline solutions lacking ATP (Guerenstein and Nuñez, 1994). ATP is found inside erythrocytes at a concentration of about 1 mM, but it is still unclear how intracellular ATP reaches the epipharyngeal sensilla triggering detection and feeding (Smith, 1979).

Salts are also crucial and necessary food components controlling feeding. Appetitive and aversive behaviours can be elicited by salt detection in both mammals and insects (Liman et al., 2014, refs. therein). Low-salt food content is appetitive for most animals, while high-salt levels are mostly rejected. Salts are main constituents of the vertebrate blood, and its ionic composition also seems to be a key factor regulating triatomine feeding, as for other blood feeders (Galun, 1967; Guerenstein and Nuñez, 1994). The optimal appetitive salt (NaCl or KCl) concentration for *R. prolixus* is around 0.15 M as long as ATP is present (Friend, 1965). Not surprisingly, this salt concentration has an osmolarity equivalent to that of the vertebrate plasma. Insects can even feed on NaBr, KBr, NaI and KI solutions containing ATP, although with a lower level of acceptance in comparison to NaCl solutions, but CaCl₂ and MgCl₂ solutions can inhibit feeding entirely (Friend and Smith, 1977). The gustatory system of *R. prolixus* thus seems to be finely tuned to salt detection and this tuning seems to control feeding decisions.

Besides ATP and salts, bitter compounds can also modulate ingestion in *R. prolixus*. Smith and Friend (1972) found that when adding the bitter compound theophylline to an appetitive solution increased the sensitivity to the phagostimulant ATP; similar effects were also proposed for other bitter substances such as caffeine. Indeed recent findings showed that the individual addition of the phenolic glycoside salicin, or an alkaloid compound such as caffeine, quinine or berberine to an appetitive solution dramatically decreased ingestion in a dose-dependent manner, eventually inhibiting

feeding altogether (Pontes et al., 2014). Bitter detection is known to modulate feeding in many different animals, including humans (Yarmolinsky et al., 2009). The fine and highly sensitive detection of bitter substances by *R. prolixus* is quite surprising, though. Mostly, phytophagous insects have GSNs dedicated to detect plant toxins that often taste bitter.

Why are bitter receptors present in a blood-sucking insect? *R. prolixus* larvae, males and females feed on the blood of vertebrates, a medium that intrinsically lacks caffeine, quinine, berberine or salicin. However, if these compounds are ingested by hosts, they can become an active part of their blood. Besides, triatomines insects evolved from predatory ancestors, in which the adaptive pressure to sense bitter compounds was probably high. In fact, triatomines can feed on hemolymph of arthropods if starved (Pontes et al., 2011). Thus, it is likely that triatomines retained this capability from their ancestors. Finally, recent findings suggested that *R. prolixus* can feed on plants similarly to what happens in other blood feeders such as mosquitoes (Díaz-Albiter et al., 2016). Mosquitoes, which feed on plants (males and females) but also on vertebrate blood (only females), also have neuronal and behavioural responses to quinine (Ignell et al., 2010; Kessler et al., 2013; Sanford et al., 2013). Therefore, bitter detection in *R. prolixus* could be simply interpreted as a mechanism that aids insects in the eventual detection of noxious substances, whether from plants, vertebrate blood, or other arthropods.

2.2.3. Intraspecific signals

As described above, triatomines exhibit a characteristic aggregation behaviour elicited by two different chemical signals (Lorenzo Figueiras et al., 1994; Lorenzo Figueiras and Lazzari, 1998a,b; Mota et al., 2014; Pires et al., 2002). Faeces deposited near or around refuge entrances serve to chemically mark these shelters (Lorenzo and Lazzari, 1996; Falvo et al., 2015). These volatile signals also attract conspecifics to the shelters and once inside, cuticular components induce arrestment on impregnated substrates via contact chemoreception (Lorenzo Figueiras and Lazzari, 1998b, Pires et al., 2002, Vitta et al., 2002). These cuticular taste signals can promote body-to-body contact, or can even remain on places previously occupied by conspecifics, in which case they are called footprints (Lorenzo Figueiras et al., 2009). The cuticular lipids of triatomines are a complex mixture of long-chain hydrocarbons, n-alkanes, branched alkanes and fatty acids (Juarez et al., 2001; Juarez and Fernandez, 2007). Behavioural experiments showed that hexane cuticular extracts produce arrestment responses similar to those elicited by footprints (Lorenzo Figueiras and Lazzari, 1998b). Octadecanoic acid (C18:0) and hexacosanoic acid (C26:0) were identified as cuticular components with a potent assembling effect in *T. infestans* (Lorenzo Figueiras et al., 2009).

Cuticular hydrocarbons also play a role in promoting sexual recognition in triatomines (Cocchiararo-Bastias et al., 2011). Behavioural experiments showed that *T. infestans* males can copulate with freeze-killed females (i.e. chemically intact), but they fail to do so if these females were previously washed with hexane. Moreover, mating can be triggered if washed freeze-killed females are impregnated with cuticular female extracts (Cocchiararo-Bastias et al., 2011). Therefore, contact pheromones mediate mate recognition in *T. infestans* and likely in other triatomine species. A female-specific fatty alcohol seems to be responsible for eliciting this behaviour (Cocchiararo-Bastias et al.,

2011). The identity and location of the taste sensilla involved in the detection of these signals still needs to be addressed.

3. The thermal sense

The thermal sense is regarded as one of the most sensitive sensory systems, allowing animals to detect subtle changes in temperature that are relevant for finding food sources and controlling their metabolism (Dusenbery, 1988). In haematophagous insects, it has been long recognized that the heat emitted by warm-blooded vertebrates is a primary guiding cue (Lehane, 2005; Lazzari, 2009).

The detection of heat necessarily involves the exchange of thermal energy between thermoreceptors and the target through mechanisms such as conduction, convection, and infrared (IR) radiation. In insects, heat exchange is facilitated by their small size and the thin cuticular layer that covers the sensory structures housing thermoreceptor cells, as a smaller receptor mass implies that less energy needs to be absorbed to increase its temperature (Dusenbery, 1988). In particular, the perception of IR has only been observed in a few animal groups, including insects within the Coleoptera and the Hemiptera. Triatomines show astonishing heat sensitivity and are capable of sensing IR radiation (Lazzari, 2009). This capability allows bugs to recognize a potential host and estimate its distance based solely on thermal information without being affected by factors such as wind, which can disrupt conduction gradients and convective currents (Flores and Lazzari, 1996; Lazzari and Nuñez, 1989; Schmitz et al., 2000; Lazzari, 2009). Triatomines use thermal information not only to find their hosts, but also for choosing resting places (Lorenzo and Lazzari, 1999), synchronize their circadian system (Lazzari, 1992), and locate blood-vessels hidden under the skin of vertebrate hosts (Ferreira et al., 2007). Surprisingly, thermal information does not play a fundamental role in blood recognition, as bugs are able to ingest blood at 3°C (Lazzari and Nuñez, 1989).

3.1. Thermo-sensitive structures

Wigglesworth and Gillet (1934) were the first to propose that the antennae of *R. prolixus* were the chief sensory organs for heat detection. Triatomines have three types of antennal sensory structures associated with this process: the coeloconic sensilla, the tapered hairs, and the cave organ (Barth, 1952). All of these structures lack cuticular pores and are less abundant than the chemosensory structures (Altner and Loftus, 1985).

Coeloconic sensilla were the first sensory structures proposed to mediate heat detection in triatomines (Bernard, 1974; Mclver and Siemicki, 1985). In *T. infestans* coeloconic sensilla can be found along the antennae, but also on the legs and different body parts, except on the proboscis (Bernard, 1974; Ferreira et al., 2007). In *R. prolixus*, they were only observed along the proximal flagellomere (Mclver and Siemicki, 1985). Externally, each sensillum is a short rounded peg inside a 2 µm deep and 6 µm diameter pit. In *R. prolixus* there are about five to eight coeloconic sensilla per antenna (Mclver and Siemicki, 1985; Zopf et al., 2014a), each containing three neurons with unbranched dendrites (Mclver and Siemicki, 1985). The dendrites of two

of these neurons extend into the peg completely filling its lumen and are in close contact with each other, while the dendrites of the third neuron are shorter and end at the base of the dendritic processes of the other two neurons (McIver and Siemicki, 1985).

The second type of sensilla housing thermoreceptive cells are the tapered hairs (Zopf et al., 2014a). There are about six to eight of these hairs in each antenna (each 14 μm long and 1.7 μm in diameter), extending close and parallel to the antennal surface between the pedicellum and the proximal flagellomere (Zopf et al., 2014a). The third type of antennal structure with a thermoreceptive function is the cave organ, located in the antennal pedicel (Barth, 1952; Lazzari and Wicklein, 1994). This structure consists of an internal cuticular invagination which terminates inside an ellipsoidal cavity. The cuticle is highly folded at the opening and extends internally through a 90 μm long channel ending in a cavity covered by numerous hairs of variable length (Barth, 1952; Catalá, 1994; Lazzari and Wicklein, 1994).

3.2. Thermal detection

The thermoreceptive cells are usually present together with other sensory neurons (commonly hygrometers) in single sensilla (Altner and Loftus, 1985; Steinbrecht, 1998). The usual pattern is a triad of sensory cells of different modalities that either respond to temperature decreases, dryness, or wetness. In addition, sensilla containing just one warm and one cold receptor cell have been reported (Altner and Loftus, 1985; Gingl and Tichy, 2001).

In *T. infestans* there are three types of neurons which show distinct responses to changes in temperature and humidity (Bernard, 1974). Recordings from coeloconic sensilla showed that two of these cells respectively increase or decrease activity with humidity changes, while the third one gradually increases activity with decreases in temperature (Bernard, 1974). Heat and humidity produced by the skin or the breath of warm-blooded animals are attractive to triatomines. The ability to detect humid sources was first examined in *R. prolixus* by Wigglesworth and Gillett (1934). These insects can locate and orient towards humid sources at room temperature, and moist heat sources can be detected from a greater distance than dry ones (Barrozo et al., 2003). Relative humidity facilitates heat exchange between the emitter (e.g. a host) and the thermoreceptors, since humid air has a higher thermal conductivity and heat capacity than dry air. Thermal responses can also be enhanced by bimodal convergence of peripheral thermosensitive and hygrosensitive inputs in the central nervous system (Lazzari, 2009).

Recently, Zopf et al. (2014a, b) reported the existence of a warm and a cold cell within coeloconic sensilla and tapered hairs of *R. prolixus*. In addition, they found that a third neuron within these sensilla responds to increases in humidity (Zopf et al., 2014a), which is consistent with the previous identification of three neurons in the coeloconic sensilla of this species (McIver and Siemicki, 1985). Both cold and warm receptor cells show stable, non-adapting, spiking activity in response to constant intensity stimuli (Zopf et al., 2014a). This was also observed in the cold, dry and moist cells of *T. infestans* (Bernard, 1974). In *R. prolixus*, the firing rate of the warm cells increased in response to air-temperature increases, while the opposite was observed in cold cells

(Zopf et al., 2014a). Thermoreceptors within both coeloconic and tapered-hairs not only responded to warm airstreams but also to IR pulses in still air (Zopf et al., 2014a). The activity of coelonic thermoreceptors in response to these stimuli was always higher than that of tapered-hairs thermoreceptors. However, when tapered-hair cells were simultaneously stimulated with moving air at different temperatures and IR pulses, they had a stronger response than coeloconic sensilla cells (Zopf et al., 2014a). These experiments thus suggest that bugs use mechanical flow information to discriminate between convective and radiant heat (Zopf et al., 2014a). This idea is also supported by the fact that a single sensillum houses two thermal receptors along a pair of mechanoreceptors (Altner and Loftus, 1985).

During host search insects are exposed to oscillating, rather than discrete, pulses of both temperature and IR, information that can inform the insects whether they are moving towards or against a warm object. Indeed, slow oscillating temperature changes (i.e. oscillation period of 600 s duration) have stronger effects on the activity of tapered-hair cells than on the activity of coeloconic thermoreceptors, while the opposite was observed in response to variations in IR (Zopf et al., 2014b). These findings suggest the presence of a combinatorial mechanism for the coding of thermal stimuli, in which the activities of those two types of thermoreceptors (tuned to air-temperature and IR, respectively) is compared and further integrated with information from antennal mechanosensory receptors (Zopf et al., 2014a, b).

Here we have only reviewed aspects of heat detection in triatomines. In mosquitoes, recent experiments which analyzed the sequential contribution of vision, CO₂ and odours in host search and finding showed that heat is an important cue mediating host landing. Also, it has been shown in mosquitoes that the cation channel TRPA1 mediates host-selective thermotaxis. In *R. prolixus*, experimental evidence indicates that a TRP channel expressed in the antennae and in other body parts is similarly involved in heat detection (Zermoglio et al. 2015). At a more central level, recent findings in the fruitfly *Drosophila melanogaster*, which were not discussed here, uncovered how signals about heat and cold stimuli are represented by neurons in higher brain centers (Liu et al. 2015; Frank et al., 2015). Details about the molecules, receptors and cell circuits mediating thermosensation in this fly, which might also underlie these processes in triatomines, are reviewed elsewhere (Barbagallo and Garrity, 2015).

4. The visual system

Despite the major role that the chemical and thermal senses play in mediating responses towards hosts, conspecifics and mates (see sections 1-3), visually-guided behaviours are very important for triatomine insects. The visual system of triatomines is well developed and adapted to a crepuscular/nocturnal lifestyle, but is also capable of rapidly adjusting to changes in environmental light conditions and of mediating rapid escape responses (Insausti and Lazzari 2002; Lazzari and Varjú 1990; Lazzari, et al., 2011; Reisenman et al., 1998; 2002). These are particularly important features in an insect which spends daytime hours hidden in dark refuges and for which hosts are also potential predators.

4.1. The visual structures

4.1.1. The compound eyes

As in all insects, the compound eyes are the main visual organs of triatomines, but adult insects also have a pair of simple camera-type eyes, the ocelli (Figure 3A-B) (Insausti and Lazzari, 2002). Compound eyes are composed of many ommatidia whose number and size increase with age, without sexual dimorphism (Settembrini, 1984). As in most insects, each ommatidia is composed of a light-gathering structure (cornea and crystalline cone), primary and secondary pigment cells, and eight photoreceptor (or retinula) cells, whose microvilli form a rhabdom where photon-absorbing visual pigments are arranged (Chapman, 1998) (Figure 3C). The axons of the retinula cells pass the basal lamina and reach the large optic lobes, which as the rest of the brain are displaced to the back of the head (Insausti, 1994). As in other insects, the optic lobes are composed of three distinct neuropils, the lamina, the medula and the lobula (Figure 3E-F).

Triatomine insects have apposition compound eyes (Muller, 1970; Reisenman et al., 2002), that is, the rhabdom extends the full length of the retinula cells between the crystalline cone and the basal lamina. Thus, each ommatidium receives light only through its own facet due to the presence of pigment cells that optically isolate ommatidia from each other (Chapman, 1998). The rhabdom of triatomines, rather than being fused as in many insects with apposition compound eyes, is open, with a ring of six rhabdomeres from retinula cells 1-6 surrounding a central pair of rhabdomeres from retinula cells 8-9 (Müller, 1970; Reisenman et al., 2002) (Figure 3C). This open rhabdom arrangement is also found in other Hemiptera, flies, and some beetles (Land et al., 1999). Several morphological changes allow triatomine compound eyes to function under a wide range of environmental light intensities (Figure 5). For instance, screening pigments within retinula and pigment cells form a “pupil” which regulates the amount of light reaching and travelling within the rhabdom (Reisenman et al., 2002). During the day the pigments form a small “pupil” and light can only reach the central rhabdomere (Figure 3C), while at night the pigments disperse and therefore the pupil widens (but the spatial resolution is decreased, see below), allowing light to reach all the rhabdomeres. In addition, light capture is further increased at night as the distance between the cornea and the rhabdom decreases. Interestingly, these rhythmic changes are under the control of an endogenous oscillator (Reisenman et al., 2002), which allows adjusting visual sensitivity in advance to the changes in environmental light conditions triatomines experience at sunrise/sunset (Figure 4C, 5).

The screening pigments accounting for the dynamic changes described above are of two kinds, a dark one (ommin) and a red one (xanthommatin); both kinds are found in primary pigment cells, while only the red one is found in retinula cells (Insausti et al., 2013). Besides their role in regulating light influx (Reisenman, et al., 2002), these screening pigments also protect sensory cells against the damage caused by both photo-oxidative stress and by the reactive oxygen species produced by blood digestion (Insausti et al., 2013).

As mentioned above, triatomines have apposition compound eyes. However, superposition compound eyes such as those found in moths are in general better suited than apposition eyes for a nocturnal lifestyle (Warrant, 1999; 2004). These eyes are highly sensitive as light can be collected through many facets (Chapman, 1998;

Greiner, 2006). Thus, crepuscular/nocturnal insects which retained apposition eyes (such as triatomines) have specializations that allow them to function under low light intensities, but at the expense of sacrificing spatial resolution. Such adaptations include a reduction in the number of facets, increments in facet diameter, changes in the curvature of facet lenses, and the movement of screening pigments described above (Greiner, 2006; Land et al., 1999; Reisenman et al., 2002). These and other dynamic changes (Reisenman et al., 2002) allow triatomine eyes to function also at the higher light intensities to which insects are exposed given their bi-modal activity pattern (Lazzari, 1992).

4.1.2. The ocelli and the ocellar system

Adult triatomines also have two well-developed ocelli located in an unusual position behind the compound eyes, looking dorsolaterally (Figure 3B), which develop gradually throughout the larval life (Insausti and Lazzari, 2000a,b). Their lenses are quite large (ca. 455 μm in diameter) but because their focal plane lies behind the retina, they cannot form focused images (Insausti and Lazzari, 2002). Interestingly, the ocelli of triatomines have several features resembling those of diurnal insects (Insausti and Lazzari, 2002; Lazzari et al., 2011), in which the function of ocelli is better understood (see Lazzari et al., 2011 for a discussion on this topic).

The ocellar corneagen and photoreceptor cells form a cup-like structure underneath the cuticular lens, and the photoreceptor axons extend into the ocellar neuropile making complex synapses (including feedback synapses) with a few thick interneurons which terminate in thoracic centers (Insausti and Lazzari 1996; Insausti and Lazzari 2002). Therefore, it is likely that the ocelli not only integrate visual information from large areas, but that are also involved in mediating fast (but unfocussed) responses related to orientation during walking and flight, as observed in other insects (Insausti and Lazzari, 1996; Insausti and Lazzari, 2002). The ocelli of triatomines, as their compound eyes, can also adapt to changes in environmental light conditions through longitudinal movements of screening pigments within the photoreceptor cells (Lazzari et al., 2011). This adaption, in contrast to that observed in the compound eyes (Reisenman et al., 2002), is a direct response to changes in light conditions, e.g. independent of the time of the day (Lazzari et al., 2011). It has been proposed that this direct response to light, together with their structural and functional features that are more typical of diurnal insects, serves the ocelli to function under varying light conditions (Lazzari et al., 2011).

In addition to these functional changes, the ocelli of triatomines have a narrow elongated pupil surrounded by a pigmentary iris. This pupil does not respond to illumination changes but rather increases in size (in correlation with the development of the cornea and the retina) after emergence to the adult stage reaching a maximum width at about three weeks of age (Insausti and Lazzari, 2000a,b). The function of the ocelli has been mostly associated with the control of flight in various insect species, but it is interesting that in triatomines –which can fly but mostly walk (see next)- these visual structures can modulate the photonegative behaviour independently of the compound eyes (Lazzari et al., 1998).

4.2. Spectral sensitivity

Although most insects species studied up to date have three spectral types of photoreceptors with peak sensitivities in the ultraviolet (ca. 350 nm), blue (ca. 440 nm) and green portion of the spectrum (ca. 530 nm) (Briscoe and Chittka, 2001; Stavenga and Arikawa, 2006), there is an increasing number of exceptions to this trichromatic condition (e.g. Arikawa et al., 1987; Wakakuwa et al., 2014; Futahashi et al., 2015). The recent finding that *R. prolixus* has three visual opsins highly similar to those found in other insects suggest that triatomines maybe trichromatic as well (Mesquita et al., 2015). Among Hemiptera, studies in aphids (which are phytophagous) demonstrated the existence of at least three photoreceptor types with sensitivities in the green (around 530 nm), blue-green (around 490 nm) and near UV (around 330-340 nm) regions of the spectrum (Kirchner et al., 2005; Farnier et al., 2014). However, it is not clear whether hemipteran insects have “true” color vision, that is, whether they can discriminate wavelengths independently of brightness (Menzel and Backhaus, 1991). With few photons available at night-time, monochromatic responses with optimally tuned visual pigments may be more useful for detecting shades and variations in light intensity.

In triatomines, behavioural studies have been useful to characterize compound eye spectral sensitivity, although none of these were designed to address whether triatomines have true colour vision. For instance, in the behavioural context of assembling, bugs respond differentially to broad-band stimuli of equal intensity in the blue, green and red regions of the spectrum (Reisenman et al., 2000). Furthermore, these spectral lights differentially modulate the response of insects to aggregation pheromones (for instance, triatomines avoid green light regardless of the presence or absence of an aggregation pheromone), indicating that visual and olfactory cues - at least in this behavioural context - interact in a spectrum-dependent manner (Reisenman et al., 2000). Thus, while these results demonstrate that insects can discriminate between spectral qualities, they do not address whether chromatic or achromatic mechanisms are involved (Reisenman et al., 2000). Other studies took advantage of the aforementioned characteristic photonegative reaction of these insects to measure their spectral sensitivity (Ward and Finlayson, 1982; Reisenman and Lazzari, 2006). Using monochromatic lights, Reisenman and Lazzari (2006) found that triatomines respond to light wavelengths ranging from near UV (ca. 357 nm) to far red (665-695), a result which agrees with findings in other nocturnal insects (Schlecht 1979; White et al. 1994). It has been shown that this visual response to far red stimuli is certainly not mediated by infrared receptors (Reisenman et al. 1998). The lower –but significant- visual response of triatomines to red light is likely accomplished through a monochromatic mechanism involving intensity-dependent stimulation of green photoreceptors (Reisenman and Lazzari, 2006), as reported for other Hemipterans (Farnier et al., 2014). The spectral sensitivity of the ocelli remains to be investigated, but in other insects these visual structures are maximally sensitive to green and UV light (Eaton, 1976; Lall and Ovid Trouth, 1989; Hung et al., 2013), which relates to their proposed role in flight control.

Studies of visual processing at many levels of the visual sensory pathway have been conducted in other insects such as flies, honeybees and dragonflies. The wealth of information available allows us a better understanding about how insects process visual information at many levels of the visual pathway. No such studies have been

conducted in kissing bugs, but population recordings from retinula cells (electroretinogram, ERG) in triatomines show responses consisting of three characteristic components (on-transient, sustained component, and off-transient, Figure 3D) described in other insects such as cockroaches, locusts and bees (Yinon, 1970; Colwell and Page 1989). Thus, for instance, the analysis of sustained component, which arises from the depolarization of retinula cells (Colwell and Page, 1989), could be used for examining several properties of the triatomine primary visual neurons, including spectral sensitivity, thresholds, eye regionalization and modulatory effects.

4.3. Visually guided behaviours

Although triatomines mostly walk, it has been long known that these insects, attracted by light sources (e.g. porch and patio lights, or lights shining through windows), disperse by flying during sunset under permissive temperatures (Vazquez-Prokopec et al., 2004; Jácome-Pinilla et al., 2015). Dispersal flights allow females to colonize new habitats, and starvation appears to be the primary stimulus that initiates flight (Lehane et al., 1992). This attraction to light sources has been exploited to trap and/or sample dispersing triatomines in the field (Vallvé et al., 1988; Noireau et al., 2000; Vazquez-Prokopec et al., 2004; Carbajal de la Fuente et al., 2007). In laboratory experiments, in which confounding factors present in the field could be controlled, Minoli and Lazzari, (2006) reported that *T. infestans* and *R. prolixus* show oriented take-off responses to white light, while *R. prolixus* also respond to UV light. This has important implications for house colonization, as true attraction to light ensures that a large majority of the flying insects reach human houses (Minoli and Lazzari, 2006; Lazzari et al., 2013). Attraction to light in dispersing triatomines may seem confounding, given their characteristic photonegative behaviour (Ward and Finlayson, 1982; Lazzari et al., 1998; Reisenman et al., 1998; Reisenman and Lazzari, 2006). Thus, triatomines have both, positive and negative oriented responses to light, and this might depend on factors such as the type of locomotion involved (flight vs. walk), the starvation status, and the stimulus pattern (i.e. a punctual light source such as a house light versus a fully illuminated environment), although these possibilities remain to be investigated (Lazzari et al., 2013).

As mentioned in the previous paragraph, *T. infestans* show a strong, stereotypic, low-threshold, light-intensity and age-dependent photonegative reaction to light (Ward and Finlayson, 1982; Reisenman et al., 1998) (Figure 5). This reaction to light might have a highly adaptive value, as it allows insects to find refuges and avoid illuminated environments where they can be exposed to predators. Accordingly, bug photonegative behaviour is much stronger at nighttime (Figure 4C), when insects are active, and is under the control of a circadian oscillator (Reisenman et al., 1998). Concomitantly with this temporal modulation of visual behaviour, a circadian rhythm in retinal morphology which increases visual sensitivity at night-time has been described in *T. infestans* (Reisenman et al., 1998; 2002). Whether further temporal modulations also happen at downstream levels of the visual sensory pathway has not been investigated.

Another behaviourally relevant visual reaction of triatomines is their response to contrasting moving objects. Bugs turn away from such objects with an escape-like response, but keep these objects in their lateral field of view (Lazzari and Varjú, 1990).

This so-called lateral fixation response is mediated by the dorsal part of the compound eyes, and provides a mechanism by which insects can keep their host/predator in sight to either approach or rapidly escape (Lazzari and Varjú, 1990).

5. Mechanoreception

Triatomine mechanosensation has been largely neglected, despite abundant evidence showing that mechanosensory responses mediate different aspects of their biology. Almost 70 years ago Autrum and Schneider (1948) showed that triatomines are sensitive to substrate vibration despite not having true subgenual organs. Vibratory communication and the role of triatomine stridulation have been analysed by different authors (Schilman et al., 2001) and reviewed in detail by Lazzari et al. (2006). Triatomines produce vibratory signals (through stridulation) during mating and under perturbation, which are propagated as mechanical waves through the insect body and the substrate. These signals are probably perceived through sensory organs located in the legs, but this remains to be investigated.

Mechanoreceptors also play a role in orientation towards odour-laden currents. In triatomines, anemotactic responses triggered by such stimuli are probably mediated by trichobotria (Lent and Wigodzinsky, 1976), proprioceptors located at each junction of the antennal articles (Insausti et al., 1999), and by the Johnston organ (Barth, 1953).

Mechanoreceptors also mediate feeding and participate in food texture evaluation. The texture properties of the substrate, evaluated by mechanosensory receptors located in the proboscis, are relevant to induce bug probing after biting warm objects (Ferreira et al., 2011). Two other mechanosensory functions associated to their haematophagous habits have been described in triatomines. Barth (1953) described a scolopidial organ in the distal part of the pedicel that would be connected to the base of the mandibles and would control stylet penetration into the host skin. In addition, a group of mechanosensory cells are associated to the distal region of the mandibles, which respond to the deflection of the stylets and could also be implicated in the penetration of stylets in their way to a blood vessel (Pinet, 1963; Bernard, 1974).

6. Molecular bases of sensory function

The molecular bases for the detection of stimuli can now be uncovered by means of functional genetics due to both, the availability of the genome sequence of *R. prolixus* and diverse techniques including genome editing tools. A brief review of the initial stage of the molecular genetics of *R. prolixus* behaviour is presented below.

6.1 Triatomine gene families related to sensory systems

There is a consensus that insect olfactory cues are detected by two different families of OSN membrane proteins: the odorant (OR) and ionotropic (IR) receptors. Therefore, it is relevant to establish roles for the diverse triatomine olfactory receptors (Mesquita et al., 2015). It is known that these insects have different responses to host odours (including CO₂) and heat depending on their age and nutritional status. Recently, a

molecular parallel to these changes has been established for the expression of olfactory co-receptors at the peripheral level in *R. prolixus* (Figure 4D), suggesting that this behavioural plasticity is based, at least partially, on changes in OSN receptor abundance (Latorre-Estivalis et al., 2015). In this way, OSNs would act as filters depending on the internal state of the insects.

The expression of several ORs and IRs has been confirmed, not only for the antennae of *R. prolixus*, but also for other tissues such as the rostrum, the tarsi and the genitalia (Latorre-Estivalis et al., 2016). Whether this indicates an olfactory role for these structures needs to be addressed. On the other hand, this expression pattern may suggest roles other than olfactory for some of these receptors.

Considering that more than 140 olfactory genes described in the genome of this species can potentially be de-orphanized, a complex scenario can be foreseen. The connection between olfactory genes and the plethora of known triatomine semiochemicals will require a multidisciplinary approach combining bioassays with molecular biology studies. It is highly desirable to determine which receptors mediate the detection of host odours, aggregation, sex and alarm pheromones, as these represent the molecular basis for such critical behavioural processes in these insects.

No orthologues of the *D. melanogaster* CO₂ receptors have been found in the genome of *R. prolixus*, as is the case for many other insect orders (Robertson and Kent, 2009). Nevertheless, as discussed previously (section 1.2.1), these bugs possess an exquisite ability to detect CO₂ and orient to airstreams presenting increased levels of this compound (Barrozo and Lazzari, 2004; Barrozo et al., 2004; Bodin et al., 2008; Taneja and Guerin, 1995). Therefore, the lack of orthologues of known CO₂ receptors suggests that other sensory proteins mediate CO₂ detection in triatomines.

It has been recently shown that *CIOR1* and *CIOR2* mediate the detection of nonanal and decanal, respectively, in *Cimex lectularius* (Liu and Liu, 2015). Interestingly, the fact that the latter has been phylogenetically related to *RproOR105* by the authors might suggest that aldehydes may be the cognate ligands, a hypothesis that deserves future evaluation.

Insects detect heat through sensory neurons expressing receptors belonging to the transient receptor potential channel (TRP) family (Montell et al., 2005), but a member of the gustatory receptor family is also involved in mediating rapid warmth avoidance in fruitflies (Ni et al., 2013). In mammals, the gene TRPV1 is responsible for the detection of noxious heat (>43 °) and capsaicin, an active constituent of chilli peppers (Palkar et al., 2015; Vriens et al., 2014). Therefore, capsaicin is normally used in experiments to determine if TRPV orthologues are involved in the detection of heat by a given species. Orthologues of this gene have been described in insects and the gene *Rprolav* has been implicated in the detection of heat in *R. prolixus* (Zermoglio et al., 2015). Other potential heat receptors, like the *R. prolixus* orthologue of TRPA1, might also be involved in warmth detection as shown for mosquitoes and fruitflies (Corfas and Vosshall, 2015; Hamada et al., 2008; McMeniman et al., 2014). Two other *Drosophila* TRPs, named *water witch* and *nanchung*, have been shown to mediate the detection of moist and dry air, respectively (Liu et al., 2007).

Insect vision is mediated by proteins of the opsin family (Arikawa and Stavenga, 2014). Insect opsins detect different wavelengths depending on their sequence, the most

common ones being quite conserved along the insect clade (Henze et al., 2012). Four opsins have been found in the genome of *R. prolixus* (Mesquita et al., 2015), including orthologues of ultraviolet, green and blue photoreceptors (which supports behavioural findings on the spectral sensitivity of these insects, section 4.2), and a pteropsin orthologue, a gene potentially related to circadian function (Velarde et al., 2005).

As described in the previous sections, the thermal, chemosensory and vision senses have relevant roles in the life of triatomines, but in all these cases the underlying molecular bases need deeper understanding and merit further studies.

6.2 Triatomine sensory information detection in the post-genomics era

The publication of the genome sequence of *R. prolixus* allowed the description of diverse gene families encoding sensory proteins. Apart from OBPs, ODEs, CSPs, ORs, IRs and TRPs, opsins, pickpocket receptors (ppks) and SNMP families have been reported. This wealth of information opened a new avenue for research on triatomine sensory physiology. The antennal expression of most of these genes has been recently confirmed with quantitative antennal transcriptomes from larvae, males and females (Latorre-Estivalis et al., unpublished results), reinforcing, as expected, that these appendages are multimodal sensory structures. One of the main features shown by such mass sequencing procedure was a dramatic increase in OR gene expression in the antennae of adult bugs when compared to those of larvae (Latorre-Estivalis et al., unpublished results). The de-orphanization of many of these genes is necessary to understand the molecular bases of well-known triatomine behaviours, knowledge that can help the development of new rational tools for the sustainable control of these epidemiologically important insects. The emergence of triatomine populations resistant to insecticides in diverse parts of Latin America (Cortez et al., 2008; Toloza et al., 2008; Vassena et al., 2000) stresses such an urgent need.

7. Concluding remarks

From a purely biological point of view, triatomines are an interesting group of insects given their lifestyle and habits, and have inspired seminal studies of insect physiology and function pioneered by Vincent B. Wigglesworth. An in-depth knowledge about how the sensory system of triatomines operates is undeniably interesting but also very important given that these insects are the vectors of the most important parasitic disease in the Americas, Chagas disease. We have highlighted here that the interplay between sensory modalities ensures that triatomines can efficiently detect resources necessary for survival. For instance, potential hosts are sources of odours, heat and water vapour, among other cues. Blood-sucking insects integrate different available inputs, giving place to unimodal and multimodal interactions which can have synergistic effects increasing, for instance, sensitivity during food search. Many aspects of sensory function in triatomines remain to be investigated. They include, for instance, how the internal state modulates visually guided behaviours, a topic that has been extensively studied in the olfactory system of these insects, how visual cues interact with other sensory cues in different behavioural contexts, etc. Ideally, we would like to know how triatomines “see” their natural world, how visual signals drive important behaviours

related to host and mate finding, avoidance of predators, and navigation. Knowledge about which gustatory stimuli drive positive or negative feeding responses will open new possibilities in the development of novel methods to control populations of vector insects like triatomines. The development of new strategies targeting the gustatory system of hematophagous insects by using anti-feedants or bitter compounds could help diminishing host-vector interactions and thus, aid in preventing vectorial transmission.

Without doubt, studies on the visual, olfactory, taste and thermal functions can contribute to the design and development of traps, as it has been done in other harmful insects, including vectors of human diseases (FAO, 1992; Maciel-de-Freitas et al., 2006). While laboratory studies where experimental conditions can be carefully controlled are critical to elucidate these issues, investigations that incorporate elements of the natural visual scenery under realistic conditions –a sensory ecology approach– are ultimately required to fully understand the function of sensory systems.

The new era of functional genetics of insect behaviour should open opportunities for the development of target-specific interference control tools such as chemical agents inhibiting key receptor function. Modulation of sensory function through the control of receptor gene expression, or that of other relevant sensory genes, may be an alternative, given the fact that plasticity in the molecular bases of sensory function seems to be relevant for these insects. The development of agents that manipulate sensory sensitivity seems to be another potential tool that deserves deeper study.

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Figure captions

Figure 1. The olfactory sense of triatomines. (A) Schematic representation of the antenna of adult *T. infestans* showing its four segments: scapus (sc), pedicellum (pe) and flagellomeres 1 and 2 (f1, f2). (B) Middle region of the first flagellomere of *R. prolixus* covered with a high density of sensilla, where two olfactory types can be recognized: the basiconic (b) and the grooved peg sensilla (gp). (C) Detail of a basiconic sensillum showing the presence of multiple pores (p) in the cuticle. (D-E) Magnification of a grooved peg sensillum. (F) Electroantennogram (EAG) responses of *T. infestans* to increasing doses (0.025, 0.25, 1.25 and 2.5 mg) of the host odour L-lactic acid. The bar beneath the trace indicates stimulus arrival. (G) Brain of *R. prolixus* showing the primary brain processing centres of olfactory information, the antennal lobes. (H) Detail of one antennal lobe and the glomeruli. A: anterior, AL: antennal lobe, an: antennal nerve, g: glomerulus, ol: optic lobes, SOG: subesophageal ganglion. Photos G-H modified from Barrozo et al. 2009.

Figure 2. The gustatory sense of triatomines. (A) Distal end of the second antennal flagellomere of *R. prolixus* showing four gustatory sensilla so-called chaetic hairs (white arrows). (B) Anterior region of the epipharynx of *R. prolixus* showing eight uniporous

epipharyngeal sensilla. (C) Detail of a single epipharyngeal sensillum of *R. prolixus*. (D) Detail of a single epipharyngeal sensillum of *T. infestans*. (E) Multiple sensory hairs in the tarsus of *R. prolixus*. (F-G) Detail of a uniporous sensilla (white arrows) in the tarsus of *R. prolixus*. (H) Single-sensillum recording of a gustatory sensory neuron housed in a chaetic sensillum of *R. prolixus* to stimulation with 10 mM KCl. The upper trace shows the excitatory response and the lower trace indicates the spikes extracted from the recording. B and C adapted from Pontes et al. 2014. a: anterior, p: posterior.

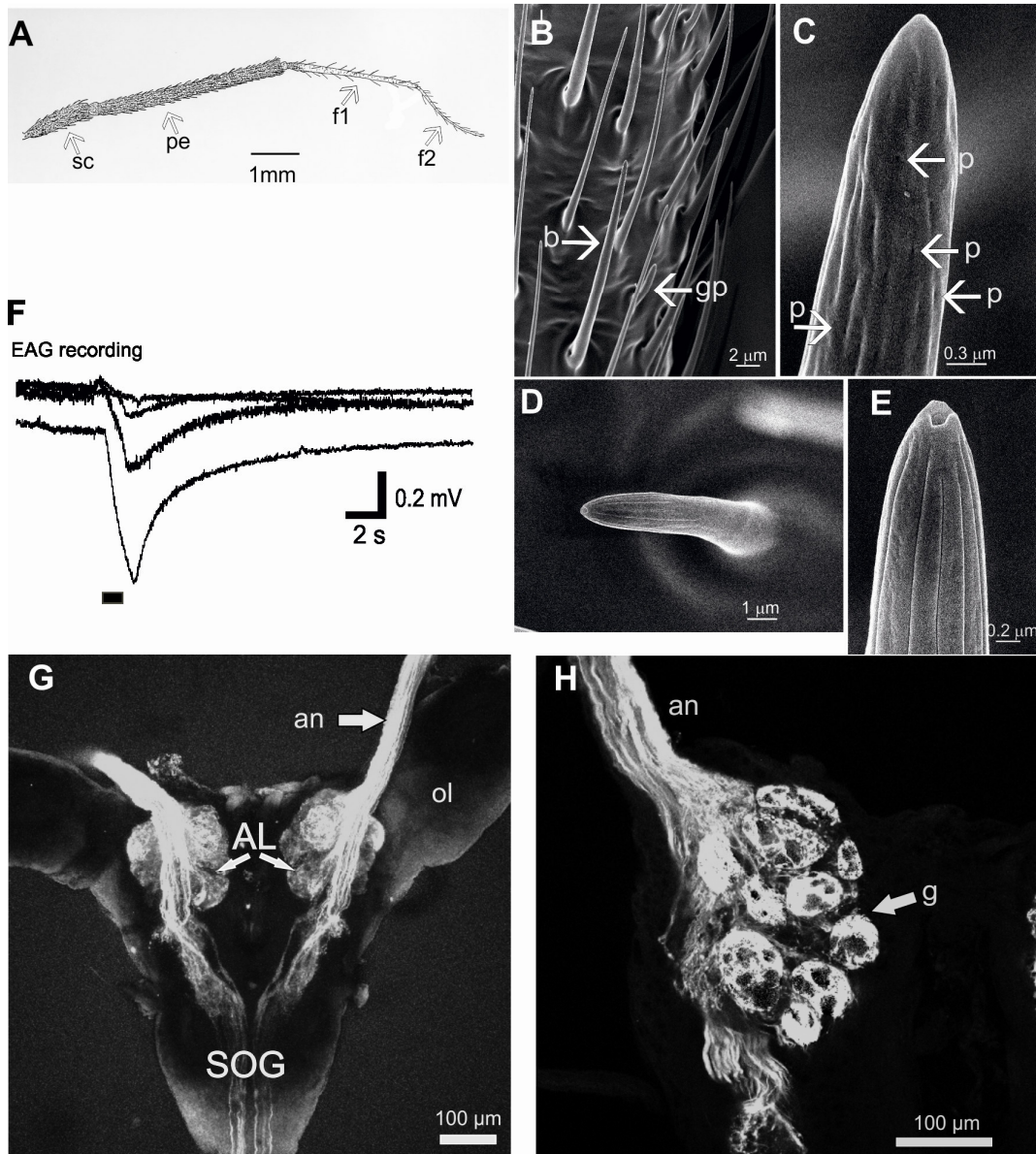
Figure 3. The visual sense of triatomines. (A-B) Top view of the head of *T. infestans*. The unique visual structures of larvae (A) are the compound eyes (white arrows), although adults (B) also have ocelli (black arrows). (C) Schematic representation (longitudinal view) of the light-adapted ommatidia of *T. infestans*. (D) Electroretinogram (ERG) response obtained from the compound eyes of *R. prolixus* to stimulation with white light (upper trace) or in absence of light stimulus (lower trace). ERGs represent the population response of retinula cells. (E) Position of the compound eyes with respect to the optic lobes. (F) Detail of the three optic lobe neuropils, the lamina, the medulla and the lobula. Photos A and B courtesy of T. Insausti, A: anterior, Bm: basal membrane, Co: cornea, Cc: crystalline cone, D: dorsal, la: lamina, lo: lobula, me: medulla, ol: optic lobe, PPC: primary pigment cell, R: retinula cells (1-6) and (7-8), Rh: rhabdomeres (1-6) and (7-8), SPC: secondary pigment cell. C,E-F adapted from Reisenman et al. 2002.

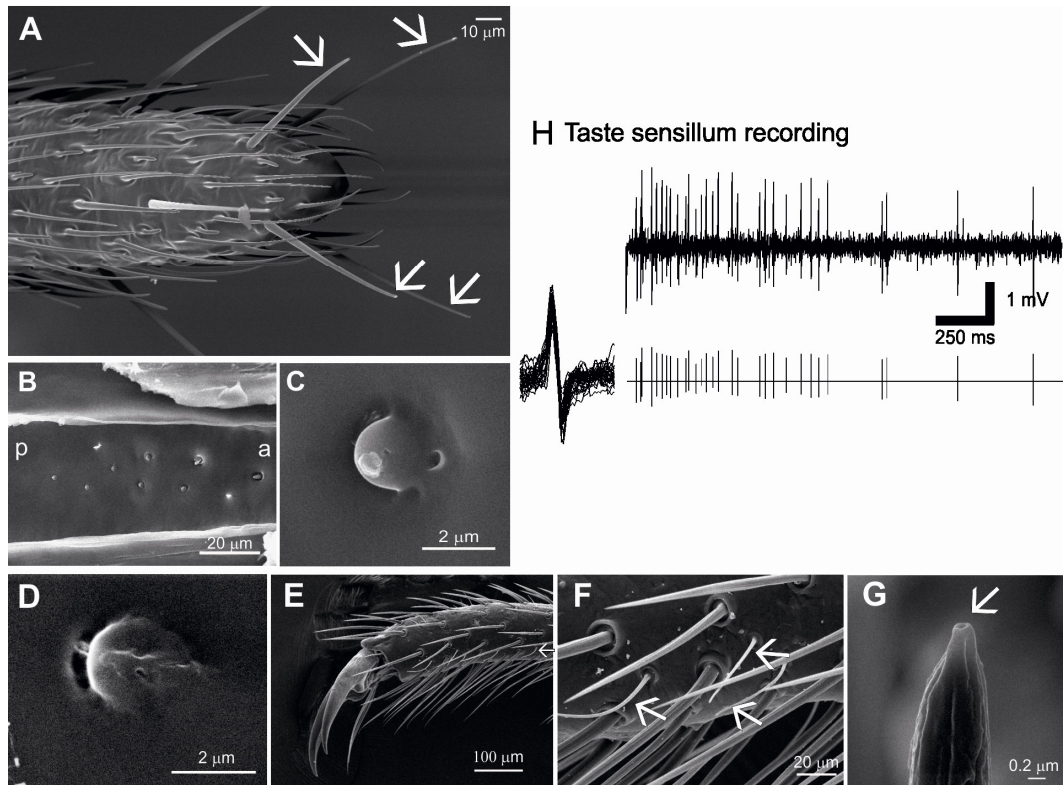
Figure 4. Sensory plasticity in triatomines. (A) EAG responses of recently fed and starved insects during the night and during the day in *R. prolixus*. The starvation status affects antennal responses to a host odour, ammonia, mostly during the nighttime, when insects search for hosts. (B) Oriented behavioural response of recently fed and starved insects in a dual-choice “T-shaped” olfactometer; one arm of the olfactometer offered a host-related odour, while the other arm offered clean air, acting as a control. While starved insects were highly attracted to the odour source, fed insects showed no preferences for either side of the maze. (C) Modulation of the photonegative behaviour of *T. infestans* by the light intensity and the time of the day (day, open circles; night, closed circles). Experiments were conducted in a rectangular arena half of which was illuminated with different intensities of white light, while the other half was kept in darkness. The preference of insects for the side of arena in darkness was much higher during the night, when the insects are normally active. (D) Gene expression variation with the feeding state of adult males of *R. prolixus*. Olfactory (*RproOrco*) and ionotropic receptors (*RproIR8a*, *RproIR76b*, *RproIR25a*) of the antenna are highly expressed in unfed insects. A-C, adapted from Reisenman 2014, Reisenman et al. 2013 and 1998, respectively and D, modified from Latorre-Estivales et al. 2015.

Figure 5. Relation between ambient light intensity and the phototactic sensitivity of insects. Light intensity variations are shown for three different microenvironments (outside a house, inside a room, and inside a crevice) during sunset in a countryside area (without interferences from artificial lights). Light intensities were measured with a radiometer equipped with a flat response detector. The horizontal lines represent the

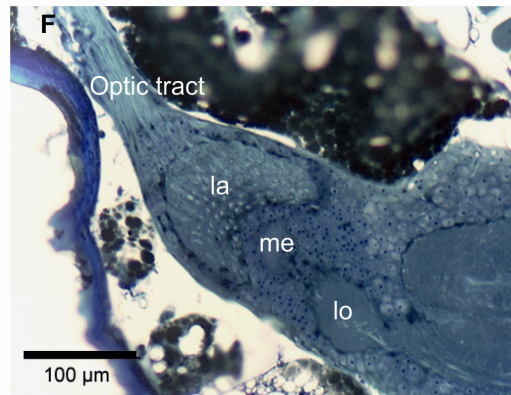
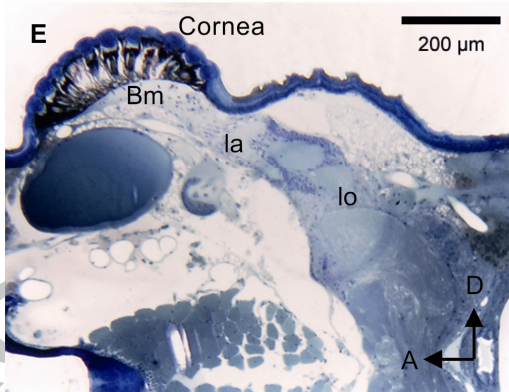
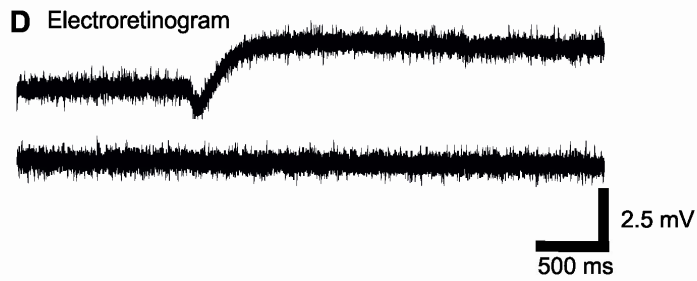
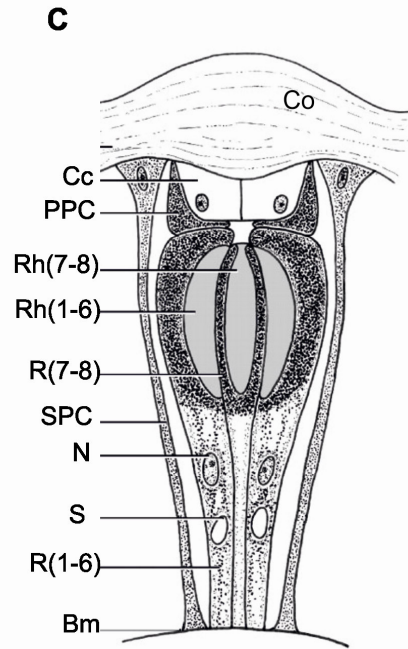
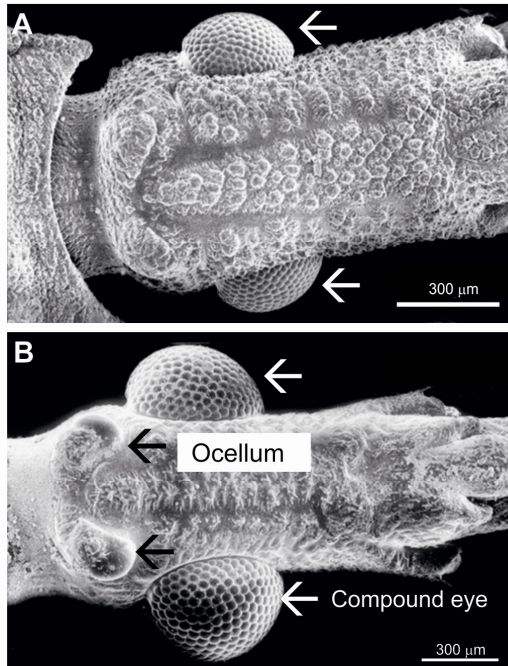
approximate photonegative behavioural thresholds of larvae and adults of *T. infestans*.
Adapted from Reisenman et al 1998.

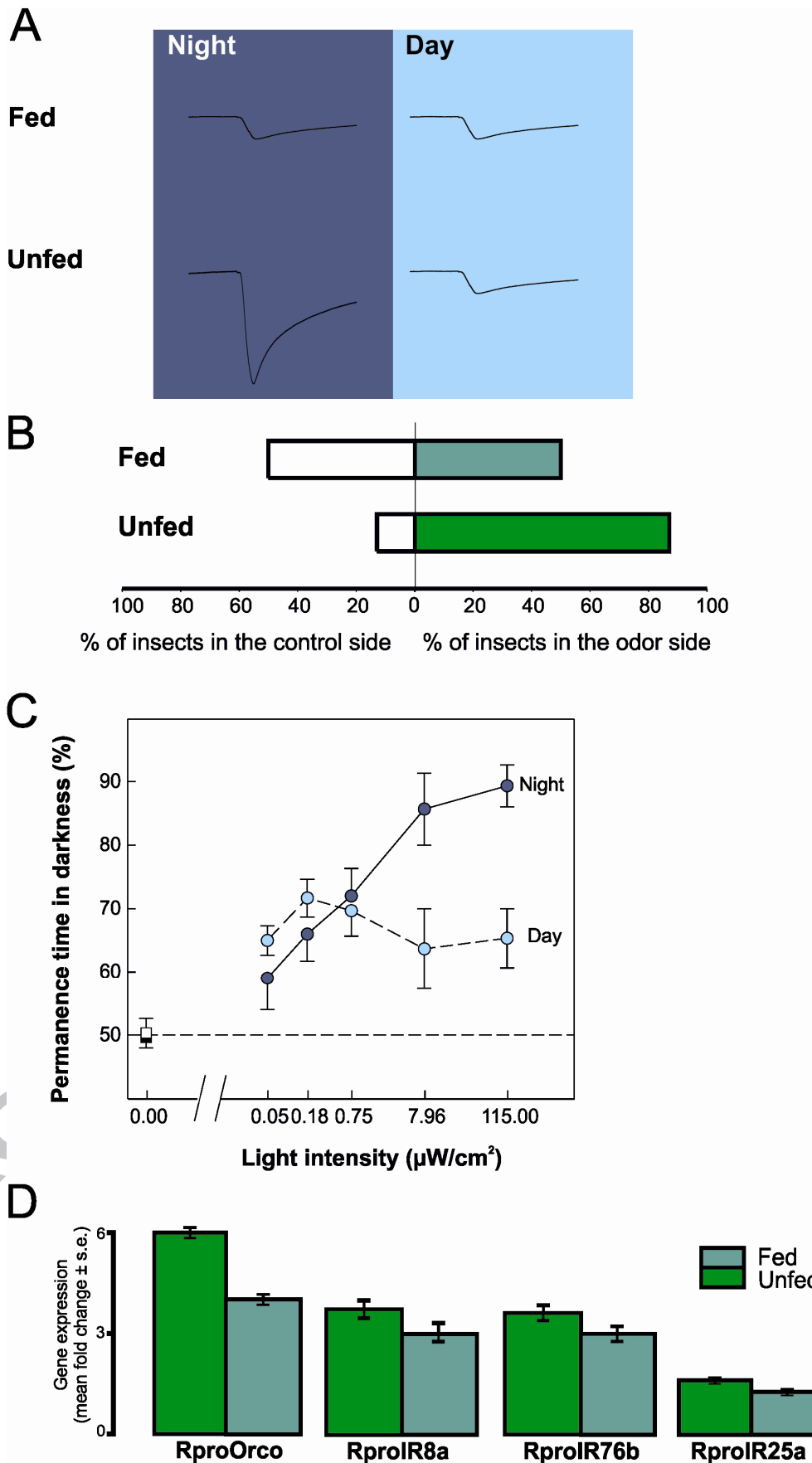
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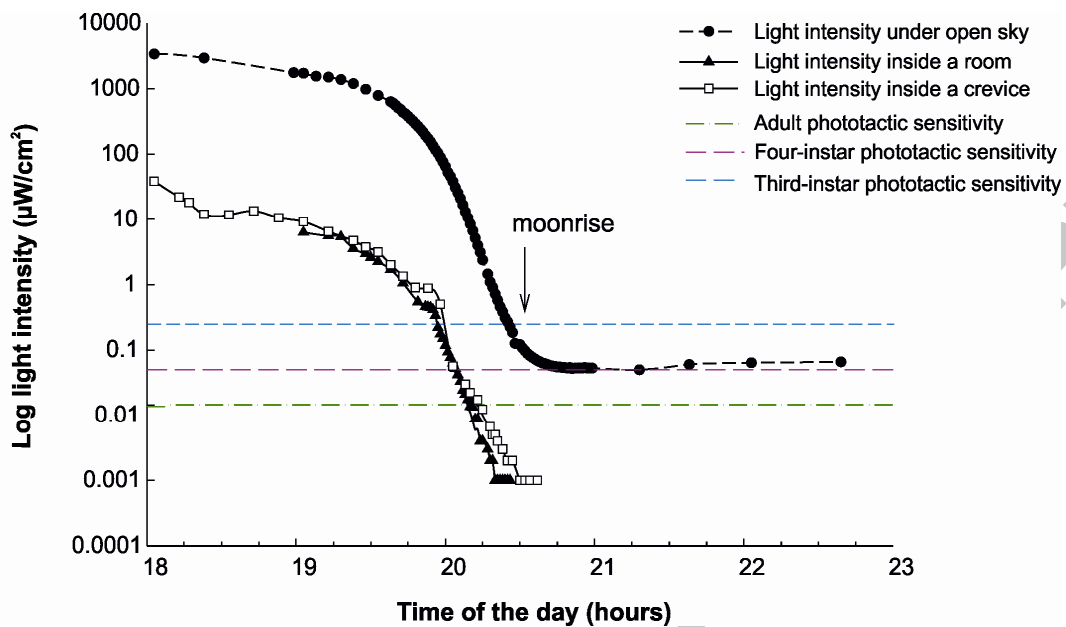


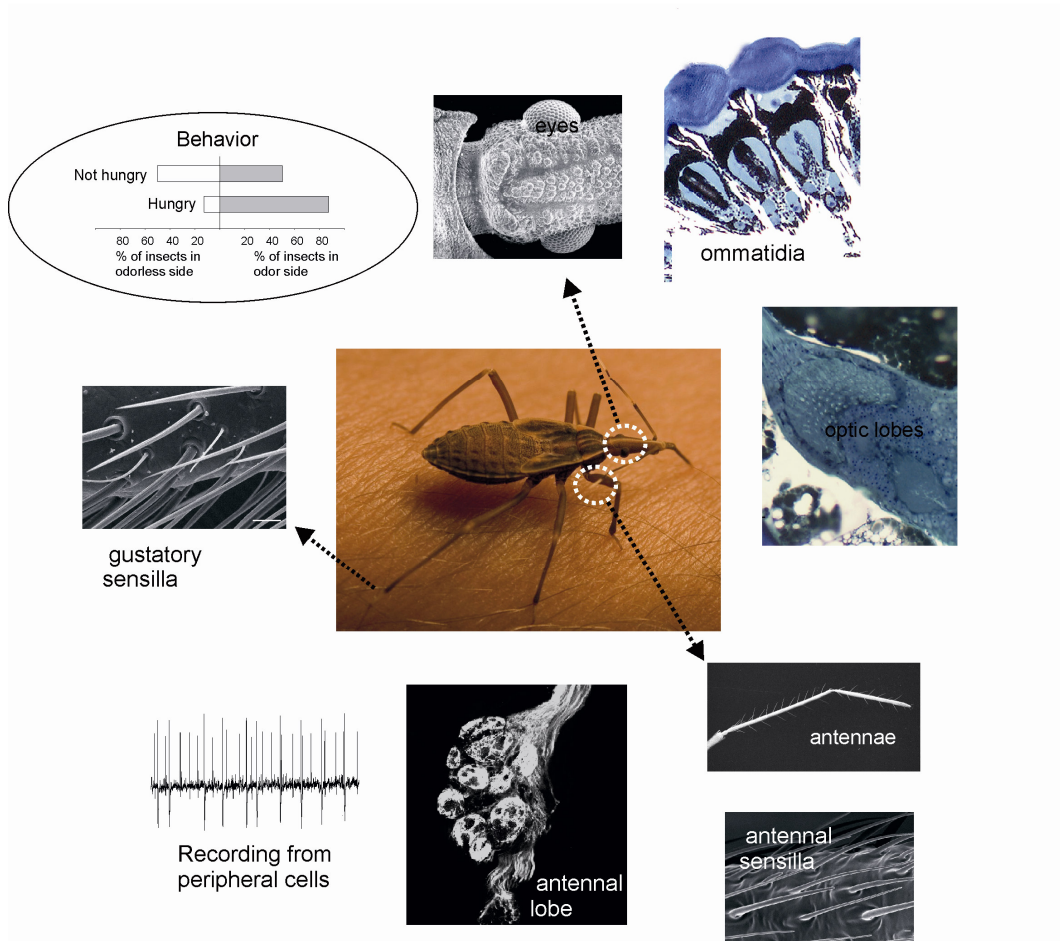


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Highlights

- Diverse triatomine behaviours rely on detecting olfactory information
- Taste plays a key role in host recognition, feeding and communication
- Perception of IR radiation: bugs have extreme thermal sensitivity
- Vision adapted to nocturnal habits, rapid adjustment to changing light conditions
- Sensory-related gene families described based on *Rhodnius prolixus* genome sequence

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