Genetic basis of triatomine behavior: lessons from available insect genomes

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Triatomines have been important model organisms for behavioural research. Diverse reports about triatomine host search, pheromone communication in the sexual, shelter and alarm contexts, daily cycles of activity, refuge choice and behavioural plasticity have been published in the last two decades. In recent times, a variety of molecular genetics techniques has allowed researchers to investigate elaborate and complex questions about the genetic bases of the physiology of insects. This, together with the current characterisation of the genome sequence of Rhodnius prolixus *allows the resurgence of this excellent insect physiology model in the* omics *era. In the present revision, we suggest that studying the molecular basis of behaviour and sensory ecology in triatomines will promote a deeper understanding of fundamental aspects of insect and, particularly, vector biology. This will allow uncovering unknown features of essential insect physiology questions for a hemimetabolous model organism, promoting more robust comparative studies of insect sensory function and cognition.*

Key words: behaviour - sensory physiology - olfaction - genes - triatomines

Triatomine behaviour as a basis for functional genetics - Kissing-bugs have become an important model organism for neuroethological studies in the last two decades (Guerenstein & Lazzari 2009, Manrique & Lorenzo 2012, Lazzari et al. current issue). The range of topics covered includes host search mechanisms (Guerenstein & Lazzari 2009), pheromone communication in contexts like mating (Manrique & Lorenzo 2012), shelter recognition (Lorenzo & Lazzari 1996) and predation risk (Ward 1981, Manrique et al. 2006), circadian rhythms and microclimatic preferences (Lazzari 1991, Roca & Lazzari 1994, Lorenzo & Lazzari 1999, Guarneri et al. 2002, 2003), state dependency (Bodin et al. 2009a, b) and diverse forms of learning (Vinauger et al. 2011a, b, 2012, 2013).

Triatomine host searching mechanisms include orientation to airstreams laden with CO_2 and other host odours (Núñez 1982, Taneja & Guerin 1995, Barrozo & Lazzari 2004), exploitation of bird and mammal emission of infrared radiation (Lazzari & Núñez 1989, Flores & Lazzari 1996, Ferreira et al. 2007) and orientation to sources of water vapour (Barrozo et al. 2003). Furthermore, the intensity of these responses depends on modulatory factors such as the phase of the daily cycle (Barrozo et al. 2004, Bodin et al. 2008), bug nutritional status (Bodin et al. 2009a) and experience (Vinauger et al. 2011a, b).

Kissing bugs also communicate through pheromones in diverse behavioural contexts (Lazzari et al. current issue). Disturbed adult triatomines emit alarm pheromones to trigger avoidance of the emission spot by conspecifics (Ward 1981, Manrique et al. 2006) and this has been suggested to mediate the avoidance of predators (Manrique et al. 2006). A pheromone also mediates triatomine aggregation inside shelters (Schofield & Patterson 1977, Figueiras et al. 1994, Lorenzo & Lazzari 1996, Pires et al. 2002b). The use of shelters during daylight hours is fundamentally driven by their strong negative phototaxis (Reisenman et al. 1998) and at a later phase by an intense thigmotactic behaviour. In addition, these insects locate bug aggregations searching for shelters marked with triatomine faeces. Once inside refuges, a contact chemical signal present in their cuticle induces their arrestment (Figueiras et al. 2009).Finally, a sex pheromone is emitted by female adults to attract males (Manrique et al. 2006, Pontes et al. 2008, Vitta et al. 2009, May-Concha et al. 2013). Sexual signals also mediate male aggregation around mating pairs, apparently promoting polyandric reproduction in some bug species (Crespo & Manrique 2007, Pontes & Lorenzo 2012). Nevertheless, the latter seems not to be generalised in the subfamily Triatominae (Pires et al. 2004).

These insects seem to evaluate the adequacy of potential refuges by their physical properties (Lorenzo & Lazzari 1999). Temperature and relative humidity range preferences apparently vary from species to species (Lazzari 1991, Roca & Lazzari 1994, Guarneri et al. 2002, 2003, Pires et al. 2002a, Schilman & Lazzari 2004). The level of
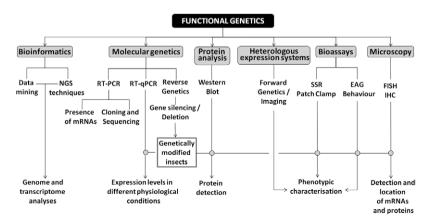
illumination and light spectral qualities of their environments clearly affect their spatial choices and the intensity of these responses seems to depend on the phase of the daily cycle (Reisenman et al. 1998, 2002). Vision also plays an important role in locomotion, flight orientation and spatial recognition by triatomines (Lazzari & Varjú 1990, Reisenman et al. 1998, 2000, Minoli & Lazzari 2006, Reisenman & Lazzari 2006, Insausti et al. 2013).

Triatomine behaviour is finely controlled by circadian clocks (Lazzari 1992, Barrozo et al. 2004, Guerenstein & Lazzari 2009). In fact, activities are distributed within two precise temporal windows: one after dusk devoted to host search and another at dawn, dedicated to shelter location, egg-hatching and ecdysis (Lazzari et al. 2013). Each activity peak is under the control of a specific endogenous oscillator that establishes the proper timing (Lazzari 1992).

There is a clear state dependency in the motivation of these insects to search for hosts, shelters and mates. Host search is modulated by age, nutritional status and reproductive condition (Bodin et al. 2008, 2009a). Recently-fed insects and gravid females avoid host associated odours (Bodin et al. 2009a). The search for refuges also depends on the nutritional state and the phase of the diel cycle (Lorenzo & Lazzari 1998). The search for reproductive mates and mating receptivity depend upon the age since the adult ecdysis and the nutritional state of the adult insects (Manrique & Lazzari 1994, Vitta & Lorenzo 2009).

The advent of next-generation sequencing (NGS), gene expression/regulation techniques and heterologous expression systems in the post-genomic era - Many insect genomes have been sequenced to date, forming a rich source of appropriate orthologues of behaviour controlling genes to initiate searches in the *Rhodnius prolixus* genome. Particularly, the genome of the pea aphid *Acyrthosiphon pisum* represents one of the best candidates for guiding BLAST searches due to their closer phylogenetic relation (The International Aphid Genomics Consortium 2010). This will be more relevant whenever a greater functional characterisation of this genome is made available. An assortment of gene sequencing, silencing, deletion and heterologous expression techniques have enabled more elaborate studies on the genetic bases of biological processes (Figure). Insect physiology benefited from this wealth of novel techniques and has shown impressive progress concomitant with the amazing potential of one particular insect model, *Drosophila melanogaster* (Table). In the last decades, it has been adopted as a main model for the study of the genetic and molecular bases of behaviour, being central to current neuroscience. The molecular mechanisms underlying circadian rhythms, plasticity and the formation of memories, sensory function and even, sexual behaviour have been studied in *Drosophila* (Table). Nevertheless, it presents limitations for neuroscience studies due its small size that restricts manipulation. For example, studies like those developed by VB Wigglesworth using *R. prolixus* as an insect model for the study of metamorphosis and neurosecretory function could only be performed thanks to an extremely practical model that allowed surgical procedures with minimal deleterious consequences. The present paper intends to propose *R. prolixus* as a new tool for the study of insect neuroscience due to these three characteristics: manipulation-friendly size, background as a classical insect physiology model and deep knowledge of diverse aspects of its behaviour. These facts, together with the recent characterisation of its genome sequence, will allow the resurgence of an excellent insect physiology model, as in Wigglesworth's time, but in the *omics* era. Next follows a series of aspects of triatomine behaviour, as well as related candidate genes uncovered for other insects, whose characterisation and study would be invaluable in *R. prolixus* and other relevant Chagas disease vectors.

The molecular basis of insect behaviour as a framework for studies on triatomines - *Sensory ecology of host searching* - Host location in triatomines is dependent on environmental and physiological conditions evaluated by their brain in order to regulate a proper expression of this behaviour. When properly motivated to feed, these insects

Workflow scheme for the study of functional genetics underlying triatomine behaviour. EAG: electroantennogram; FISH: fluorescence *in situ* hybridisation; IHC: immunohistochemistry; NGS: next-generation sequencing; qPCR: quantitative polymerase chain reaction; RT: reverse transcription; SSR: single sensillum recording.

TABLE

Main gene targets underlying insect behaviour and sensory physiology

Protein family	Physiological function reported	Insect model	Genes	References
Odourant receptors	Host detection	*Anopheles gambiae, Aedes aegypti* and *Culex quinquefasciatus*	Diverse	Hallem et al. (2004), Bohbot et al. (2007), Syed and Leal (2009)
	Oviposition site detection	*Cx. quinquefasciatus*	*CqOr2*	Pelletier et al. (2010)
	Pheromone detection	*Apis mellifera, Bombyx mori* and *Drosophila melanogaster*	Diverse	Sakurai et al. (2004), Benton et al. (2007), Patch et al. (2009)
	Repellent detection	*Ae. aegypti* and *D. melanogaster*	*AaOr2, AaOr7, AaOr8* and *DmOr59b*	Ditzen et al. (2008), Bohbot et al. (2011)
Ionotropic receptors	Detection of volatile amines and acids	*D. melanogaster*	Diverse	Benton et al. (2009), Silbering et al. (2011)
Gustatory receptors	CO_2 detection	*An. gambiae* and *D. melanogaster*	*DmGr21a, DmGr63a, AgGr22, AgGr23* and *AgGr24*	Jones et al. (2007), Lu et al. (2007)
	Bitter compound detection	*D. melanogaster*	*DmG66a, DmGr22e, Dm93a* and *DmGr33a*	Thorne et al. (2004), Moon et al. (2006)
	Sugar detection	*B. mori* and *D. melanogaster*	*BmGr-9, DmGr5a, DmGr64a, DmGr64f* and *DmGr61a*	Dahanukar et al. (2001), Thorne et al. (2004), Sato et al. (2011)
	Pheromone detection	*D. melanogaster*	*DmGr68a, DmGr32a* and *DmGr33a*	Bray and Amrein (2003), Miyamoto and Amrein (2008), Montell (2009)
Transient receptor potential (TRP)	Visual transduction	*D. melanogaster*	TRP, TRP-γ and TRPL	Hardie and Minke (1992), Xu et al. (2000)
	Cool avoidance	*D. melanogaster*	TRP and TRPL	Rosenzweig et al. (2008)
	Mechanotransducer-proprioception	*D. melanogaster*	*nompC*	Walker et al. (2000), Yan et al. (2013)
	Thermal sensitivity	*An. gambiae* and *D. melanogaster*	TRPA1, *painless* and *pyrexia*	Tracey Jr et al. (2003), Lee et al. (2005), Wang et al. (2009)
	Thermal preference	*D. melanogaster*	TRPA1	Hamada et al. (2008)
	Electrophile sensitivity	*An. gambiae* and *D. melanogaster*	TRPA1	Kim et al. (2010), Kwon et al. (2010)
	Thermal/chemical sensor	*Apis mellifera*	TRPA1	Kohno et al. (2010)
	High-temperature nociception	*D. melanogaster*	TRPA1 and *painless*	Tracey Jr et al. (2003), Xu et al. (2006)
	Courtship behaviour	*D. melanogaster*	*painless*	Sakai et al. (2009)
	Hygroreception	*D. melanogaster*	*nanchung* and *water witch*	Liu et al. (2007)

Protein family	Physiological function reported	Insect model	Genes	References
Mechanically activated cation channel	Mechanical nociception	D. melanogaster	piezo	Coste et al. (2010)
Degenerin/ epithelial sodium channel/ pickpocket (ppk)	Water detection	D. melanogaster	ppk28	Cameron et al. (2010), Chen et al. (2010)
	Courtship behaviour	D. melanogaster	ppk23 and ppk29	Hasemeyer et al. (2009)
	Salt detection	D. melanogaster	ppk11 and ppk19	Liu et al. (2003)
Others	Vision	A. mellifera and D. melanogaster	Rh1-Rh8 and AmUVOP	Townson et al. (1998), Yamaguchi et al. (2010)
	Foraging behaviour	Bombus terrestris, D. melanogaster and Schistocerca gregaria	foraging	Osborne et al. (1997), Ben-Shahar (2005), Lucas et al. (2010)
	Circadian clock control	D. melanogaster	clock, cycle, period and timeless	Sandrelli et al. (2008)
	Learning and memory	A. mellifera	stripe	Lutz and Robinson (2013)
	Learning and memory	D. melanogaster	CrebB	Yin et al. (1994)

need to locate warm-blooded animals for a blood meal. As mentioned above, this task is performed detecting host signals through an array of sensory channels that include the detection of CO_2, diverse odours, heat and water vapour. Once host detection is achieved, these insects approach the animal and finally need to find an appropriate biting site to pierce a blood vessel. All these behavioural steps depend on the detection of stimuli at sensory neurons in the periphery of their nervous system.

Chemoreceptor mediated host searching - CO_2 is considered a long distance host cue promoting both the activation of resting triatomines and their orientation through odour modulated anaemotaxis (Barrozo & Lazzari 2004). The physiological and molecular bases of detection of this host cue are unknown for triatomines, but relevant progress has been made to uncover a similar mechanism in two dipterans (Jones et al. 2007, Lu et al. 2007). According to these reports, both *Drosophila* and *Anopheles gambiae* detect CO_2 with heterodimers composed of gustatory receptors (GRs) that are co-expressed in specific olfactory receptor neurons (ORNs). These receptors (*DmGr21a* and *DmGr63a*) are expressed in the antennae of *D. melanogaster* and the maxillary palps of *An. gambiae* (*AgGr22, AgGr23* and *AgGr24*). These GRs belong to three ancient lineages of GRs also found in moths and beetles (Robertson & Kent 2009). Surprisingly, these gene lineages have not been found in other arthropods like aphids, lice, honey bees, water fleas and black-legged ticks. Nevertheless, some of these arthropods are known to detect CO_2, suggesting that they evolved a different molecular mechanism to detect this cue. The study of the molecular bases of CO_2 detection by triatomines is therefore relevant and will rely on diverse bioassays already developed.

Host odours other than CO_2 play a fundamental role in the orientation of triatomine bugs and their detection is probably mediated by proteins belonging to two different families: the odourant receptors (ORs) and the ionotropic receptors (IRs). In insects, ORs present seven transmembrane domains characteristic of all members of this protein family (Vosshall et al. 1999). In the ORNs expressing them, these receptors need to be co-expressed together with a phylogenetically conserved chaperon protein currently named "odourant receptor coreceptor-Or-Co" for all insects (Larsson et al. 2004, Vosshall & Hansson 2011). ORs are divergent proteins with low sequence identity between insect species and their roles cannot be predicted by sequence homology alone. Olfactory sensory neurons express different receptor genes, such that individual neurons are functionally distinct (Table). Numbers of ORs are extremely variable in insects, ranging from only 10 in the human louse to 265 in the flour beetle (TGSC et al. 2008, Kirkness et al. 2010).

A second family of insect ORs includes IRs, recently described in *Drosophila*, which present three transmembrane domains and constitute cation channels (Benton et al. 2009). These proteins have been suggested to mediate olfaction and taste in protostomes, an apparently ancient function for detecting chemical signals from the environment (Croset et al. 2010). IRs have been proposed to

act as dimers or trimers of subunits co-expressed in the same neuron, which comprise individual odour-specific receptors and one or two broadly expressed coreceptors (Abuin et al. 2011). The suggested coreceptor units for IRs have thus been named IR25a, IR8a and IR76b. The ORNs expressing IRs are restricted to chemoreceptor hairs characterised by being double-walled, wall pore sensilla (e.g., grooved-pegs) and appear to have a relatively conserved role for detection of a restricted set of odourants in different insects (Pophof 1997, Diehl et al. 2003, Yao et al. 2005, Qiu et al. 2006). According to Silbering et al. (2011), odourant detection by IRs represents an insect olfactory subsystem that evolved in parallel to OR based olfaction. Numbers of IRs also show a relevant degree of variation ranging from 12 in the human body louse to 95 in *Aedes aegypti* (Croset et al. 2010).

The identification of receptor genes mediating host odour detection in the *R. prolixus* genome along with an analysis of olfactory-driven behaviour in these insects may enable researchers to understand the mechanisms linking host recognition and triatomine behaviour. These receptors and their ligands need to be characterised using a multidisciplinary approach including the study of gene expression, its physiological modulation and the electrophysiological properties of the ORNs expressing them (Figure). A deeper comprehension of the olfactory physiology underlying triatomine host detection would permit the design of antagonists to block these functions, both for OR and IR proteins involved.

Thermo and hygroreceptor mediated host searching - Triatomines make use of their highly developed thermal sense to detect potential hosts. These bugs detect the heat emitted by warm-blooded animals in the form of infrared radiation (Lazzari & Núñez 1989, Schmitz et al. 2000). The antennae of these insects house infrared detection organs (Lazzari & Wicklein 1994, Flores & Lazzari 1996) and are also critical for the bilateral integration of thermal information necessary to approach hosts (Flores & Lazzari 1996). Triatomine thermoreceptor neurons have been poorly studied and information on their electrophysiological properties is very limited (Bernard 1974). The physiology of thermoreceptor neurons and their receptor proteins should be studied in these insects, which represent one of the most practical models to determine the molecular basis of heat perception in animals. Several genes belonging to the transient receptor potential A (TRPA) subfamily are known to mediate thermoreception in *D. melanogaster, An. gambiae* and *Apis mellifera*. The TRPA proteins belong to the TRP superfamily, which is composed of seven subfamilies of transmembrane protein channels with a relevant role in diverse sensory modalities including vision, taste, smell, thermo and mechanosensation (Table). The members of the TRPA subfamily are characterised by presence of six transmembrane domains and large numbers of ankyrin motifs in the N-terminal domain (Montell 2005). Orthologous sequences in the genome of *R. prolixus* need to be identified. Thermoreceptor genes with functional roles such as TRPA1, *pyrexia* and *painless* have been described in *Drosophila* (Tracey Jr et al. 2003, Lee et al. 2005, Wang et al. 2009).

Water vapour has been implicated as a host signal used by triatomines during their approach to blood-meal sources (Barrozo et al. 2003) and the molecular basis of its detection have been well studied in *D. melanogaster* (Liu et al. 2007). Briefly, vinegar flies detect air humidity levels by means of two different TRP proteins belonging to the TRPV subfamily, *nanchung* (involved in detecting dry air) and *water witch* (required to detect moist air). An evaluation of possible orthologues of these genes in the genome of *R. prolixus* would allow functional studies on their role in host location.

Host recognition and biting - After their final approach, triatomines need to recognise surface properties on the potential host in order to trigger biting responses properly. These can be reinforced by stimuli perceived through diverse sensory channels, but fundamentally through thermoreception and contact chemoreception. The first is mediated by antennal thermodetection structures recognising warm surfaces and even warmer blood vessels (Flores & Lazzari 1996, Ferreira et al. 2007). The second can be mediated by proteins belonging to at least two different families, the GRs and pickpocket receptors (ppks). Insect GRs are membrane proteins generally dedicated to detect non-volatile substances (Table). These are G protein-coupled receptors presenting seven transmembrane domains that mediate the recognition of substances present on substrates and are expressed in the cilia of neurons housed inside contact chemoreceptor sensilla (Clyne et al. 2000). Pickpocket receptors belong to the Degenerin/epithelial sodium channel gene family and present two transmembrane helixes, two short intracellular domains and a large cysteine-rich extracellular loop (Ben-Shahar 2011). In *Drosophila*, ppk receptors have been related with the detection of water, e.g *ppk28* (Cameron et al. 2010, Chen et al. 2010) and salt, e.g. *ppk11* and *ppk19* (Liu et al. 2003). Their presence in the genome of *R. prolixus* should be assessed and their potential role in host recognition in triatomines evaluated.

Pheromones: the molecular basis of chemical communication processes - As already mentioned, pheromones are used by triatomines to exchange information in diverse behavioural contexts. The substances composing them are most probably detected by triatomine ORs, IRs, GRs and ppks which still need to be characterised. Potential agents blocking their functions may become alternatives for triatomine control, which suggests that the identification of genes coding for these receptors would be extremely relevant to allow their manipulation. In the case of sex pheromone detection, any receptors showing exclusive expression in adults, males or female triatomines should be main research targets and may be identified through NGS techniques such as RNAseq (Grosse-Wilde et al. 2011, Pitts et al. 2011, Bengtsson et al. 2012).

Spatial and temporal orientation - Negative phototaxis is a common behavioural trait of most triatomine bug species (Lazzari et al. 1998, Reisenman et al. 1998, 2000, Reisenman & Lazzari 2006). This behavioral feature induces avoidance of open areas during daylight hours (Lorenzo & Lazzari 1996, Mota & Lorenzo 2012),

which is mediated both by their compound eyes and ocelli (Lazzari et al. 1998, Reisenman et al. 1998). The sensitivity of behavioural responses mediating negative phototaxis in these insects varies in a circadian manner (Reisenman et al. 1998) and the migration of screening pigments in both visual organs is also under the control of a circadian clock (Reisenman et al. 2002). In contrast to their robust negative phototaxis, light sources become attractive to triatomines when adults initiate flight in search for new habitats, food and mates (Noireau & Dujardin 2001, Vazquez-Prokopec et al. 2004, Minoli & Lazzari 2006).

Insect visual pigments, as those of vertebrates, are photoreceptors composed of an *opsin* protein and a light-sensitive chromophore derived from retinal (Terakita 2005). Since most organisms synthesise a single type of chromophore, the diversity of visual pigment absorption spectra essentially depends on the opsin structure. Thus, the spectral sensitivity of a given species is controlled by the type of *opsins* existing in its genome and expressed in the retina cells of its visual organs (Briscoe & Chittka 2001). *R. prolixus* offers an excellent model to study the molecular basis of vision in nocturnal arthropods. Characterisation of its visual receptor genes would enable understanding their relation to behaviour.

Little is known about the mechanisms of photoreception and visual processing in triatomines. Some behavioural studies suggest the ability of these bugs to discriminate between distinct spectral properties of visual stimuli (Reisenman et al. 1998, 2000, Minoli & Lazzari 2006, Reisenman & Lazzari 2006), but it remains unclear whether triatomine vision relies on chromatic and/or achromatic mechanisms (Briscoe & Chittka 2001). True colour vision depends on subtractive interactions between at least two photoreceptor types (Menzel & Backhaus 1991), but so far the number of photoreceptor types in triatomine visual organs has not been reported. The study of *opsin* gene expression in triatomines could be combined with behavioural and neurobiological approaches to provide insights into the visual processing mechanisms displayed by these insects (Figure).

The expression of the triatomine behaviours listed so far is under a strict control of circadian clocks (Lazzari et al. 2004). This affects host search activity, egg hatching, moulting, the expression of thermopreference and the use of shelters (Lazzari et al. 2004). The study of the molecular bases of circadian rhythms has been one of the most elaborate areas in insect physiology in the last decades (Sandrelli et al. 2008). A complex network of genes controlling the circadian expression of insect behaviour, including main roles like those of *clock*, *cycle*, *timeless* and *period* (Table), has been characterised in *Drosophila* (Sandrelli et al. 2008). It is probable that orthologues of these and other clock genes exist in *R. prolixus* and their identity should be determined through bioinformatic searches. Likewise, their expression cycling profiles should be clarified to allow experimental studies on their control of relevant biological features of triatomine biology such as oscillations in olfactory sensitivity.

Motivation: modulation and plasticity - The levels of expression of locomotor activity in triatomines seem extremely plastic, varying from almost null in immature insects to intense in starved individuals. This particular aspect of triatomine behaviour makes their physiology a cyclic process of long starvation intervals interspersed with short gaps of blood repletion that trigger a cascade of neuroendocrine events promoting moulting. This discrete separation allows clearly associating experimental manipulation to gene expression alterations, establishing cause-effect relations not easily attributable for *Drosophila* or other classic models that feed continuously. The underlying physiological processes regulating locomotor activity have not been studied in detail and these may involve mechanisms regulating gene expression. In the last decade, the study of a gene named *foraging* (*for*), encoding a cGMP-dependent protein kinase, has allowed a better comprehension of the genetic basis of locomotion in several insects (Reaume & Sokolowski 2011). For example, individuals showing two distinct profiles of locomotor activity controlled by different alleles of the *for* gene, called *rovers* and *sitters*, exist in *D. melanogaster* (Osborne et al. 1997). For locusts, changes in the expression levels of this gene are associated with behavioural shifts characteristically triggered at high population densities (Lucas et al. 2010). In addition, variations in the expression of the *for* gene have been indicated to promote nurse honey bees to become foragers (Ben-Shahar 2005). The existence, function and regulation of this gene in the *R. prolixus* genome and its potential relation to the ample regulation of locomotor activity in triatomines deserve to be explored.

Brain production of diverse neuropeptides has been described in *R. prolixus* and their roles have been related to the control of bug physiology (Ons et al. 2009). Nevertheless, their impact on triatomine behaviour has not been analysed and their potential on the modulation of behaviour needs to be addressed. Many aspects of triatomine behaviour have been shown to be extremely plastic. Therefore, the study of the genetic and molecular bases of behaviour modulation in triatomines is necessary.

In recent years, several reports have shown that *R. prolixus* is capable of diverse forms of learning (Vinauger et al. 2011a, b, 2012, 2013, Minoli et al. 2013). These include simple habituation (Vinauger et al. 2013), associative learning with positive or aversive rewards (Vinauger et al. 2011a, b) and operant conditioning (Vinauger et al. 2013). All these facts indicate that this species might represent a friendly model to study molecular aspects of learning. Memory related genes, such as *CrebB* (Yin et al. 1994) or *stripe (*Lutz & Robinson 2013) have already been described in other insects and their characterisation in triatomines would allow a deeper understanding of behavioural plasticity.

Behaviour modulation due to parasite infection is another relevant issue not properly evaluated for triatomines. Parasite-host associations are unapparent elements in an ecological community and affect competition relations intra and inter species, the distribution and abundance of species and even community composition

(Horwitz & Wilcox 2005). In this way hosts cannot be considered alone, as their relation with the environment and conspecifics necessarily includes their natural parasites. The ability of parasites to manipulate host phenotypes facilitating transmission is an important and well known paradigm in the study of the evolutionary biology of host-pathogen interactions (Thomas et al. 2005, Moore 2013). Changes in vector behaviour have been reported in different systems (Molyneux & Jefferies 1986, Schaub 1989, 1992, Killick-Kendrick & Molyneux 1990, Alekseev 1991, Hurd 2003, Lefevre & Thomas 2008). Generally, these alterations affect feeding behaviour, fundamentally when parasites are transmitted by vector bites, as in African trypanosome infected tsetse flies (Jenni et al. 1980), *Leishmania*-infected sandflies (Beach et al. 1985) and *Plasmodium*-infected mosquitoes (Ribeiro et al. 1985, Rossignol et al. 1986, Koella et al. 1998). *R. prolixus* has its feeding behaviour affected when infected by *Trypanosoma rangeli* (Garcia et al. 1994). Authors suggest that this parasite interferes the synthesis of antihemostatic molecules during salivary gland infection, but Paim et al. (2013) showed an unspecific reduction of stored proteins in the salivary glands in the presence of *T. rangeli*. Other behavioural effects on vectors, such as alterations in locomotory activity in *Ae. aegypti* infected by dengue virus (Lima-Camara et al. 2011) and changes in foraging and defecation in *Mepraia spinolai* infected by *Trypanosoma cruzi* (Botto-Mahan et al. 2006) have been eventually reported, but many aspects of infected vector behaviour are still unstudied.

In spite of the significant advances described here for triatomine behaviour studies, little is known about whether trypanosome infection affects bug behaviour. The few studies concerning behavioural modifications in triatomines infected by trypanosomes suggest that such alterations may occur (Garcia et al. 1994, Botto-Mahan et al. 2006). Triatomines host several parasite species, such as *T. cruzi*, the causative agent of Chagas disease and *T. rangeli*. Alterations in triatomine behaviour caused by these parasites could affect their distribution or even increase parasite transmission rates. In case trypanosomes are capable of inducing changes in triatomine behaviour, the molecular mechanisms triggering this would need to be characterised.

The study of the molecular basis of behaviour in *R. prolixus* would allow a better understanding of fundamental features of vector biology. Besides, it would enable accessing relevant aspects of insect physiology mostly unknown for models other than classic holometabolous insects, like dipterans and moths. This would encourage comparative approaches to study insect sensory function, as well as cognition, at the molecular, cellular and organism level.

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