



# Host-seeking: How triatomines acquire and make use of information to find blood

Pablo G. Guerenstein<sup>a,b</sup>, Claudio R. Lazzari<sup>c,\*</sup>

<sup>a</sup> ARLDN, University of Arizona, Tucson, USA

<sup>b</sup> CICYTTP - CONICET, Diamante, Argentina

<sup>c</sup> Institut de Recherche sur la Biologie de l'Insecte, UMR 6035 CNRS - Université François Rabelais, Tours, France

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## ABSTRACT

The evolution of triatomine bugs towards haematophagy has demanded different types of adaptations, i.e., morphological, physiological and behavioural. In fact, haematophagy evolved as a secondary adaptation facilitated by frequent vertebrate contact. As derived from other Heteroptera, probably from an entomophagous group, some main morphological pre-adaptations, as piercing mouthparts and sucking pumps were already present. Thus, the most important novel acquisitions of triatomines include physiological and behavioural traits to obtain and handle the blood meal. In this review, we discuss how the sensory system and the behaviour of triatomines have been shaped by natural selection to accomplish the tasks of finding a vertebrate host and getting access to its blood. The feeding behaviour of triatomines is presented in its spatial and temporal context. Finally, some methods to study these topics are described.

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## 1. The problems

Living in close association with a vertebrate host has several advantages for blood-feeding insects. The nest or burrow offers a stable environment in terms of temperature and relative humidity, where the animal is less exposed to predators and near of potential food sources. However, adopting haematophagy implies to be able to solve several major problems; among them, those concerning obtaining food, blood, that is neither exposed, nor at a fixed location. Instead, blood circulates in vessels under the skin of mobile hosts, which may play, at the same time, the role of predators. Feeding on blood also demands a number of physiological adaptations, such as the ability to digest that blood, to cope with parasites present in an otherwise sterile food, to handle the oxidative stress produced by the highly reactive heme radicals, and to establish symbiotic associations in order to gather essential elements absent or in lower amounts than necessary in the blood. Water balance is another important issue to be solved. A blood meal is rich in water and it can represent several times the weight of the starved insect. So, haematophagous insects are submitted to hygric stress due to the excess of water just after feeding and to the opposite (water deficit) if starved for a long time.

The present review synthesizes our knowledge about the behavioural strategies that allow triatomines to efficiently feed on a living vertebrate host. We have included some methodological insights aimed at facilitating a deeper understanding of the different experimental approaches used and the significance of experimental data, as well as to aid readers interested in investigating the different facets of host finding in blood-sucking insects.

## 2. The sensory tools

Blood-sucking insects dedicate many of their sensory abilities to detect and follow the physical and chemical signals emitted by their hosts; triatomines are not an exception. In triatomines, apart from the visual system, which is well developed but devoted to other tasks, such as the control of phototaxis and the synchronization of circadian clocks (Lazzari et al., 1998; Reisenman et al., 1998, 2000); all the other sensory systems are directly involved in host finding. As other insects, triatomine bugs bear in their antennae a diversity of hair-like cuticular structures housing sensory cells, the sensilla, distributed along the four antennal segments, from proximal to distal, the scapus, the pedicel and the flagelus composed by two articles. The external and internal morphological characteristics of the cuticular hairs, together with the functional properties of the receptor cells that they house, determine the nature of the stimuli they are able to detect, i.e., their specific modality. Thus, a sensillum may be mechanosensitive, chemosensitive, thermosensitive, hygrosensitive, or even combine modalities. Triatomines are halfway between ectoparasites living on their host body (as fleas and lice), and those being confronted to search their host on a rel-

\* Corresponding author at: Institut de Recherche sur la Biologie de l'Insecte, Faculté des Sciences et Techniques, Avenue Monge, Parc Grandmont, 37200 Tours, France Tel.: +33 2 47 36 73 89; fax: +33 2 47 36 73 89.

E-mail address: [claudio.lazzari@univ-tours.fr](mailto:claudio.lazzari@univ-tours.fr) (C.R. Lazzari).

actively vast area, like tsetse flies and mosquitoes. The association of triatomines to vertebrate nests or human housings keeps them near but not on their potential food sources. This gradient in the association of haematophagous insects to their vertebrate host has been correlated to the number of sensory structures devoted to locate such a host bear by their antennae. This number is lower in insects closely associated to the host than in those that spend most time at some distance from it: lice 10–20, fleas about 50, bedbugs 56, *T. infestans* 2900 and stable flies nearly 5000 (Lehane, 2005). Even though this correlation seems to be quite evident if we compare, for instance, lice and mosquitoes or even closer relatives as bedbugs and triatomines (Lehane, 2005), it should be applied cautiously. Firstly, because the total number of sensory structures may reflect the insect's sensitivity but also the diversity of cues that it is able to detect, and so not all of them obligatorily associated to the search of a host. Secondly, because the number of outer cuticular structures does not necessary reflect sensitivity or diversity of cues. The reason for this is that a given sensillum can house different numbers of sensory cells.

In the antennae of Triatominae, 16 different types of sensilla have been identified, 13 on the surface of the antenna and 3 into the pedicelum (Insausti et al., 1999). These numbers include 8 types of mechanosensory sensilla, 5 types of chemosensory, 1 class of mechano-chemoreceptor, 1 type of thermo-hygroreceptor and 1 type whose function remains speculative. This knowledge is the result of morphological, ultrastructural and physiological studies by different authors (Wigglesworth and Gillet, 1934; Barth, 1952, 1953; Mayer, 1968; Bernard, 1974; Justo and Tramezzani, 1977; McIver and Siemicki, 1984, 1985; Catalá, 1994; Catalá and Schofield, 1994; Lazzari and Wicklein, 1994; Taneja and Guerin, 1997; Insausti et al., 1999; Guerenstein and Guerin, 2001; Diehl et al., 2003). The bad news is that, unfortunately, each author has adopted a different nomenclature for these structures, and to find the correspondence is not always a simple task for the reader (for a review, see Insausti et al., 1999).

After the pioneer work of Bernard (1974), describing the functional properties of several types of sensilla responding to different modalities, most of the physiological work has focussed on olfac-

tory sensilla. Table 1 synthesizes the information on host odor constituents (odourants) that stimulate antennal olfactory receptor cells (ORCs) in olfactory sensilla of triatomines.

It is interesting to note that the spatial pattern of chemosensory sensilla in the adult pedicelus shows marked differences between species or even populations of the same species. For example, the Tribe Rhodniini can be distinguished from Triatomini because of the lack of chemosensory sensilla in the pedicelus of the former (Catalá, 1997; Gracco and Catalá, 2000). On the other hand, the Triatomini show species-specific variability of chemosensory sensilla types in the adult pedicelus (Catalá, 1997; Gracco and Catalá, 2000), and it has been proposed that the density and number of types of chemosensory sensilla in this antennal segment is positively correlated with the habitat range of each species (Catalá, 1997; Carbajal de la Fuente and Catalá, 2002). Thus, for example, adults of *Triatoma infestans*, a predominantly domestic bug, shows a reduction in the density of those sensilla in the pedicelus whereas the adult *T. sordida*, a species that tends to occupy more diverse habitats and has greater capacity for active dispersal, carries more such sensilla in that segment (Catalá, 1997). In addition, differences in the pattern of chemosensory sensilla have been found between sylvatic and domestic populations of *T. infestans* (Catalá and Dujardin, 2001). Moreover, *T. sordida* and *T. dimidiata* are examples of species that show sexual dimorphism in the pedicelus because males show a higher density of basiconic thin-walled sensilla (Catalá, 1997; Catalá et al., 2005). In Rhodniini, the number of chemosensory sensilla on the flagellum varies between species (Catalá and Schofield, 1994).

### 3. The host cues

Vertebrate hosts are a source of plenty of sensory cues that haematophagous insects are able to detect. This includes visual, mechanical, thermal, hygric and chemical signals produced by the hosts or by the associated microbial flora. In the case of triatomines, three types of cues have been shown to be directly involved in host-seeking. These are heat, moisture and odours (the latter including CO<sub>2</sub>, which we strictly consider an odour for the purposes of this

**Table 1**

Host odourants found active on triatomine olfactory receptor cells (ORCs). Responses consisted on an increase in spike rate unless otherwise indicated. Grooved pegs (GPs) and basiconic sensilla (BS) (Guerenstein and Guerin, 2001) in this table were named basiconic and trichoid thin-walled sensilla respectively by Catalá (1997). Apart from the odourants found active in particular sensilla, EAG recordings indicated response to C<sub>3</sub>, C<sub>5</sub> and C<sub>6</sub> n-carboxylic acids (Barrozo, 2003). Results are from nymphs of *T. infestans* unless otherwise indicated.

ORC type	Best stimuli known	Other, less effective, stimuli known	References
GP type 1 - ORC1	Ammonia	Methyl-, Dimethyl-, Ethyl-amine	Taneja and Guerin, 1997; Guerenstein and Guerin, 2001; Diehl et al., 2003
GP type 1 - ORC3	Isobutylamine	Isopentylamine (no other C <sub>4-5</sub> amine tested) <sup>a</sup>	Guerenstein, 1999; Diehl et al., 2003
GP type 2 - ORC1	Isobutyric acid	Butyric, Isovaleric, 2-methylbutyric acids	Bernard, 1974; Taneja and Guerin, 1997; Guerenstein and Guerin, 2001; Diehl et al., 2003
GP type 2 - ORC2	Ammonia	Methyl-, Dimethyl-, Ethyl-amine	Bernard, 1974; Taneja and Guerin, 1997; Guerenstein and Guerin, 2001; Diehl et al., 2003
GP type 2 - ORC3	Isobutylamine	3-Methyl 2-butylamine, 2-butylamine, butylamine, isopentylamine <sup>a</sup>	Guerenstein, 1999; Diehl et al., 2003
GP type 3 - ORC1	C <sub>4-5</sub> aliphatic amines <sup>b</sup>		Guerenstein, 1999; Diehl et al., 2003
GP type 3 - ORC3	Isobutylamine	2-Butylamine, 3-Methyl 2-butylamine, Butylamine, Isopentylamine <sup>a</sup>	Diehl et al., 2003
BS Aldehyde receptor <sup>c</sup>	Heptanal, Octanal, Nonanal	None	Guerenstein and Guerin, 2001
BS terpene receptor 1	α(+) pinene, (-) limonene	None	Guerenstein, 1999
BS terpene receptor 2	α(+) pinene	None	Guerenstein, 1999
BS terpene receptor 3	Terpinen-4-ol	α(+) terpineol	Guerenstein, 1999
BS heterocyclic-aromatic receptor (adults)	Pyridine, furan	None	Mayer, 1968

<sup>a</sup> Weak responses to Methyl-, Dimethyl-, Ethyl-amine were also obtained.

<sup>b</sup> Responses consisted on a decrease in spike rate.

<sup>c</sup> A nonanal-excited cell was also found in basiconic sensilla of adults of *T. infestans* and in nymphs and adults of *Rhodnius prolixus* and *Dipetalogaster maxima*.

review). Mechanical cues are indirectly concerned, as responsible for odour-triggered anemotaxis (to follow an airstream) and the response to pulsed odour plumes, as for example, those originated by host breathing. If triatomines are spontaneously anemotactically negative (e.g., Taneja and Guerin, 1995, 1997) or positive (Barrozo et al., 2003; Barrozo and Lazzari, 2004a,b) is not fully clear. In any case, it should be emphasized that they show a strong tendency to walk upwind (positive anemotaxis) when an airstream is laden with an attractive cue.

### 3.1. Host odours: the long-range guides

The detection of host odours activates triatomines, which tend to increase their locomotor activity (Núñez, 1982; Taneja and Guerin, 1995, 1997; Guerenstein and Guerin, 2001). In a domestic habitat the spontaneous or odour-induced increase in locomotor activity may make the insects leave their refuge and display long-range attraction behaviour if the appropriate attractive odours are present (see Section 4). Both behavioural responses, activation and attraction, can be evoked by natural host odour blends or by single constituents (odourants) of those blends.

As in virtually every hematophagous insect, one such odourant is carbon dioxide (CO<sub>2</sub>; Guerenstein and Hildebrand, 2008). It is known that odours from a source travel through the air in discrete packets so that at a certain distance from that source those packets of odour interspersed with clean air are detected as intermittent stimuli. For a flying insect encountering such an 'odour plume' the frequency of stimulation can be high, and that frequency increases as the insect approaches the source. Thus, mosquitoes can only orientate toward sources of CO<sub>2</sub> if this odourant is presented in an intermittent (pulsed) manner (Geier et al., 1999). In contrast, *T. infestans* nymphs are attracted by a CO<sub>2</sub> source under both, continuous or pulsed stimulation, although the latter becomes repellent at high frequency (Barrozo and Lazzari, 2006). It is unknown if this interspecific difference is, for example, related to the fact that triatomines walk while searching for a host, or because in their habitat, odour may disperse in a more homogeneous way (it could be expected that at night, especially in the domestic and peridomestic habitats, atmospheric turbulence is low and odour plumes will be more continuous; see Lehane, 2005). The oriented response of triatomines towards CO<sub>2</sub> is modulated by an endogenous circadian rhythm (Barrozo et al., 2004a). The insects respond to CO<sub>2</sub> only at the beginning of their scotophase, when they search for food (Barrozo et al., 2004a; Barrozo and Lazzari, 2004a), and it has been shown that the temporal pattern of response to different odourants varies according to the temporal allocation of the behavioural context to which each odour is associated with (Lazzari et al., 2004; Bodin et al., 2008a). On the contrary, the starvation time (as time after ecdysis) does not modulate this response in the range 20–60 days (Barrozo and Lazzari, 2004a). It should be noted that, as in at least some mosquitoes (Gibson and Torr, 1999; Bosch et al., 2000; Bernier et al., 2003; Smallegange et al., 2005), CO<sub>2</sub> is not a necessary stimulus to attract triatomines (Núñez, 1982; Taneja and Guerin, 1997; Guerenstein and Guerin, 2001; Barrozo and Lazzari, 2004a,b) although it is sufficient to evoke such attraction (Núñez, 1982; Taneja and Guerin, 1995; Guerenstein and Guerin, 2001; Barrozo and Lazzari, 2004a).

Other host odourants also evoke behavioural responses per se. Those responses may just include activation as in the case of nonanal, an odourant present in chicken, human and other vertebrate odours (Guerenstein and Guerin, 2001 and references therein). In addition, odourants that evoke just attraction include isobutyric acid and 1-octen-3-ol (Guerenstein and Guerin, 2001; Barrozo and Lazzari, 2004a), both constituents of human sweat odour (Cork and Park, 1996). Finally, both activation and attraction

are evoked by ammonia, an odourant present in sweat and urine of vertebrates (Taneja and Guerin, 1997 and references therein). Single odourants are effective only within a narrow range of concentrations (e.g., Guerenstein and Guerin, 2001; Barrozo and Lazzari, 2004a), except for CO<sub>2</sub> (Barrozo and Lazzari, 2004a).

In nature, the insects are confronted to mixtures of odours which often include common constituents. Responding uniquely to such mixtures, consisting on several constituents presented at particular proportions, should help increase the certainty that the odour source is (or is not) the expected host (Lehane, 2005). Thus, natural odour mixtures are more efficient in attracting triatomines than single odourants (e.g., Barrozo and Lazzari, 2004b), and some synthetic mixtures also evoke a higher response respect to that to its constituents when presented singly (Table 2). While work with single odourants emphasized the importance of CO<sub>2</sub> in triatomine host attraction (e.g., Barrozo and Lazzari, 2004a), work with mixtures of odourants highlighted the role of L (+) lactic acid (L-LA), a human skin odourant (Barrozo and Lazzari, 2004a,b). L-LA is not attractive by itself although when combined with CO<sub>2</sub> a synergistic attraction is observed (Barrozo and Lazzari, 2004a). Thus, the response threshold for CO<sub>2</sub> when presented alone (ca. 300 parts per million–ppm) decreases when combined with L-LA to ca. 75 ppm. Moreover, a mixture of L-LA and C<sub>3–5</sub> n-aliphatic carboxylic acids (present in human sweat and skin odour; Cork and Park, 1996; Bernier et al., 2000), at a particular odourant ratio, is attractive even when none of the constituents evoke a response when presented alone at the same, lower, or even higher concentrations, i.e., a synergistic effect is observed. Finally, when the latter mixture is combined with sub-threshold amounts of CO<sub>2</sub>, an additional synergistic effect is evinced, making the mixture L-LA + C<sub>3–5</sub> carboxylic acids + CO<sub>2</sub> as attractive as the odour of a mouse, at least, under laboratory test conditions (Barrozo and Lazzari, 2004b; Table 2). Attraction to synthetic odour mixtures usually occurs within a narrow range of concentrations and ratios (Barrozo and Lazzari, 2004a,b). In mosquitoes, a mixture of L-LA and CO<sub>2</sub> also acts in a synergistic way (e.g., Geier and Boeckh, 1999), and the mechanistic bases of this phenomenon are being unveiled. Thus, a short pulse of CO<sub>2</sub> instantly sensitizes the olfactory system to human skin odours for >10 s (Dekker et al., 2005). In mosquitoes the synergy between L-LA and CO<sub>2</sub> occurs at the CNS level because those two odourants are perceived by different sensory organs (see Guerenstein and Hildebrand, 2008). Synergistic effects in neuronal responses to CO<sub>2</sub> plus other odourants at the central level have been observed in the antennal lobe of a moth (Guerenstein et al., 2005). Also, interactions between responses to odourants at the peripheral sensory level have been observed in, for example, beetles and moths (Nikonov and Leal, 2002; Ochieng et al., 2002). A better understanding of the interactions between the information about different odourants in the olfactory system of triatomines may help develop more efficient synthetic attractant blends for them.

As mentioned, CO<sub>2</sub> is known to be used as a host cue by a number of hematophagous insects. Moreover, the ORCs of different hematophagous insects including triatomines appear to respond to similar or even the same host odourants. Thus, virtually all host odourants triatomines are known to detect are also detected by other blood-sucking insects. The list includes nonanal (triatomines: Guerenstein and Guerin, 2001; mosquitoes: Du and Millar, 1999), L-LA (triatomines: Barrozo and Lazzari, 2004a; mosquitoes: e.g., Geier and Boeckh, 1999), ammonia (triatomines: Taneja and Guerin, 1995; mosquitoes: e.g., Meijerink et al., 2001), 1-octen-3-ol (triatomines: Barrozo and Lazzari, 2004a; mosquitoes: Takken and Knols, 1999), short-chain carboxylic acids as butyric acid (triatomines: Guerenstein and Guerin, 2001; Barrozo and Lazzari, 2004b; Barrozo, 2003; mosquitoes: e.g., Pappenberger et al., 1996; Knols et al., 1997), C<sub>4–5</sub> aliphatic amines as isopentylamine (tri-

**Table 2**

Synthetic odour mixtures tested in behavioural studies. Increased activation and attraction refer to the behavioural response evoked by the mixture respect to that evoked by the constituents alone (i.e., additive or synergistic effects). Negative results are of limited value because only one or a limited number of proportions and concentrations have been tested. Results from nymphs of *T. infestans* unless otherwise indicated. L-LA: L (+) lactic acid; C<sub>3-6</sub> refers to n-aliphatic carboxylic acids.

Mixtures tested	Increased activation	Increased attraction	References
L-LA + CO <sub>2</sub>	No	Yes <sup>b</sup>	Núñez, 1987 <sup>a</sup> ; Barrozo and Lazzari, 2004a
L-LA + C <sub>3</sub>	No Data	Yes <sup>b</sup>	Barrozo and Lazzari, 2004b
L-LA + C <sub>4</sub>			
L-LA + C <sub>5</sub>			
L-LA + C <sub>3</sub> + C <sub>4</sub> + C <sub>5</sub>			
L-LA + C <sub>6</sub>	No Data	No	Barrozo and Lazzari, 2004b
C <sub>3</sub> + C <sub>4</sub> + C <sub>5</sub>			
C <sub>3</sub> + C <sub>4</sub> + C <sub>5</sub> + CO <sub>2</sub>			
L-LA + C <sub>3</sub> + C <sub>4</sub> + C <sub>5</sub> + CO <sub>2</sub>	No Data	Yes <sup>b,c</sup>	Barrozo and Lazzari, 2004b
CO <sub>2</sub> + Ammonia	Yes	Yes	Rose, 1998; Otálora-Luna et al., 2004 <sup>a,d</sup>
Isobutyric acid + Nonanal	No	No	Guerenstein and Guerin, 2001
Isobutyric acid + Ammonia			
Isobutyric acid + CO <sub>2</sub>			
1-octen-3-ol + CO <sub>2</sub>	No	No	Barrozo and Lazzari, 2004a

<sup>a</sup> Tested on nymphs of *R. prolixus*.

<sup>b</sup> Synergistic effect.

<sup>c</sup> Attraction similar to that evoked by mouse odour.

<sup>d</sup> Direct comparison with the response evoked by the constituents alone is not obvious.

atomines: Diehl et al., 2003; mosquitoes: Pappenberger et al., 1996), and terpenes as  $\alpha$ (+) pinene (triatomines: Guerenstein, 1999; mosquitoes: Bowen, 1992). Many other host odourants known to be detected by hematophagous insects have not been thoroughly tested or not tested at all in triatomines yet, and it could be expected that at least some of them are also active in these insects. The long list includes indole, 3-octanol, oxobutyric acid, acetophenone, 6-methyl-5-hepten-2-one, and acetone, among many others (Kwon et al., 2006; Meijerink et al., 2000; Syed and Leal, 2007; Gibson and Torr, 1999; Logan and Birkett, 2007). In mosquitoes, the behavioural role of some of these odourants is unclear because when added to an odour mixture a single odourant increases attraction in some tests whereas it makes the mixture repellent/unattractive in others, and these contradictory results were also obtained with L-LA (see Logan and Birkett, 2007). Thus, a compound presented at an inappropriate ratio in a blend may appear as a repellent (i.e., it may work as a repellent in that odour context). In addition, the relevance or effect of a certain odourant within a mixture could depend on which are the other constituents of that mixture. For example, an odourant could be necessary to make a certain mixture attractive, and have a less important role in another mixture, composed of other constituents.

### 3.2. Heat: a main cue for triatomines

Even when the heat emitted by the host body is widely recognized as an orienting cue for many blood-sucking insects, its role rests relatively unknown for the vast majority of these insects because most investigators are more interested in chemical than in thermal cues. This is not the case for triatomines, whose thermal sense and the use they do of thermal cues have been the object of diverse studies.

The behavioural sensitivity of triatomines to heat is extremely high. They react to extremely weak heat energy. In the experiments by Lazzari and Núñez (1989a), significant responses were obtained when small heat sources were used and only a few  $\mu$ Watt/cm<sup>2</sup> reached the insect (Lazzari and Núñez, 1989a; Lorenzo et al., 1999a,b). In terms of detection range, this means that they would be able to perceive a human face from a maximal distance

of 2 m, and a dog from several meters. If confirmed, these results would indicate that triatomines possess one of the highest thermal sensitivities known in animals up to date.

Triatomines remains as the only group of blood-sucking insects where the ability to perceive the infrared radiation emitted by the host body has been demonstrated (Lazzari and Núñez, 1989a; Schmitz et al., 2000). But, what this means in terms of ability to locate a host? To answer this, some thermodynamics concepts are necessary. The transfer of heat between two bodies at different temperatures may occur according to three different physical processes, i.e., conduction, convection and radiation. When heat conduction energizes atoms, it increases their vibration and this vibration spreads through the material. In our case, the material is the air, and the result is the formation of a temperature gradient around the body of a host, which could be used as an orientation cue by the insects.

Convection concerns the heat exchange with a moving fluid. When the fluid (air) is heated by conduction from the body, its temperature increases and it becomes less dense. Subsequently, it starts to ascend away from the heat source. When its temperature drops back, its density increases and thus, it descends. For some hematophagous insects, convection is not only a matter of heat transfer, but also of the production of ascending airstreams that transport host odours. Mosquitoes, for example, seem to make use of convective currents to approach a host (Lehane, 2005). It should be noted that convective currents are only useful as a cue when the insects approach from just above a host.

The third mechanism, radiation, involves the emission and absorption of radiant heat of wavelengths corresponding to the infrared region of the electromagnetic spectrum. This exchange does not require a conducting material or the movement of fluid. Any object which temperature is above the absolute zero (0 K or -273 °C) emits infrared radiation of a wavelength corresponding to its temperature. At present, the capacity to perceive radiant heat has been found in just three groups of animals: snakes, pyrophilic Coleoptera and Triatominae. For a blood-sucking insect, detection of radiation implies the ability to assess, from any relative position, the heat emitted by a potential host, without perturbation by wind, which disrupts conduction gradients and convective currents. Cur-

ously, this ability has been rarely investigated in other insects, being assumed that they use warm air gradients (i.e., conduction) or convective currents as cues, even when experimental evidence is also lacking for these mechanisms.

Heat receptor organs are found mainly on the antennae of triatomines (Bernard, 1974; Insausti et al., 1999; Lazzari and Wicklein, 1994). These organs are actively moved following particular patterns in the presence of a source of heat (Flores and Lazzari, 1996). These movements, together with the bilateral integration of antennal inputs play a key role in the location and evaluation of a distant heat source by triatomines. Standing bugs scan a wide portion of the environment by moving the antennae and, once walking, they maintain the chosen course by focusing the goal with the antennae, whose angle of aperture depends on the solid angle subtended by the thermal source (Flores and Lazzari, 1996; Flores, 2005). The integrity of the two antennae is necessary for a correct orientation towards the heat source (Wigglesworth and Gillet, 1934; Flores and Lazzari, 1996). Unilateral antennectomy results in initial straight trajectories towards the goal, always followed by a sharp deviation towards the side of the intact antennae at a fairly close and constant distance from the source. This failure to reach the goal evinces the existence of a control system switching between two orientation mechanisms (Dusenbery, 1992). *Telotaxis*, for which the input of only one bilateral organ suffices, guides orientation up to ca. 1.0–1.5 cm from the source, when the turn occurs, whereas *tropotaxis*, which relies on the integration of information gathered by bilateral sense organs, guides the final approach. The integration of bilateral information by tropotaxis by an antennectomized insect results in a greater stimulus intensity reaching the intact antenna, and consequently a turn towards that side occurs in order to try to equalize the bilateral inputs.

*T. infestans* can discriminate the temperature and distance from a heat source, independently of the source's emitting area, using solely thermal information, a behaviour that could be related to their ability to perceive radiant heat (Lazzari and Núñez, 1989a). Radiation is a less diffuse cue than heat conduction or convection; consequently, the necessary information to estimate the distance to a thermal source may be derived from the differential stimulation received in different portions of the antennae. Particularly, the angle of incidence, area, temperature, would provide useful information towards that aim. How do we know that *T. infestans* is capable of discerning sources at different temperatures as well as at different distances? Roughly speaking, a distant (or small) burning source and a close (or big) tepid source are well discriminated by these bugs (Lazzari and Núñez, 1989a; Lorenzo et al., 1999a,b). How they obtain and make use of thermal information for this task is a puzzle that is still being studied.

In triatomines, the importance of heat is not only highlighted by the high sensitivity of their thermal sense (Lorenzo et al., 1999a,b) or by their ability to evaluate the thermal properties of a distant source; the biological roles of this cue also emphasize its relevance. Indeed, heat is the only host-associated signal, both necessary and sufficient to induce the bugs to display the proboscis extension response (PER) and to bite an object. As mentioned above, odours are very important cues for the orientation of the bugs. However, a source of odours at room temperature is usually not bitten. Conversely, bugs will try to bite any odourless object which temperature is around that of a living host (Lorenzo et al., 1999a,b). Objects at a temperature outside the range between a couple of degrees above ambient levels and ca. 47 °C, would not be considered as a potential host (Lorenzo et al., 1999a,b).

It is worth to mention that our knowledge about how triatomines acquire and make use of thermal information comes from a couple of species (mainly *R. prolixus* and *T. infestans*) which feed on warm-blooded vertebrates. Nevertheless, some triatomines

obtain food from reptilians or even other insects, i.e., formally, cold-blooded animals, a fact raising the question about the role of heat in this species. Albeit it can be argued that even these animals can increase its body temperature by basking or metabolic activity, we cannot exclude that the mechanism triggering the PER could be based on different stimuli. Indeed, Wigglesworth and Gillet (1934) indicated in their classical work that when both antennae are ablated to *R. prolixus*, the bugs started to follow moving objects and when in proximity, they extended their proboscis apparently trying to bite. They interpreted this visual pursuit as a primitive behaviour associated to a predatory ancestral origin.

The way in which triatomines make use of thermal information is not only interesting from a fundamental point of view, but also because of their practical consequences. Indeed, thanks to the fact that bugs tend to bite any warm objects, it is possible to rear them using simple artificial feeders, demanding only to increase the temperature of the blood some degrees above ambient levels to make the bugs feed (Núñez and Lazzari, 1990).

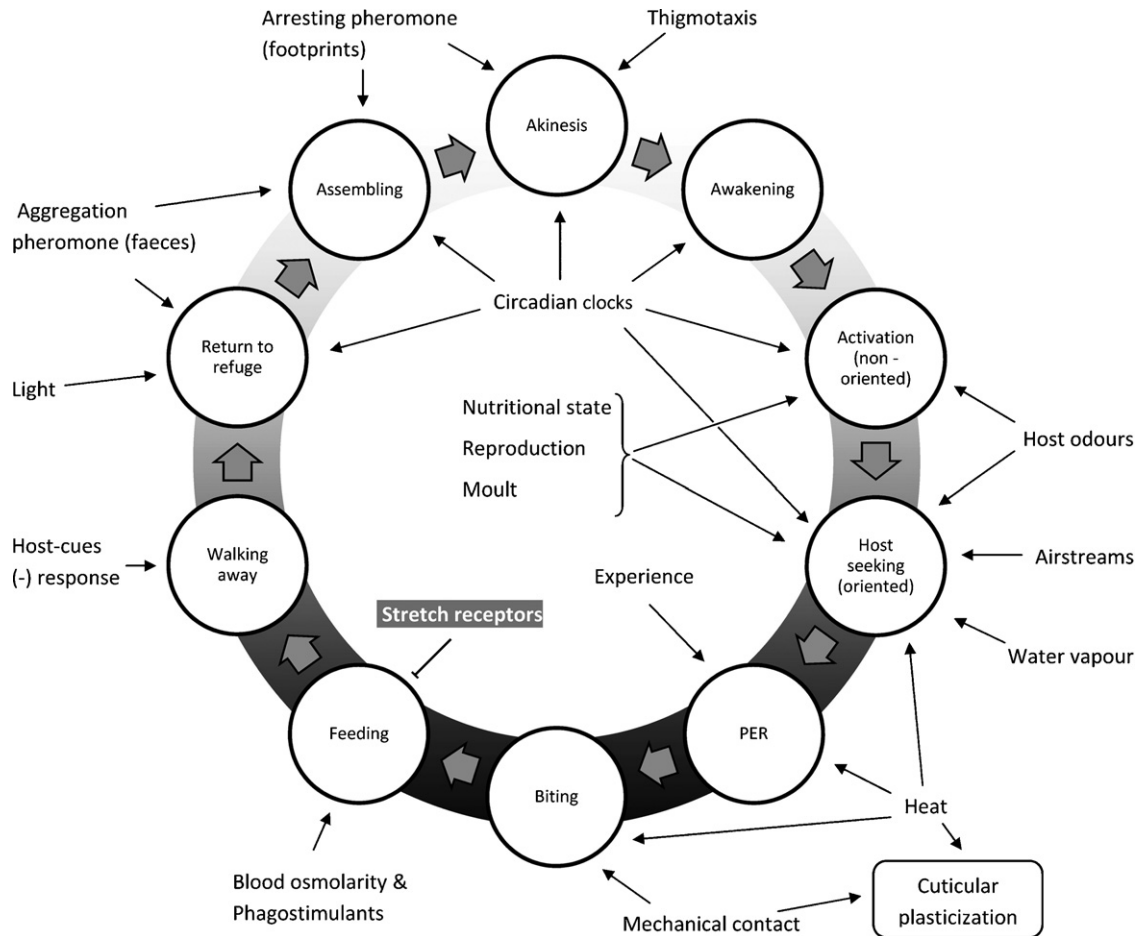
It should be emphasized that in a natural context, triatomines are not exposed to single cues, such as a particular odour or heat, but to combinations of multiple cues. The extreme sensitivity of triatomines to heat may be further increased by central integration of heat information with information about other cues. For instance, it has been shown that water vapour, which constitutes a close-range orientation cue by itself; increases the response of triatomines to heat (Barrozo et al., 2003). However, if the enhanced response is due to integration of different sensory inputs by the insect's brain or to a physical phenomenon (moist air transports more heat than dry air) is by the moment unclear.

Recently, heat has been attributed an additional important role in triatomine food-seeking behaviour: to locate blood vessels below the skin (Ferreira et al., 2007). By analyzing the biting behaviour of bugs over a live host, it has been shown for the first time that the insects do not direct their proboscis randomly over the skin, but directly towards a vessel. When the host skin was replaced by a heated metal plate with a vessel-shaped heat source on it, the temperature of both being controlled independently, the same precision in finding the warmest zone was observed, suggesting that heat discontinuities over the skin might guide biting. Moreover, the same authors found that biting the warmest zone requires bilateral integration of the thermal inputs of both antennae. If this integration is abolished or the antennal inputs experimentally altered, the bugs miss the target or do not display the PER at all, suggesting that, if present, rostral thermoreceptors would not be involved in guiding the biting behaviour (Ferreira et al., 2007).

#### 4. A typical night in the triatomines' life

Host finding and blood-sucking are just components of a complex behavioural repertoire triatomines display during the night. Therefore, for a better understanding of the place that these behaviours occupy in the context of the general activity of the insects, a summary of the behaviours that would be displayed in a night when a bug feeds is provided here (see Fig. 1 and references therein).

Triatomine bugs, particularly those species relatively closely associated to the human habitat, spend most of the daytime in akinesis and hidden in their refuges. During this time, their diurnal hosts are active. The akinesis behaviour is controlled by external cues, such as assembling pheromones and tactile stimulation by the substrate and conspecifics (i.e., the bugs show thigmotaxis), in combination with an internal motivational state of inactivity, controlled by the insect's circadian system (i.e., internal clocks). At dusk, when their hosts are resting, the bugs awake and leave their refuges to adventure into an environment abundant in cues reveal-



**Fig. 1.** Diagram showing the series of events occurring in a typical night when a triatomine bug feeds, indicating the effect of endogenous or motivational (inside the ring of circles) and exogenous (outside) factors upon the different behaviours. During the day hours the insect remains immobile (i.e., in “akinesis”; Wigglesworth and Gillet, 1934) inside its refuge, until the circadian system drives its awakening (Lazzari, 1992). The animal starts moving, eventually leaving its refuge (Lorenzo and Lazzari, 1998). The exposition to host odours, the sensitivity for which is modulated by a circadian clock (Barrozo et al., 2004b) and dependent of the physiological state (Bodin et al., 2008b), activates the insect, increasing its chances to find guiding cues towards a potential food source. The synergisms within unimodal and between multimodal cues (Taneja and Guerin, 1995; Barrozo et al., 2003; Barrozo and Lazzari, 2004a,b), the sensitivity which is modulated by endogenous factors, facilitate the orientation of the insect towards a host. When in proximity, the heat emanated by the host body triggers the proboscis extension response (PER); heat is the only cue both necessary and sufficient to evoke the PER, a response modulated by the individual experience (Lallement, 2007), and biting (Lazzari and Núñez, 1989a; Flores and Lazzari, 1996). At this point the animal is confronted to find a blood vessel “hidden” under the host’s skin (solenophagy), a task guided by antennal thermoreceptors (Ferreira et al., 2007). The mechanical contact with the warm skin surface evokes the insertion of the piercing mouthparts and the plasticization of the abdominal cuticle of larvae, which allows the bug to take a big blood meal (Janowski et al., 1998). Once blood is located, its osmolarity and chemical compounds (such as adenosine nucleotides and salts) but not its temperature, allows blood recognition, triggering feeding (Friend and Smith, 1977; Guerenstein and Núñez, 1994; Lazzari and Núñez, 1989b). The stimulation of abdominal stretch receptors (Anwyl, 1972; Chiang and Davey, 1988) stops feeding when the animal has gorged. After feeding, the insect walks away from the host, to protect itself from antiparasitic host behaviours. Walking away could be driven by the same cues involved in host location, in this case evoking repellence (Bodin et al., 2008b). The insect remains outside a shelter until dawn (Lorenzo and Lazzari, 1998), when it returns to its refuge guided by volatiles released by the bug’s faeces (aggregation pheromone) as chemical landmarks of the access to refuges (Lorenzo and Lazzari, 1996), the sensitivity to which is modulated by the natural light cycle (Bodin et al., 2008a). This aggregation pheromone (Schofield and Patterson, 1977), together with an arresting one left by footprints (Lorenzo Figueiras and Lazzari, 1998) and thigmotaxis keeps the insects in akinesis inside the refuge during the day hours, protecting them from predators.

ing the presence of vertebrate hosts. Volatile odourants released by the host skin and breath diffuse and are transported by airstreams. The first physiological effect of these chemicals is to activate the bugs to walk around in order to increase the probability of finding cues capable to orient them to a potential host. The bimodal stimulation of antennal receptors by mechano- (air movement) and chemical (odours) stimuli triggers the odour-modulated anemotaxis (upwind orientation). Provided that airstreams are laden with odours after they blow over the host’s body, just walking facing the stream, represents a simple solution to come close to the host. As the bug approaches the host, cues of additional modalities (e.g., water vapour and heat) may be detected, assigning an appetitive value to the complex, multimodal signals. It should be mentioned that some host odourants are associated to more than

one context in the biology of triatomines. Certain compounds, such as valeric, butyric and isobutyric acid are present in pheromonal blends (alarm, sex, aggregation; e.g., Schofield, 1979a,b; Cruz López et al., 1995; Rojas et al., 2002; Guerenstein and Guerin, 2004; Manrique et al., 2006), in addition to being emitted by hosts. The unimodal and multimodal convergence of stimuli allows a parsimonious use of sensory organs, in particular chemoreceptors, but needs the integration of several cues to define a particular context. In this case, host odourants (including CO<sub>2</sub>) and heat, would determine a feeding context.

Once near or over a host, the insect must find an adequate place to bite and to find a blood vessel. For this, as mentioned above (Section 3.2), heat plays a key role. On the one hand, as previously indicated, heat is the only cue both, necessary and sufficient to

evoke the PER which brings the bug's mouthparts in contact with the host's skin (Flores and Lazzari, 1996). On the other hand, the thermal sense is responsible for the choice of the biting place. A random search by repeatedly biting the host skin could bring the bug to eventually find a blood vessel. Nevertheless, this strategy increases the risk to be detected by the host. Triatomines would be able to detect temperature discontinuities in order to direct their bites to blood vessels, before any contact of their proboscis with the host skin (Ferreira et al., 2007).

When the proboscis of a triatomine nymph contacts a warm surface, not only biting is triggered, but also a plastic change in the mechanical properties of their abdominal cuticle (plasticization; Bennet-Clark, 1962). This process takes place every time the insect tries to feed, notwithstanding whether or not the insect succeeds in drawing some blood (Ilanowski et al., 1998; Melcon et al., 2005). The result is that the abdomen is capable of storing a significant amount of blood.

Once the stylets reach the interior of a vessel, it is the blood osmolarity and the presence of phagostimulants which will confirm the insects that acceptable blood is available and, therefore, that blood-sucking could start (Friend and Smith, 1977; Guerenstein and Núñez, 1994). Interestingly, triatomines do not evaluate blood temperature. Changing this temperature during feeding does not affect their behaviour (Friend and Smith, 1977). Moreover, the bugs can even be induced to feed on cold blood (6 °C) by appropriately heating the antennae (Lazzari and Núñez, 1989b).

If not perturbed, a blood meal may reach 5–10 times the insect's body weight, and sometimes even more. The signal to stop sucking blood is provided by abdominal stretch mechanoreceptors (Anwyl, 1972; Chiang and Davey, 1988). Once detached from the host skin, the insect walks away from the host in order to avoid being detected and eventually killed. Recent evidence reveals that the same cues allowing the insects to orientate towards a host may have a repulsive role depending on the nutritional state of the bug (Bodin et al., 2008b).

The recently fed bug should return to a refuge in order to find protection and rest during the day. Nevertheless, this does not happen immediately after feeding, which is usually accomplished at the beginning of the night, but at dawn (Lorenzo and Lazzari, 1998). During the time before dawn, the insect exhibits no responsiveness to aggregation signals (Lorenzo Figueiras and Lazzari, 2000; Bodin et al., 2008a), allowing the copious diuresis that follows feeding to occur outside shelters.

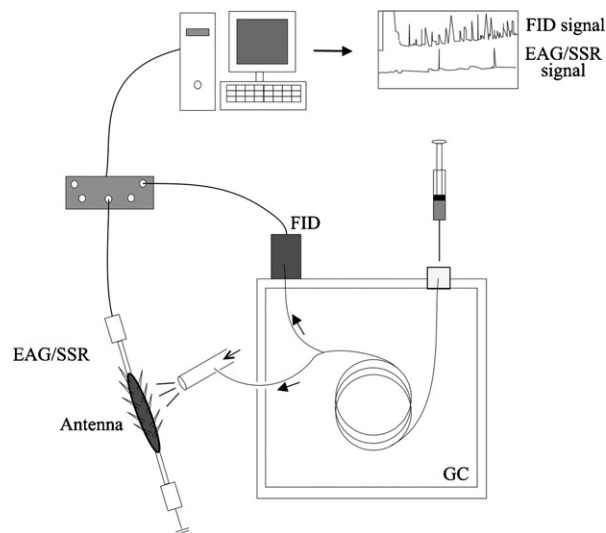
At dawn, bugs start returning to their shelters guided by chemical landmarks present in their faeces, and which are mainly found near the accesses to refuges (Lorenzo and Lazzari, 1996). These substances constitute one of the aggregation pheromones of triatomines (Schofield and Patterson, 1977; Lorenzo Figueiras et al., 1994). A second one, associated to the cuticle, is left behind on walked surfaces, and promotes assembling and arresting of the insects (Lorenzo Figueiras and Lazzari, 1998). These two pheromones, together with thigmotaxis, keep the insects inactive inside shelters during daytime. The internal circadian clock determines the moment to awake to start again a new cycle of activity (Lazzari, 1992).

## 5. The methods of study

The study of how triatomines acquire and make use of information to find blood sources requires a diversity of experimental approaches. For both sensory and behavioural studies on olfaction, it is important to use natural host odour as one of the stimuli to be tested. For this, headspace extracts of the odourants that constitute the host odour mixtures should be made. To make such an extract, odour is collected using a variety of techniques that include the use

of synthetic adsorbents (e.g., Tenax, Porapak) for air entrainment (e.g., Guerenstein and Guerin, 2001) and solid-phase microextraction (SPME, see Guerenstein and Guerin, 2004 and Manrique et al., 2006 for its use with triatomine odours), among other methods. In the former case, the adsorbent is eluted with an appropriate solvent and usually several microlitres of odour extract solution are obtained. In the latter, the odour mixture is absorbed by and kept in an SPME cartridge. In order to identify the odourants that constitute the odour mixture, the odour extract solution (or the SPME cartridge) can be injected into a gas chromatograph coupled to a mass spectrometer (GC–mass spectrometry, GC–MS). In contrast to SPME, the use of odour extract solutions gives the possibility to inject the sample several times and, in addition, to keep part of the extract for physiology or behaviour tests with the insects. On the other hand, the use of SPME has the advantage that no solvent is injected into the GC and, therefore, even very volatile constituents can be resolved and identified. However, for SPME, each GC injection requires a new odour collection.

Vertebrate odours are composed by hundreds of odourants. However, only a fraction of them are detected by the insects. To identify those odourants in an efficient manner, electrophysiological techniques that record the responses of ORCs are often used. Those techniques usually include the electroantennogramme (EAG, which represents the summation of the receptor potentials of a fraction of the ORCs on the antenna; Lorenzo et al., 1999a,b; Fontan et al., 2002) and single sensillum recording (SSR, which records the spiking activity of ORCs within a single sensillum, in addition to the sensillum receptor potential) (Mayer, 1968; Bernard, 1974; Taneja and Guerin, 1997; Guerenstein, 1999; Guerenstein and Guerin, 2001; Diehl et al., 2003). Whereas the advantages of the EAG include its simplicity of use and the ability to record the responses of several ORCs simultaneously, SSR is a more reliable and sensitive technique, and allows a systematic and complete functional characterization of all ORCs of an insect. The disadvantages of SSR



**Fig. 2.** Diagram of the gas chromatography coupled electroantennogramme or single sensillum recording set up (GC–EAG or GC–SSR). A sample of headspace host odour extract is injected into a gas capillary column in the oven of a gas chromatograph. After chemical separation is reached, the effluent is split between the GC detector (e.g., a flame ionization detector, FID) and the airstream to the electrophysiological preparation. Both the signal from the FID and from the electrophysiological preparation are fed into a computer. The time lag between the two signals is compensated by adequate software. The electrophysiological response/s indicate/s the FID peak/s to which the olfactory system responds to (i.e., the 'active' peak/s). Identification of the active peaks (odorants) is later accomplished by means of gas chromatography coupled to mass spectrometry.

include recording of responses from only one or a few ORCs simultaneously (therefore, it is time consuming), and that it is technically more challenging.

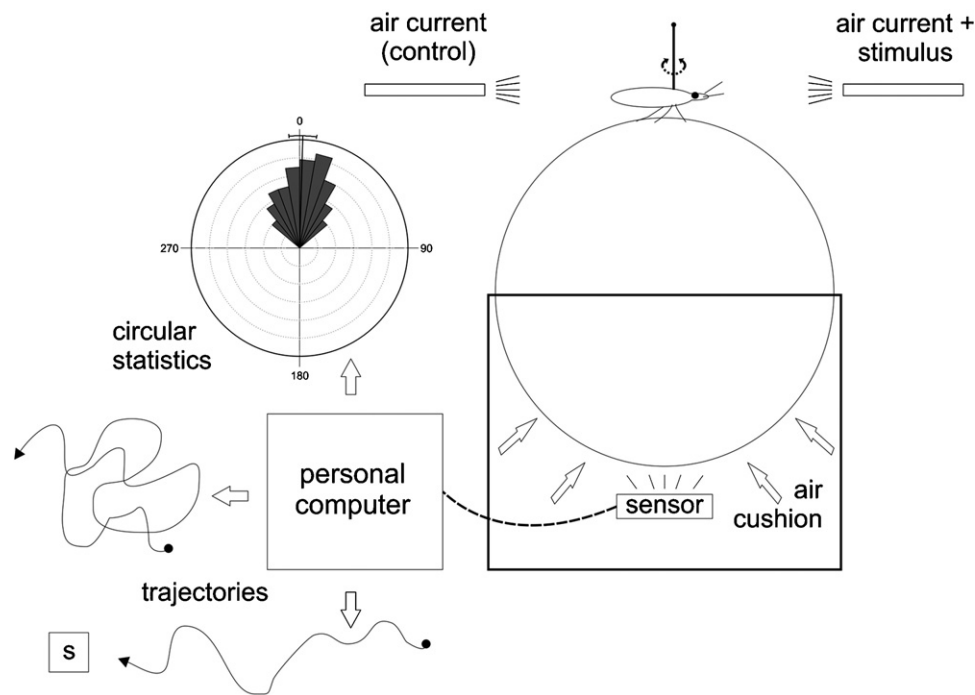
The olfactory stimuli for electrophysiology studies can consist on synthetic versions of odourants previously identified as constituents of host odour. As those electrophysiology recordings are usually long lasting, several odourants at different concentrations can be screened in a single experiment. However, a more efficient way to identify the active components of host odour consists on coupling a GC to an electrophysiology preparation (usually an EAG or SSR, Fig. 2; Guerenstein and Guerin, 2001). For this, an extract of host odour is injected into the GC, and the effluent is split between the GC odour detector (e.g., a flame ionization detector, FID) and the electrophysiological preparation. Then, the retention times of the GC (FID) peaks that evoked a physiological response are established. Identification of those odourants can be reached by means of GC–MS of the same extract sample. Thus, the insect's olfactory system serves as a bio-detector of the active constituents of the mixture. GC–EAG/SSR analysis of synthetic versions of the active odourants serves to confirm their identity (Guerenstein and Guerin, 2001).

Spontaneous behavioural activity (e.g., non-oriented appetitive search) and behavioural responses to host odours are studied using a variety of devices. In order to measure spontaneous activ-

ity and activation by host odours different actographs are used. One device (rocking actograph) consists on a cylindrical, horizontal, small chamber in which the insect is placed. The chamber lays on a pivotal line so that it could slightly fall to the left or right. An electrical circuit, closed only when the chamber lays on one side (e.g., right), records the displacements of the chamber and hence, of the insect (the bug moving across the midline turns on or off the electrical contact; Constantinou, 1984; Settembrini, 1984; Lazzari, 1992).

Another device consists on a wheel-cage actograph. In this case, the insect is placed in a vertical chamber. A glass capillary shaft, crossing the chamber at its mid height is the rotating axis of the cage. Movements of an insect result in rotation of the chamber, which is converted into electrical pulses by means of a lateral shutter that interrupts the light to an infrared-beam detector (Núñez, 1987).

Activation by odours is also studied using a number of other devices in which attraction can also be investigated. These devices, known as “locomotion compensators”, include the Kramer sphere, Dahmen sphere (and its modification by Lazzari) and olfactometers. In the Kramer sphere, a walking insect is kept at the apex of a sphere with the help of two servomotors. A sensor above the insect detects its movements and drives the servomotors to compensate the position of the sphere to maintain the insect at the apex (the insect's rotation movements, however, are not compensated). The  $x$ -,  $y$ -



**Fig. 3.** Experimental arrange to record the oriented response of triatomines to carbon dioxide and other chemicals under open-loop conditions. In this case, the locomotion compensator consists on a hollow Styrofoam sphere suspended by a vertical airstream (Dahmen, 1980; Barrozo and Lazzari, 2004a). The bug is fixed by its back to a freely rotating wire centred at the apex of sphere. The insect can walk and rotate freely, but cannot change its distance to the air outlet. An optic sensor placed in the opposite pole of the sphere registers the walking paths, and that information is fed into a computer (Barrozo and Lazzari, 2004a). A pump forces room air through a charcoal filter and a flowmeter to generate a clean airstream to be laden with the test odours. The pathways followed by the insects are analyzed by means of circular statistics (Batschelet, 1981; Fisher, 1993; Zar, 1998), since standard “lineal” statistical analysis is not applicable. The mean walking angle displayed by each insect along the experimental time is computed and subsequently, for every experimental group a mean angle and the length of the resultant mean vector ( $r$ ) are calculated. The relative position of the stimulus delivery current is conventionally designated as  $0^\circ$  and the control current as  $180^\circ$ . Whereas walking angles extend from  $0$  to  $360^\circ$ ,  $r$  varies between  $0$  and  $1$  ( $0$  indicating a non-defined mean direction and  $1$  a straight path to a given direction). The statistical evidence of directness is tested following the Rayleigh test (Batschelet, 1981; Zar, 1998), being  $H_0$ , the sampled population is uniformly distributed around a circle, versus  $H_a$ , the population does not follow a uniform circular distribution; circular uniformity implies no mean direction. When the data deviate significantly from uniformity, the  $V$ -test (Zar, 1998) is carried out to assess whether the mean angle calculated from the sample is statistically distant from the stimulus direction ( $0^\circ$ ). The pathways can be also tested for eventual bimodal axial directions (i.e., walking in opposite directions vs. randomly) by means of the Rao's spacing test (Fisher, 1993). Additionally, for an easier visualization of the data, an orientation index is calculated, multiplying the cosine of the group mean angle ( $gma$ ) by the length of the mean resultant vector ( $r$ ). The orientation index varies between  $-1$  and  $1$ ,  $-1$  indicating orientation away from the stimulus and  $1$  orientation towards the stimulus location (Barrozo and Lazzari, 2004a,b, 2006; Bodin et al., 2008a).



coordinates of the insect's displacements are tracked indirectly by two sensors that record the movements of the sphere. Thus, parameters related to the locomotor behaviour can be measured. These include average walking speed and total distance walked. Attraction is assessed by analyzing the walking angles respect to an airstream carrying the odour stimulus and calculating the percent displacement in the upwind direction (Taneja and Guerin, 1995, 1997; Guerenstein and Guerin, 2001; Otálora-Luna et al., 2004). The Dahmen sphere (Fig. 3) uses the same principles. However, whereas the perspex sphere of the Kramer device rests on the servomotors, the hollow Styrofoam sphere of the Dahmen device is suspended by a vertical airstream. In the latter case, the insect is tethered to a freely rotating stiff wire centred at the apex of the sphere. When walking the insect displaces the sphere, which movement is recorded by an optical sensor (Barrozo and Lazzari, 2004a,b, 2006; Barrozo et al., 2004a; Bodin et al., 2008a).

The olfactometers include a dual-choice device designed taking into account behavioural particularities of triatomines like *T. infestans* (Guerenstein et al., 1995), that is, it exploits the tendency of the bugs to let themselves fall from ceilings when detecting host odours as coming from below. The device consists of a rectangular arena with three plastic tubes connected to it. One tube acts as refuge and starting place, and the insects could leave this tube and reach the arena by climbing onto a piece of cardboard. The other two tubes, at the opposite side of the arena, act as test (odour stimulus) and control capture-tubes. The insects can drop into the capture-tubes but cannot escape from them. An airstream from the odour source (and its control) reaches the starting tube through the capture-tubes. In this case, activation is quantified as the percentage of insects caught in both capture-tubes relative to the total number of insects in the experiment. Attraction is quantified as the percentage of insects caught in the test tube relative to the number caught in total. The olfactometer differs from the sphere devices in that the insects can change their position respect to the stimulus source (close-loop design for translation). On the contrary, in the sphere devices the insects are always at the same distance from the airstream exit (open-loop design for translation) allowing a better control of the stimulation conditions.

Finally, attraction can also be quantified by using odour-lured trap devices (Guerenstein et al., 1995). A trap prototype in use consists on a circular access ramp from which the bugs can drop into a container from which they cannot escape. At the centre, there is a plastic receptacle containing the odour source from which volatile odourants diffuse. Odourants descend to the floor of the trap where they reach a series of exit slits in the rim of an inverted cup. The odours then diffuse up to the dark lid of the trap device. When host-seeking insects reach the upper edge of the ramp they detect the odour as coming from below. The bugs drop in only when odour reaches them from below. Attraction is quantified as in the case of the olfactometers (above).

The electrophysiological techniques (EAG and SSR) as well as the devices described to test behavioural responses can also be used for experiments in which the stimulus is different from odours (e.g., heat, water vapour).

The research here summarize shows that in addition to their role as Chagas disease vectors, triatomines are excellent models for studying basic physiological and behavioural mechanism and adaptations associated to haematophagy. We hope that the information provided here will encourage future research on them.

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