Sensory and behavioural responses of *Rhodnius prolixus* and *Triatoma infestans* to vertebrate odours and to volatiles from triatomine faeces

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(Réponses sensorielles et comportementales de *Rhodnius prolixus* et de *Triatoma infestans* aux odeurs des vertébrés et aux substances volatiles des fèces des triatomes)

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IMPRIMATUR POUR LA THÈSE

Réponses sensorielles et comportementales de Rhodnius prolixus et Triatoma infestans aux odeurs des vertébrés et aux substances volatiles des fèces des triatomes.

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La Faculté des sciences de l'Université de Neuchâtel sur le rapport des membres du jury,

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autorise l'impression de la présente thèse.

Neuchâtel, le 2 juillet 1996

Le doyen:

R. Dändliker

R. Dändliker

To my grand-parents, parents, brother and sister

Without God we cannot. Without us God will not.

St. Augustine

Experience, the name men give to their mistakes.

Oscar Wilde

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GENERAL INTRODUCTION

No one fancies being bitten by insects and undoubtedly the mere thought of a 2-3 cm long one piercing its needle-like proboscis through the skin and sucking out 200-300 mg of blood in 10-15 minutes is frightening. But in certain poorer quarters of South and Central America many people and animals are often visited in their sleep by such insects. These insects are known by their common name of "kissing bugs" as they frequently feed via the mucous membranes of the mouth and eyes.

A brief understanding of systematics and distribution of these bugs, of their habits and habitats, life-cycle, and the fact that they are medically and economically important insects would be useful before entering the realms of their behavioural world and the physiology of their senses. In the studies presented here an attempt is made to understand some sensory and behavioural aspects of host- and refuge-seeking by these bugs.

1. Triatomine systematics and distribution

The bugs used in these studies are hemimetabolous insects with piercing/sucking mouth parts and belong to the order of Hemiptera which consists of two sub-orders: Homoptera and Heteroptera. All Homoptera and many Heteroptera feed on plant fluids. Some Heteroptera are predaceous, feeding on body fluids of other arthropods and vertebrates. Two families of this sub-order contain blood-sucking insects: Cimicidae or bed-bugs (all haematophagous), and Reduviidae, commonly known as assassin bugs. Most Reduviidae are predators of other insects, but one sub-family, the Triatominae, are adapted to feed on vertebrate blood. There are more than 115 known species of Triatominae.

Triatomine bugs are mainly distributed in the American continent and some Caribbean islands, though a few species have been reported also from Africa, Asia and Australia (Lent & Wygodzinsky, 1979; Kalshoven, 1970; Else *et al.*, 1977 and Monteith, 1974). The geographic distribution of the model insects for my studies, *Rhodnius prolixus* and *Triatoma infestans*, is shown in Figure 1. *Triatoma infestans* is mainly distributed over the southern countries of the South American continent : Chile, Argentina, Uruguay, Paraguay, Bolivia, Brazil and Peru, and is still spreading. *Rhodnius prolixus* occurs in the northern part of South America, and in

Central America, mainly in Venezuela, Colombia, Guyana, the French Guyana, Surinam, Guatemala, Honduras and El Salvador.

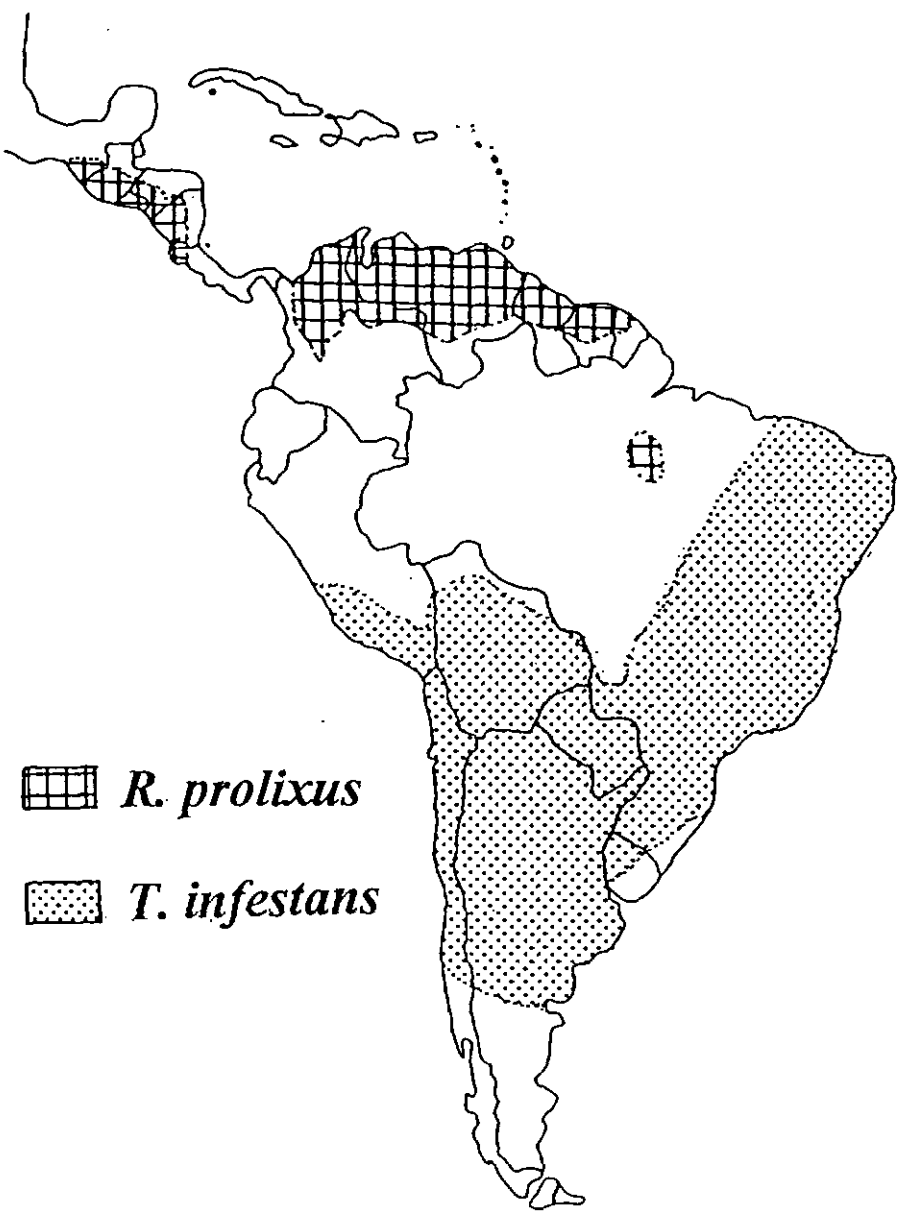

Figure 1 Distribution of two triatomine vectors of Chagas' disease *Rhodnius prolixus* and *Triatoma infestans* in Latin America

2. Habitats and habits of triatomines

All species of Triatominae are sylvatic in origin, their natural habitats primarily associated with the nests or burrows of a wide variety of small mammals and birds, and occasionally with lizards (Lent & Wygodzinsky, 1979). Some species, however, have become closely associated with man's domestic and peridomestic environment. They usually inhabit animal houses such as stables and corrals, chicken and pigeon coops, guinea-pig and rabbit houses, whereas human houses become infested only if they have unplastered or cracked walls, thatched roofs or other corners and crevices where the bugs can easily find refuge. *T. infestans* is almost exclusively a domestic and peridomestic species. The sylvatic habitat of this species amongst rock-piles associated with wild guinea-pigs is known only from the Cochabamba valley of Bolivia which is supposed to represent the original focus of the species (Schofield, 1994). *R. prolixus* still maintains its sylvatic ecotopes such as palm tree crowns and birds nests in addition to inhabiting domestic and peridomestic habitats.

Most triatomines are nocturnal in habit. They are found aggregated in their refuges during the day-time becoming active at night when they search for a host if hungry and in need of a blood-meal (Lent & Wygodzinsky, 1979). Settembrini (1984) studied the circadian rhythms of locomotor activity in *T. infestans* males in an actograph. Under 12:12 h L:D regime bugs showed a nocturnal unimodal locomotor activity-rhythm beginning with the onset of the scotophase and ending with the onset of the photophase. The rhythm persisted clearly for at least 8 cycles under regimes of constant light or darkness and adaptation to a reversed L:D regime occurred after 2-4 days, indicating that the locomotor activity in these bugs is endogenously controlled. Later, experiments by Lazzari (1992) revealed a bimodal pattern in the spontaneous daily locomotion activity in *T. infestans* exhibiting two peaks in activity, one at the beginning of the scotophase and the other at the beginning of the photophase. The two activity peaks could be correlated with the food-searching habit at dusk (beginning of scotophase) and with the refuge searching habit at dawn (beginning of photophase). The environmental temperature preference in *T. infestans* also varies with a daily rhythm and is dependent on the levels of starvation (Lazzari, 1991). From records of the positions of adult bugs in an arena with a thermal gradient, he found that during the scotophase the bugs moved to lower

temperatures in the gradient and suggested that a preference for lower environmental temperatures during the night is related to host-seeking since it augments the temperature difference between the source (vertebrate host) and the background. He also showed that higher temperatures were preferred soon after feeding to stimulate digestion and fluid loss, but with increasing starvation lower temperatures were preferred as it is presumably more suitable for conserving energy and water. Similar experiments with these bugs in a humidity gradient have shown that *T. infestans* prefers low (0%) humidity (Roca & Lazzari, 1994). Only the adult stages of these bugs are winged, the nymphs are wingless and incapable of flying. The adults of some triatomine species are known to fly over 100-200 km distances when dispersing from one habitat to another (Schofield *et al.*, 1991). Such dispersive flights occur more often at high temperatures (*ca.* 30°C) and conditions of low nutritional status (Lehane *et al.*, 1992), but if sufficient food sources are available the bugs generally do not leave a given habitat (Lent & Wygodzinsky, 1979). Though a more common means of dispersal is carried out passively by man via use of roofing materials such as palm fronds and straw, and by transporting the bugs and their eggs in furniture, clothes and other household goods (Lent & Wygodzinsky, 1979). Migratory birds can also serve as dispersal agents for the eggs and young stages of some nest-dwelling species such as *Rhodnius prolixus* (Lent & Wygodzinsky, 1979; Schofield, 1979).

3. Triatomine life-cycle

Triatominae have a typical hemimetabolous life-cycle, from eggs through five nymphal instars to adult males and females. All nymphal and adult life-stages of triatomine bugs are obligate haematophagues, most species feed at night on their sleeping hosts and they have a relatively long life-cycle and low rate of reproduction. Fertilized females generally lay a few eggs at a time and each female can lay 100-600 eggs during her adult life of 3-12 months, depending on species and quantity of blood ingested. The eggs generally hatch within 10-30 days after oviposition and the first instar nymphs are ready for their first blood-meal 2-3 days later, but can survive 2-3 weeks without feeding. The blood-meals are large, each nymph takes about 8-9 times its own weight and adults about 2-4 times. The later nymphal instars can withstand starvation for weeks or even months. Usually a single replete blood-meal of 20-30

minutes is sufficient to initiate moulting to the next nymphal stage, but 2-5 smaller meals are often taken by second to fifth instar nymphs before moulting. Under laboratory conditions at 26-27°C and 70% RH and with weekly feeding, *R. prolixus* can complete the cycle from egg to adult in 2-3 months while *T. infestans* takes 5-6 months.

4. Triatomines as vectors of Chagas' disease: medical and economic importance

Triatomine bugs transmit the protozoan parasite *Trypanosoma cruzi*, the causative agent of American trypanosomiasis or Chagas' disease. When an infected bug is feeding on a vertebrate host it drops its faeces containing the infective forms of *T. cruzi* onto the host skin. The parasite, thus released, penetrates the abraded skin, mucous membranes or any wound. After local multiplication at the site of entry the parasite enters the blood-stream and subsequently migrates to the tissues, especially muscle, where it multiplies and completes its development. After some time the parasite again enters the blood-stream and is infective to triatomine bugs who pick them up in the blood when feeding. The rest of the parasite's development is then completed in the vector's body and the cycle continues. The bugs, once infected, retain the infection for life.

Chagas' disease is a serious public health problem in the South American continent. Over half of the known species of Triatominae have been naturally or experimentally infected with *T. cruzi*, and because of their similar behaviour and physiology all species are regarded as potential vectors. This disease occurs mainly in rural areas with poor housing conditions and especially affects children and young adults, resulting in chronic suffering and high mortality. The World Health Organization estimates indicate that, over 24 million persons are infected and 65 million are at risk in the endemic areas (Schofield *et al.*, 1987). In addition to disease transmission, blood loss due to the bugs' feeding habits can cause chronic anaemia in the people living in infested houses. Average costs of supportive treatment and surgical interventions for chronically ill patients have been estimated at around US$ 40 million per year (Schofield & Dias, 1991).

No vaccine is available, and except in the very early stage of infection, there is no effective chemotherapy, leaving vector control as the only alternative. Insecticidal

control is the only method that has been extensively applied, but an effective environmentally acceptable and economically viable control method still remains to be developed.

5. Host-seeking in Triatominae

The triatomines are obligate blood-feeders at all life-stages so, each individual insect has to find a host several times in its life-time. From the studies on other blood-sucking arthropods it is known that several sensory modalities can play a role in host-finding and feeding on a host. Vision and olfaction are the most important senses reported to be involved in activation and orientation to a host over long distances. At shorter distances, in addition to visual and olfactory stimuli, temperature plays an important role. Heat and humidity are important stimuli required to initiate probing and feeding. *R. prolixus* has been a model for insect physiologists for almost a century, but behaviourally the triatomines have not been studied extensively.

In flying insects, vision plays an important role in host-seeking (Sutcliffe, 1987). Triatomines are reported to use flight only as adults when dispersing in search of a new habitat (Schofield *et al.*, 1991). The role of directed flight or responses to visual stimuli during host-seeking by these bugs is not known. Though dark stationary visual objects are approached by triatomines as a hiding place, moving visual stimuli have been reported to alert the animal and are hence associated more with defence rather than with host-seeking. Lazzari and Varjú (1990) made experiments with tethered *T. infestans* adults walking on a styropore Y-maze and showed that the bugs turn away from approaching or rotating objects, but hold them within the lateral part of their visual field. This may be an escape strategy where the bug perceives any fast moving object as a potential enemy and runs away from it, but while doing so it does not loose sight of the object completely by turning a 180°. Instead, it keeps the object at an angle of *ca.* 120° laterally. In another study, also with tethered *T. infestans* adults, the bugs avoided a directional light stimulus by turning away from it (Ward & Finlayson, 1982).

Olfaction has always been suggested as an important modality in host-seeking by triatomine bugs but there is no clear-cut study showing an oriented response to odour alone by individual bugs. Vapours from a hamster elicited an increase in

locomotor activity of *R. prolixus* nymphs (Núñez, 1982). He investigated activation of these bugs in a wheel-cage actograph and showed that CO_2 on its own was not effective, but when tested with stimuli from the cage where a hamster was earlier kept the bugs were activated, indicating that CO_2 and some other volatile stimuli were required to activate the bugs. The same author reported oriented responses of groups of *R. prolixus* nymphs to CO_2, human forearm and vapours over a hamster, with or without CO_2, under daylight conditions in a T-shaped olfactometer. Although olfaction seems to be involved in the orientation of the bugs in Núñez's study, the possibility that visual stimuli and group-effects affected the results cannot be excluded. Guerenstein *et al.* (1995), captured more *T. infestans* nymphs in a trap baited with baker's yeast; a source of numerous volatiles including CO_2. Aggregation of groups of *T. infestans* adults in black-painted boxes near the source of stimulation (a pigeon or a mouse) and within 12 h of the start of exposure is also reported (MacCord *et al*, 1986), but in this study the sensory modality involved was not at all clear.

The role of temperature in addition to odour in attraction of triatomines to hosts at short distance has been investigated as early as 1934 by Wigglesworth and Gillett who carried out some preliminary experiments to study the mechanisms of short distance (*ca.* 4 cm) orientation by *R. prolixus* adults with blackened eyes to a warm glass tube, and to the same tube at room temperature covered with fresh mouse skin. They concluded that the bug was attracted to its host mainly by the warm air emanating from it and only partly by the smell. These same authors also carried out some ablation experiments to demonstrate the role of antennae in host-seeking in *R. prolixus*. With one ablated antenna the bugs walked with a bias towards the side with the intact antenna when responding to the mouse-skin covered tube; Bugs deprived of both antennae no longer responded, indicating antennae to be the main sense organs used in locating the stimulus source (Wigglesworth & Gillett, 1934). These authors concluded that in normal insects the direction of the source of stimulus was usually inferred only after the antennae had been extended in every direction in turn and that "orientation consisted in a reflex pursuit of the antennae into the zone of optimal stimulation". In this context, the role of antennal guidance was also remarked upon by Núñez (1982) in that he also described an 'awakening' response of the

R. prolixus nymphs in the presence of stimulus during which the bugs erected their antennae sensing the air with sudden and brief movements, then the bugs turned to face the air-stream and advanced upwind in his olfactometer. He also showed probing responses of these bugs to warm or moist sources at close range. Later Lazzari and Núñez (1989a) conclusively established that starved *T. infestans* nymphs respond to radiant heat alone, are capable of discriminating between sources at different temperatures, and concluded that heat possibly plays a role in host-finding at short range. These bugs are also equipped with heat-sensitive sensilla coeloconica on the antennae (Bernard, 1974). In addition, hairs in a cavity, termed the cave-organ as it is located inside a cuticular pouch on the distal part of pedicel of the antennae, has also been reported to contain sensory units which respond electrophysiologically to thermal stimuli (Lazzari & Wicklein, 1994).

In the presence of CO_2 in heated air *T. infestans* show increased activation and probing (Wiesinger, 1956). Lazzari and Núñez (1989b) have investigated the effect of temperature on feeding by *T. infestans* using an artificial feeder. From their experiments, which allowed an independent control of temperature of the feeder surface and of the blood contained in it, they concluded that proboscis extension and probing were evoked by the temperature of the feeder surface while the blood temperature influenced the number of bugs actually feeding.

6. Use of refuges by Triatominae

After a blood-meal, the considerable increase in body weight makes the bugs less agile. Reaching the safety of a refuge is then a primary concern as at this stage they are easy targets for predators. Most triatomine bugs live aggregated in the vicinity of their hosts and establish their refuges in animal stalls or cracks and crevices in the walls of human dwellings (Lent & Wygodzinsky, 1979). Apart from being a safe haven not too far away from the food source, the refuge also brings the sexes together and promotes mating and gene transfer. In addition, the younger stages require the presence of the older bugs since they pick up a symbiont indispensable for development from their faeces via coprophagy (Baines, 1956; Harington, 1960; Schaub *et al.*, 1989). The very young stages also obtain their blood-meal by cannibalising on the older fed bugs (Ryckman, 1951; Schaub *et al.*, 1989). Observing

the behaviour of *T. infestans* nymphs in and around artificial refuges, Lorenzo and Lazzari (1996) have recently demonstrated that these bugs exhibit a stereotypic behaviour in which they preferentially defecate just outside the refuge. This results in faecal accumulation near the entrance of refuges. These authors also showed aggregation of bugs at refuges marked with faeces, suggesting a role of faeces as a cue for refuge selection. The potential of faeces as an assembly or aggregation factor has been quite extensively investigated. Schofield and Patterson (1977) released a group of 50 *T. infestans* and *R. prolixus* nymphs in a choice arena (16 cm dia.) and two hours later found more bugs on the faecal contaminated papers than on the clean control ones. This aggregation can be mediated by contact chemostimulation as well as via odour since it has been shown that the biased distribution of groups of *T. infestans* nymphs in favour of the faeces in an olfactometer after 15 min is influenced by papers impregnated with dry faeces (Lorenzo Figueiras *et al*, 1994). Freshly deposited faeces (collected during and 3 h after feeding) were however rejected. Similarly, aggregation of five species of Triatominae on their own faeces and that of other triatomines has been reported (Cruz-López *et al*., 1993) and Cruz-López and Morgan (1995) showed that nymphs of *T. infestans* and *T. mazzottii* are arrested on polar extract of their faeces. All these studies support the hypothesis of Lorenzo and Lazzari (1996) that these bugs might use faecal deposits to mark refuges.

7. Excretion in insects

Excretion is the separation and elimination of unwanted substances present in the blood and retention or re-absorption of useful constituents in order to maintain a constant internal environment in the body. Malpighian tubules are the main organs of excretion in insects. The urine produced by the Malpighian tubules is discharged into the intestine at the junction of the mid-gut and hind-gut where it is mixed with the residue of food from the mid-gut, these together form the excreta or faeces of the insects (Wigglesworth, 1972). Insects generally excrete nitrogen as uric acid from the Malpighian tubules and are classified as uricotelic animals as against ureotelic animals like mammals and aquatic vertebrates with urea as their main excretory product and ammonotelic animals which are mainly aquatic and excrete ammonia. Very often the excretion of other purines such as xanthine, hypoxanthine, adenosine and guanine and

enzymatic by-products of uric acid i.e., allantoin and allantoic acid are also included in uricotelism (Cochran, 1985). Though uric acid is the main nitrogenous waste product of insects their excreta also contain small quantities of urea, inorganic salts and pigments. In blood-sucking and plant-sucking insects, immediately after a meal there is an abundance of water which is eliminated under the influence of diuretic hormone. This process produces crystal-clear urine which is known as the diuretic product. Later, when less water is available, the urine is concentrated. In *R. prolixus* adults the complete digestion of blood requires 5-6 weeks (Wigglesworth, 1931). The excretion consists of three phases: 1) immediately after feeding, the insect voids its gut releasing the black residue of its previous meal, 2) for the next few hours it excretes a clear colourless fluid, the diuretic product, 3) then over a period of weeks urine is excreted in the form of a pultaceous mass which dries as a yellow powder. Briegel (1986) divides similar products of mosquito excretion into faeces, diuresis products and urine, respectively.

8. Olfaction in haematophagous insects

Most animals require information from their environments in order to acquire the basic necessities of life such as food, shelter, mates, and information on danger. Man has evolved to perceive the world around him mainly via visual stimuli and sounds and the senses of smell and taste, though well developed, have taken second place. In contrast, for lower organisms, the world around them is filled with an array of chemicals which are perceived via olfactory and gustatory senses. Most of our knowledge on olfaction in insects is based on the studies with non-haematophagous species such as, moths, cockroaches and honey-bees, but is often generalised for all insects. In these insects olfactory processes, involved mainly in pheromone perception, have been extensively studied employing different behavioural, electrophysiological and histological techniques. The sense of olfaction is mainly located on the antennae, but also partly on the mouth-parts in some groups, in the form of small organs called sensilla. These sensilla are comprised of three elements: a hair-, peg- or plate-like cuticular structure and below it, embedded between the epithelial cells are sensory cells and accessory cells, i.e., trichogen, tormogen and thecogen cells. The sensory or receptor cells housed in the sensilla are responsible

both for reception of information from outside and its transfer to the brain for further processing. Olfactory sensilla are usually characterised by numerous pores in the shaft wall of the hair (wall-pores) as opposed to terminal-pore sensilla which are gustatory (Zacharuk, 1985; Altner & Prillinger, 1980). Sensilla without pores (no-pore) usually house thermo-, hygro- or mechano-receptive cells (Zacharuk, 1985). However, all sensilla can house receptors for more than one modality *e.g.*, terminal-pore hairs very often also contain mechanoreceptors and wall-pore hairs can contain thermoreceptors. During development of olfactory sensilla, two of the accessory cells, the trichogen and tormogen cells, form the hollow cuticular hair-shaft as well as its cavity, the sensillum lymph space, filled with a lymph with unusually high K^+-ion concentration. This high K^+-ion concentration, actively maintained by ion pumps in the accessory cells, provides a positive transepithelial potential between the sensillum lymph space and haemolymph space. The olfactory receptor cells within the sensilla are bipolar neurons with their dendrites extending into the hair and the axons reaching the central nervous system forming the antennal nerve. The dendrite of the olfactory receptor neuron is separated into an inner and outer dendritic segment by a short ciliary structure. The outer dendritic segment is surrounded by the sensillum lymph while the inner dendritic segment and the soma of the olfactory receptor neuron are surrounded by the third accessory cell; the thecogen cell. Odour molecules diffuse into the interior of the sensillum via the pores present on the cuticle of the hair-shaft to reach the dendritic membrane, the site of stimulus transduction (Stengl *et al.*, 1992). How the mostly hydrophobic odorants pass through the aqueous sensillum lymph and reach the perceptive structures of the receptor cells is still unclear. For the pheromone-detecting sensilla of moths there are two main theories: 1) waxy pore tubules have been suggested to represent a direct conduit for odour molecules to reach the dendritic membrane without ever entering the sensillum lymph. However, the number of such pore tubules is relatively small and most of them are thought to end blindly in the lymph and not at the dendritic membrane (Kaissling, 1986); (2) sensillum lymph has been found to contain high concentrations of water-soluble proteins that bind pheromones. These pheromone-binding proteins (PBPs) are assumed to dissolve the hydrophobic pheromones in the sensillar lymph by forming pheromone-PBP complexes (Kaissling, 1986). These complexes may rapidly traverse the distance

between the pore and the receptive sites on the dendritic membranes. For sensilla detecting other odour molecules such as food odour, general odour-binding proteins (GOBPs) are suggested to be involved (Laue *et al.*, 1994). Spoke canals, filled with secretion, have been suggested as conducting medium for more polar odour molecules in the grooved-peg sensilla of cockroaches (Altner *et al.*, 1977) and bed-bugs (Steinbrecht & Müller, 1976) but not much is known about the exact mechanism of conduction to the dendritic membrane.

At the receptor sites in the dendritic membrane the odour molecules are assumed to interact with as yet unidentified receptor proteins. This activated odour-receptor complex is thought to result in opening of ion channels either directly or via second messenger cascades. This causes a local influx of K^+-ions into the dendrite and a depolarisation across the dendritic membrane occurs, *i.e.*, the receptor potential, which then spreads to the spike-generating region of the neuron and generates action potentials or spikes. Stimuli that cause a depolarisation and increased firing rate of a receptor cell are called excitatory stimuli while those causing hyperpolarisation and decreased firing are called inhibitory stimuli. Both the receptor potential and the generated action potentials can be measured using extra-cellular electrophysiological techniques (described below). The global picture of receptor potentials from several receptors all showing the same specificity to an odour stimulus on the antennae is known as the electroantennogram (EAG) which can be readily recorded (Schneider, 1957).

The olfactory information from the neuron is transmitted via the chemosensory fibres along the antennal nerve to the olfactory glomeruli in the deutocerebrum or antennal lobes, the first synapse in the system. After initial processing, the information is then projected onto the mushroom bodies and other centres in the protocerebrum which are the centres for pre-motor processing and integration.

By comparison with agriculturally important pests of crops such as moths, the olfactory system has not been investigated in much detail in haematophagous arthropods. Few studies are complete and these have focused on the characterisation of responses from receptors in sensilla at the periphery. Bloodsucking insects like mosquitoes and tsetse flies are attracted to hosts over long distances by olfactory stimuli (Sutcliffe, 1987). After feeding, other behaviours such as search for suitable

oviposition sites in mosquitoes are also, at least partly, mediated by odours (Bentley & Day, 1989). In mosquitoes, *Aedes* and *Culex* spp., olfactory receptor cells on the antennae are known to perceive host associated volatiles such as, fatty acids (Davis & Sokolove, 1976; Davis, 1977; Davis, 1984; Bowen, 1995) and ammonia (Davis, 1977). CO_2-sensitive receptors are located in clubbed sensilla on the maxillary palp of mosquitoes of various speceis (Kellogg, 1970; Grant *et al.*, 1995). Electrophysiological responses to natural and synthetic oviposition attractants (Mordue (Luntz) *et al.*, 1992; Davis, 1977) and to plant volatiles (Davis, 1977; Bowen, 1992) have also been reported for mosquitoes of *Aedes* and *Culex* spp. Receptor cells in the sensilla on the antennae of tsetse flies *Glossina* spp. perceive host-associated volatiles such as, CO_2 (Bogner, 1992; Den Otter & Van der Goes van Naters, 1992), 3-methyl-phenol and p-cresol (Bogner, 1992; Den Otter & Van der Goes van Naters, 1992), 1-octen-3-ol (Den Otter & Van der Goes van Naters, 1992) acetone (Bogner, 1992; Den Otter & Van der Goes van Naters, 1992), 2-butanone and propanal (Bogner, 1992). Electroantennogram responses to host urine-associated phenolic compounds have also been reported (Den Otter, 1991). Similar EAG responses of stable flies *Stomoxys calcitrans* to the host odour components 1-octen-3-ol and 3-methylphenol have also been reported (Schofield *et al.*, 1995). EAG responses have also been recorded to volatiles from parous female (in the host-seeking phase) biting midges *Culicoides impunctatus* by other parous females, but the active compounds have not been identified (Blackwell *et al.*, 1994). In another species, *Lutzomyia longipalpis* (sandfly) the dipteran haematophagous vector of Leishmaniasis, oviposition attractants hexanal and 2-methyl-2-butanal have been identified from host faeces by linking the electrophysiological preparation to the effluent of a gas chromatograph (GC) (Dougherty *et al.*, 1995). These authors also obtained responses to pinenes, and benzaldehyde from receptors in a wall-pore sensilla on the antennae of these flies. EAG responses of the screw-worm fly *Cochliomyia hominivorax* were recorded to a range of electrophysiologically active volatiles isolated from the fluid in the wounds occupied by the larvae of this parasite such as carboxylic acids, aliphatic and aromatic alcohols, phenols, indoles, naphthalene and δ-valerolactam (Cork, 1994). The same technique was also used to isolate host derived volatiles perceived by olfactory sensilla on the tarsi of first pair of

legs of another arthropod, the ixodid tick *Amblyomma variegatum*. A variety of vertebrate volatiles have thus been identified including, aliphatic and aromatic aldehydes, and lactones (Steullet & Guerin, 1994a; Steullet & Guerin, 1994b), fatty acids, nitrophenols and ammonia (Steullet & Guerin, 1994b), hydrogen sulphide (Steullet & Guerin, 1992b) and CO_2 (Steullet & Guerin, 1992a).

9. Olfaction in Triatominae

The antenna of the Triatominae consists of four segments: a basal short and stout scape, followed by a long pedicel and two flagellar segments (Fig. 2). Many different types of hairs occur on the antennae of triatomines and not all are olfactory sensilla. Wigglesworth and Gillett (1934) described four types of hairs on the flagellar segments of *R. prolixus* antennae employing light microscopy, and already suggested that one of them could be olfactory in function. Later, employing scanning and transmission electron microscope techniques nine types of hairs were described from the antennae of *T. infestans* (Bernard, 1974) and *R. prolixus* (Catalá & Schofield, 1994). Three of these are described as wall-pore sensilla with an inflexible socket and probably olfactory in function (Bernard, 1974; Table 1). The thin-walled sensilla basiconica (type E) are abundant on both segments of the adult flagellum but only occur on the terminal segment of the nymphal flagellum of both *Rhodnius* and *Triatoma* (Fig. 2). They can also be found on the ventral side of the adult pedicel in *Triatoma*. They are *ca.* 25 μm and 60 μm long in *Triatoma* and *Rhodnius*, respectively, and are innervated by approximately 15 sensory cells in *Rhodnius* (Catalá & Schofield, 1994). Their estimated number on an antenna of adult *Triatoma* is *ca.* 550 (Bernard, 1974; Table 1). A second type, also sensilla basiconica (type F) are short 6-10 μm long and 1 μm dia. hairs in *Triatoma* and 12-18 μm long in *Rhodnius* with 12 longitudinal grooves on their surface. They are present on both segments of the adult flagellum but only on the terminal segment of the nymphal flagellum of both *Rhodnius* and *Triatoma* (Fig. 2). These are grooved peg-like sensilla supposed to contain *ca.* 3 dendrites in *Triatoma* (Bernard, 1974) and 5-6 neurons in *Rhodnius* (Catalá & Schofield, 1994). Their number on an antenna of adult *Triatoma* has been estimated at around 350 (Bernard, 1974; Table 1). A third type, thick-walled sensilla trichoidea (type D; Bernard, 1974) are 35 μm long in *Triatoma* and 75-80 μm

in *Rhodnius* with just visible longitudinal grooves on the surface. Their distribution on the antenna is similar to that of the sensilla basiconica type F, but their estimated number (*ca.* 2000) on an antenna of *Triatoma* is high (Bernard, 1974; Table 1).

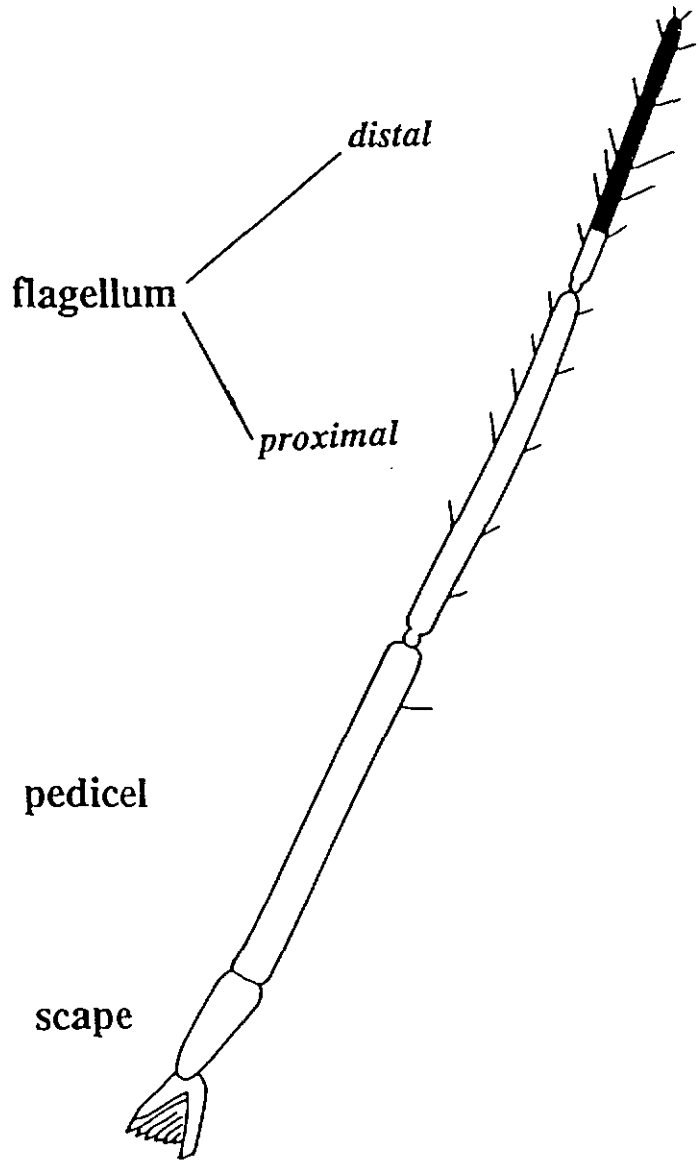

Figure 2: Schematic representation of an antenna of *Triatoma infestans* nymph. The antenna consists of a short scape proximally, a pedicel and two flagellar segments distally. The three types of wall pore sensilla are present exclusively on the distal flagellar segment indicated by the blackened area (from Bernard, 1974).

Table 1

Published data available from morphological and electrophysiological investigations on three types of olfactory sensilla (wall-pore sensilla with inflexible sockets) on the antenna of *Triatoma infestans*.

Type*	Wall Charcteristic	Total no. on adult antenna	Length (μm)	Basal dia. (μm)	Dendrites observed	Responses obtained to	Ref
Basiconica (Type E)	Thin-walled	~550	~18-26	~2	~80	direct breathing, fatty acids & amyl acetate	Mayer, 1968 Bernard, 1974
Basiconica (Type F)	Double-walled with longitudinal grooves	~350	~6-10	~1	~3	fatty acids, ammonia & humidity	Bernard, 1974;
Trichoidea (Type D)	Thick-walled with longitudinal grooves	~2000	~35	~1.5	~1-2	no data-	Bernard, 1974

* all three types are present on both flagellar segments of the adult antennae, but only on the distal segment of the nymphal antennae. Type E is also present on the ventral side of the adult pedicel.

Mayer in 1968 first recorded single-unit activity using tungsten electrodes from single-walled sensilla basiconica (type E, Bernard, 1974) of *T. infestans* and reported that "direct breathing" on the insect caused an increase in impulse frequency of two cells. Later, Bernard (1974) made electrophysiological recordings employing glass electrodes from the base of sensilla and described three (type D, E & F, described above) sensilla as chemoreceptors. He obtained responses to synthetic chemical stimuli such as, pyruvic acid, L-lactic acid, D-lactic acid and amyl acetate from the olfactory sensilla type E. The double-walled sensilla basiconica (type F) he split into two types (Ff1 and Ff2) depending on their electrophysiological responses, but only one type (Ff1) was reported to respond to olfactory stimuli such as, pyruvic acid, n-butyric acid, lactic acid and ammonia. Receptors within sensilla type D did not respond to any olfactory stimuli tested in Bernard's (1974) study though unit activity could be recorded.

In the present study an attempt is made to understand the role of certain olfactory stimuli in the attraction of triatomine bugs to host odours and to the volatiles from their own faeces employing electrophysiological and behavioural techniques. A servosphere or locomotion compensator is used to study the detailed behaviour of *T. infestans* and *R. prolixus*. Most studies showing orientation of blood-sucking insects are made in wind-tunnel or olfactometers. Frequently, the sensory modalities involved have not been clearly defined. My studies on the locomotion compensator under controlled conditions permitted me to test responses of triatomine bugs to odour alone. In addition, this method allowed me to monitor details of the behaviour of these bugs before, during and following stimulation with volatile stimuli.

10. Methodologies employed

Locomotion compensator: All behavioural experiments in this study have been made on a locomotion compensator (Fig. 3), the working principle of which is described in Results 1. In short, it works in such a way that the walking insect is maintained at the same position in space by compensating for any displacement made by the insect in the x-y plane. This method of studying behaviour gives various advantages over other more commonly employed bioassays in olfactometers and wind tunnels. In many behavioural studies involving orientation the end result, *i.e.*, the number of insects

approaching or reaching a source is scored. This limited approach does not permit evaluation of the mechanisms and behavioural components involved in the approach to the goal (Kennedy, 1977). In contrast, the locomotion compensator monitors every move of the insect at regular intervals therefore, various parameters of locomotion, such as speed and turning can be independently evaluated. Since the animal is maintained by the compensator at the same position in space, video recordings of the animal in close-up view can readily be made and this allows monitoring of non-locomotory elements of the behaviour of the animals.

The technique used allows independent control of the stimuli affecting various sensory modalities involved in orientation mechanisms and attraction of an animal to a stimulus source. Since the animal stays at one position, it is easier to control and measure all environmental factors accurately in space and time. In wind tunnels, for example, flying insects can leave the odour plume, whereas on the servosphere it is the experimenter who dictates when the test insect is exposed to the odour. This possibility to maintain a constant environment and change it in time rather than in space without the experimental animal being able to influence by itself its exposition to it is usually not feasible in other bioassays. In my case, I could isolate the animal from any thermal radiation and air turbulences in a zone of *ca.* 12.5 cm dia. employing a metal-foil hollow cylinder with an inlet and outlet for a conditioned air-stream. The animal was thus tested for responses to changes in its olfactory environment only while running in an air-stream of constant velocity, temperature and humidity. In addition, the environment was devoid of any mechanical stimuli other than the homogenous surface of the sphere. The animal had full freedom to walk in all directions for an unlimited length of time without ever encountering any physical barrier such as walls or edges as can happen in most other behavioural bioassays. In addition these bioassays are often undiscriminating where very often the long and short distance stimuli are combined without the control of experimenter. Since *R. prolixus* and *T. infestans* are predominantly walking insects (nymphs are wingless and the winged adults rarely fly under lab conditions) this experimental set-up was well suited to study the behavioural responses to odour stimuli.

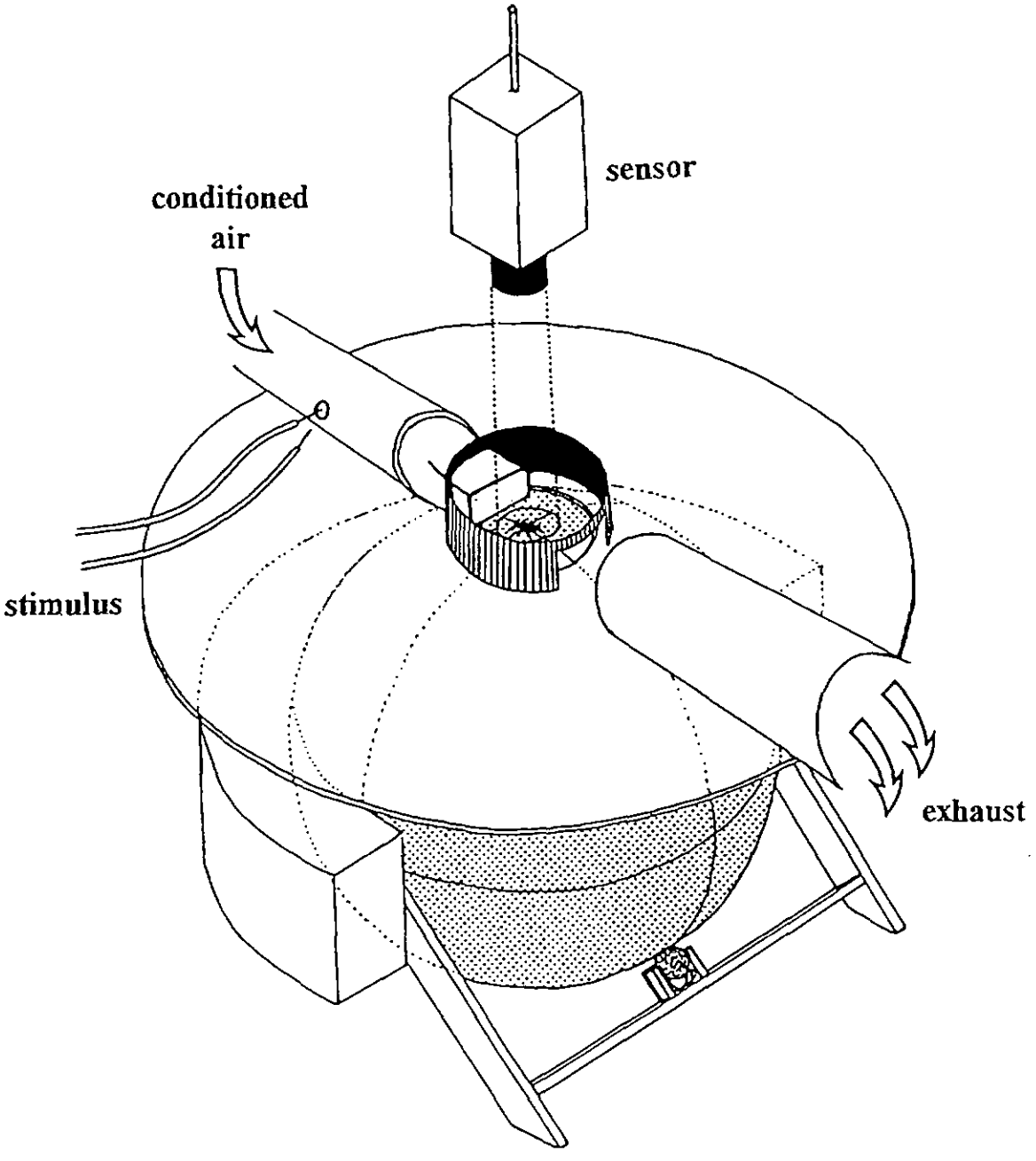

Figure 3 The set-up for behavioural studies consisting of a locomotion compensator (a perspex servosphere linked to a position sensor) and a horizontal air-stream delivery with stimulus injection and exhaust. The insect walks on the apex of the sphere and is surrounded by a metal-foil cylinder. Experiments are done in complete darkness.

Most olfactometer and wind tunnel bioassays require direct observations by the experimenter. This necessitates presence of the observer who can himself become a stimulus and some light is required in order to make observations, thus adding visual cues. In contrast, the studies on the locomotion compensator allowed experiments to be performed in the dark, with observations made at a distance on a computer linked to the servosphere and via video images supplied by an infra-red sensitive camera.

Another advantage of using the locomotion compensator is the possibility to monitor the off-response behaviour of the animal. Most bioassays assume that once an insect has perceived the stimulus it will go straight to the source, which is not necessarily the case. In nature an animal can very often lose the odour plume due either to a physical obstacle or due to its own misjudgement of direction. Incorporation of an end-control or after-stimulation observation period allowed me to study the behaviour in a similar situation *in vitro*.

The treatment of the insects before experiments needs attention to ensure that what are otherwise "physiological black boxes" all receive equal pre-conditions to render the sample tested physiologically more homogenous. Responsiveness to host associated volatiles was ascertained with starved bugs all of whom subdued a uniformly long acclimatisation period on the servosphere before presenting the stimulus. In addition, inter-individual variation was reduced by maintaining constant rearing conditions.

Electrophysiology: Electrophysiological methods are used peripherally in arthropod sensory physiology to pick up electrical events such as receptor potentials and action potentials generated at the level of receptor cells. This way sensitivity and specificity of odour perception can be evaluated. An electrophysiological technique to obtain olfactory responses measured as voltage shifts over the length of an insect antenna or the electroantennogram (EAG), was first introduced by Schneider in 1957. The next step was the recording of responses from individual olfactory receptor cells in a single sensillum (Morita & Yamashita, 1961; Schneider & Boeckh, 1962). Since then, these two techniques have been widely utilised in the study of sensory physiology in insects and other arthropods. Though behavioural studies allowing for proper control of the environment are often sufficient to discriminate between different modalities, they are

usually time-consuming and require large number of insects to obtain reliable results. By contrast, electrophysiological techniques, measuring peripheral responses from for example, the olfactory system, allow quick screening of several compounds at different doses on antennae or single sensilla. Information thus obtained can tell whether the insect can perceive the products or not. In addition, such electrophysiological preparations can be used as a detector system in the effluent of a gas chromatograph to selectively isolate compounds from complex natural extracts (Arn *et al.*, 1975). However, it still remains to be seen if the information perceived has any behavioural significance to the animal. Thus, an approach combining the two methods, measuring sensory input and behavioural output, is preferable.

In insect olfactory sensilla, the dendrites of receptor cells bathe in the sensillum lymph which is separated from the haemolymph in the antenna by the sensory epithelium and its basal membrane. Electrophysiological recordings can be made either extracellularly or intracellularly. In extracellular recordings, a recording electrode contacts the sensillum lymph whereas the reference electrode is inserted in the haemolymph space on the other side of the sensory epithelium and the transepithelial potential between the two electrodes is measured. The recordings can be made from single sensilla, *i.e.*, without interference from other neighbouring sensilla as they are electrically well isolated from each other. The electrical resistance between the extraepithelial sensillum lymph spaces of neighbouring sensilla is estimated to be in the order of 100 MΩ (Kaissling, 1995). Two types of electrodes are commonly used: simple tungsten wire electrodes or Ag/AgCl electrodes in a glass capillary filled with a physiological ringer. Tungsten electrodes are suitable only for AC recordings of nerve impulses as they tend to distort DC signals, *i.e.*, slow potential shifts by cutting off low frequencies. By contrast, glass electrodes permit recording of shifts in potential and action potentials associated with receptor cells. In addition, though recordings with tungsten electrodes can be very stable and long-lasting once achieved, the receptor cells can be more easily destroyed while making contact. With particularly long sensilla, as in moths, where manipulation is easier, the sensillum lymph is contacted by the recording electrode via the cut tip of the sensillum (Kaissling, 1995). In this study, all single sensillum recordings have been made employing pulled glass capillary Ag/AgCl electrodes with a tip diameter of *ca.* 2 µm,

by simply contacting the base of the porous wall of sensilla on the antennae of *T. infestans* nymphs.

A global picture of the receptor potentials, or the electroantennogram (EAG), can be recorded with both electrodes making electrical contact with the haemolymph space of the antenna. The two electrodes are placed preferably at the two ends of the same antenna to avoid interference by potentials from muscle cells. The EAG is useful to compare the effects of chemical compounds acting on a population of similar receptor cells occurring in large numbers on the antenna.

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ORIGINAL PAPER

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Oriented responses of the triatomine bugs *Rhodnius prolixus* and *Triatoma infestans* to vertebrate odours on a servosphere

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Abstract Oriented responses of both *R. prolixus* and *T. infestans* adults were recorded on a servosphere to mouse-odour, one of its components (CO_2), and to rabbit urine-odour. The volatiles were delivered in an air-stream under controlled conditions which excluded other sensory modalities. In stimulus-free air the triatomines walked preferentially downwind in straight bouts interrupted by stops or periods at relatively low speeds, all of variable duration. In odour-laden air, bugs maintained their typical walking habit but switched from negative to positive anemotaxis. The characteristic response to odour onset was to stop, sample the air with the antennae, turn upwind *in situ*, and then walk off in the direction of the source for at least a few seconds, i.e., odour mediated anemotaxis. Mouse-odour caused *T. infestans* to increase its speed to 5.3 cm s^{-1}. Both species continued with the upwind response for some time after odour delivery ceased, but the crosswind component of the tracks was more prominent during this period – an effort, we presume, by the bugs to re-contact an odour plume. This investigation provides unequivocal evidence for host finding in triatomines by olfactory cues alone.

Key words *Rhodnius* · *Triatoma* · Behaviour · Host-odours · Orientation

Introduction

Triatoma infestans (Klug) and *Rhodnius prolixus* Stål are among the most important vectors of American trypanosomiasis or Chagas' disease, a protozoan infection of man and other mammals caused by *Trypanosoma cruzi* (Chagas). These haematophagous bugs, belong to the subfamily *Triataminae (Heteroptera: Reduviidae)* and are commonly called kissing bugs. They inhabit palm trees, birds' nests, and burrows of small mammals in the wild, and peridomestically the crevices and corners of human dwellings and animal quarters. Though a great deal has been achieved in understanding the basic physiology of these bugs (Wigglesworth 1972), limited attention has been paid to their sensory physiology and host-finding behaviour. A preliminary indication that both temperature and olfactory cues might be used in host location was provided by Wigglesworth and Gillett (1934), who studied close range host orientation in *R. prolixus* with blackened eyes. They reported responses to a warm glass tube and to the same tube at room temperature covered with fresh mouse skin. Later Wiesinger (1956) reported increased activation and probing by *T. infestans* in the presence of CO_2 in heated air, and Núñez (1982) investigated activation and orientation responses of *R. prolixus* to CO_2, and to human forearm and hamster odours. However, in neither of the two latter experimental set-ups were conditions controlled to the extent that one could discriminate between the contribution of odour vis-à-vis that of other modalities such as thermal or visual cues to the overall activity recorded. Lazzari and Núñez (1989) have conclusively established that *T. infestans* responds to radiant heat alone and is able to discriminate between sources at different temperatures, concluding that this sensory modality could play an important role in host-finding at short-range.

Evidence for the perception of odours from vertebrates by triatomines has also been obtained from electrophysiological recordings from olfactory sensilla of these bugs. Antennal receptors of *T. infestans* respond to human breath (Mayer 1968), and to such vertebrate volatiles as lactic acid, pyruvic acid, *n*-butyric acid and ammonia (Bernard 1974). Despite this, no clear-cut study has yet been made to establish the

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contribution of host-odour on its own to host finding from a distance by triatomines. We therefore set out to study the responses of *R. prolixus* and *T. infestans* to vertebrate volatiles as delivered to the bugs in an air-stream under controlled conditions on a servosphere. Apart from establishing unequivocal attraction of the bugs to the volatiles, these experiments permit us to describe some aspects of the walking behaviour of these triatomines.

Materials and methods

Insects

Colonies of the triatomine bugs *R. prolixus* and *T. infestans*, (hereafter referred to as *Rhodnius* and *Triatoma*) originating from long-standing cultures at the Swiss Tropical Institute, Basel, are maintained at 26 ± 1°C, 85 ± 5% RH and a 12:12 h L:D cycle in an incubator. They are fed at regular intervals on anaesthetised Guinea-pigs. Equal numbers of adult bugs of both sexes were used in all experiments. Since adult triatomines can fly, their wings were immobilised by attaching the hemielytra together with a piece of transparent tape. Prior to all tests, *Triatoma* was starved for 4–5 weeks and *Rhodnius* for 8–10 weeks after the last pre-imago blood-meal. Each bug was allowed an acclimatisation period of 8–10 min in the air-stream on the servosphere (see below) before recording any responses. All tests were carried out during the dark-phase of their daily cycle, and no more than 15 bugs were tested on any single day.

Servosphere

A servosphere or Kramer sphere (Kramer 1976) was used to record responses of the triatomine bugs to various host odours. This apparatus functions in such a way as to keep the moving insect at the same position in space, in our case at the apex of a perspex sphere (50 cm dia). The sphere is mounted so that it can be rotated around two orthogonal axes in the horizontal plane by two low inertia servo-motors. A beam of filtered incandescent light (filter cut-off 780 nm) from a position sensor fixed 26 cm directly above the apex of the sphere illuminated a circle 4.0 cm dia. A small disc (*ca.* 2.0 mm^2) of retroflective material (3 M, No. 7610, Switzerland) glued to the pronotum of the bug reflected light back to the sensor. The reflected light supplied the position sensor with information on the bug's movements and this was used to drive the servo-motors which compensated the sphere's position to maintain the insect at the apex. The *xy* coordinates of displacements made by the animal were recorded at 0.1 s intervals by two incremental pulse-generators mounted on the sphere's equator, both working at a resolution of 0.1 mm. These 0.1 s samples constituted the instantaneous component vectors of the track. Coordinates were supplied to a SAM II 68 K computer (KWS Inc. Ettlingen, Germany) for subsequent analysis of tracks. Records of the movements of individual insects lasted 6 min, comprised of 3 consecutive two min periods, i.e., control, test and end-control.

Stimulus delivery

All stimuli were delivered to the bugs walking on the apex of the sphere in a charcoal-filtered air-stream maintained at 25°C and 90% RH. Conditioning of the air-stream was achieved by a computer-operated humidity/flow controller (Syntech, The Netherlands) submerged in a water-bath (Neslab, U.S.A.) at 25°C. The desired relative humidity of the air leaving this apparatus is obtained by regulating the relative proportions of two air-flows through the system by means of mass flow controllers. Temperature is first controlled by passing the two flows through temperature exchangers. One flow then passes through a saturation vessel filled with distilled water. The two flows are finally mixed at a fixed ratio in order to obtain the desired humidity at the apex of the sphere. Output flow from the mixing chamber is set by means of a voltage to flow converter, working in tandem with the mass flow controllers. This conditioned-air was delivered to the apex of the sphere in a stainless-steel water-jacketed tube (35 mm i.d.). The jacket served to circulate fluid from the water-bath around the air-stream to avoid condensation. This tube contained a plug of stainless-steel wool, and an aluminium honey-comb baffle (1.2 cm thick with cells measuring 4.0 mm across) at its mouth to remove air-turbulence. The tube also bore a 7.0 cm long rectangular metal-foil extension (2.8 cm high and 4.5 cm wide) ending 3 cm from the apex of the sphere. This served to deliver the air-flow tangentially to where the bugs walked and at the required width to cover the maximum scanning range of the bugs' antennae (*ca.* 2.6 and 3.4 cm, respectively, for *Rhodnius* and *Triatoma*, i.e., twice the length measured from the reflective foil to the tip of a fully stretched antenna). The air-flow was maintained at a speed of 0.1 $m\,s^{-1}$ as measured by an anemometer (Thermo-air, Schiltknecht Messtechnik, Switzerland, response time is < 1 s). Temperature and humidity of the air-flow at the apex of the sphere were measured with a thermo-electric hygrometer (Hygro-Air II, Schiltknecht Messtechnik, Switzerland, response time 1 s).

Odour stimuli were introduced continuously for 2 min into the conditioned air-stream via silicone tubing (4.0 mm i.d.) connecting the vessel containing the odour source (see below) to a syringe needle (1.2 mm i.d.). This needle was inserted through a rubber septum in the wall of the stainless-steel tube upwind of the steel wool and baffle at 26 cm from the apex of the sphere. The charcoal-filtered air-flow could be switched between control and test flows (see below) by means of computer-controlled solenoid valves (Stimulus Control System, Syntech, The Netherlands).

Metal-foil (0.06 mm thick) standing 12.0 cm high on a table surrounded a zone (12.5 cm dia) around the apex of the sphere. This served to prevent infrared radiation, particularly from the experimentalist, from reaching the bug. The metal-foil cylinder had openings at opposite sides of the base to permit passage of the conditioned air-stream over the apex of the sphere. Upon exiting the cylinder, the conditioned air was collected into an extraction tube (13 cm dia) which lay in one line with the air-stream at 16 cm from the apex of the sphere. This tube conveyed odour-laden air (suction rate ca. 3 l/s) through a charcoal filter (Camcarb, Camfil, Sweden) before returning it to the room. The area around the sphere was kept dark with light-shielding black curtains, and room temperature was maintained at 23–25°C.

Stimuli

Mouse-odour was tested by blowing charcoal-filtered air (500 ml/min) over two albino Swiss mice held on sawdust in separate desiccators (2.5 l) connected in parallel to the conditioned air-stream. The mice were placed in the desiccators 4–6 h before testing bug responses. Ventilation was achieved by placing wire-mesh over the evacuation port during the period of odour build-up in the desiccators. Air blown (500 ml/min) over sawdust dampened with a few ml water contained in two glass bottles (1.0 l) served as control. Odour of rabbit-urine was tested by blowing charcoal-filtered air (250 ml/min) through 200 ml of urine taken some hours earlier from collection-trays under cages holding New Zealand white rabbits. The urine was held in a 1 l gas-wash bottle and was heated to 37°C in a water-bath for 1 h before and during the test, since heated urine elicited a stronger response than when cold. The control consisted of the same set-up but with 200 ml water in the

gas-wash bottle. CO_2 was tested by introducing the pure gas from a cylinder at 50 ml/min into the conditioned air-flow to give a concentration of 0.6% (the level in mouse-odour) at the apex of the sphere as measured by CO_2 indicator-tubes (± 5–10% error; Drägerwerk, Germany). Charcoal-filtered air (50 ml/min) introduced into the conditioned air-flow served as control. Introduction of the odour stimuli changed neither the temperature nor humidity of the conditioned air-flow as measured with the thermo-electric hygrometer.

Track analysis

Walks of both *Rhodnius* and *Triatoma* on the servosphere are not continuous, but occur in bouts interrupted by stops or periods of relatively low speed, all of variable duration. At the start and towards the end of these walking bouts or subtracks (see below) the bugs walk very slowly with some irregular movements, leading to inaccurate measurements of the angles associated with them by the servosphere. Reducing the sampling from the basic 0.1 s rate of the system did not help in any way to overcome the problem, as the frequency and duration of the small erratic movements was too irregular to be smoothed in this way. Instead, reduction in the sampling rate distorted the real walking behaviour of the bugs by causing overlaps between the walk and stop periods of some tracks, and occluded some important aspects of the behaviour. Above all, data reduction had the effect of merging what constituted some real but short walking bouts. The matter was resolved by keeping the basic sampling rate of the system and discarding all instantaneous displacements smaller than 0.5 mm (the mean instantaneous displacement was 3.0–5.0 mm). Most inaccurate measurements were associated with such small movements at the start and end of walking bouts, and, in any event, the contribution of such movements to the overall distance travelled by the bugs was negligible.

The course angles to wind ($-180°$ to $180°$, $0°$ being straight upwind) were analysed using subtracks. A subtrack is defined as any walking bout of a second or more. Since subtracks tend to be relatively straight, the circular mean (mean Φ, Batschelet 1981) of the instantaneous course angles was calculated for each subtrack and is referred to hereafter as the subtrack-angle. The absolute values of these subtrack-angles (dependent variables) were then grouped into 3 angle-classes, i.e., "upwind" (0 to $\leq 60°$), "crosswind" (> 60 to $\leq 120°$), and "downwind" (> 120 to $\leq 180°$). The frequencies of the subtrack-angles in the upwind and crosswind bins were then compared for control versus test, and control versus end-control periods, using the binary response model of the Linear Logistic Procedure (SAS Software, USA.). The model considers each subtrack to have a binary response per bin, i.e., the response Y_{sb} for subtrack s and bug b is 1 if the subtrack-angle is in the specified bin, otherwise it is 0. If, P_{sb} is the probability of having a response 1 for subtrack s of bug b, then our additive model is:

$$\ln [P_{sb}/(1 - P_{sb})] = \beta_b + \alpha T_s \qquad (1)$$

where β_b is the effect of the bug, α is a coefficient expressing the stimulus effect, and T_s is the period variable ($T_s = 0$ for control, and $T_s = 1$ for test or end-control). That is, $\ln [P_{sb}/(1 - P_{sb})] = \beta_b$ for control, and $\ln [P_{sb}/(1 - P_{sb})] = \beta_b + \alpha$ for test and end-control periods. α is therefore an overall expression of the stimulus effect which is added in test or end-control to the effect due to the individual bugs' behaviour for the periods being considered. The maximum likelihood estimates of α and β_b were computed by the iteratively reweighted least squares algorithms in SAS. The computed value of α with its standard error and associated Wald chi-square value indicated whether a test or end-control was significantly different from the control period in the specified bin. In addition, using the values of α and β_b thus calculated, transformation of formula (1) permitted calculation of the subtrack-angle probability (P_{sb}) in the bin, predicted by the model as:

$$P_{sb} = \exp(\beta_b + \alpha \cdot T_s)/[1 + \exp(\beta_b + \alpha \cdot T_s)] \qquad (2)$$

Stimulus effects on both *Rhodnius* and *Triatoma* for upwind and crosswind bins were ranked by comparing values of α and the associated standard-errors which were obtained during test and end-control periods, employing the method generally used for comparing means of two samples with normal distributions. The ranking was applied to treatment effects on each species as well as to inter-species responses to the same treatment.

The instantaneous course angles were expressed as vectors of length 1, and a straightness index r for each subtrack was computed as the mean of these unit vectors. Since r serves as a measure of dispersion, ranging in value from 0 to 1, higher values indicate straightness and lower values circuitousness (Batschelet 1981).

To find out if the insects walked significantly more in the upwind direction ($-60°$ to $60°$) during test or end-control periods than during the control, the percent displacement upwind for the period was calculated for each bug and compared using Wilcoxon's signed rank test for paired replicates. Similarly, to establish if there was an increase in the bugs' walking speed due to a treatment, the mean speed per period was calculated for each bug by pooling the data for all subtracks made by a bug during that period. These speeds were then compared pairwise between periods (Wilcoxon's signed rank test for paired replicates). Antennal grooming, made during stops, was monitored from the video recordings (see below). Unless otherwise indicated, means are presented with 95% confidence intervals throughout.

Turn analysis

Major changes in walking direction, such as those which occur in response to odours of vertebrates, were only made by both *Rhodnius* and *Triatoma* when they stopped. Since characterisation of these turns was not possible with the servosphere data, they were analysed for *Triatoma* responding to rabbit urine-odour from video recordings (Panasonic VCR NV-180, Japan). These video records were made during all experiments with a black and white infrared-sensitive camera (Canon Ci 20PR, Japan) positioned over the conditioned air-delivery tube at a distance of ca. 80 cm from the apex of the sphere and pointing at an angle of 70° to horizontal. In order to describe the upwind turns made by the bugs, the positions of the longitudinal body-axis *vis-à-vis* due upwind were transcribed from the video screen onto a plastic sheet during a turn. The size of the turn was calculated as the difference between the angle to wind after the bug stopped and the angle to wind before the bug began to run continuously again for at least 3 s. The biggest upwind turns ($\geq 30°$) made by 11 bugs anywhere during the 2 min control period (where the bugs walk preferentially downwind) were compared with the first upwind turns made by the same bugs after onset of rabbit urine-odour (Wilcoxon-Mann-Whitney test). In addition, a frame-by-frame analysis of the horizontal component of antennal movements made during such turns was undertaken. Each time the bug changed the position of its body-axis and/or of the antenna on the side to which it turned, the angle of this antenna to the longitudinal body-axis was also transcribed onto the plastic sheet. The largest of these angles for each turn was compared for control versus test situation (Wilcoxon-Mann-Whitney test).

Results

Walking behaviour of triatomines on the servosphere

Rhodnius and *Triatoma* both walked in straight bouts (subtracks) interrupted by stops, or periods at relatively

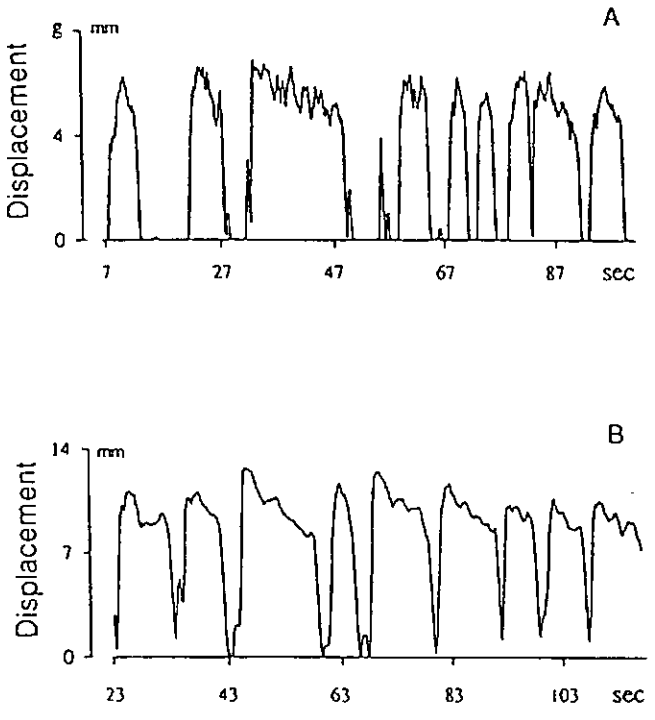

Fig. 1 Displacement of two *Triatoma* (A and B) as sampled every 0.1 s during a 90 s walk on the servosphere. Triatomines walk in bouts which start at speeds higher than the mean, and decelerate thereafter. These bouts are separated by stops or periods at much lower speed (< 5 mm s^{-1}), both of variable duration. These records, which are typical, were made while the bugs walked in a pure air-stream (0.1 m s^{-1})

low speeds, all of variable duration (Fig. 1). Subtrack durations ranged from extremes of one second to about a minute with a mean of 5.0 ± 0.4 s ($n = 500$). The mean straightness index of the subtracks was 0.968 ± 0.004 ($n = 514$) for *Rhodnius*, and 0.959 ± 0.004 ($n = 1073$) for *Triatoma*. Subtrack speed ranged from extremes of 5 mm s^{-1} to about 150 mm s^{-1} with a mean of 33 ± 1.48 mm/s^{-1} for *Rhodnius* ($n = 452$), and 48 ± 1.68 mm s^{-1} for *Triatoma* ($n = 543$). Though the instantaneous speed was relatively constant for individual subtracks, a trend was observed in comparatively longer subtracks whereby the bugs made an abrupt start at a high speed which gradually decreased to end in an abrupt stop. This tendency was more prominent in some than in other subtracks even of the same insect (Fig. 1). The bugs made most changes in walking direction at low speeds or on the spot between two successive subtracks. Three *Rhodnius* and thirteen *Triatoma* groomed during stops for durations ranging from 1 s to over a min with medians of 25 and 11.5 s, respectively. These groomings occurred at random during the 6 min recordings. Both *Rhodnius* and *Triatoma* walked preferentially downwind in clean air (Fig. 2). A few bugs of both species demonstrating a persistent tendency to walk upwind during the acclimatisation period were excluded from the tests.

Though the tracks made by the triatomines were rather straight, the actual path is a zigzag with a period varying from 1.11 to 3.33 s as recorded by the sphere (Fig. 3). Added to this mode of displacement of variable amplitude is a wobble in the gait with a period of 0.2–0.5 s. This unsteadiness was clearly visible from the video records as a transverse see-saw of the abdomen combined with a left-right swinging of the body-axis, evidently due to the bugs' six-legged mode of locomotion.

Responses to stimuli

The characteristic response of both *Rhodnius* and *Triatoma* to all host odours tested, i.e., CO_2, mouse-

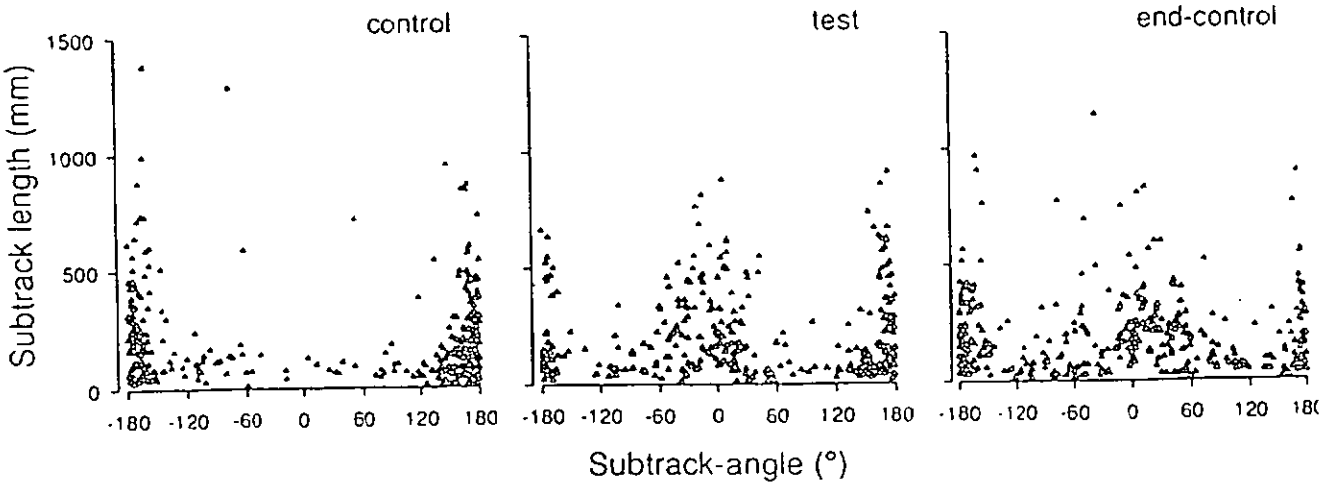

Fig. 2 Scatter graphs of subtrack lengths plotted against subtrack-angles for the successive control, test, and end-control periods of experiments to determine *Triatoma*'s response to rabbit urine-odour ($n = 22$). The bugs walked essentially downwind during the initial control period in the clean air-stream. The proportion of subtracks in the upwind direction increased in response to the odour of rabbit urine. Even after odour delivery ceased, *Triatoma* continued to show an upwind response and the proportion of crosswind (61°–120°) subtracks increased

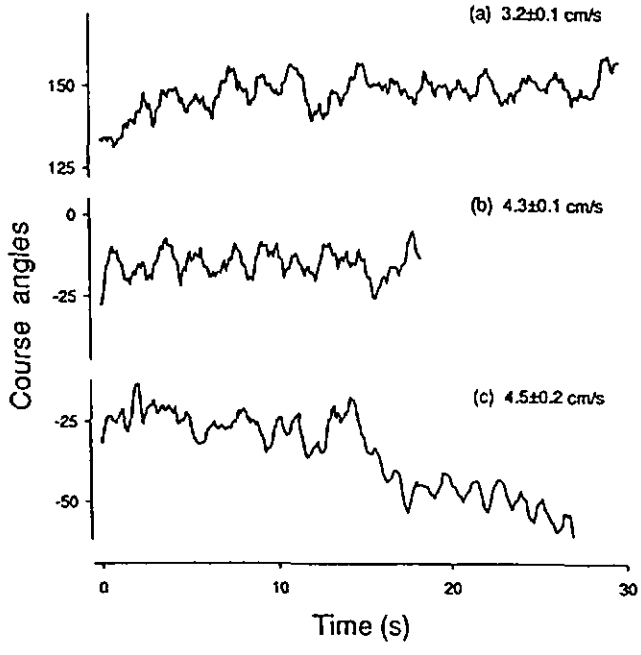

Fig. 3 Course angles with respect to wind (0°) of consecutive 0.1 s vectors of subtracks made by 3 different *Triatoma* during control (a), test (b) and end-control (c) periods of experiments with rabbit urine-odour. These detailed representations of the angles described by the bugs, after applying a running mean over 5 vectors, clearly demonstrate the oscillations left and right of relatively straight paths. The examples shown aptly represent the variation observed. The underlying wobble due to the animal's gait is also visible. Mean speed per subtrack is indicated in each case

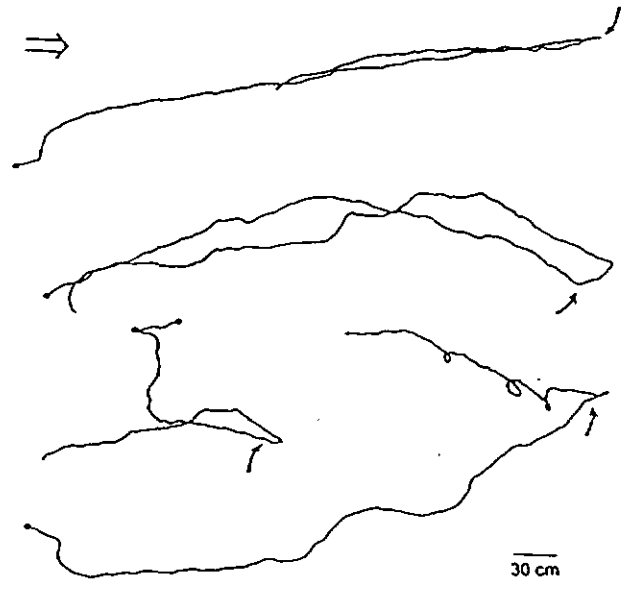

Fig. 4 Tracks made by four *Triatoma* on the servosphere depicting their responses to the odour of rabbit urine. The tracks started (●) with the bugs walking downwind (open arrow top left) in a pure air-stream (0.1 $m s^{-1}$). After odour of rabbit urine was added (bold arrows), the bugs turned to walk upwind. Stops made by the bugs in their tracks, including those made to turn upwind (see Fig. 7), are not detailed here

odour and rabbit urine-odour, was a sharp upwind turn after onset of stimulus delivery (Fig. 4). The fastest upwind turns occurred within ten seconds of the application of odour, but some bugs took as long as one min or more to respond and a few showed no response. The binary response model of the linear logistic procedure shows a good fit to the data (Fig. 5). For both *Rhodnius* and *Triatoma* all the odours tested had a significant effect ($P < 0.05$) on the course taken by the bugs during the test and end-control periods as indicated by the upwind α values (Table 1). The percent upwind displacement by both *Rhodnius* and *Triatoma* was significantly higher during the test period for all stimuli tested ($P < 0.05$). This was also true during the end-control ($P < 0.05$) following delivery of mouse-odour and rabbit urine-odour to *Triatoma* (Fig. 6).

Subtracks were invariably fewer and shorter in the crosswind direction in all control and test situations, with the exception of *Triatoma*'s response to CO_2. However, upon loss of all the odours tested, both *Rhodnius* and *Triatoma* undertook significantly more crosswind subtracks (Fig. 2). The effect on displacement in this direction during the end-control was significant for all stimuli tested on both species, but only for *Triatoma* responding to CO_2 during the test period (Table 1).

Triatoma showed significantly stronger attraction, as indicated by higher upwind α values, to mouse-odour and rabbit urine-odour than to CO_2 ($P < 0.05$); effects were not significantly different ($P > 0.05$) during end-controls (Table 1). No difference between treatments was recorded for *Rhodnius*. Comparison of stimulus effects between *Rhodnius* and *Triatoma* indicated that the upwind response of *Triatoma* to mouse-odour was the strongest and significantly higher than that of *Rhodnius* ($P < 0.05$). *Triatoma* also showed an increase in speed when responding to mouse-odour ($P < 0.05$), an effect not seen with this species to any other stimulus, nor for *Rhodnius* to any treatment. However, *Rhodnius* did walk for a significantly longer time in response to CO_2 ($P < 0.05$). The stimulus effects on crosswind displacement were not different for either *Rhodnius* or *Triatoma*.

Upwind turns in response to stimuli

Most of the acute turns made by *Triatoma* and *Rhodnius* occurred within 10 s of odour onset, after the bugs had stopped. Only 8 of the 11 *Triatoma* tested for a response to rabbit urine-odour made upwind turns greater than 30° anywhere during the control period, whereas the first turns upwind to odour made by all 11 bugs during test (8 of which occurred within 10 s of odour onset) were significantly greater (Fig. 7). Nine of these turns were greater than 90°, including 4 which were over 145°; only one turn exceeded 90° in controls.

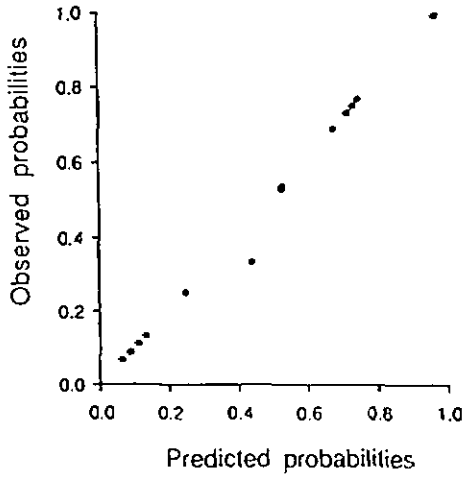

Fig. 5 Probabilities of upwind walks (P_{ub}) by 14 *Triatoma* responding to mouse-odour, as predicted by the binary response model of the logistic procedure in SAS (see text) plotted against the observed values for the same bugs. The high correspondence of the observed to predicted values clearly demonstrates the goodness of fit of the model to the data

When initiating an upwind turn in response to rabbit urine-odour, *Triatoma* first moved its head and/or swung the antennae in the upwind direction without moving the body, taking the antenna on the turn side more upwind. It then followed by turning the rest of the body upwind. The bug brought the body-axis upwind either in one big turn or slowly in a series of smaller consecutive turns in the same direction. In either case, the complete turn never exceeded 45 s in duration. Once a direction was taken as a result of such a turn, then the bug usually walked in this direction for at least a couple of seconds. The mean of the widest angle of the turn-side antenna to the body-axis measured during the above mentioned turns was 90.91° ± 14.25 ($n = 11$) during test as compared to 64.75° ± 7.94 ($n = 8$) during control ($P < 0.001$). These data are typical for turns made in response to odour by both species.

Discussion

An oriented response by *Rhodnius* and *Triatoma* to mouse-odour, one of its components (CO_2), and to rabbit urine-odour has been established. Since the bugs were starved, the behaviour may be considered as appetitive, in response to stimuli associated with a food source. The responses of the two species are remarkably similar.

Triatomines normally show thigmotactic behaviour, preferring to stay in crevices except when seeking a blood-meal. Since we handled the bugs to put them on the sphere, an acclimatisation period of 8–10 min was necessary before starting an experiment. After being released on the sphere, both *Rhodnius* and *Triatoma* ran fast in all directions during the first minute and then took to walking in a preferred direction, usually downwind, at a constant speed. The preferential downwind running response (negative anemotaxis) of both *Rhodnius* and *Triatoma* in a clean air-flow might be interpreted as an attempt by the triatomines to leave the exposed area to seek a refuge. Negative anemotaxis in response to an air-flow at roughly the same wind-speed as that used here has also been reported for the German cockroach on the servosphere, another insect which prefers shelter (Bell and Kramer 1979).

Onset of odour changed the negative anemotactic behaviour of the triatomines into a predominantly positive one. The bug first stopped walking and moved both head and antennae laterally and/or vertically, it then turned the head and the antennae in the upwind direction and this was followed by an upwind turn by the rest of the body. After making the turn, the triatomine then continued to walk in the upwind direction for at least a few seconds. This upwind orientation is evidently triggered by the arrival of the odour since such turns and the upwind response were not observed in clean air. The response is guided by the wind direction to which the bugs orient, i.e., odour mediated anemotaxis. This positive anemotactic response to odour is all the more significant considering the persist-

Table 1 Host-odour effects on upwind and crosswind orientation by *Rhodnius* and *Triatoma* as estimated by the stimulus effect α (α_1 for test, α_2 for end-control) computed with the binary response model of the logistic procedure in SAS (see text). Upwind is 0°–60°, and crosswind 60–120°. Stimulus effects (± SE) followed by an asterisk indicate a significant difference from control, and different letters signify differences between two stimuli on a species ($P < 0.05$). The response to mouse-odour during the test period is the only significantly different ($P < 0.01$) response between the two species to the three odour stimuli tested

	Rhodnius			Triatoma		
	CO_2	Mouse-odour	Rabbit urine-odour	CO_2	Mouse-odour	Rabbit urine-odour
Upwind						
α_1	2.04 ± 0.5*	2.5 ± 0.43*	2.81 ± 0.55*	2.14 ± 0.34*[b]	5.04 ± 0.74**	3.88 ± 0.42**
α_2	–	1.1 ± 0.22*	0.82 ± 0.22*	1.16 ± 0.19*	1.7 ± 0.41*	1.59 ± 0.18*
Crosswind						
α_1	0.73 ± 0.38	– 0.42 ± 0.37	0.59 ± 0.33	0.64 ± 023*	– 0.2 ± 0.22	– 0.13 ± 0.28
α_2	–	0.44 ± 0.2*	0.74 ± 0.17*	0.36 ± 0.12*	0.3 ± 0.12*	0.36 ± 0.13*

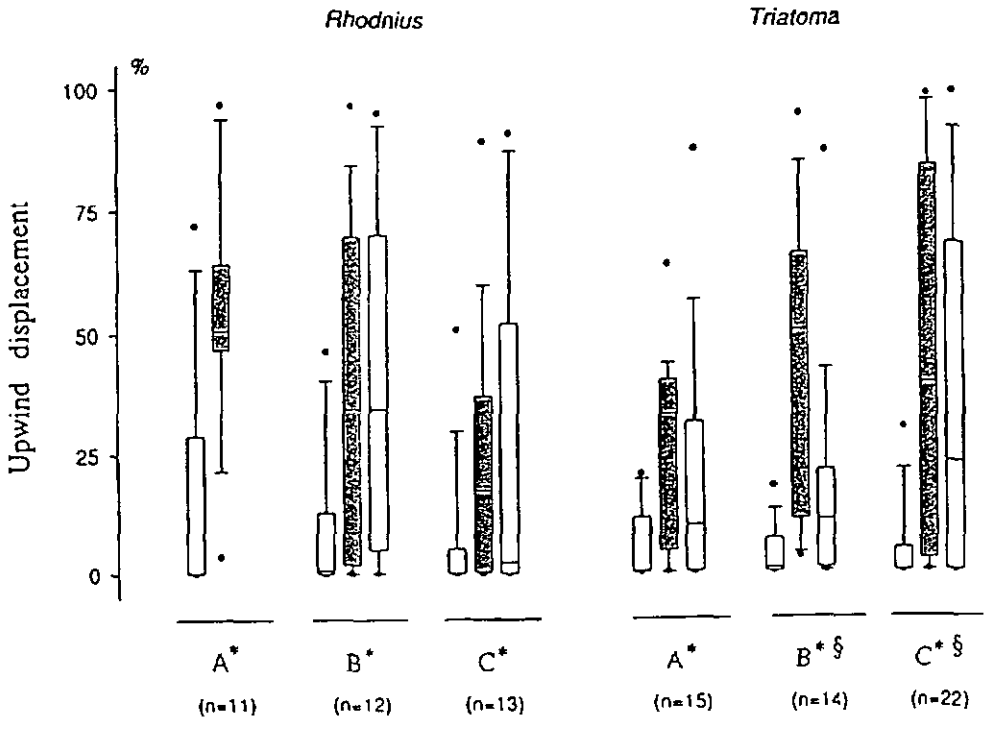

Fig. 6 Box plots of the upwind displacement (60° either side of due upwind) as % of the total for the period made by *Rhodnius* (left) and *Triatoma* (right) in response to 0.6% CO_2 (A), mouse-odour (B) and rabbit urine-odour (C). Responses to each treatment are divided into test period (*stippled*) and control periods which preceded and followed it (respectively, left and right of the stippled box). End-control data are missing for *Rhodnius* with CO_2. The line within a box marks the median, the lower and upper boundaries of a box indicate the 25th and 75th percentiles, error bars below and above a box indicate the 10th and 90th percentiles, and the 5th and 95th percentiles are shown as circles. The asterisk after a treatment letter indicates that upwind displacement was significantly higher ($P < 0.05$) in test than in the control period which preceded it, and § indicates a significantly higher upwind displacement for the end-control compared to the initial control period (Wilcoxon signed-rank test)

ent downwind running by the bugs during the control. Most bugs responded by turning once, whereas others responded repeatedly by turning upwind after having veered downwind or crosswind. The fact that the bugs walk less often and for a shorter duration in the crosswind direction during control and test periods could be associated wih these anemotactic responses, i.e., negative in odour-free and positive in odour-laden air.

Antennal movements almost always preceded an upwind turn in our experiments. The antennae were moved either in unison or independent of one another. Sometimes the bugs even raised the anterior part of the body while stretching the antennae vertically and somewhat backwards, usually in unison. Although we did not measure antennal movements along all axes, measuring the maximum horizontal component of the turn-side antenna to the body-axis during a turn shows that the biggest shifts in the angle of the antennae accompany turns in response to odour. Antennal movements are not, however, related to the size of such turns. Even when walking due upwind the bugs stop either to make course corrections or to sample the air with lateral and vertical movements of the head and antennae. These movements of head and antennae can be as big as those observed during upwind turns, and can even be made, on occasion, while running. Antennal movements have already been reported for *Rhodnius* in response to warmth and odour (Wigglesworth and Gillett 1934) and for *Triatoma* in the presence of hosts and a heat source (Núñez 1982; Lazzari and Núñez 1989). Increased angular deflection of antennae with respect to the body-axis has also been reported for the American cockroach in response to stimulation with its sex and aggregation pheromones (Rust et al. 1976).

Both *Rhodnius* and *Triatoma* continued to walk in the upwind direction for some time after stimulus delivery ceased, but rarely continued upwind throughout the complete end-control period. This upwind walk during the end-control may be attributed to pursual of the upwind direction taken during the test period. However, some bugs which walked crosswind or downwind towards the end of the test period, showed a renewed upwind response after stimulus delivery ceased, suggesting a chemically triggered upwind response to disappearing odour. Some adaptation may have occurred following exposure of the bugs for 2 min to host odour. However, the test period had to last this length of time considering how long it took some bugs of both species to respond (> 1 min); pulsing the odour did not improve the situation for delayed respondents. An upwind response after odour delivery ceased was not

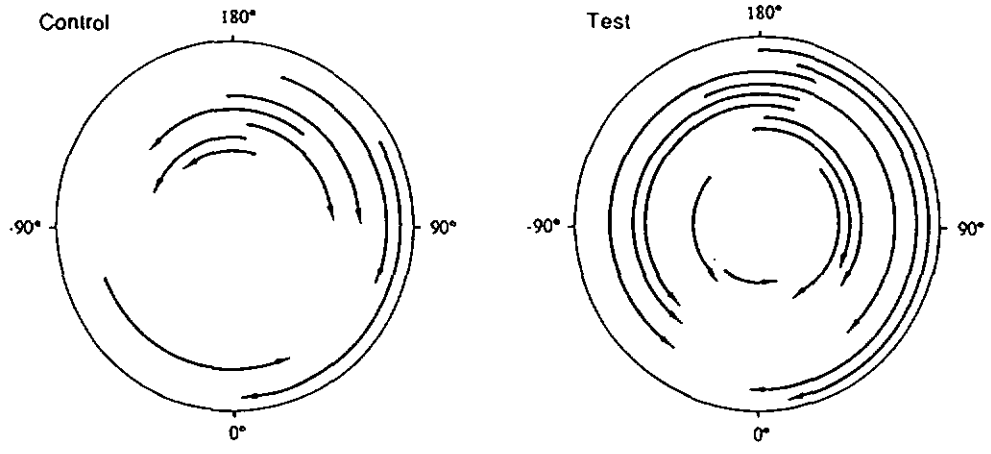

Fig. 7. Arcs representing the first upwind turns made by 11 *Triatoma* in response to rabbit urine-odour (test) as compared to the biggest upwind turns of at least 30° made anywhere during the preceding control period. *Triatoma* walks predominantly downwind in pure air. Each arc represents the size, and the arrow indicates the direction of the turn made by a bug; 0° is straight upwind. The turns made by the eleven bugs are concentrically arranged in ascending order. Only 8 bugs made a few upwind turns bigger than 30° in control compared to all 11 who frequently reoriented upwind in response to rabbit urine-odour

recorded for granary beetles (Tobin and Bell 1986), but the cockroach *B. craniifer* maintained the walking direction induced by an air-stream even after it ceased to blow (Bell and Kramer 1979). In addition to walking more upwind during the end-control than during control, the bugs also made more subtracks in the crosswind direction after the host odours ceased to be delivered. The higher component of crosswind displacement during the end-control can be associated with a search strategy by the bugs to re-contact odour. An odour could be accidentally lost under natural conditions either due to an obstacle, the bugs' tendency to walk straight, or due to a shift in wind direction. The higher crosswind component of walks during the test period by *Triatoma* responding to CO_2 could be explained as a response to an incomplete stimulus incapable of inducing a strong upwind response.

The general tendency of both *Rhodnius* and *Triatoma* to walk in bouts which are very straight seems to be an innate characteristic of triatomine displacement on the ground for it is always maintained, i.e., before, during and after stimulation with an attractive odour. Triatomines normally live in refuges and crevices associated with the host's abode, e.g. hen houses, and are nocturnal in behaviour. In the context of the niche we may therefore suggest a searching strategy on the part of bugs where they leave their refuge when hungry, sample the air, turn if required, i.e., in the direction of a host stimulus, run straight in the chosen direction, and stop again after some time to make any necessary corrections. The bugs might even integrate this series of essentially straight subtracks and use the information during their return trip. There is evidence that foraging ants integrate their tortuous outgoing paths and return home along straight paths (Müller and Wehner 1988). Evidently, it would be useful for the bug to respond to directional stimuli from the outset with a straight walk to the host, for the same return walk will bring it safely back when laden with the blood-meal to the security of the refuge. Returning to a refuge after a blood-meal is probably not an easy task for triatomines: fifth instars can increase in weight by a factor of 6 to 8 when fully engorged. This strategy of walking straight at a high speed in bouts of random duration, intercepted by stops when the bug samples the environment with its antennae, could also help to reduce the chance of being captured by a predator. A similar walking behaviour with intermittent stops has been reported for the American cockroach, which stopped for shorter periods but more frequently in the presence of pheromone (Bell and Kramer 1980). We did not observe any consistent trend in the frequency or duration of the stops made by the triatomines in response to the stimuli tested. The fact that the bugs have to stop to turn is probably related to the high speeds at which they walk ($33 \pm 1.48\ mm\ s^{-1}$ for *Rhodnius* and $48 \pm 1.68\ mm\ s^{-1}$ for *Triatoma*). If the bugs turned at their mean walking speed, the size of the curve required to bring them upwind to the odour might quite easily take them out of the odour plume.

The zigzag in the walking tracks of both *Rhodnius* and *Triatoma* is similar to that reported for the American cockroach walking on the servosphere (Bell and Kramer 1979). The triatomines show this zigzag during walks in any direction and for all situations tested, i.e. before, during, and after odour delivery. This idiothetically controlled mechanism of displacement superimposes a wobble in the bugs' body while they run, which is undoubtedly due to their six-legged mode of locomotion (Delcomyn 1985). The origin of the zigzag may be purely mechanical to allow the animal to run at a high speed in a fixed direction despite the wobble. But an internally driven sinusoidal mode of displacement might have the added advantage of providing the central controller for running with more meaningful in-

formation (in terms of integration) from sensory organs such as antennae which, incidentally, bear thermo- and hygro-receptors in addition to chemoreceptors. That is, instead of perception of that which arises from unsteadiness in the walk, the zigzag could serve to regularly provide the central controller with an independent signal from left and right of the chosen course.

Evidence for host odour perception by haematophagous arthropods is provided by reports on the presence of olfactory receptors for CO_2 in tsetse flies (Bogner 1992), stable flies (Warnes and Finlayson 1986), and ticks (Steullet and Guerin 1992a); receptors for hydrogen sulphide from breath in ticks (Steullet and Guerin 1992b); lactic-acid receptors in mosquitoes (Davis 1984) and *Triatoma* (Bernard 1974); ammonia receptors in ticks (Haggart and Davis 1979; Steullet and Guerin 1994b) and *Triatoma* (Bernard 1974; our own unpublished data); fatty acid receptors in ticks (Steullet and Guerin 1994a) and in *Triatoma* (Bernard 1974); and receptors for a range of other volatiles of rabbit, steer, and human origin in tsetse flies and ticks (Bursell et al. 1988; Steullet and Guerin 1994a, b). Although the literature abounds with reports on the behavioural responses of haematophagous arthropods to vertebrates, few investigators have taken the effort to discriminate between the relative contribution of different modalities to the attraction observed, i.e., the influence of odour as against that of heat or visual cues. Yet other reports have depended on the responses of groups of insects, ignoring the influence of individual respondents on each other. Despite this, responses by individual *Triatoma* to radiant heat alone have been recorded (Lazzari and Núñez 1989). In addition, responses of triatomines to conspecifics and their own faeces have been documented (Baldwin et al. 1971; Schofield and Patterson 1977; Figueiras et al. 1994). Here we have taken care to test responses of individual *Rhodnius* and *Triatoma* to host odours under strictly defined conditions. Even though the experiments were made in total darkness, we cannot say with certainty that an object associated with the experimental set-up in the vicinity of where the bugs ran was not visible to these night-active insects. However, if this were the case, a response to it, independent of the wind and wind plus odour effects reported here, should have been recorded. This was not the case. On the contrary, it was only on the arrival of host-odours that the triatomines were caused to break from what was essentially a downwind walk in pure air to an upwind response in odour-laden air. This provides unambiguous evidence for attraction of these haematophagous bugs to host odours alone. It vindicates the observations by Wigglesworth and Gillett (1934) who concluded from short range (ca. 4 cm) experiments in the absence of air-currents that odour of mouse-skin at room temperature was attractive, since the moisture component of the fresh bait did not attract their blinded bugs.

Triatoma showed the strongest response to mouse-odour and a weaker one to CO_2, indicating that components of mouse-odour other than CO_2 serve in the attraction observed. Mouse-odour also induced a significant increase in speed (positive orthokinesis) in *Triatoma*, and the percent displacement upwind was higher after mouse-odour and rabbit urine-odour were turned off than before the bugs were exposed to the stimuli. Although the responses of *Rhodnius* to the three odour stimuli were not different, the response profile tended to confirm what was recorded for *Triatoma*. The strong response to mouse-odour could be attributed to its relative completeness as a source of information for these haematophagous bugs. The response to the stale urine-odour is significant considering its ammonia content, and *Triatoma* does possess ammonia receptors on the antennae (see above).

To conclude, the observed oriented responses of *Rhodnius* and *Triatoma* to vertebrate odours in an air-stream indicate that perception of olfactory cues alone is sufficient to attract these bugs to a host.

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Ammonia attracts *Triatoma*: behavioural and neurophysiological data

Introduction

Triatoma infestans (Klug) belongs to the sub-family Triatominae (Heteroptera: Reduviidae) and is one of the most important vectors of American trypanosomiasis or Chagas' disease. This haematophagous bug is prevalent in South America, predominantly inhabiting domestic habitats where they take refuge in the cracks and crevices of dwellings. Peridomestically, they are associated with animal shelters such as chicken coops, guinea-pig runs and goat corrals.

Most triatomines are nocturnally active, feeding on their reposing hosts, after which the triatomines return to refuges where they spend daylight hours. This *modus vivendi* is in fact quite adaptive: The hallmark of bug-infested dwellings is an increase in the quantity of faecal droppings on the walls harbouring refuges (Gürtler *et al.*, 1995), suggesting that bugs regularly return to the same refuge. Indeed, it has recently been demonstrated that bugs leave the refuge to defecate at the entrance, and that such entrance-marked refuges are preferentially occupied (Lorenzo & Lazzari, 1996). Through the predictability of the resting sites of hosts in the domestic and peridomestic settings, triatomines will soon become accustomed to the path taken for a blood-meal from the refuge. Marking a specific refuge with faeces would further serve to inform other bugs of the suitability of its location in the vicinity of hosts, as defecation occurs primarily in a few days after a blood-meal (Wigglesworth, 1931). Aggregation within specific refuges would promote gene transfer, transmission of symbionts needed for development of young stages via coprophagy (Harington, 1960; Baines, 1956; Schaub *et al.*, 1989), and cannibalism, where unfed nymphs feed on engorged individuals (Ryckman, 1951; Schaub *et al.*, 1989). The latter phenomenon

could be expected to be of most benefit for the small blood requirement of the youngest stages which have not yet learned the way to the host.

Considering the success of triatomines as parasites, we may postulate them to possess certain behavioural and sensory attributes which would permit the bugs to adapt to, and fully exploit, the relatively constant spatial separation between host and refuge. *T. infestans* can react to irradiated heat and even differentiate between bodies at different temperatures (Lazzari & Núñez, 1989a). Previous studies in this laboratory with starved bugs on a servosphere have shown that, on their own, host odours as well as vapours of host urine and the respiratory product CO_2 all cause *Rhodnius prolixus* and *Triatoma infestans* (hereafter, *Rhodnius* and *Triatoma*) to walk upwind towards the source (Taneja & Guerin, 1995). The basis of this odour-mediated anemotaxis is perception of chemostimuli via olfactory receptors on the antennae. The latter are activated during orientation: Bugs walk predominantly downwind in clean air with the antennae stretched forward in line with the body axis. Upon stimulation with host volatiles the bugs stop, turn the head and antennae upwind, and this is followed by an upwind turn by the rest of the body (Taneja & Guerin, 1995). Our first electrophysiological recordings from the antennae of these bugs revealed an olfactory receptor within grooved-peg sensilla which readily responded to a volatile liberated by stale host urine and wetted triatomine faeces. Wetted faeces produces this volatile in such quantities that it is readily identified as ammonia. Here we describe the neurophysiological responses of olfactory receptors in the grooved-peg sensilla on the antenna to NH_3, the identification of this volatile from a variety of biological sources vital to the bugs, attraction of *Triatoma* to NH_3, and the functional significance of such responses for triatomines.

Materials and methods

Insects: A colony of *Triatoma* is maintained at 26°C, 75% RH and 12:12 h L:D cycle in a climate chamber. The bugs are regularly fed on heparinised cow-blood using the *in-vitro* feeding technique described by Núñez & Lazzari (1990). Fifth instar nymphs were used in all experiments described here. Behavioural experiments were made with nymphs which were starved for 6-8 weeks after moulting, and electrophysiological recordings were made from nymphs 1-4 weeks post-ecdysis.

Locomotion compensator: A servosphere (Kramer, 1976) was used to record the responses of the *Triatoma* nymphs to biological media and ammonia. This apparatus functions in such a way as to keep the walking insect at the same position in space, *i.e.*, at the apex of a perspex sphere (50 cm dia.), to which the stimulus delivery tube (see below) is directed as described previously (Taneja & Guerin 1995). All tests were carried out in the dark, during the scotophase of the bugs' daily cycle. The room temperature was maintained at 23-25°C, and a charcoal-filtered air-stream (0.1 ms^{-1}) at 25°C and 90% RH was continuously delivered tangentially to the apex of the sphere. Prior to recording behavioural responses, each bug was allowed an acclimatisation period of at least 8-10 min on the sphere. After this, 6 min records of individual bug's behaviour were made, comprising 3 consecutive two min periods, *i.e.*, control, test, and end-control.

Track analysis: The x-y co-ordinates of displacements made by the *Triatoma* nymphs were recorded every 0.1 s at a resolution of 0.1 mm on a SAMII 68 K computer (KWS Inc. Ettlingen, Germany) via pulse generators responding to 0.1 mm displacements of the servosphere. These co-ordinates of displacements, constituting the instantaneous component vectors of tracks with a length and a direction (course angle) with respect to wind (0° is straight upwind), were analysed (after discarding all instantaneous displacements smaller than 0.5 mm) on a PC with specially developed track analysis software. To ascertain if the bugs walked significantly more in the upwind direction (-60° to 60°) in response to stimulation, the percent upwind

displacement for each period was calculated for each bug and compared using the two-tailed Wilcoxon signed rank test for paired replicates (Siegel & Castellan, 1988). Latency in response was investigated by comparing the percent upwind displacement during the first and second minutes of the test and end-control periods with the corresponding parts of controls. Since presence of an olfactory stimulus could also influence the walking speed of the nymph, this was analysed by calculating the mean speed for each bug after eliminating all walking bouts of less than 1 s (relevant walking bouts are continuous displacements of at least 1s and defined as subtracks, *cf.* Taneja & Guerin, 1995). Speeds of such subtracks were then compared between the different periods for each bug using the two-tailed Wilcoxon signed rank test for paired replicates.

Electrophysiology: A bug was mounted (either dorsally or ventrally) on a glass slide with a layer of double-sided sticky-tape (Tesa, Germany) on a black background. The legs, thorax and head of the bug were further fixed with fine grain, normal setting, dental zinc cement (De Trey, Germany) and strips of adhesive tape. The antenna, which consists of a short scape proximally, a long pedicel and two flagellar segments distally, was gently pressed onto double-sided sticky tape using a fine needle and further fixed with thin strips of adhesive tape over the pedicel and the proximal flagellar segment. To prevent body desiccation, a wad of wet cotton-wool was placed on the bug's abdomen. The slide with the bug was then mounted under the microscope (Kombistereo M3Z, WILD, Switzerland) in such a manner that at least the two antennal flagellar segments were bathed in a humidified (95-100% RH) air-stream (24±2°C) delivered to the preparation at 1 ms^{-1} via a glass tube (6 mm I.D.). As reference, a chloridised silver electrode in a drawn-out glass capillary (tip dia. *ca.* 4 µm) filled with a triatomine ringer solution (composition: 161 mM NaCl, 2.6 mM KCl, 5 mM $CaCl_2$, 2 mM Tris buffer, and 4 g glucose; Bernard, 1974) was inserted at the base of the scape. A second silver electrode in another drawn-out glass capillary (tip dia. *ca.* 2 µm) was used for recording from the base of basiconic grooved-peg sensilla present on the terminal flagellar segment of the nymph's antenna (type Ff; Bernard, 1974). Good single unit activity of olfactory receptors can be readily recorded from within the sensilla in this manner, and the set-up also sufficed

for electroantennogram (EAG) recordings from the antenna. Micromanipulators (Leitz, Switzerland) permitted accurate positioning of the electrodes. The recording electrode was connected to a high impedance ($10^{12}\Omega$) preamplifier (x10, Syntech, The Netherlands), and this signal was fed from the preamplifier into an AC/DC amplifier (UN-03, Syntech, The Netherlands), amplified again (50-100 times) and recorded on a tape in a DAT recorder (Biologic, France). Alternatively, the signal from the main amplifier could be recorded directly on the hard disk of a PC via a 16-bit analogue-digital IDAC card (Syntech, The Netherlands), and monitored simultaneously with an oscilloscope (Tektronix 5103, USA). The taped recordings were replayed and recorded on the PC via the DAS16 analogue-digital board (MetraByte Corporation, USA) or via the IDAC board also containing a 16-bit analogue-digital converter (Syntech, The Netherlands). Action potentials were analysed using the spike analysis programmes SAPID (Smith *et al.* 1990) and AutoSpike (Syntech, The Netherlands).

Stimuli and stimulus delivery for behavioural recordings: Triatoma nymphs were allowed to deposit excrement for 20-30 days from feeding on filter paper discs (9 cm dia., Schleicher & Schuell, Merck, Switzerland) which are used routinely to adsorb any liquid waste products in triatomine culture vials. The bugs were in constant contact with these papers between blood-meals. These papers, with deposits of adsorbed faeces and other excretory products, were regularly removed and stored in 1 l gas-wash bottles at room temperature (hereafter referred to as dry faecal-papers). This material has a wholesome food-like odour to the human nose. The faecal-papers in some of these bottles were saturated with distilled water, kept overnight at room temperature, and stored thereafter at 4°C (hereafter referred to as wetted faecal-papers). Bottles containing wetted faecal-papers were brought to room temperature before use. A few hours after wetting, this material releases a strong nauseous odour to the human nose. Both dry and wetted faecal-papers (*ca.* 60 g before wetting) were tested on the servosphere by blowing charcoal-filtered air (240 ml/min) over them in a gas-wash bottle. Dry and wetted filter paper discs, never brought into contact with bugs or their faeces, served as controls.

To provide an artificial source of ammonia, serial dilutions of aqueous NH_3 (Fluka, Switzerland) were made in distilled water and stored at 4°C. Three doses were tested on the servosphere by blowing charcoal-filtered air over 2, 24 and 235 µmole NH_3 applied to filter paper discs (12.5 cm dia.) in a 1 l gas-wash bottle. Distilled water applied to similarly sized discs served as controls. In addition, the intermediate dose of 24 µmole was also tested at 35% RH in the air-stream flowing over the bugs on the servosphere. The different RH levels were achieved by regulation of the relative proportions of the wet and dry air-flows through the computer operated humidity/flow controller (Syntech, The Netherlands) which has been previously described (Taneja & Guerin, 1995).

The stimulus delivery system to the apex of the sphere was the same as described earlier (Taneja & Guerin, 1995). The vapours from the gas-wash bottles were introduced via teflon-tubing (2 mm I.D. x 4 mm O.D.) attached to a syringe needle which was inserted at 26 cm from the apex of the sphere through a rubber septum in the wall of a stainless-steel tube which conducted the main air-stream to the apex of the sphere. The odours were introduced into the tube upwind of steel wool and a baffle at the exit of the tube. Computer controlled solenoid valves switched the charcoal-filtered air-flow between control and test flasks for the consecutive 2 min control, test and end-control periods.

Stimuli and stimulus delivery for electrophysiological recordings: Dry (1 g) and wetted faecal-papers (*ca.* 1.0 g before wetting) were tested by passing charcoal-filtered air over them in 5 ml polypropylene syringes as stimulus cartridges. The needle of the syringe was introduced into the air-stream in which the flagellar segments bathed (above) via a rubber septum-covered hole in the wall of the glass tube 23 cm from the preparation (*cf.* Steullet & Guerin, 1992a). Air over clean dry or wetted filter papers served as control. Log dilutions of aqueous NH_3 were made in distilled water and 14 doses of NH_3 (0.03-59 µmole) were applied to filter paper strips (*ca.* 0.5x5 cm) and placed in the polypropylene cartridges. Stale rabbit-urine was also tested by applying 5 µl to a filter paper strip. Distilled water alone was applied to the filter paper strips in controls. Stimulation consisted of flushing 1 ml of compressed air in 1 s through the stimulus cartridge via a teflon stopper connected via

teflon tubing (2 mm I.D. x 4 mm O.D.) to solenoid valves in a stimulus delivery system (ST-05, Syntech, The Netherlands). To compensate for any major change in temperature or humidity occurring in the air-stream flowing over the sensillum other than that due to the introduction of the stimulus, the 1 s stimulus puff interrupted a complementary air-flow of 1 ml/s which flowed continuously into the air-stream. In adaptation experiments, filtered air (120 ml/min) was passed for 100 s over filter paper discs (12.5 cm dia) in 1 l gas-wash bottles to which different doses of aqueous NH_3 in the range of 13-235 µmole, equivalent to those employed in behavioural tests, were applied.

Different doses of the following synthetic chemicals were also tested as above to study the response profiles of receptors located in the grooved-peg sensilla: methyl-amine (dose range: 0.13-13 µmole), dimethyl-amine (dose range: 0.09-9 µmole), trimethyl-amine (dose range: 0.17-17 µmole), ethyl-amine (dose range: 0.15-15 µmole), and diethyl-amine (dose range: 0.14-14 µmole) dissolved in distilled H_2O, in addition to propyl-amine, iso-propyl-amine, butyl-amine, hexyl-amine, octyl-amine, acetone, formic acid, acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid, iso-valeric acid, pyruvic acid, lactic acid, propanol, pentanol, iso-pentanol, 1-hexanol, 1-octen-3-ol, propanal, butanal, hexanal, heptanal, octanal, trans-2-hexenal, vanillin, 4-heptanone, 2-nitrophenol, 4-methylphenol, methyl-salicylate, hexyl-acetate, dimethyl sulphide, sodium sulphide and limonene, all dissolved in dichloromethane (Merck, analytical grade); dichloromethane and distilled water served as solvent blanks. Initially, a 10 µg aliquot of each stimulus was tested (after evaporation of the organic solvent). When a receptor responded to a chemical, graded doses of the product (100 ng, 1µg, 10 µg and 100 µg at stimulus source) were tested. All chemicals except methyl-amine, dimethyl-amine, trimethyl-amine, and ethyl-amine were more than 98% pure. Three min were allowed for stimulus evaporation within the cartridge prior to delivering the volatile to the preparation. CH_4 (neat from the mains) and CO_2 (from a gas cylinder) were also tested by diverting the gas-flow through the stimulus cartridge.

Identification and quantification of ammonia and carbon dioxide: The nauseous vapour released continuously from the wetted faecal-papers and stale

rabbit-urine was identified as ammonia employing a simple colorimetric test kit for gross detection and quantification of dissolved NH_3 in the 0.5-10 ppm range (Aquamerck, Merck, Switzerland). For this, the vapour was collected by bubbling 5 ml stimulus air through 1 ml water. NH_3 levels in dry faecal-papers were too low to be detected by this method. A more sensitive method was used for accurate colorimetric identification and quantification of ammonia in the 0-1000 ppb range using a flow-injection analysis and gas-diffusion technique (Tecator Instruments, Sweden) where the standard and unknown aqueous samples (200 µl) containing dissolved NH_3 are injected sequentially into a carrier stream which is mixed with sodium hydroxide. The mixed stream then passes along a teflon membrane in a gas diffusion cell, where the ammonia gas formed diffuses through the membrane into an indicator stream. The resulting colour change of the indicator is measured at 590 nm from which final measurements for the unknowns are computed relative to the standard curve in µg/l (ppb). To accurately calibrate the amount of NH_3 released from biological substrates which were employed in behavioural and electrophysiological experiments, the vapours released were bubbled through distilled water. Measurements of amounts released in the behaviour experiments were made from dry (60 g) and wetted faecal-papers (60 g prior to wetting) in 1 l gas-wash bottles, stale rabbit-urine (200 ml heated to 37°C in a 1 l gas-wash bottle, which proved attractive in an earlier study; Taneja & Guerin, 1995), four doses of aqueous NH_3, *i.e.*, 2, 24, 235 and 2353 µmole applied to filter paper discs in 1 l gas-wash bottles, and two controls with clean dry and wetted filter paper discs in 1 l gas-wash bottles. Two 100 ml gas-wash flasks connected in series (the second flask employed to monitor any breakthrough from the first) and containing 100 ml distilled water were used as traps through which 480 ml air was bubbled in 8 min (flow rate, 1 ml/s) from all the stimuli except the dry faecal-papers. From the latter, where the amount of NH_3 released was lower, the same volume of air was bubbled through two 10 ml flasks connected in series containing 10 ml water. To establish the stability of the release rate of NH_3 from 235 µmole and 24 µmole sources of aqueous NH_3 and wetted faecal-papers in 1 l gas-wash flasks over the two minute period of delivery in behaviour experiments, measurements were made of the release rate for the first and the last 30 s by bubbling 120 ml of air into two 100 ml flasks with distilled water as above. To quantify the

amount of NH_3 delivered to the *Triatoma* electrophysiological set-up, 1 ml of air over 0.06, 0.6, 5.9 and 59 µmole aqueous NH_3 and distilled water (control) applied to filter papers in the 5 ml stimulus cartridges was evacuated from 10 individual cartridges at each dose and bubbled through two 10 ml gas-wash flasks connected in series. The amounts of NH_3 in these aqueous solutions were quantified by the Tecator® colorimetric method. As the amounts quantified from different substrates were subsequently diluted by factors of *ca.* 24 and 42 in the stimulus delivery systems to the servosphere and electrophysiology set-ups, respectively, the amounts indicated hereafter for these bioassays are quantities in the stimulus delivery air-streams.

To ascertain if the production of NH_3 from the wetted faecal-papers had a bacterial or enzymatic basis, both the bacteria and the possible substrate for enzymatic action were successively removed from aqueous washes of the wetted faecal-papers. In the bacterial assay, a 30 ml water wash of 60 g wetted faecal-papers was filtered under sterile conditions over an acrylic filter (pore size 0.2 µm, Acro50A, Gelman Sciences, Switzerland) to remove any bacteria. The filtrate was then allowed to dry on sterile filter paper discs under sterile conditions to evaporate any dissolved ammonia from the filtrate. These dried filter papers were then re-wetted with sterile distilled water, held overnight and analysed for any NH_3 release using the Aquamerck® test kit. In the enzyme assay, 40 ml distilled water was added to 10 ml of the filtrate just described, this solution was then filtered under pressurised nitrogen through a PM10 membrane filter (cut-off at 10 KD) using an Amicon ultrafiltration cell (Model 8050, Amicon, Switzerland). The 2 ml residue was re-dissolved in 48 ml water and re-filtered. This process was repeated 3 times to wash out any residual dissolved ammonia or substrate. Then 5 µl, 50 µl, and 500 µl of 10 mM urea and uric acid solutions in distilled water were added to 100 µl aliquots of the residue. The mixtures were brought to 1 ml by adding distilled water, incubated at 27°C for 3-4 h, and then analysed for any NH_3 release as above. A similar assay was carried out with residue and substrate employing 50 mM Tris buffer (pH 8) instead of distilled water.

In addition to the ammonia measurements, CO_2 release from the gas-wash bottles containing dry and wetted faecal-papers and stale rabbit-urine was measured with CO_2 indicator-tubes (± 10% error; Dragerwerk, Germany).

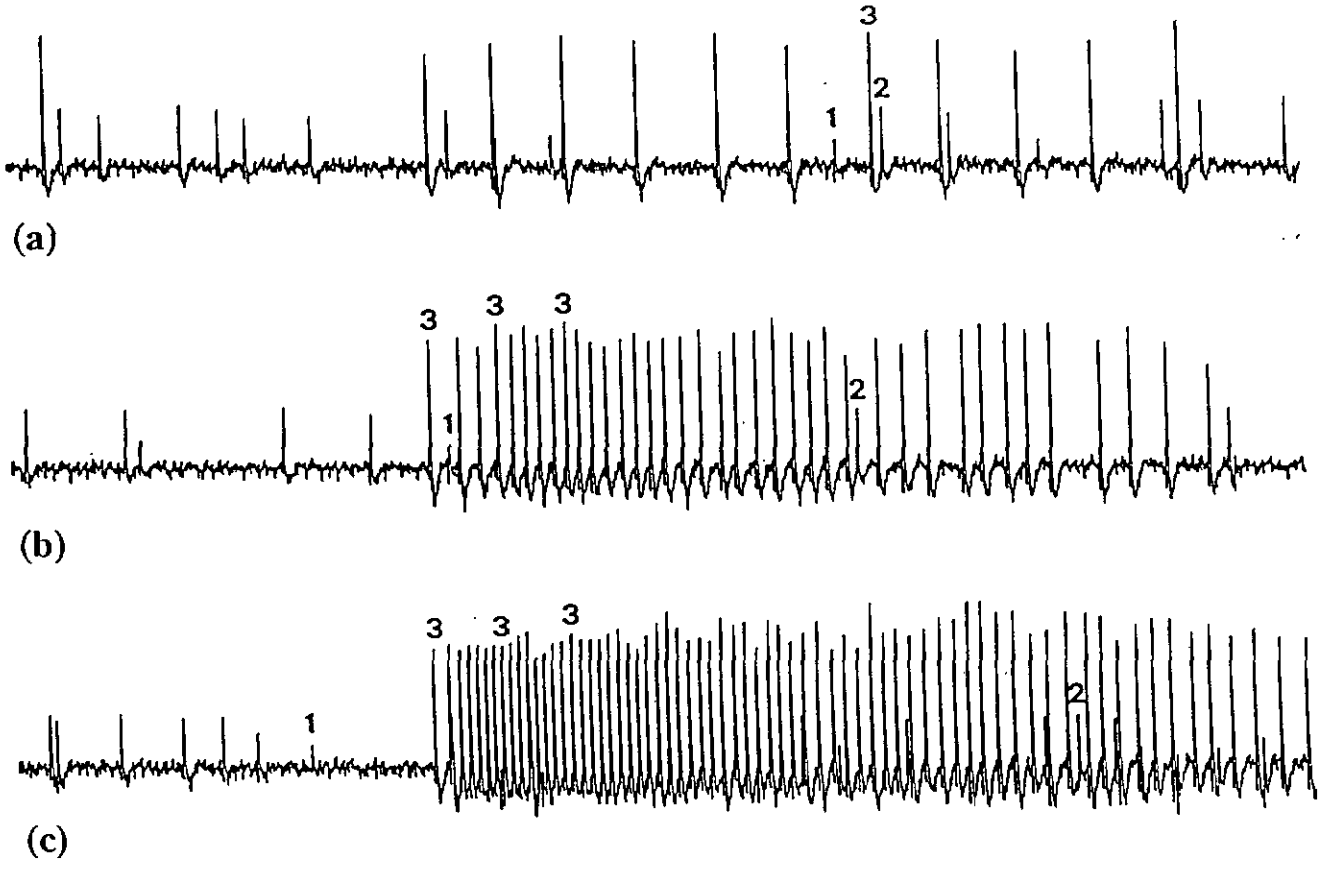

Figure 1: Responses of an olfactory receptor within a grooved-peg type-1 sensilla (GP1) on the antenna of a *Triatoma* nymph to NH_3 at (a) 2.6 ppb, (b) 5.5 ppb and (c) 20.4 ppb in air reaching the preparation. Recordings of spontaneous activity from the GP1-type sensilla typically reveals three receptors with small (**1**), intermediate (2) and big (3) amplitude spikes. Receptor 3 is most sensitive to NH_3 and also responds to methyl-, dimethyl-, ethyl-, and diethyl-amines but at thresholds 10 times higher. No adequate stimuli have been identified for receptors **1** and 2. Horizontal bar indicates 1 s stimulation and the big spike in trace (a) has an amplitude of *ca.* 2.5 mV.

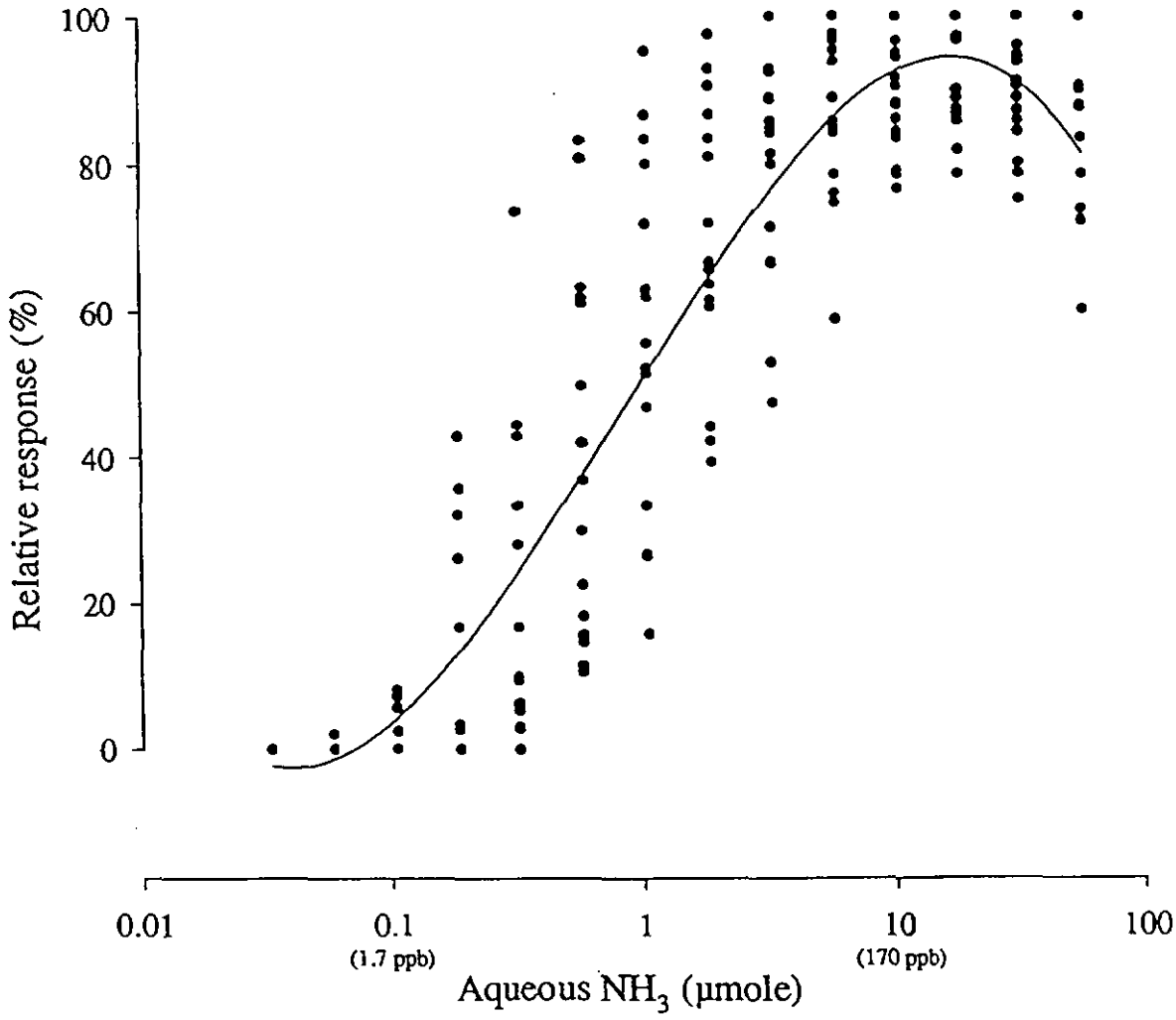

Figure 2: Dose-response relationship of NH_3-excited receptors on antennae of 15 *Triatoma* nymphs. For this, each electrophysiological preparation of a single double-walled grooved-peg sensilla type-1 housing the receptor was subjected to stimulation for 1 s with NH_3 vapour released from increasing doses of aqueous NH_3 on filter papers. Spikes were counted in the first 1 s of the response. Since the spontaneous activity of the NH_3-responding unit varies with time, spikes occurring in a 1 s control period preceding each stimulation were subtracted from the response. The responses to the different doses of NH_3 delivered were normalised with respect to the strongest response (100%) for each bug. The dose-response is log-linear over the dose range 1.7 to 200 ppb NH_3; At higher doses the response plateaues. (The trend line was drawn by linear regression using least squares to fit the data to a polynomial of order 3, $y = a + bx + cx^2 + dx^3$; SigmaPlot for Windows, Jandel Scientific, USA)

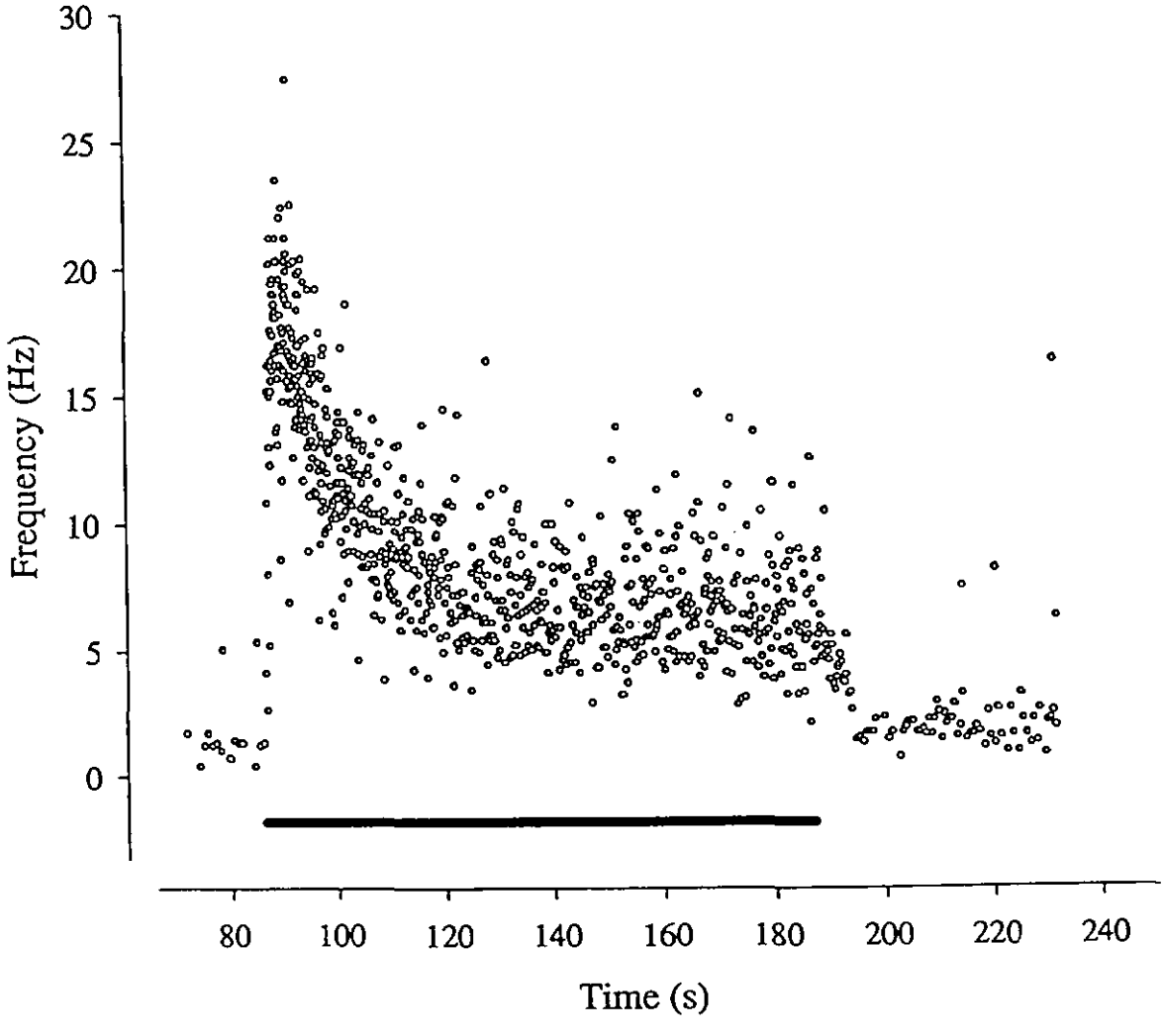

Figure 3: The instantaneous frequencies (Hz), as derived from the inter-spike interval, of a NH_3-excited receptor in a grooved-peg type-1 sensilla on the antenna of a *Triatoma* nymph in response to continuous stimulation for 100 s (black bar) with 3.4 ppb NH_3. The interspike interval of the responding unit is readily separated from other cells that fired, based on clear amplitude differences (*cf.* Fig. 1). After an initial phasic response, the unit continues to fire at a lower rate throughout the period of stimulation but at a level clearly higher than the spontaneous levels recorded before and after stimulation. This response is typical.

Results

Electrophysiology: Records of spontaneous activity from the base of grooved-peg sensilla on the antenna of *Triatoma* nymphs typically yields a spike train with three receptor cells, clearly distinguishable on the basis of different spike amplitudes: a small spike, an intermediate spike and a big amplitude (2-3.5 mV) spike, all firing at a spontaneous mean frequency of *ca.* 2-3 Hz (Fig. 1). The NH_3-excited receptor with the biggest amplitude spike was extremely sensitive to vapours over 1 g wetted faecal-papers and 5 µl stale rabbit-urine, causing an increase in the mean firing rate to *ca.* 25 Hz and 27 Hz, respectively. An attempt to trap the volatiles released from the wetted faecal-papers on a column of the porous polymer Porapak Q® (50-80 mesh, Millipore Corporation, USA; Steullet & Guerin, 1992b) was unsuccessful, but bubbling the vapour through a water column in a gas-wash flask proved an efficient trap for the stimulant. The smell of the trapped material to the human nose already indicated ammonia as the probable stimulus for the receptor. Half-log dose increments of NH_3, *i.e.*, 0.2, 0.6 and 2.0 µmole on filter paper in the stimulus cartridge cause increases in the firing rate of the responding receptor to 10 Hz, 37 Hz and 64 Hz, respectively (Fig. 1). The responses of such NH_3-excited receptors on the terminal flagellar segment of antennae of 15 different *Triatoma* nymphs indicate a dose-response function which is linear over the dose range 1.7 to 200 ppb in the stimulus-delivery air-stream, after which it plateaues (Fig. 2). The threshold of the responding receptors lay at *ca.* 0.1 µmole l^{-1} in air, equivalent to 1.7 ppb. This NH_3-receptor also responded to stimulation with methyl-, dimethyl-, ethyl-, and diethyl-amine, but at a threshold some 10 times higher than that of NH_3. No response was recorded from this receptor to any other synthetic chemicals tested. Neither did NH_3 or any of these products prove adequate to stimulate the other receptor cells within the grooved-peg sensillum housing the big-amplitude NH_3-excited receptor. When stimulated continuously for 100 s with 0.1-1.0 µmole NH_3 l^{-1} (2 to 17 ppb), the NH_3-excited receptor continued to fire tonically above the spontaneous level following a drop in frequency from an initial phasic response (Fig. 3). This sensillum is referred to hereafter as grooved-peg sensillum type-1 (GP1).

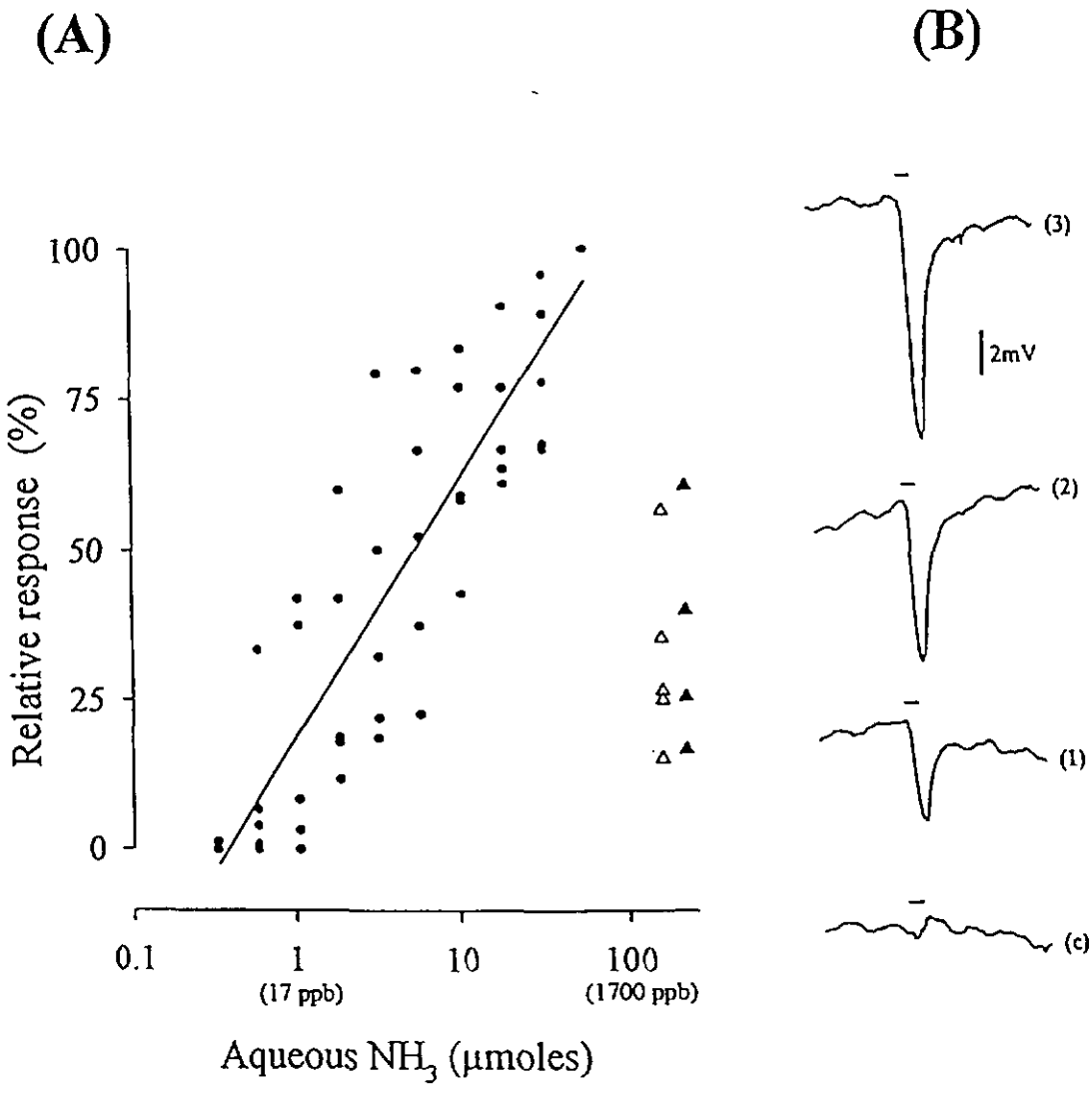

Figure 4: Electroantennogram responses of *Triatoma* nymphs to NH_3. **A)** Responses of antennae from 5 *Triatoma* nymphs to 1 s stimulation with NH_3 vapour released from increasing doses of aqueous solutions of NH_3 on filter paper. After subtracting the response due to the control (water only), the responses to the different doses of NH_3 were normalised with respect to the strongest response (100%) for each bug. The normalised responses of these bugs to 1.0 g wetted faecal-papers (triangles) and 5 µl of stale rabbit-urine applied to filter papers in stimulus cartridges (dark triangles) are indicated alongside. (The trend line fitted by linear regression is of first order using least squares method, y = a + bx; SigmaPlot for Windows, Jandel Scientific, USA). **B)** Electroantennogram traces from one bug to the control (c), (distilled water) and three doses of NH_3, *i.e.*, 51 ppb (1), 136 ppb (2) and 180 ppb (3) in the air reaching the preparation.

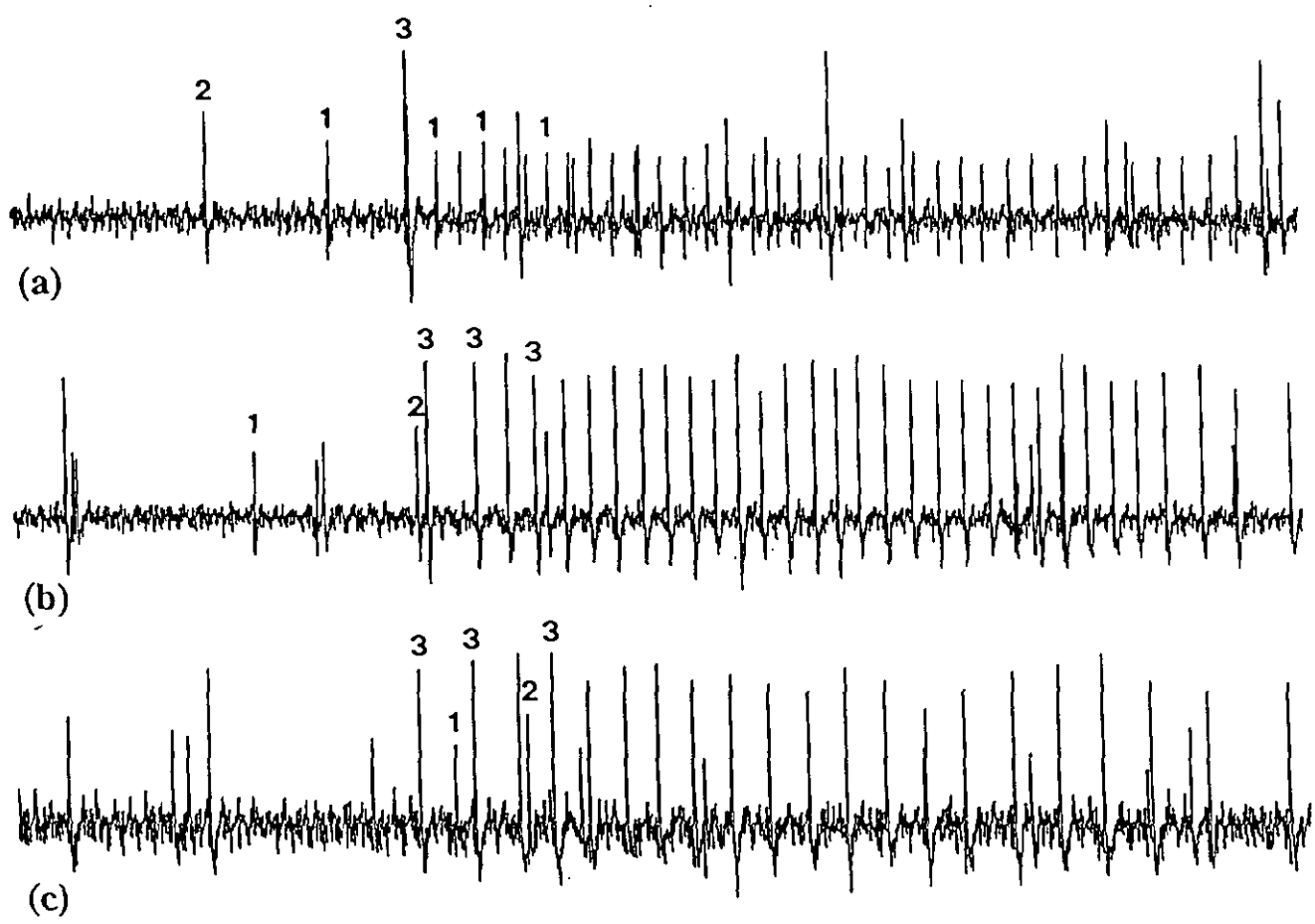

Figure 5: Responses of olfactory receptors within a grooved-peg type-2 sensilla (GP2) on the antenna of a *Triatoma* nymph to (**a**) 5.5 ppb NH_3 (**b**) a concentrate of host-odour extract and (**c**) 100 ng iso-butyric acid in the stimulus cartridges. Recordings of spontaneous activity from GP2-type sensilla typically reveals three receptors with small (**1**), intermediate (2) and big (3) amplitude spikes. Receptor **1** is most sensitive to NH_3 and less so to methyl-, dimethyl-, ethyl- and diethyl-amines. Receptor 3 is most sensitive to host odour and one of its constituents, iso-butyric acid. Horizontal bar indicates 1 s stimulation and the big spike in the trace (a) has an amplitude of *ca.* 2 mV.

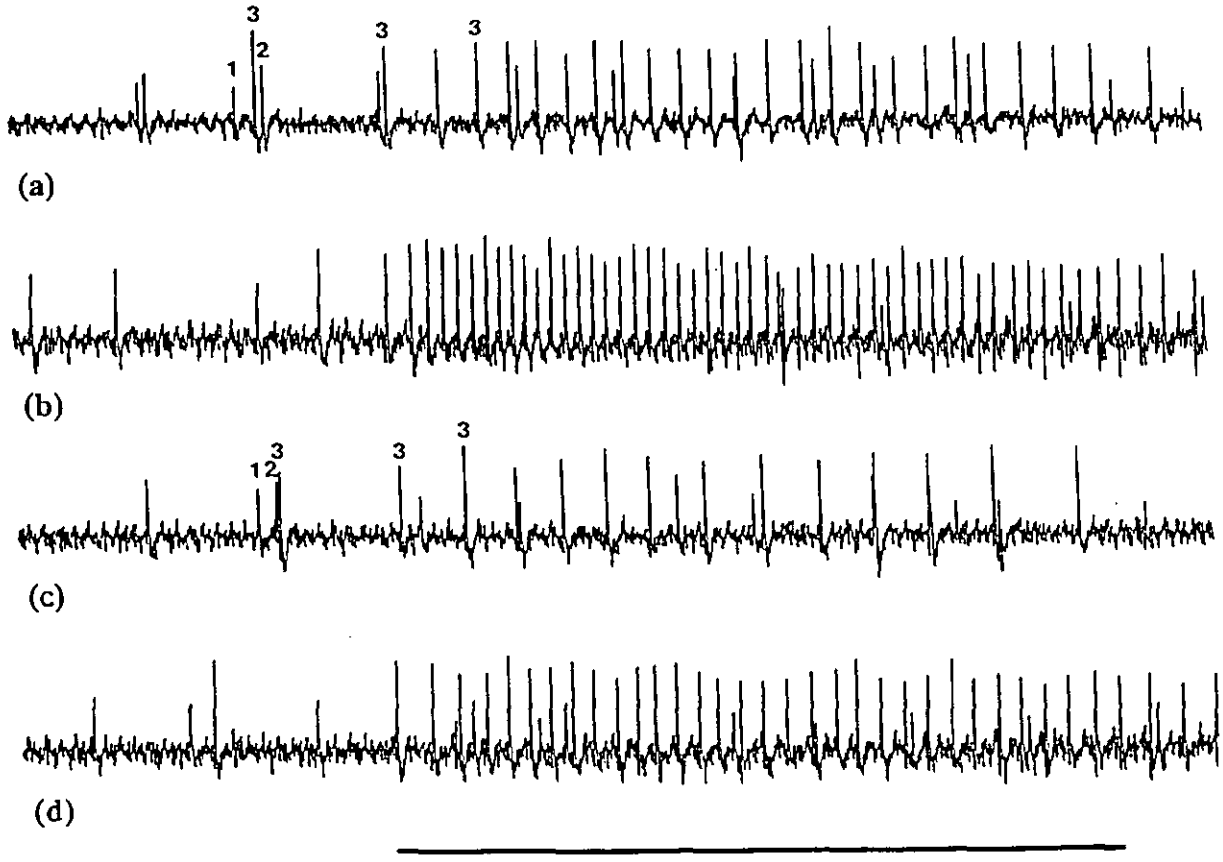

Figure 6: Responses of an olfactory receptor within a grooved-peg type-2 sensilla (GP2) on the antenna of a *Triatoma* nymph to (a) butyric acid, (b) iso-butyric acid, (c) valeric acid and (d) iso-valeric acid (each at 1.0 μg in the stimulus cartridge). This is the same preparation as in figure 5 with small (**1**) intermediate (2) and big (3) amplitude spikes. Receptor 3 is most sensitive to iso-butyric acid followed by iso-valeric acid, (b and d) and both are more active than their n-isomers (a and c). Such responses were reproducible. Receptor 1 is the one depicted in figure 5 responding to NH_3. The Horizontal bar indicates 1 s stimulation and the big spike in the trace (a) has an amplitude of *ca.* 2 mV.

The overall density of the NH_3-excited receptors within these sensilla on the antennae of *Triatoma* nymphs is sufficiently high to permit recordings of strong electroantennogram responses to NH_3. Mean depolarisations of *ca.* 4 mV were recorded for 3 μmole l^{-1} in air (51 ppb), rising to *ca.* 10 mV for 32 μmole l^{-1} (544 ppb). The response function of the responding antennae is linear over the dose range 5-544 ppb delivered in stimulus air-stream, similar to that recorded for the single receptors (Fig. 4A). The EAG response levels to vapours from 1.0 g wetted faecal-papers and 5 μl stale rabbit-urine on filter paper in the stimulus cartridges correspond to doses of between 2 and 6 μmole NH_3, or 34 and 102 ppb, respectively (Fig. 4B).

Though, all the grooved-peg sensilla basiconica on the antenna of *Triatoma* are morphologically similar, and the spontaneous activity indicated the presence of three receptor cells with different spike amplitudes in all such sensilla investigated, the receptors within different sensilla differ in their response characteristics. In the GP1-type described above, the receptor with the biggest spike amplitude responded to NH_3, certain amines, vapour from stale rabbit-urine and wetted faecal-papers. However, another sub-type (GP2) showed a higher mean spontaneous activity of the cells firing (*ca.* 4-5 Hz) and the cell with the biggest spike amplitude (2-3.5 mV) in this sensillum responded to iso-butyric acid (Fig. 5). This cell also responded to a host odour extract as trapped on Porapak Q® (Steullet & Guerin, 1992b; Steullet & Guerin 1994a; Steullet & Guerin, 1993) from animal rooms containing mice and rabbits (Fig. 5). Though less sensitive, this big-amplitude cell also responded to butyric acid, valeric acid, iso-valeric acid, pyruvic acid, lactic acid and vanillin (Fig. 6). This receptor did not respond to NH_3 but the cell with the smallest spike amplitude in this GP2-type sensillum did (Fig. 5). This cell also responded strongly to vapours from stale rabbit-urine, wetted faecal-papers and to methyl-, dimethyl-, ethyl-, and diethyl-amine at a lower sensitivity, a response profile similar to the big amplitude receptor in the GP1 sensillum described above. We made recordings from at least 10 such sensilla on different *Triatoma* nymphs.

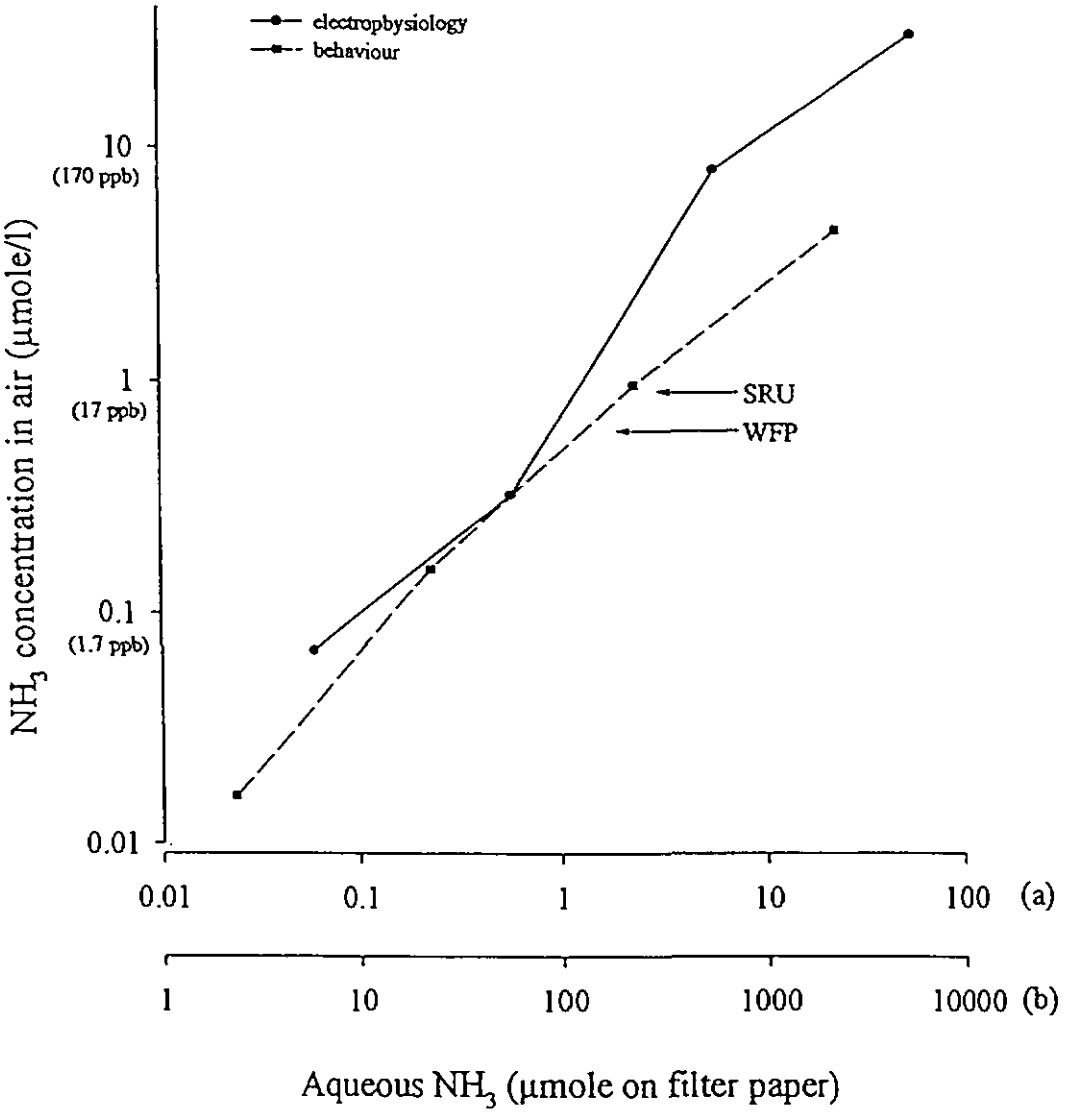

Figure 7: Plots of the release rate of NH_3 into the stimulus-air (µmole l^{-1}) reaching sensory organs in electrophysiology set-up and nymphs on the servosphere from different amounts of aqueous NH_3 (µmole) applied to filter paper in 5 ml stimulus cartridges and 1 l gas-wash bottles, respectively. The release rate was measured over 4 increasing log doses for both the electrophysiology and behavioural experiments using the Tecator® flow-injection and gas-diffusion technique (*cf.* Materials & Methods). The upper abscissa (a) represents amounts of NH_3 in the stimulus cartridges employed for 1 s electrophysiological stimulations and the lower one (b) amounts in the gas-wash bottles employed for 2 min stimulations on the servosphere. The amount of NH_3 released in ppb is also indicated in parentheses on the ordinate. Each point on the plots represents the mean of 2-3 measurements. For comparative purposes, the amounts of NH_3 released from 60 g wetted faecal papers (WFP) and 200 ml stale rabbit-urine (SRU) in 1 l gas-wash bottles are also marked on the graph.

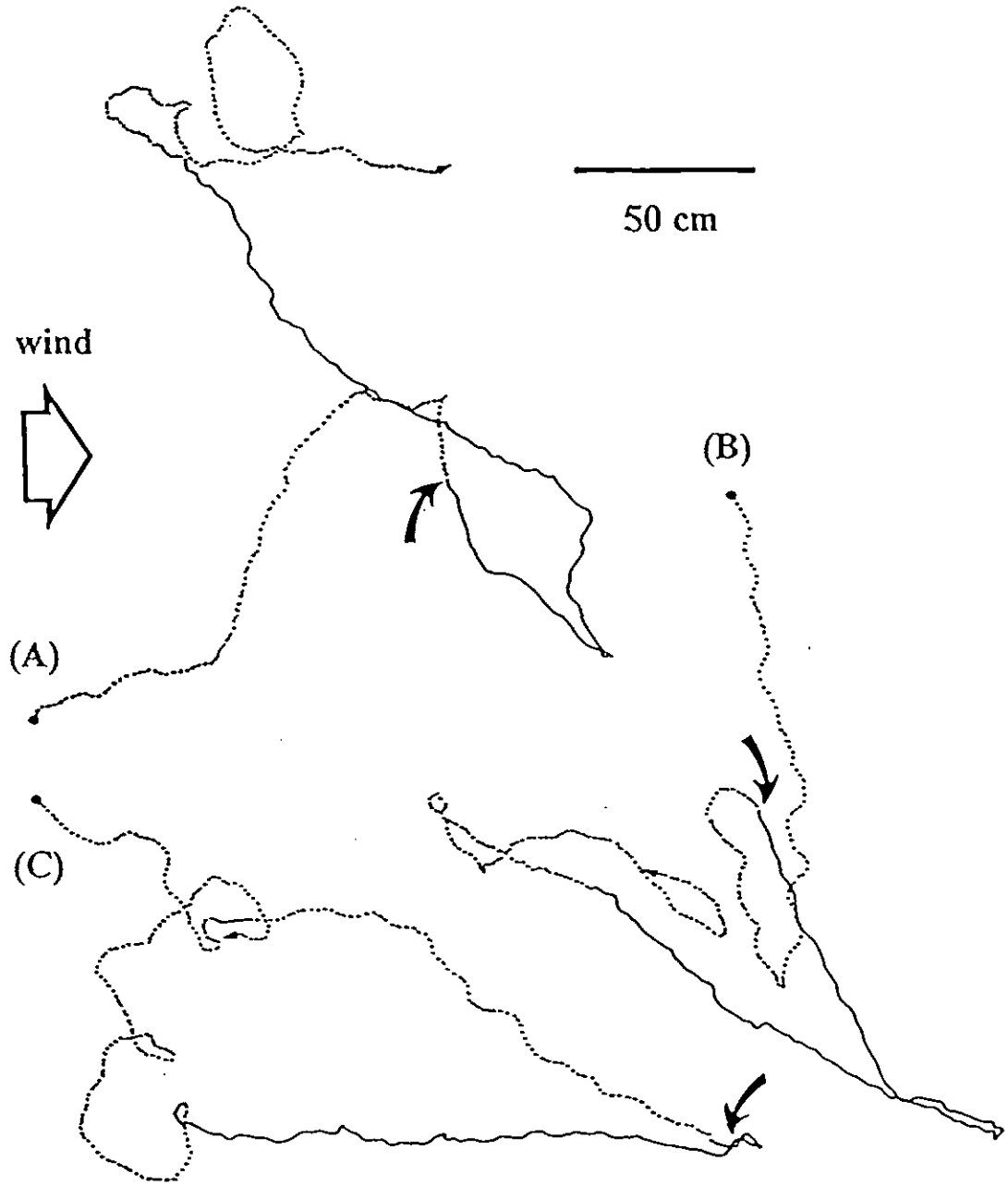

Figure 8: Tracks made by three *Triatoma* nymphs on the servosphere in response to different doses of NH_3: 0.3 ppb (A), 3 ppb (B) and 17 ppb (C) in the air-stream (0.1 ms^{-1}) reaching the bug. Records consisted of 2 min consecutive control, test and end-control periods. The tracks started (•) with the bugs walking mostly downwind in a pure air-stream (dotted line). After NH_3 was added (bold arrows), the bugs turned to walk upwind (almost immediately at the highest dose, and with a delay at the lower doses). After cessation of NH_3 delivery into the air-stream (dotted line) the bugs frequently continued their upwind course. Stops made by the bugs in their walks are not detailed in these tracks. Open arrow at top left indicates wind direction.

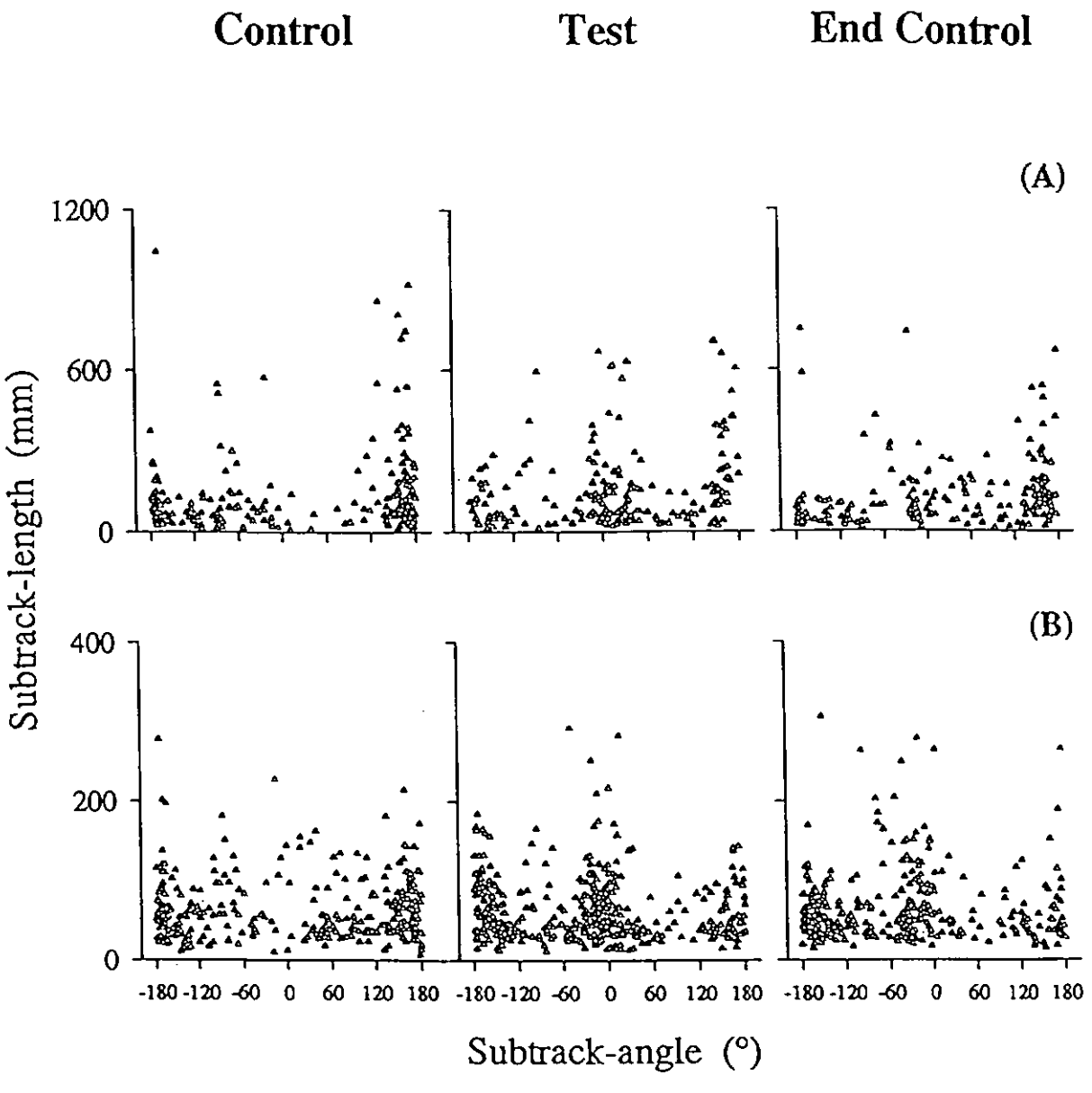

Figure 9: Scatter graphs of subtrack-lengths plotted against subtrack-angles for successive 2 min control, test and end-control periods of experiments to determine *Triatoma* nymphs' responses to **A**, 60 g wetted faecal-papers in a gas-wash bottle (n=11) and to **B**, 17 ppb NH_3 vapours released from aqueous NH_3 on filter paper in a gas-wash bottle · (n=12). A subtrack-angle is the circular mean (mean Φ, Batschelet, 1981) of the instantaneous course angles calculated for each subtrack (a walking bout of a second or more, *cf.* Taneja & Guerin, 1995). Bugs usually walked downwind during the initial controls in clean air. The proportion of subtracks in the upwind direction, *i.e.*, a cone of 60° on either side of due upwind (0°) is significantly higher ($p<0.01$) for the test periods for both treatments. Walks in the upwind direction are also significantly higher ($p<0.01$) during the end-control following delivery of the NH_3 vapour. The ordinate scales are different as bugs tested in **B** walked less.

Identification and quantification of ammonia and carbon dioxide: The colorimetric Aquamerck® test-kit showed that the vapour over wetted faecal-papers and stale rabbit-urine contained large quantities of ammonia. After this initial identification, the more specific and sensitive flow-injection analysis and gas diffusion technique indicated that 60 g of wetted faecal-papers released *ca.* 256 ppb NH_3 whereas 200 ml stale rabbit-urine released 394 ppb. The amount of NH_3 released from 60 g dry faecal-papers, by contrast, was extremely low at *ca.* 0.12 µmole l^{-1} or 2 ppb. The amount of NH_3 released from clean dry and wetted filter papers was estimated at *ca.* 0.3 ppb. This analytical method for NH_3 also permitted us to quantify the amount of ammonia in the stimulus-air reaching the bugs in both the electrophysiology and behaviour set-ups from the doses of aqueous NH_3 employed. The release rate was linearly related to the amounts of aqueous NH_3 applied with a slope of *ca.* 1 (Fig. 7). Almost all the NH_3 released was trapped in the first flask of the in-series water traps (<1% of the amount trapped in the first measured in the second from the 235 µmole NH_3 source and wetted faecal-papers). The release rate of NH_3 in the first and last 30 s periods of 2 min flushing with air over the 235 µmole and 24 µmole sources of aqueous NH_3 and wetted faecal-papers in the gas-wash bottles was the same, indicating a constant release rate of NH_3 over two minute period of delivery to the bugs on the servosphere from such sources.

Removal of any bacteria from the wetted faecal-paper wash did not affect NH_3 liberation from the dried and re-wetted filtrate. In the enzyme assay, addition of urea or uric acid to the ultrafiltration residue of the wetted faecal-paper wash did not restore NH_3 release. Analysis of the CO_2 content of the vapours directly released from 60 g wetted faecal-papers and 200 ml stale rabbit-urine indicated *ca.* 0.25% CO_2 from the 1 l gas-wash bottles. By contrast, the amount of CO_2 released from similarly held 60 g dry faecal-papers was not above ambient (0.04%).

Behaviour: Records of walks performed by *Triatoma* nymphs on the servosphere were similar to those described earlier for *Rhodnius* and *Triatoma* adults (Taneja & Guerin, 1995) in that the bugs walked in straight bouts (subtracks) interrupted by

stops, or periods at relatively low speeds, all of variable duration. The nymphs walked invariably downwind during the initial control period. In response to biological substrates releasing NH_3 or to stimulation with NH_3 vapour alone, the nymphs responded by turning sharply upwind (Fig. 8). A shift in distribution of the subtrack-angles was recorded from the nymphs from essentially downwind runs before stimulation to upwind runs following stimulus delivery (Fig. 9). This resulted in an increase in the % upwind displacement by *Triatoma* nymphs during the 2 min period of stimulation in response to all the NH_3-releasing biological and artificial substrates tested; this increase was significant for wetted faecal-papers and the two higher doses of NH_3 from the synthetic source ($p<0.01$; Table 1). The bugs also continued to respond with upwind walks after cessation of delivery of these vapours as indicated by a higher % upwind displacement during the end-control period than in control, but the significance of the response was again dose dependent (Table 1).

Analysis of the behavioural data shows a latency in the response of the *Triatoma* nymphs depending on the stimulus dose. Comparing the % upwind displacement during the first and second minute segments of test periods with the corresponding segments of controls revealed that the % upwind displacement was significantly higher during the first minute of stimulation with wetted faecal-papers (*ca.* 10 ppb NH_3 released in the stimulus air) and the highest dose of NH_3 (*ca.* 17 ppb) delivered to the servosphere (Table 2, Fig. 10 A) . The response was delayed at the lower doses of NH_3, only becoming significant ($p<0.01$) for the 3 ppb dose in the second minute of stimulation (Table 2, Fig. 10 B). This response profile was mirrored in the end-control period where upwind displacement continued throughout for strong stimuli, but was only manifested during the first minute after stimulus-off for weaker stimuli (Table 2, Fig. 10). The analysis of speed showed that the bugs walked faster in the first minute of the test period than in the corresponding minute of control ($p<0.05$), but only while responding to the two higher doses of NH_3.

The behavioural responses of the bugs to the intermediate dose (3 ppb) of NH_3 at 35% RH demonstrated that NH_3 perception was not interfered with at this low RH as the bugs showed the typical response of stopping, turning and walking upwind to follow the odour. The percent upwind displacement was significantly higher ($p<0.05$) during both 2 min test and end-control periods than the preceding control

period, as with the same dose of NH_3 at 90% RH (above). But at 35% RH there was no latency in the response as compared to the same dose tested at 90% RH (Fig. 10 B), i.e., the response was stronger in the first minute of the test period (Fig. 11).

Table 1: Increase in % upwind displacement by *Triatoma* nymphs in response to stimulation with odours from triatomine faecal-papers, and different amounts of NH_3 vapour. Each record consisted of 2 min consecutive control, test and end-control periods. The increase in upwind displacement, defined here as % of the total displacement in a cone of 60° on either side of due upwind (0°), in the test and end-control periods for each bug is with respect to that recorded for the initial control period. Values presented are medians of the increases, 'n' is the number of bugs tested for each treatment, and levels of significance were established with the two-tailed Wilcoxon signed rank test for paired replicates: ** $p< 0.01$, * $p< 0.05$, n.s. not significant.

	Dry faecal-papers (n=10)	Wetted faecal-papers (n=11)	NH_3 (17 ppb) (n=12)	NH_3 (3 ppb) (n=10)	NH_3 (0.3 ppb) (n=10)
Test over control	10.37 n.s.	42.12 **	37.16 **	25.29 **	17.62 n.s.
End control over control	3.34 n.s.	22.41 *	28.32 **	15.90 *	7.95 n.s.

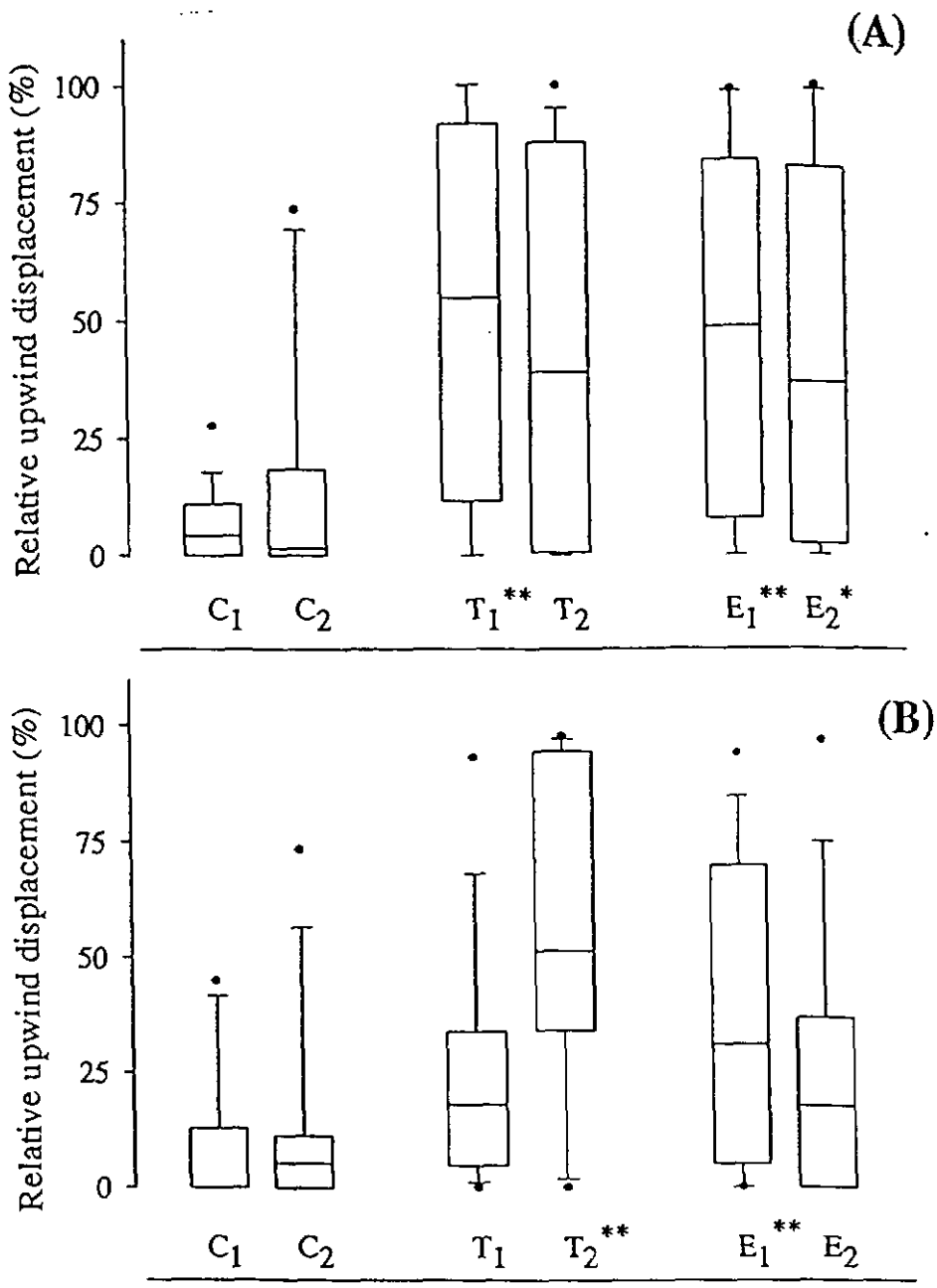

Figure 10: Data demonstrating dose dependency in the latency of the response of *Triatoma* to NH_3 vapour. Box plots of distance walked upwind (60° either side of due upwind) by *Triatoma* nymphs as a % of the total distance walked for different periods of experiments with NH_3 vapour in the stimulus air: (A) 17 ppb (n=12), and (B) 3 ppb (n=10). Each record consisted of 2 min consecutive control, test and end-control periods. To demonstrate latency, upwind displacement is plotted separately here for the first- and second-minute segments of each period, *i.e.*, C_1 and C_2 control, T_1 and T_2 test, and E_1 and E_2 end-control periods. The asterisk after a segment letter indicates that the % upwind displacement was significantly higher (** $p<0.01$ and * $p<0.05$) in that minute than in the corresponding minute of the control (two-tailed Wilcoxon signed rank test for paired replicates). The lines within a box mark the median, the lower and upper boundaries of a box indicate 25th and 75th percentiles, bars below and above a box indicate the 10th and 90th percentiles, and points represent data beyond these limits.

Table 2: Analysis of the change in the percent upwind displacement in a cone of 60° on either side of due upwind (0°) by *Triatoma* nymphs in response to stimulation with odours from triatomine faecal-papers, and different amounts of NH_3 with view to detecting any latencies in responses. Each record consisted of 2 min consecutive control, test and end-control periods. The change in % upwind displacement in the first- and second-minute segments T_1 and T_2 of test, and E_1 and E_2 of end-control periods for each bug is expressed as the difference with that recorded for the corresponding segments C_1 and C_2 of initial control period. Values presented are medians of the changes, 'n' is the number of bugs tested for each treatment, and levels of significance for each treatment were established with the two-tailed Wilcoxon signed rank test for paired replicates: ** $p< 0.01$, * $p< 0.05$, n.s. not significant.

	Dry faecal-papers (n=10)	Wetted faecal-papers (n=11)	NH_3 (17 ppb) (n=12)	NH_3 (3 ppb) (n=10)	NH_3 (0.3 ppb) (n=10)
Test period 1 versus Control period 1	10.16 n.s.	32.22 *	52.6 **	17.98 n.s.	4.8 n.s.
Test period 2 versus Control period 2	8.90 n.s.	6.83 n.s.	13.2 n.s.	30.85 **	9.8 n.s.
End control period 1 versus Control period 1	0.73 n.s.	25.22 *	41.15 **	26.23 **	4.66 n.s.
End control period 2 versus Control period 2	-0.51 n.s.	0 n.s.	14.56 *	-0.36 n.s.	0.57 n.s.

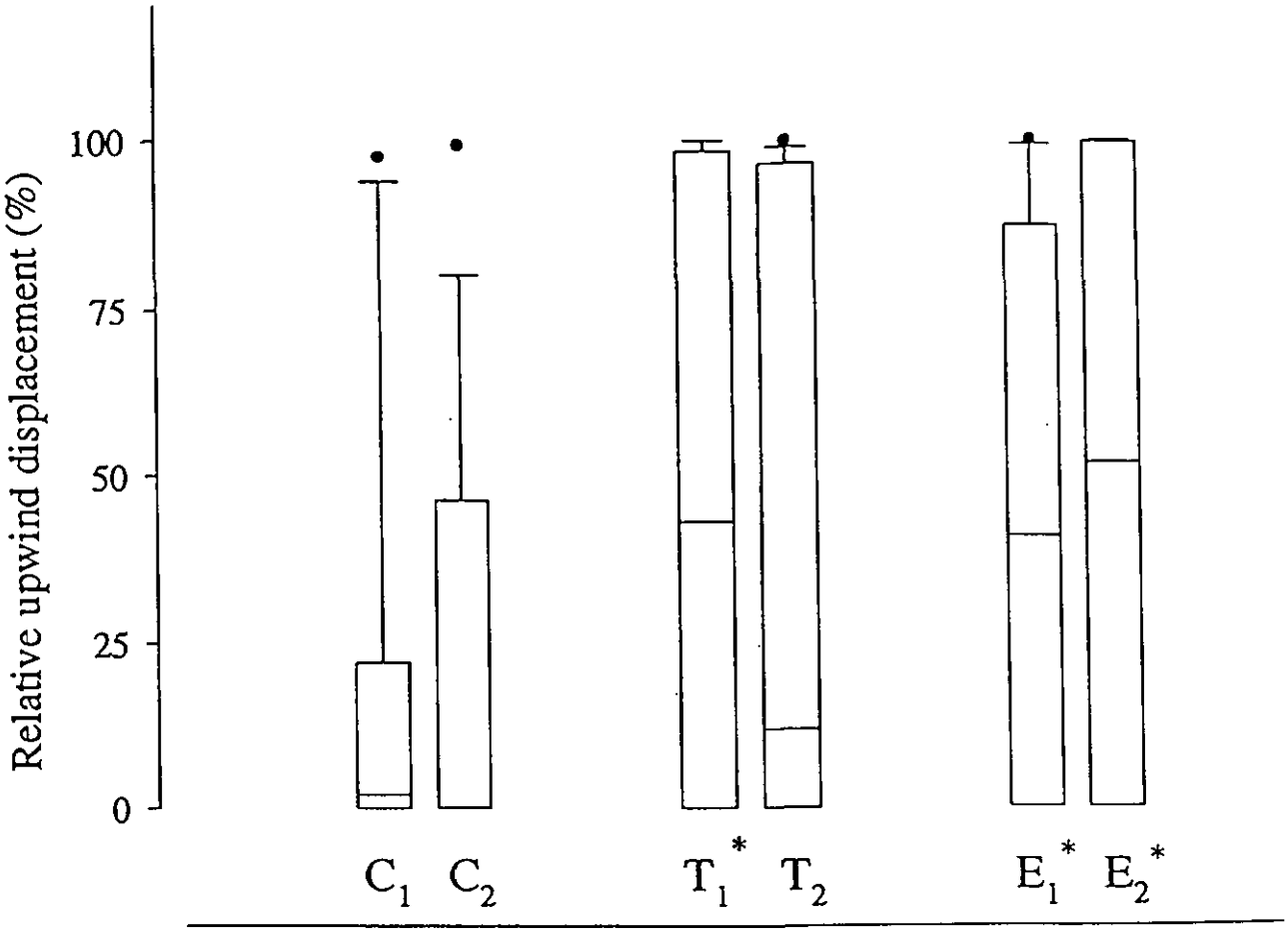

Figure 11: Data demonstrating the attraction of 3 ppb NH_3 to *Triatoma* nymphs when presented in an air-stream at 35% RH. Box plots are presented of the distance walked (60° either side of due upwind) as a % of the total distance walked by 14 bugs for the first- and second-minute segments of each period ,i.e., C1 and C2 control, T1 and T2 test, and E1 and E2 end-control segments. The asterisk indicates that the % upwind displacement was significantly higher ($p<0.05$) in that segment than in the corresponding segment of the control (two-tailed Wilcoxon signed rank test for paired replicates). For details of data presentation see figure 10. Whereas the overall attractivity of 3 ppb NH_3 is confirmed at 35% RH, the latency of the response changes as the bugs respond to the NH_3 vapour at the reduced RH of 35% from the

outset of the 2 min test period (compare with the response to 3 ppb at 90% RH, Fig. 10 B), and the end-control response is also stronger.

Discussion

The data presented here clearly demonstrates *Triatoma*'s ability to perceive and differentiate between different amounts of NH_3 released into the air from attractive biological substrates via specialised olfactory receptors on the antennae. *Triatoma* responds anemotactically to NH_3 vapours presented alone in an air-stream. These findings have a number of significant aspects. Both the sensory and behavioural thresholds at *ca.* 2 ppb NH_3 in the stimulus-delivery air represents a very high sensitivity of the bugs to this product. This sensitivity could already account for the increase in upwind displacement which was at first recorded for 60 g dry faecal-papers from which *ca.* 0.1 ppb NH_3 reached the nymphs on the servosphere. The increase in upwind displacement recorded here for dry faecal-papers, though persistent, does not indicate significance due to the low number of replicates. To underline the correspondence between the sensory and behavioural data, it is shown here that the strength of *Triatoma*'s sensory response and attraction to sources of NH_3 depends on the dose of NH_3 delivered to the bug.

Wetting 60 g triatomine faecal-papers causes a dramatic increase in the amount of NH_3 released by a factor of 100 within a few hours. Some 10 ppb NH_3 reached the bugs from this substrate. Vapours from just 1 g of such wetted faecal-papers from either nymphs or adults of *T. infestans* and *R. prolixus* in the stimulus cartridge is sufficient to elicit a clear response from the NH_3-receptor in GP1 sensilla despite a dilution factor of *ca.* 42 in the stimulus delivery system. The responses to quantities of NH_3 some 15 times higher (150 ppb), as delivered by the stimulus air from aqueous NH_3, are still well within the linear part of the response function of both the single receptors and the electroantennogram. Doses of NH_3 over a similar range injected into the air-stream passing over the bugs on the servosphere cause attraction when delivered from either 60 g wetted faecal-papers or 235 µmole aqueous NH_3 in gas-wash bottles. Both the electrophysiological and behavioural responses remain linear over two log doses, indicating correspondence between the

sensory and behavioural responses of *Triatoma* to this volatile. CO_2 is attractive to triatomines at levels of *ca.* 0.5% in air and higher (Taneja & Guerin, 1995), but the quantity (0.25%) of this product released simultaneously with NH_3 from the wetted faecal papers is not sufficient to have affected the attraction observed as it was diluted by a factor of 24 to near ambient levels in the stimulus delivery system to the servosphere.

The behavioural response of these bugs to stimulation with NH_3 vapours from faecal-papers and to NH_3 alone on the servosphere is similar to that described earlier for *Rhodnius* and *Triatoma* adults responding to host volatiles (Taneja & Guerin, 1995). Onset of stimulus changed the negative anemotactic behaviour of the *Triatoma* nymphs recorded here in air alone into a predominantly positive one: Upwind turning and walking was always preceded by a stop and active movements of the antennae. Since three log doses were tested in the behaviour tests, it allowed us to uncover a dose-dependent behavioural latency, *i.e.*, the lower the dose delivered, the longer it took the bugs to respond. More bugs respond and the % upwind displacement is higher in the first minute of stimulus delivery than in the second with 60 g wetted faecal-papers and at the highest dose of NH_3 (17 ppb) delivered to the servosphere, where they also walk faster. At the intermediate dose (3 ppb) of NH_3 this effect was only recorded during the second minute of stimulation on the servosphere, though a response was already indicated by an increase in speed during the first minute. One might argue that a behavioural response after such prolonged exposure to the stimulus is surprising. However, we have already documented the requirement to expose some bugs for a long period to host odours before a behavioural response can be induced (Taneja & Guerin, 1995), hence the long stimulation period employed. Moreover, single-unit electrophysiology experiments where single sensilla were exposed for 100 s to a range of NH_3 doses (2 to 17 ppb), the range found to be attractive in behavioural tests, indicate that the NH_3-excited receptors do not adapt completely. A slow adapting NH_3-sensitive neuron is also reported from the tick *Rhipicephalus sanguineus* (Haggart & Davis, 1980). At the lower dose of *ca.* 3.0 ppb NH_3 delivered to the servosphere, the bugs do show stops during which they swing their antennae upwind from the outset of the test period, but they only undertake upwind displacement after prolonged exposure to the stimulus.

Apparently there are some time-based sensory phenomena underlying the recruitment of a sufficient number of receptors peripherally or some CNS mechanisms which are invoked at low NH_3 doses in the air before upwind displacement can be induced. In any event, the latency observed in the behavioural response at low doses of NH_3 can be considered as part of the overall dose dependency of the response of *Triatoma* to NH_3.

The end-control response to NH_3 is also similar to that recorded earlier for *Triatoma* responding to host volatiles, *i.e.*, they continue to walk upwind during the end-control period after adequate stimulation (Taneja & Guerin, 1995). In experiments where bugs respond faster to the presence of the stimulus (ammonia from wetted faecal-papers or the highest dose of aqueous NH_3), pursuance of the source already wanes during the test period. Interestingly, turning the stimulus off caused such bugs to turn abruptly upwind in the end-control and upwind displacement continued for some time. At the intermediate dose of aqueous NH_3, this end-control response is still present but proportionally weaker, underlining, once again, the dose dependency of *Triatoma*'s response to NH_3.

NH_3-sensitive receptor on the antennae of *T. infestans* has been reported earlier by Bernard (1974) who described two types of olfactory sensilla on antenna of *Triatoma*. Both are wall-pore sensilla, present on both segments of the adult flagellum, but only on the terminal flagellar segment of nymphs. One type, a thin-walled sensilla basiconica (type E; Bernard, 1974) is *ca.* 25 µm long x 2 µm dia. The other type, a double-walled sensilla basiconica (type F; Bernard, 1974) is the basiconic grooved-peg sensilla from which recordings are reported in this paper. These are short 6-10 µm long 1 µm dia hairs with three dendrites (Bernard, 1974). A similar type of sensilla occurs on the antennae of another heteropteran bug *Cimex lectularius*, the bed-bug (Steinbrecht & Müller, 1976), and on other haematophagous arthropods such as mosquitoes (McIver, 1982; Cribb & Jones, 1995) and on the Haller's organ of ticks (Hess & Vlimant, 1986).

Not all grooved-peg sensilla on the antennae of *Triatoma* nymphs responded similarly to the stimuli tested here, indicating a clear-cut difference between two sub-populations. The first sub-type, GP1, with three spontaneously firing cells has a NH_3-excited receptor with a big amplitude spike. This receptor is also sensitive to

other amines but at a sensitivity 10 times lower than that to NH_3. The second sub-type of grooved-peg sensilla from which recordings were made, the GP2-type, also has three spontaneously firing cells but here the NH_3-excited receptor has a small amplitude spike. The cell with the biggest amplitude spike in this sensillum responds to short-chain fatty-acids and to host odour extracts. Bernard (1974) has also reported differences in response profile for the same grooved-peg sensilla. He obtained responses to synthetics such as pyruvic acid, butyric acid, lactic acid and ammonia from one grooved-peg type sensilla in his studies (Ff1), most likely the same as the GP2-type in our study, as he also described activation of two different receptors by ammonia and butyric acid. On this basis Bernard (1974) did differentiate a functionally separate population of grooved-peg type sensilla (Ff2) on the antennae of *Triatoma*. Though he did not obtain a response to any volatiles tested, he did report the presence of a hygroreceptor in the Ff2 type. Similar inhomogeneity in the response profiles is also recorded for two functionally distinct classes of fatty-acid receptors in grooved-peg sensilla of mosquitoes (Bowen, 1995). It is of interest that grooved-peg double-walled sensilla which invariably house olfactory receptors for n-acids and amines in arthropods (Altner *et al.*, 1977) is confirmed here for *Triatoma*. NH_3-sensitive neurons in similar grooved-peg sensilla have also been reported from other blood-sucking arthropods such as ticks (Steullet & Guerin, 1994b; Haggart & Davis, 1980;) and mosquitoes (Kellogg, 1970), and fatty-acid sensitive receptor cells have been reported from grooved-peg sensilla of ticks (Steullet & Guerin, 1994b), mosquitoes (Davis, 1977) and *Periplaneta* (Altner *et al.*, 1977; Sass, 1978). The fact that it is possible to record strong EAGs to NH_3 in a dose dependent manner from *Triatoma*'s antennae is evidence for the presence of populations of depolarised receptors.

Ammonia has been identified in effluvia from vertebrates such as in saliva, eccrine sweat, and urine (Albone, 1984; Lentner, 1981). Attraction and aggregation responses to NH_3 sources have already been documented for a range of both haematophagous and non-haematophagous arthropods such as horse-flies *Hybomitra lasiophthalma* (Hribar *et al.*, 1992), the human body louse *Pediculus humanus* (Mumcuoglu *et al.*, 1986), the cockroach *Blatella germanica* (Sakuma & Fukami, 1991), the flour mite *Acarus siro* (Levinson *et al.*, 1991a), and fruit flies

(Bateman & Morton, 1981; Mazor *et al.*, 1987; Robacker & Warfield, 1993). The reliable source of NH_3 from vertebrates employed in this study is stale rabbit-urine. NH_3 will likewise be released in large quantities from the excretory products of animals in the usual peridomestic niche occupied by the triatomines, *i.e.*, chicken coops and animal stalls. Attractants for tsetse flies have already been identified in vertebrate urine (Hassanali *et al.*, 1986, Owaga *et al.*, 1988; Saini, 1990).

The role of a species' own excretory products serving as an aggregation cue has been documented for haematophagous arthropods such as the human body louse (Wigglesworth, 1941; Mumcuoglu *et al.*, 1986), soft ticks (Leahy *et al.*, 1973; Otieno *et al.*, 1985; Dusbábek *et al.*, 1991), and hard ticks (Leahy *et al.*, 1983; Otieno *et al.*, 1985), and the honey-bee ectoparasite *Varroa jacobsoni* (Donzé & Guerin, 1994) as well as for the flour mite *Acarus siro* (Levinson *et al.*, 1991b), the cockroach (Roth & Cohen, 1973; Sakuma & Fukami, 1991), and boll weevils (Mitchell *et al.*, 1975). A series of nitrogen-containing aromatic volatiles isolated in vapours over faecal-papers from *Triatoma* spp. proved unattractive (Cruz-López & Morgan, 1995). Although, these authors did not isolate ammonia from the faeces, they did indicate that a polar extract of the faeces was attractive.

Uric acid is the main excretory product of triatomines as is the case for most terrestrial insects. Free ammonia is toxic and large quantities of water are required for its elimination. Therefore, ammonia is often an important medium for nitrogen excretion in aquatic arthropods but is seldom important as an excretory substance in terrestrial animals (Wigglesworth, 1972; Cochran, 1985). Though ammonia was reported by Wigglesworth (1931) to be absent from the excretory products of *R. prolixus* analysed directly at different stages of excretion, Harington (1961) has reported presence of ammonia in the excreta of *R. prolixus* and *T. infestans* collected from filter papers in the jars containing fifth instar and adult bugs. This indicates that ammonia is not a direct product of excretion but appears in the faeces, possibly as a breakdown product, at a later stage outside the body of the animal. Removal of any bacteria from aqueous washes of the wetted faecal-papers used in our study had no effect on the subsequent release of NH_3 from the filtrate and the possibility that NH_3 release is due to the enzymatic breakdown of urea or uric acid is unlikely in view of the findings presented here. The concurrent release of CO_2 and stability of NH_3

release rate from the wetted faecal-papers over a long time may indicate breakdown of an excretory product. Preliminary investigation of the filtrate has revealed the presence of diverse ammonium salts on the faecal-papers (unpublished data). Salts such as ammonium carbonate, ammonium chloride, ammonium sulphate, ammonium nitrate and ammonium phosphate are known to cause aggregation of *P. humanus* (Mumcuoglu *et al.*, 1986) and the soft tick *Argas persicus* (Otieno *et al.*, 1985).

Triatomines spend most of their life aggregated in refuges, leaving only when hungry to search for a blood-meal (Lent & Wygodzinsky, 1979). Living in a refuge is highly advantageous to the bugs as it provides protection, enhances mate finding and thus increases reproductive success. However, there are other advantages. A special case documented for triatomines is the transfer of symbionts via coprophagy and cannibalism (Ryckman, 1951; Schaub *et al.*, 1989). These symbionts, which are essential for triatomine development, are mainly transferred via coprophagy on wet faeces (Schaub *et al.*, 1989). Juvenile stages of fleas have also been reported to perform coprophagy (Lewis, 1995): The larvae of these insects do not parasitise hosts but fulfill their blood requirement by feeding on the faeces of the blood-sucking adults which in the case of fleas consists of remnants of digested blood of previous meals, and droplets of virtually undigested blood. The results presented here show how triatomines are attracted to their own faeces. Considering the importance of the refuge, it is therefore not surprising to find that they are marked with faeces at the entrance and that such marked refuges are preferred by the bugs (Lorenzo & Lazzari, 1996). When defecating, the bugs show a stereotypic behaviour: after emerging from the refuge, the bugs turn 180°, walk backwards and defecate down the wall (Lorenzo & Lazzari, 1996). Defecating outside the refuge is all the more significant since the faeces also serves as the mode of transmission for triatomine pathogens (Schaub *et al.*, 1989; Schaub *et al.*, 1992). Defecating outside would therefore serve the dual purpose of keeping the refuge as clean as possible while simultaneously permitting re-wetted faeces to serve as a guide to these night active bugs returning from a blood-meal or to guide those bugs newly seeking a viable refuge. Aggregation responses and attraction of triatomines to their own faeces has already been documented (Lorenzo Figueiras *et al.*, 1994). These authors also demonstrated a daily rhythm in the aggregation/dispersion behaviour of *T. infestans* with aggregation

occurring at dawn and dispersal at dusk when bugs leave to seek hosts. *Triatoma* establishes refuges in dry environments (Roca & Lazzari, 1994), so the faster response to NH_3 at 35% RH than at 90% RH reported here is interesting in this context, and may serve to underline the use of NH_3 in refuge selection at low humidities. We have shown here that water is the limiting factor for NH_3 release from faeces but fresh defecation on old faecal-markings would reduce this constraint. This would render refuges occupied by regularly feeding bugs more apparent by the presence of NH_3 vapour.

In conclusion, it could be that NH_3 represents the parsimonious use of an infochemical in *Triatoma*, having a different behavioural role depending on the physiological state of the bugs, *i.e.*, affecting host attraction in the company of other stimuli when hungry and refuge selection when replete.

This study has established that triatomines are attracted to host odours as well as to vapours from their own faeces, underlining the sensory and behavioural adaptations of this parasite for its hosts and its own excretory products. Since the study has concentrated on the responses of triatomines to volatiles, it shows that olfaction is an important sensory modality for these bugs. CO_2, visual stimuli and heat have often been suggested as stimuli in the attraction of haematophagous arthropods (Sutcliffe, 1987; Waladde & Rice, 1982; Núñez, 1987). Although the role of CO_2 on its own as an activation stimulus is well established, clear-cut evidence for orientation to it remains controversial (Sutcliffe, 1987; Gillies, 1980; Takken, 1991). The behavioural evidence presented here for oriented responses by triatomines to CO_2 on its own indicates that these bugs are equipped with sensory organs and a CNS system which can process the sensory input to result in an oriented motor response. A CO_2-sensitive receptor still remains to be discovered in triatomines. A much stronger attraction was recorded from adult *Triatoma* and *Rhodnius* to whole animal odours and to stale host urine, both of which contain CO_2. However the CO_2 content of urine was near ambient after a dilution by a factor of *ca* 24 in the air-stream reaching the bug. This indicates that there are other volatiles involved in attraction of these bugs to their vertebrate hosts. Clearly these volatiles need to be isolated and identified in order to understand fully the sensory and behavioural basis of host finding in triatomines. In this context the single-unit responses recorded from a receptor in the GP2-type sensillum is very pertinent. This receptor also responds to short-chain fatty acids and in particularly to iso-butyric and iso-valeric acids. Fatty acids have recently been implicated in the attraction of *Anopheles gambiae* to Limburger cheese (Knols & De Jong, 1996), the odour of which is similar to that of human feet, the chosen site for feeding by these mosquitoes on man (De Jong & Knols, 1995).

Perception of NH_3 by receptors in two types of grooved-peg sensilla on the antenna of *Triatoma* nymphs is very likely to contribute to the attraction of these nymphs to stale host urine. In addition to these NH_3-excited receptors in GP1 and GP2 sensilla described in Results 2, I twice observed inhibition of a large amplitude spike in what must constitute another sub-population of these grooved-peg sensilla (Table 1). This observation has recently been confirmed in our laboratory

(Guerenstein, personal communication). CO_2-excited and CO_2-inhibited receptors have also been found in the capsule of Haller's organ in ticks (Steullet & Guerin, 1992a). This same NH_3-inhibited receptor was excited by formic acid, acetic acid, propionic acid, and propyl-amine. None of these products nor the other synthetic or natural volatiles tested excited the receptors with intermediate or small amplitude spikes in these two sensilla. Another type of grooved-peg sensilla encountered was similar to the GP2-type where the receptor with the small amplitude spike responded to ammonia, methyl-, dimethyl-, ethyl-, and diethyl-amines, stale rabbit-urine and wetted faecal-papers, but there was no response from the receptor with the largest spike amplitude in this additional grooved-peg sensillum type to fatty-acids and host-odour as observed for GP2-type. I made recordings from at least eight such sensilla. In this fourth GP-type, neither did the receptors with the large or intermediate amplitude spikes respond to any of the natural or synthetic volatiles tested. There is therefore evidence for at least four functionally different sub-populations of grooved-peg sensilla on the antennae of *T. infestans*, all with three spontaneously firing receptors (Table 1).

Nitrogen along with carbon and hydrogen is a basic element for life on this planet and it is in all proteinaceous material which many organisms require to develop and reproduce. Ammonia is very often an end-product of decomposition of proteinaceous matter and as such could have served as a cue for a source of protein and food to insects during evolution. It is still used by certain insects like fruit-flies to find proteinaceous material (Bateman & Morton, 1981). It is very likely that triatomines were entomophagous before evolving to the blood-sucking habit. Most of the other heteropterans in the family Reduviidae have stayed with the entomophagous habit and also possess piercing-sucking mouth-parts. It is possible that before developing the blood-feeding habit these bugs fed on other insects in the nests and burrows of vertebrates. Ammonia could already have played a role in such a niche in the attraction of the triatomines to feeding sites since it is released from decomposing excretory material of both insects and vertebrates. It is a general view that the haematophagous habits in many blood-sucking parasites followed on their close association with vertebrates (Lehane, 1991).

Table 1

Response profiles of four types of functionally different grooved-peg sensillae (GP) on the antennae of nymphs of *Triatoma infestans*. Each with three receptors with different spike amplitudes.

Receptor type	Sensilla type			
	GP-1 type	GP-2 type	GP-3 type	GP-4 type
Big spike amplitude	Type A-excitation	Type B-excitation	-	Type A-inhibition, Type C-excitation
Medium spike amplitude	-	-	-	-
Small spike amplitude	-	Type A-excitation	Type A- excitation	-
	-			-

Type A excitation by ammonia, wetted faecal-papers, stale rabbit-urine, methyl-, dimethyl-, ethyl-, and diethyl-amines
Type A inhibition by ammonia, wetted faecal-papers, stale rabbit-urine, methyl-, dimethyl-, ethyl-, and diethyl-amines
Type B excitation by host-odour, isobutyric acid, butyric acid, valeric acid, isovaleric acid, pyruvic acid, lactic acid, and vanillin
Type C excitation by formic acid, acetic acid, propionic acid, and propylamine

The attraction of starved adults of *Triatoma* and *Rhodnius* to stale urine, if caused by the release of ammonia alone, could represent a basic response to ammonia on the part of the bugs in order to escape from the exposed situation on the servosphere to a place indicating life, and hence a refuge, in the vicinity of a host. But in itself it is very likely that vertebrate urine contains other volatiles in addition to NH_3 which the bugs have the capacity to perceive in order to deduce the association with the potential presence of a host. Other host-associated stimuli would include humidity and heat at a close range. Starved bugs under conditions of water deprivation display a probing response and drink water and saline solutions (Wigglesworth & Gillett, 1934; Núñez, 1982; Guerenstein & Núñez, 1994; our own unpublished observations). Apart from the smell of urine serving as direct evidence for presence of a host, presence of urine or other excretory products releasing NH_3 is the hallmark of regularly occupied resting sites of vertebrates.

Faeces could contain other less volatile gustatory chemostimuli. Liquid faeces soon after feeding will contain haem and other undigested material from blood. This will still have nutritive value for bugs and this might be why coprophagy is indulged in by hungry bugs. Larvae of another blood-sucking insect, the fleas are known to fulfil their blood requirement by feeding on the faeces of adults which contains both digested and undigested blood (Lewis, 1995). This could explain the aggregation on the faeces so frequently documented (Cruz-López *et al.*, 1993; Schofield & Patterson, 1977; Lorenzo Figueiras *et al.*, 1994). The role for as yet uninvestigated contact chemostimuli in the final stages of host and refuge selection via contact chemoreceptors on the antennae, legs and mouth-parts should not be neglected. In any event it is significant that small molecules such as CO_2 and NH_3 have been demonstrated here to influence the behaviour of triatomines, commonly known as kissing bugs, because of their habit of biting near the mouth and mucous membranes such as around the eyes. Human breath contains 3-4% CO_2 (Steullet & Guerin, 1992a) and saliva some 40-400 μmole l^{-1} of ammonia (Lentner, 1981). The use of NH_3 and CO_2 as cues is adaptive as it helps the bugs to reach these predilection sites where feeding can be carried out efficiently as the mucous membranes are easy to pierce and feed through.

GENERAL DISCUSSION

In triatomines ammonia could play a parsimonious role as an infochemical, an infochemical being a chemical that conveys information in an interaction between two individuals, evoking in the receiver a behavioural or physiological response that is adaptive to either one of the interactants or to both (Dicke & Sabelis, 1988). The parsimonious use of one compound mediating different chemical interactions is common in insects (Blum, 1996). It has been clearly demonstrated that triatomines seek out dry environments, strongly preferring the lowest humidity (0%) in a humidity gradient (Roca & Lazzari, 1994). In this context, it is appropriate to add that the enhanced attractivity of 3 ppb NH_3 tested at the lower RH of 35% argues for use of NH_3 at such humidities in refuge selection. By contrast, perception of NH_3, in addition to the many other volatiles released by vertebrate hosts, at higher humidities could signify a food source for hungry bugs. Placing the faeces outside the refuge as documented by Lorenzo & Lazzari (1996), would have the added advantage of avoiding exposure of the bugs to persistent release of NH_3 from faeces, reducing the risk of adaptation, and thus allowing the sensory system to remain tuned for the perception of amounts signalling a host in the vicinity.

The capacity to perceive ammonia and ammonium ion is widespread in the animal kingdom from unicellular slime-molds (Bonner *et al.*, 1988; Bonner, 1994) through acarids (Haggart & Davis, 1979; Levinson *et al.*, 1991a) and insects (Mazor *et al.*, 1987; Mumcuoglu *et al.*, 1986; Sakuma & Fukami, 1991; Mitchell *et al.*, 1975) to humans. The threshold for ammonia perception for humans is 53 ppm (Stecher, 1968) and its vapours at high concentrations are toxic. This basic capacity of perception has been employed as a behavioural guide by various arthropods such as the flour mites *Acarus siro* (Levinson *et al.*, 1991a), the Mediterranean fruit flies *Ceratitis capitata* (Mazor *et al.*, 1987), the human body louse *Pediculus humanus* (Mumcuoglu *et al.*, 1986), the cockroaches *Blattella germanica* (Sakuma & Fukami 1991), the house cricket *Acheta domesticus* (McFarlane *et al.*, 1983) and the boll weevils *Anthonomus grandis* (Mitchell *et al.*, 1975). Triatomines seem to have fully incorporated and exploited it in their life-style, showing a sensitivity to *ca.* 1 ppb, some 50,000 times lower than that of humans. Since ammonia is hydrophilic its transport and transduction is likely to be as NH_4^+ ion. Ammonia can be found dissolved in the aqueous body constituents as NH_4^+ ion, and the ammonia content of

human blood is 30 μmole l^{-1} (Lentner, 1984), and Chinese oak silkworm larva contains *ca.* 7 mg ammonia per 100 ml of haemolymph (Spector, 1956), so presence of internal NH_4^+- receptors to maintain a balanced internal milieu is possible. It is very likely that these internal receptors have often been exteriorised in evolution in order to obtain information from the external milieu too.

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SUMMARY

Rhodnius prolixus and *Triatoma infestans* are haematophagous heteropteran bugs belonging to the Family Reduviidae, sub-family Triatominae and are distributed in the Central and South-American continent. They are of major public health importance in South and Central America as they are the vectors of a protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas'disease or American trypanosomiasis.

Oriented responses and walking behaviour of both *R. prolixus* and *T. infestans* were recorded on a locomotion compensator. A servosphere or locomotion compensator is an apparatus that functions in such a way as to keep the walking insect at the same position in space, *i.e.*, at the apex of a perspex sphere to which the stimulus delivery tube carrying a conditioned air-stream is directed. The triatomines typically walked in straight bouts with a slight zig-zag, interrupted by stops or periods at relatively low speeds, all of variable duration. This basic walking behaviour was observed throughout 6 min records of each bug comprising three consecutive two min periods, *i.e.*, control, test and end-control. The mean walking speed of *R. prolixus* adults was *ca.* 3 cm/s and that of *T. infestans ca.* 5 cm/s.

Observations on orientation behaviour of these triatomine bugs on the locomotion compensator were used to test the role of host odours and volatiles from their own faeces in their host- and refuge-seeking behaviour. The volatiles tested were whole mouse-odour, one of its components CO_2, and rabbit urine-odour with *R. prolixus* and *T. infestans* adults, and odour of triatomine faeces and NH_3 (one of the odour components of triatomine faeces and vertebrate urine) with *T. infestans* nymphs. These volatiles were delivered in an air-stream under controlled conditions of constant

temperature, humidity and darkness. Any change in the behaviour of the bugs observed could thus be considered as a response to the presence of the odours.

In the stimulus-free air-stream the bugs walked preferentially downwind, *i.e.*, they showed negative anemotaxis, but this switched to a positive anemotaxis in odour-laden air. The characteristic response of these triatomines to odour onset was to stop, sample the air with the antennae, turn upwind and then walk off in the direction of the source, *i.e.*, odour mediated anemotaxis. The changes in walking direction were always made on the spot during a stop or at low walking speeds between the walking bouts or subtracks at relatively high speeds. The bugs made biggest shifts in body axis when responding to odour delivery. Some bugs also showed a continued upwind response to attractive volatiles after the odour delivery ceased, *i.e.*, an after-response. The response of *T. infestans* adults to whole mouse-odour was stronger than that of *R. prolixus* to the same stimulus. In addition, the response of *T. infestans* to whole mouse odour and to volatiles from vertebrate urine was stronger than its response to CO_2 alone at amounts (0.6%) present in the whole mouse-odour.

NH_3 was identified and quantified from biological attractants, *i.e.*, host urine and triatomine faeces, and the attractivity of this compound was demonstrated on the locomotion compensator. A latency in attraction to NH_3 was recorded in responses of *T. infestans* nymphs to ammonia which was dependent on NH_3 concentrations and influenced by relative humidity. The response to volatiles from wetted faecal-papers releasing 10 ppb NH_3 in the conditioned air was similar to the response recorded to the highest dose of NH_3 tested (17 ppb).

Electrophysiological recordings from olfactory sensilla on the antennae of *T. infestans* nymphs demonstrated the presence of NH_3-sensitive receptors within small

grooved-peg sensilla. These receptors responded strongly to volatiles from triatomine faeces and from host urine, as well as to synthetic methyl-, dimethyl-, ethyl-, and diethyl-amines at much higher thresholds. On continuous stimulation with ammonia, the NH_3-excited receptors did not adapt completely.

Grooved-peg sensilla are present exclusively on the distal flagellar segment of the antennae of *T. infestans* nymphs. Four functionally different types of such sensilla were identified based on responses of the three receptors with different spike amplitudes recorded from all such sensilla. In addition to NH_3-sensitive receptors these grooved-peg sensilla also house receptors sensitive to other host-odour components such as fatty acids.

The number of NH_3-sensitive receptors was sufficient to permit electroantennogram (EAG) recordings from the antennae of *T. infestans* nymphs and both EAG and single-unit responses to NH_3 were dose-dependent over the range 1.7 to 200 ppb, which covered the range of doses to which *T. infestans* nymphs responded behaviourally. Quantification of ammonia showed that the release rate of NH_3 into the conditioned air-stream reaching the bugs in the electrophysiology set-up and on the locomotion compensator from different amounts of aqueous NH_3 applied to filter papers was linear.

This study demonstrates that *Rhodnius prolixus* and *Triatoma infestans* are attracted to host odours and to volatiles from their own faeces.

RÉSUMÉ

Rhodnius prolixus et *Triatoma infestans* sont des punaises hétéroptères et hématophages. Ils appartiennent à la famille Reduviidae, sous-famille Triatominae et ils sont distribués dans le continent de l'Amérique du Sud et Centrale. Elles sont d'importance majeure pour la santé publique en Amérique du Sud et Centrale, car elles sont vectrices d'un parasite protozoaire *Trypanosoma cruzi*, l'agent transmettant de la maladie de Chagas ou trypanosomiase d'Amérique.

Les réponses orientées et le comportement de marche de *R. prolixus* et *T. infestans* ont été enregistrés sur un compensateur de locomotion. Une servosphère ou compensateur de locomotion est un appareil qui fonctionne de façon à maintenir l'insecte marchant dans la même position dans l'espace, c'est-à-dire, sur l'apex d'une sphère en plexiglas, oú le tuyau qui amène l'air avec les stimuli est orienté. La marche typique des triatomes consiste en bouts droits avec un léger zig-zag interrompu par des arrêts ou des périodes de vitesse réduite, tout de durée variable. Ce comportement de base a été observé pendant 6 minutes d'enregistrement de chaque insecte comprenant les 3 périodes consécutives de 2 minutes, c'est-à-dire, le contrôle, le test et le dernier contrôle. La vitesse moyenne des adultes de *R. prolixus* était 3 cm/s et celle de *T. infestans* 5 cm/s.

Les observations du comportement d'orientation de ces punaises sur le compensateur de locomotion ont été utilisées pour tester le rôle des odeurs d'hôte et des substances volatiles venant de leurs propres fèces dans leur comportement de recherche d'un hôte ou d'un abri. Les substances volatiles testées sur les adultes de *R. prolixus* et *T. infestans* étaient l'odeur complète de souris, un de ces composant: le CO_2, et l'urine de lapin et celles testées sur les nymphes de *T. infestans* étaient l'odeur des fèces des triatomes et NH_3 (un des composant volatiles des fèces des triatomes et

de l'urine d'hôte). Ces substances volatiles ont été délivrées dans un courant d'air dans des conditions contantes de température et d'humidité et à l'obscurité. Ainsi, chaque changement de comportement de ces punaises observé était donc une réponse à la présence d'odeurs.

Dans l'air sans stimulus les punaises marchaient de préférence dans le vent, c'est-à-dire elles montraient un anémotaxis négatif, mais cette préférence a été changée dans un anemotaxis positif dans l'air portant l'odeur. La réponse caractéristique de ces punaises au commençement de la livraison d'odeur était de s'arrêter, de échantillonner l'air avec ces antennes, de tourner contre le vent, puis de marcher dans la direction de la source, c'est-à-dire un anemotaxis conditionné par l'odeur. Le changement dans la direction de marche était toujours fait pendant un arrêt sur place ou en marchant à vitesse réduite entre les bouts de marche à grande vitesse: les sous-trajets. Les plus grandes réorientations de l'axe du corps ont été faites en répondant au livraison d'odeur. Quelques punaises ont continué de marcher contre le vent même après l'arrêt de livraison d'odeur, c'est-à-dire une après-réponse. La réponse des adultes de *T. infestans* à l'odeur complète de souris était plus forte que celle de *R. prolixus* au même stimulus. En outre, la réponse de *T. infestans* à l'odeur de souris et aux substances volatiles d'urine des vertébrés était plus forte qu'au CO_2 seul à 0.6%. Cette quantité de CO_2 est la mème dans l'odeur complète de souris.

NH_3 a été identifié et quantifié dans des attractants biologiques c'est-à-dire l'urine d'hôte et les fèces des triatomes, et l'attractivité de ce composant a été démontré sur le compensateur de locomotion. Un délai dans l'attraction de NH_3 a été enregistré dans les réponse des nymphes de *T. infestans* à l'ammoniaque qui était dépendant de la concentration de NH_3 et influencé par l'humidité. La réponse aux

substances volatiles des papiers de fèces humides qui relachent 10 ppb de NH_3 dans l'air conditionné a été semblable à la réponse enregistrée au plus haut dosage de NH_3 testé (17 ppb)

Les enregistrements électrophysiologiques sur les sensilles olfactives sur les antennes des nymphes de *T. infestans* ont démontré la présence de récepteurs sensitifs à NH_3 situés dans des sensilles courtes à sillons. Ces récepteurs ont répondu fortement aux substances volatiles des fèces des triatomes, à celles dans l'urine d'hôte et aussi à un seuil plus haut aux méthyl-, diméthyl-, éthyl-, et diéthyl-amines synthétiques. En stimulant continuellement avec l'ammoniaque les récepteurs exités par NH_3 ne se sont pas complètement adaptés.

Les sensilles courtes à sillons sont présentes exclusivement sur la partie distale du flagellum des antennes des nymphes de *T. infestans*. Quatre types fonctionellement différents de ces sensilles ont été identifiés basés sur les réponses enregistrés des trois récepteurs avec des spikes de différentes amplitudes. En plus des récepteurs sensitifs à NH_3, ces sensilles courtes à sillons abritent aussi des récepteurs sensitifs aux autres composants de l'odeur d'hôte comme les acides gras.

Le nombres de ces récepteurs sensitifs a NH_3 était suffisant pour permettre l'enregistrement de l'électroantennogramme (EAG) sur les antennes des nymphes de *T. infestans*. Les réponses de l'EAGs et celles des unités seules, à NH_3 étaient dépendantes des dosages dans une gamme de 1.7 à 200 ppb, qui couvre la gamme de dosage à la quelle les nymphes de *T. infestans* répondent comportementalement. La quantification de l'ammoniaque a montré que le taux de décharge de NH_3 dans le flux d'air conditionné qui arrive aux punaises dans l'électrophysiologie et sur le

compensateur de locomotion depuis différentes quantités de NH_3 aqueux appliquées sur des papiers filtres était linéaire.

Cette étude démontre que *R. prolixus* et *T. infestans* sont attirés par des odeurs d'hôte et par des substances volatiles de leurs propres fèces.

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Erratum:

In chapter 1 of the results the reference to Lorenzo Figueiras *et al.* (1994) is mistakenly cited as Figueiras *et al.* (1994).