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Ammonia attracts the haematophagous bug *Triatoma infestans*: behavioural and neurophysiological data on nymphs

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Abstract 1) Nymphs of the haematophagous bug *Triatoma infestans* (Heteroptera: Reduviidae) are attracted to volatiles from their own faeces on a servosphere. 2) Biological substrates attractive to triatomines release NH_3: wetted triatomine faecal papers release NH_3 at 256 ppb NH_3 from a 60-g source and stale rabbit urine at 394 ppb from 200 ml. Ammonia released from aqueous NH_3 also attracts bugs at doses of 3 ppb and 17 ppb on the servosphere. 3) Bugs typically show negative anemotaxis in a stimulus-free air-stream on the servosphere. At onset of stimulation with ammonia from either biological substrates or aqueous NH_3 the bugs stop, move their antennae, turn and walk upwind, i.e. odour-mediated anemotaxis. 4) At lower NH_3 doses a latency in attraction is recorded, but this latency disappears when the relative humidity of the stimulus delivery air-stream is dropped from 90 to 35%. 5) Electrophysiological recordings from single olfactory sensilla on antennae of *Triatoma* nymphs reveal two different types of NH_3-excited receptors, both within grooved-peg sensilla. The responses of one of these receptor cells to NH_3 has been studied in detail and shows that the action potential discharge rate is dose-dependent over the range 2–200 ppb. 6) The amplitudes of electroantennograms recorded from *Triatoma* nymphs to NH_3 are dose dependent over the range 5–550 ppb.

Key words *Triatoma infestans* · Ammonia · Olfaction · Electrophysiology · Attraction

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 where it takes refuge in the cracks and crevices of human dwellings. Peridomestically, this bug is 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 and 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 (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 to the youngest stages which have not yet learned the way to the host and which have relatively small blood requirements.

Considering the success of triatomines as parasites, we may postulate that they 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 and Núñez 1989). Previous studies in this laboratory with starved bugs on a servosphere have shown that, on their own,

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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 and Guerin 1995). The basis of this odour-mediated anemotaxis is detection 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 and 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* was maintained at 26 °C, 75% RH and 12:12 h L:D cycle in a climate chamber. The bugs were regularly fed on heparinised cow-blood using the in-vitro feeding technique described by Núñez and 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 diam.), to which the stimulus delivery tube (see below) is directed as described previously (Taneja and 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 m s^{-1}) at 25 °C and 90% RH was continuously delivered tangentially to the apex of the sphere. The orifice of this delivery system measured 2.8 cm high and 4.5 cm wide, and ended 3 cm from 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 three consecutive 2-min periods, i.e. control, test, and end-control in order to record the behaviour of the bugs before, during and after stimulus delivery, respectively.

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 diam. (Schleicher & Schuell, 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^{-1}) over them in a 1-l gas-wash bottle and injecting the effluvium into the air-stream which blew over the bugs at the apex of the sphere. 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 μmol NH_3 applied to filter paper discs (12.5 cm diam.) in a 1-l gas-wash bottle. Distilled water applied to similarly sized discs served as controls. In addition, the intermediate dose of 24 μmol 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 and Guerin 1995).

The stimulus delivery system to the apex of the sphere was the same as described earlier (Taneja and Guerin 1995). The vapours from the gas-wash bottles were introduced via Teflon-tubing (2 mm i.d. × 4 mm o.d.) attached to a syringe needle which was inserted 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.

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, 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 (arbitrarily defined as a cone of 60° either side of due upwind) 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 and 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 the presence of an olfactory stimulus could also influence the walking speed of the nymphs, 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 1 s and defined as subtracks; cf. Taneja and 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 m s^{-1} via a glass tube (6 mm i.d.). As reference, a chloridised silver electrode in a drawn-out glass capillary (tip diam. ca. 4 μm) filled with a triatomine ringer solution [composition: 161 mmol·l^{-1} NaCl, 2.6 mmol·l^{-1} KCl, 5 mmol·l^{-1} $CaCl_2$, 2 mmol·l^{-1} 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 diam. 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). Clear 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} Ω) preamplifier (×10, Syntech, The Netherlands), and this signal was fed from the preamplifier into an AC/DC amplifier (UN-03, Syntech, The Netherlands), low-pass filtered, amplified again (50–100 times) and recorded on a tape in a DAT recorder (Biologic, France). The taped recordings were replayed and recorded on a PC via the DAS16 analogue-digital board (MetraByte Corporation, USA) or via an 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). Electroantennogrammes were recorded directly on the hard disk of a PC via the 16-bit analogue-digital IDAC card (Syntech, The Netherlands).

Stimuli and stimulus delivery for electrophysiological recordings

Dry (1.0 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 which served as stimulus cartridges. The plunger of the syringe was replaced by a rubber seal with a 2 mm hole at the 5-ml mark in the barrel to provide a confined volume into which volatiles could evaporate. 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 and 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 μmol) were applied to filter paper strips (ca. 0.5 × 5 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 (above) by attaching a Teflon stopper to the syringe barrel which in turn was connected via Teflon tubing (2 mm i.d. × 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^{-1} which flowed continuously into the air-stream. In adaptation experiments, filtered air (120 ml min^{-1}) 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 μmol, 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 μmol), dimethyl-amine (dose range: 0.09–9 μmol), trimethyl-amine (dose range: 0.17–17 μmol), ethyl-amine (dose range: 0.15–15 μmol), and diethyl-amine (dose range: 0.14–14 μmol) 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, 1 μg, 10 μg and 100 μg at stimulus source) were tested. All chemicals 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 and Guerin (1995)], four doses of aqueous NH_3, i.e. 2, 24, 235 and 2353 μmol 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^{-1}) 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 μmol and 24 μmol sources of aqueous NH_3 and wetted faecal-papers in 1-l gas-wash flasks over the 2-min period of delivery in behaviour ex-

periments, 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 µmol aqueous NH_3 and distilled water (control) applied to filter papers in the 5-ml stimulus cartridges was evacuated from ten individual cartridges at each dose and bubbled through two 10-ml gas-wash flasks connected in series which served as traps. The amounts of NH_3 in these aqueous solutions were quantified by the Tecator colorimetric method and from this the amount of NH_3 in the stimulus air was calculated. Since violatile stimuli 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 air reaching the bugs.

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 redissolved in 48 ml water and re-filtered. This process was repeated three times to wash out any residual dissolved ammonia or substrate. Then 5 µl, 50 µl, and 500 µl of 10 $mmol \cdot l^{-1}$ 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 $mmol \cdot l^{-1}$ 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).

Results

Behaviour

Records of walks performed by *Triatoma* nymphs on the servosphere were similar to those described earlier for *Rhodnius* and *Triatoma* adults (Taneja and 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. 1). A shift in distribution of the subtrack-angles was recorded for the nymphs from essentially downwind runs before stimulation to upwind runs following stimulus delivery (Fig. 2). 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

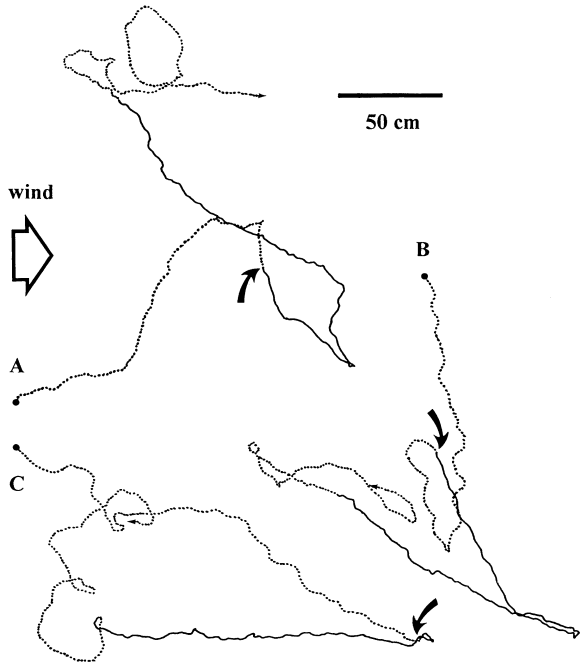


Fig. 1A–C Tracks made by three *Triatoma* nymphs on the servosphere in response to different doses of NH_3: 0.3 ppb **(A)**, 3 ppb **(B)** and 17 ppb **(C)** in the air-stream (0.1 m s^{-1}) reaching the bug. Records consisted of 2-min consecutive control, test and end-control periods. The tracks started (●) 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

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 into the stimulus air) and the highest dose of NH_3 (ca. 17 ppb) delivered to the servosphere (Table 2). 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. 3A). 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. 3A). The analysis of speed showed that the bugs walked faster in the first minute of the test period than in the corre-

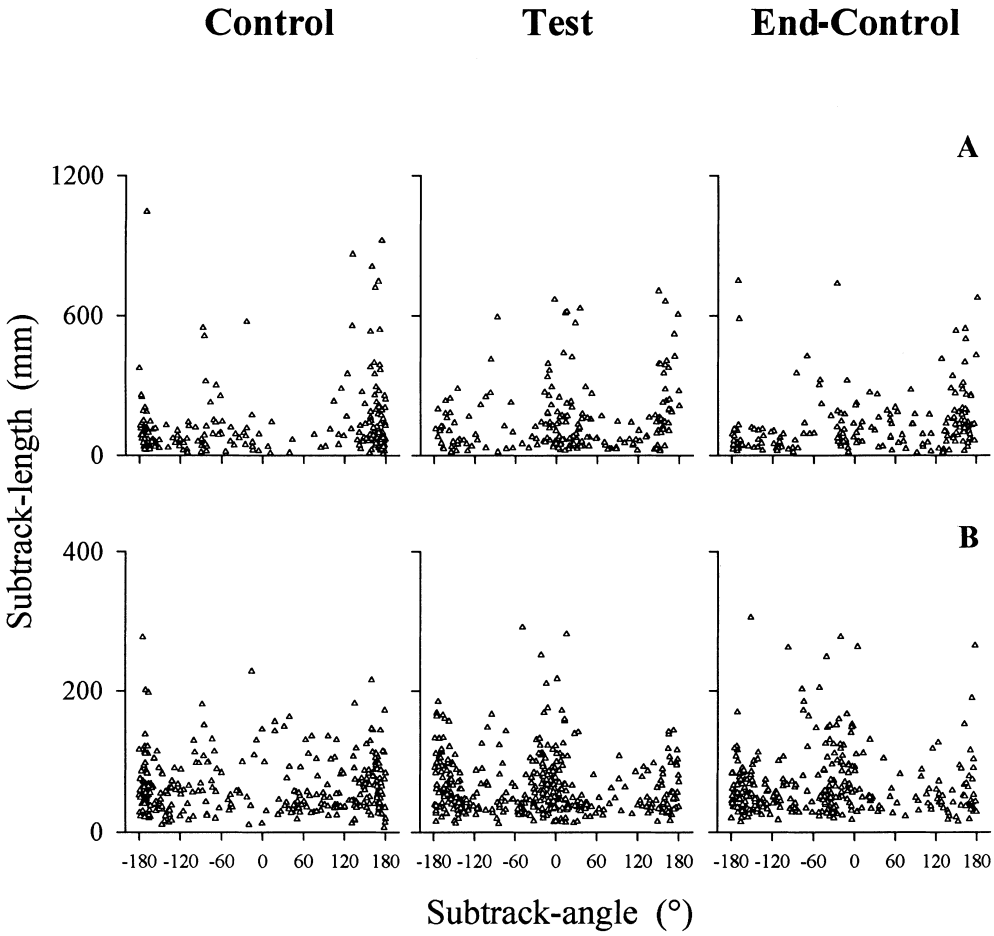


Fig. 2A, B Scatter graphs of subtrack-lengths plotted against subtrack-angles for successive 2-min control, test and end-control periods of experiments to determine *Triatoma* nymphs' responses to **A,** 60 g wetted faecal papers in a gas-wash bottle ($n = 11$) and to **B,** 17 ppb NH_3 vapour released 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 and 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

sponding 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 detection 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); however, at 35% RH there was no latency in the response as compared to the same dose tested at 90% RH, i.e. the response was stronger in the first minute of the test period (Fig. 3).

Electrophysiology

Records of spontaneous activity from the base of grooved-peg sensilla on the antenna of *Triatoma*

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 experiment 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

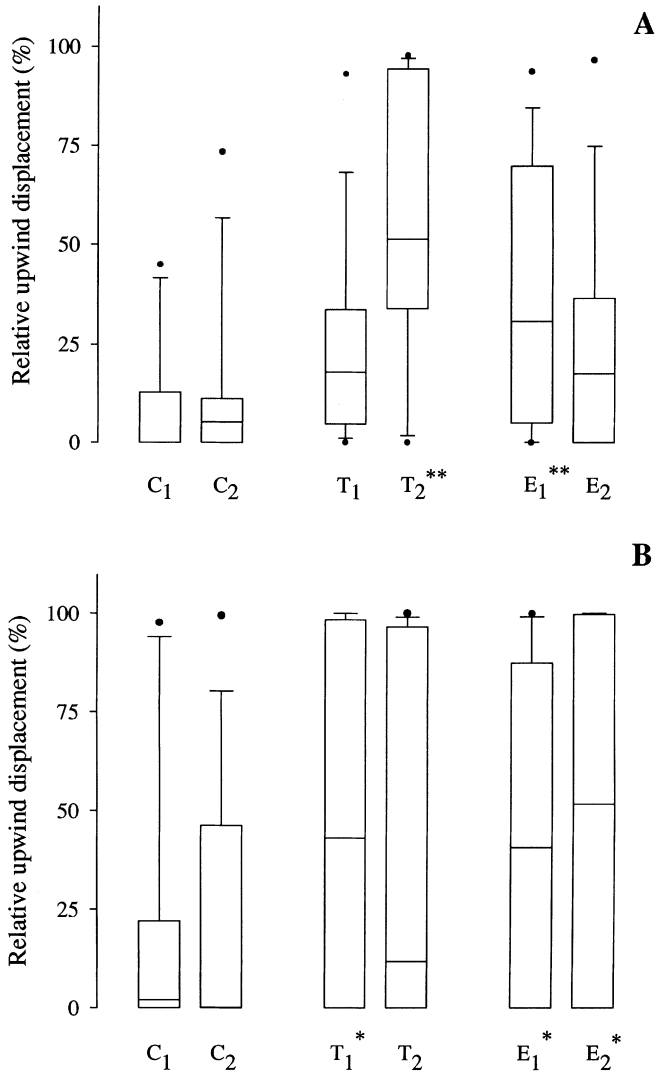
	Dry faecal papers ($n = 10$)	Wetted faecal papers ($n = 11$)	NH_3 (17 ppb) ($n = 12$)	NH_3 (3 ppb) ($n = 10$)	NH_3 (0.3 ppb) ($n = 10$)
Test over control	10.37 n.s.	42.12 *	37.16 *	25.29 *	17.62 n.s.
End control over control	3.34 n.s.	22.41 **	28.32 *	15.90 **	7.95 n.s.

* $P < 0.01$, ** $P < 0.05$, n.s. not significant

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 a view to detecting any latencies in responses. Each experiment 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 recording periods for each bug is expressed as the difference with that recorded for the corresponding segments C_1 and C_2 of the 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:

	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.

* $P < 0.01$, ** $P < 0.05$, n.s. not significant



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 large amplitude (2–3.5 mV) spike, all firing at a few Hz (Fig. 4). The receptor cell with the largest amplitude spike was extremely sensitive to NH_3, and vapours over 1 g wetted faecal-papers and 5 µl stale rabbit-urine caused an over tenfold increase in the firing rate. 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 and 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 in-

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Fig. 3A, B Data demonstrating latency in the response of *Triatoma* nymphs to NH_3 vapour at a high relative humidity. 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 3 ppb NH_3 vapour in the stimulus air: **(A)** 90% RH ($n = 10$), and **(B)** 35% RH ($n = 14$). Each experiment 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 of these period. i.e. C_1 and C_2 control, T_1 and T_2 test, and E_1 and E_2 end-control segments. 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. Whereas 3 ppb NH_3 is attractive at both humidities, the latency of the response shows a shift, i.e, the bugs only responded after 1 min at 90% RH **(A)** but responded to the NH_3 vapour at 35% RH throughout the 2- min test period **(B)**. The end-control response is also stronger at 35% RH

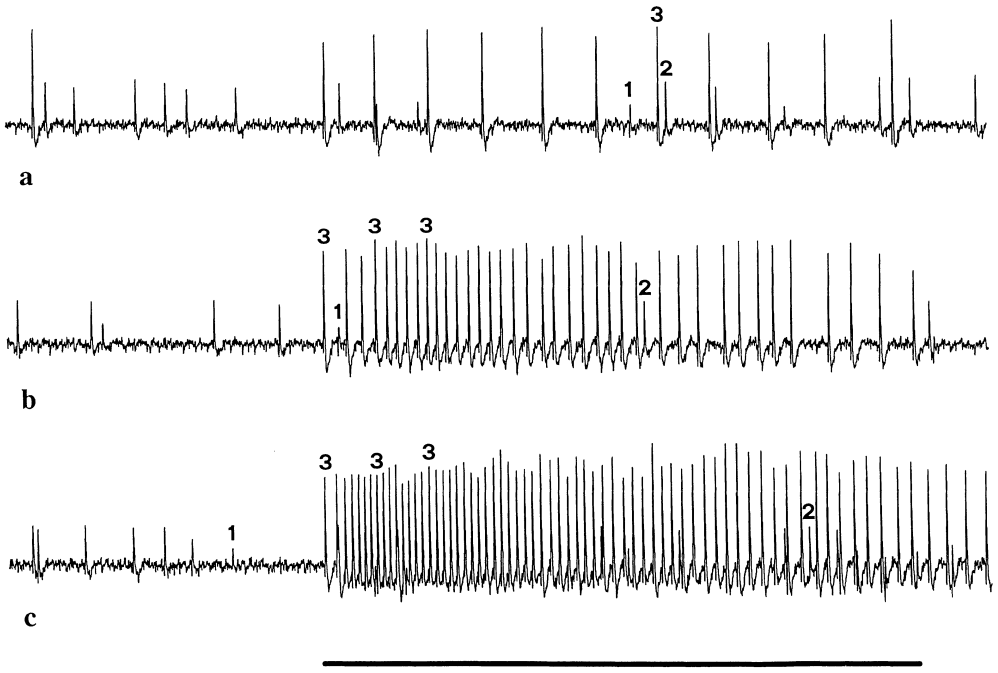

Fig. 4A–C Responses of an olfactory receptor within a grooved-peg type-1 sensillum (GP1) on the antenna of a *Triatoma* nymph to NH_3 at **(A)** 2.6 ppb, **(B)** 5.5 ppb and (**C**) 20.4 ppb in air reaching the preparation. These increments in the amount of NH_3 passing over the sensillum caused an increase in the firing rate of the NH_3 sensitive receptor from the spontaneous level of ca. 2 Hz to 10, 37 and 64 Hz, respectively. 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, most sensitive to NH_3, also responds to methyl-, dimethyl-, ethyl-, and diethyl-amines but at thresholds ten 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

dicated ammonia as the probable stimulus for the receptor. Increments in the amount of aqueous NH_3 on the filter paper in the stimulus cartridge caused clear increases in the firing rate of this receptor (Fig. 4). The responses of individual 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 1.7–200 ppb in the stimulus-delivery air-stream, after which it plateaues (Fig. 5). As indicated by the fitted curve, the threshold of the responding receptors lies at ca. 0.1 $\mu mol \cdot l^{-1}$ 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 ten 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 evoke a response from the other receptor cells within the grooved-peg sensillum housing the large-amplitude NH_3-excited receptor. This sensillum housing the NH_3 receptor cell with the large spike amplitude is referred to hereafter as grooved-peg sensillum type-1 (GP1). When stimulated continuously for 100 s with 0.1–1.0 μmol NH_3 l^{-1} (2–17 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. 6).

Triatoma nymphs also showed electroantennogram responses to NH_3. Mean depolarisations of ca. 4 mV were recorded for 3 $\mu mol \cdot l^{-1}$ in air (51 ppb), rising to ca. 10 mV for 32 $\mu mol \cdot l^{-1}$ (544 ppb). The response function of the responding antennae is linear over the dose range 5–544 ppb delivered in the stimulus air-stream, similar to that recorded for the single receptors (Fig. 7A). 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 μmol NH_3, or 34 and 102 ppb, respectively (Fig. 7B).

Although the external morphology of all the grooved-peg sensilla basiconica on the antenna of *Triatoma* is the same as indicated by light and scanning electron microscope examination, and the spontaneous activity indicated the presence of three receptor cells with different spike amplitudes in all such sensilla investigated, receptors within individual 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 (GP-2) showed a higher mean spontaneous activity of the cells firing (ca. 4–5 Hz) and the cell with the largest spike amplitude (2–3.5 mV) in this sensillum responded to isobutyric acid (Fig. 8). This cell also responded to a host odour extract as trapped on Porapak Q (Steullet and Guerin 1992b, 1994a) from animal rooms containing mice and rabbits (Fig. 8). This receptor did not respond

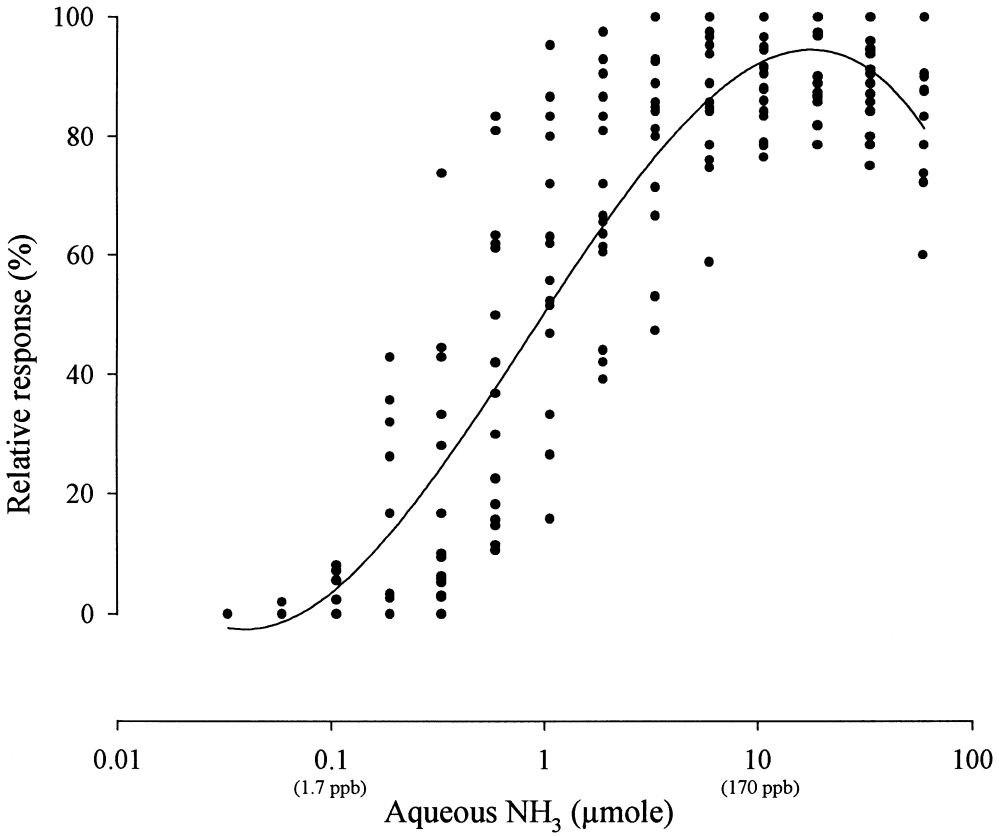

Fig. 5 Dose-response relationship of NH_3-excited receptors on antennae of 15 *Triatoma* nymphs. For this, each electrophysiological preparation of a single double-walled grooved-peg sensillum type-1 housing the receptor was subjected to stimulation for 1 s with NH_3 vapour released from increasing doses 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 1.7–200 ppb NH_3; at higher doses the response plateaues. A trend line was fitted

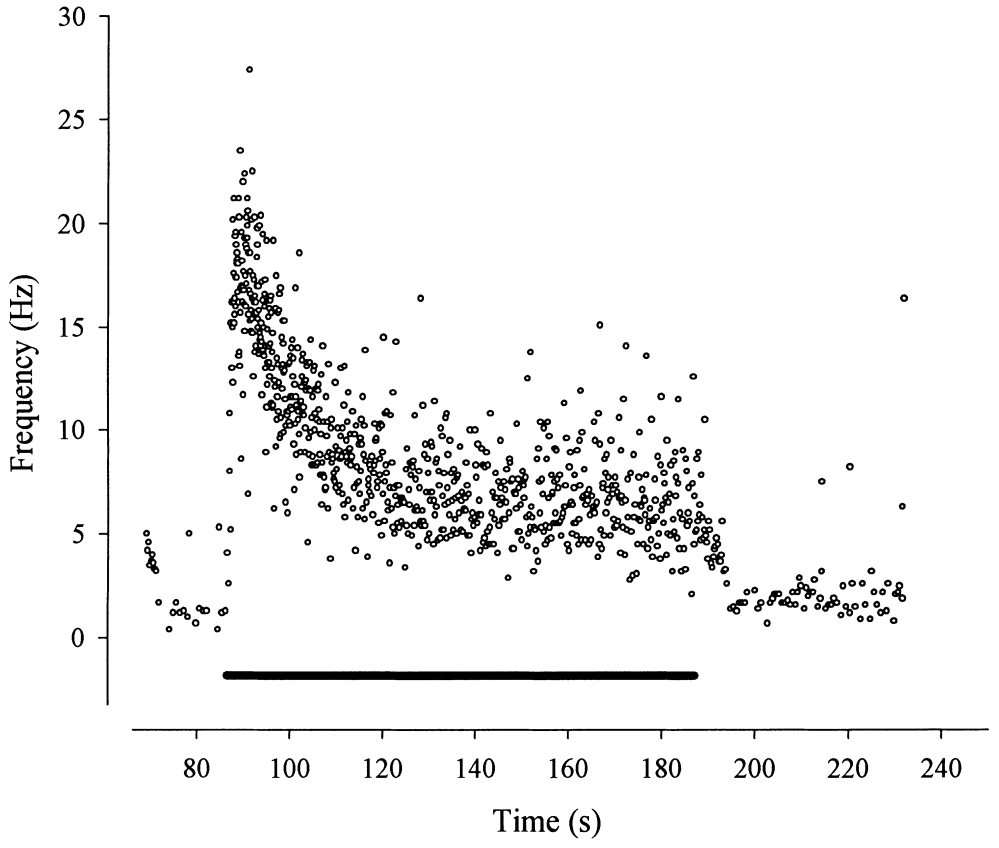

Fig. 6 The instantaneous frequencies (Hz), as derived from the inter-spike interval, of a NH_3-excited receptor in a grooved-peg type-1 sensillum on the antenna of a *Triatoma* nymph in response to continuous stimulation for 100 s (*black bar*) with 3.4 ppb NH_3. The interspike interval of the responding 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

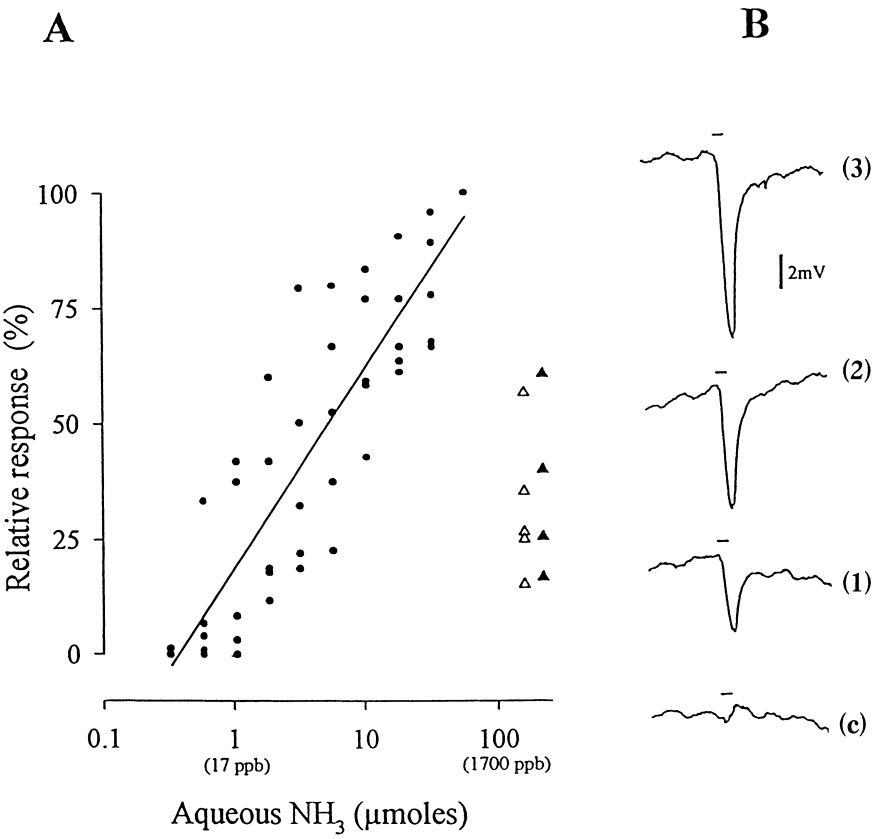

Fig. 7A, B Electroantennogram responses of *Triatoma* nymphs to NH_3: **A** responses of antennae from five *Triatoma* nymphs to 1 s stimulation with NH_3 vapour released from increasing doses in aqueous solutions of NH_3 on filter paper. After subtracting the response due to the control (water only), 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. A trend line was fitted; **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. *Horizontal bars* indicate 1-s stimulation

to NH_3 but the cell with the smallest spike amplitude in this GP2-type sensillum did (Fig. 8). This cell also responded strongly to vapours from stale rabbit-urine, wetted faecal-papers and to methyl-, dimethyl-, ethyl-, and diethyl-amines at a lower sensitivity, a response profile similar to the large amplitude receptor in the GP1 sensillum described above. We made similar recordings from ten such sensilla on different *Triatoma* nymphs.

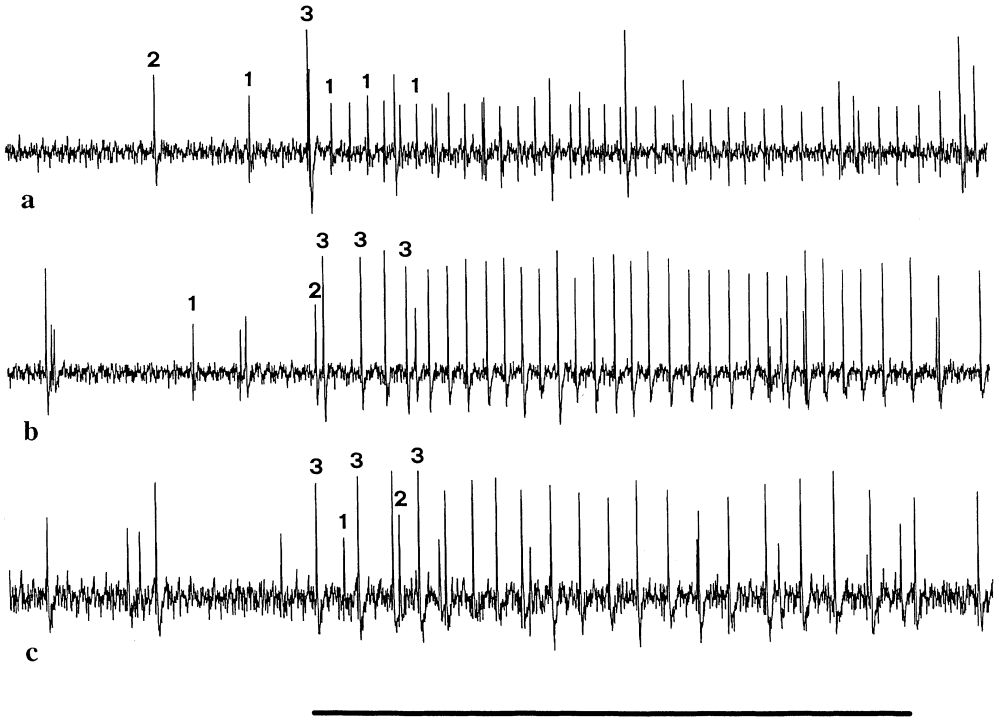

Fig. 8A–C Responses of olfactory receptors within a grooved-peg type-2 sensillum (GP2) on the antenna of a *Triatoma* nymph to **(A)** 5.5 ppb NH_3 , **(B)** a concentrate of host-odour extract, and **(C)** 100 ng isobutyric acid in the stimulus cartridges. Recordings of spontaneous activity from GP2-type 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, isobutyric acid. *Horizontal bar* indicates 1-s stimulation and the big spike in the trace **(a)** has an amplitude of ca. 2 mV

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 $\mu mol \cdot l^{-1}$ 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. 9). 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 µmol 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 µmol and 24 µmol 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 a 2-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%).

Discussion

Attraction of nymphs to ammonia

The data presented here clearly demonstrate *Triatoma*'s ability to perceive and differentiate between different amounts of NH_3 released into the air from attractive

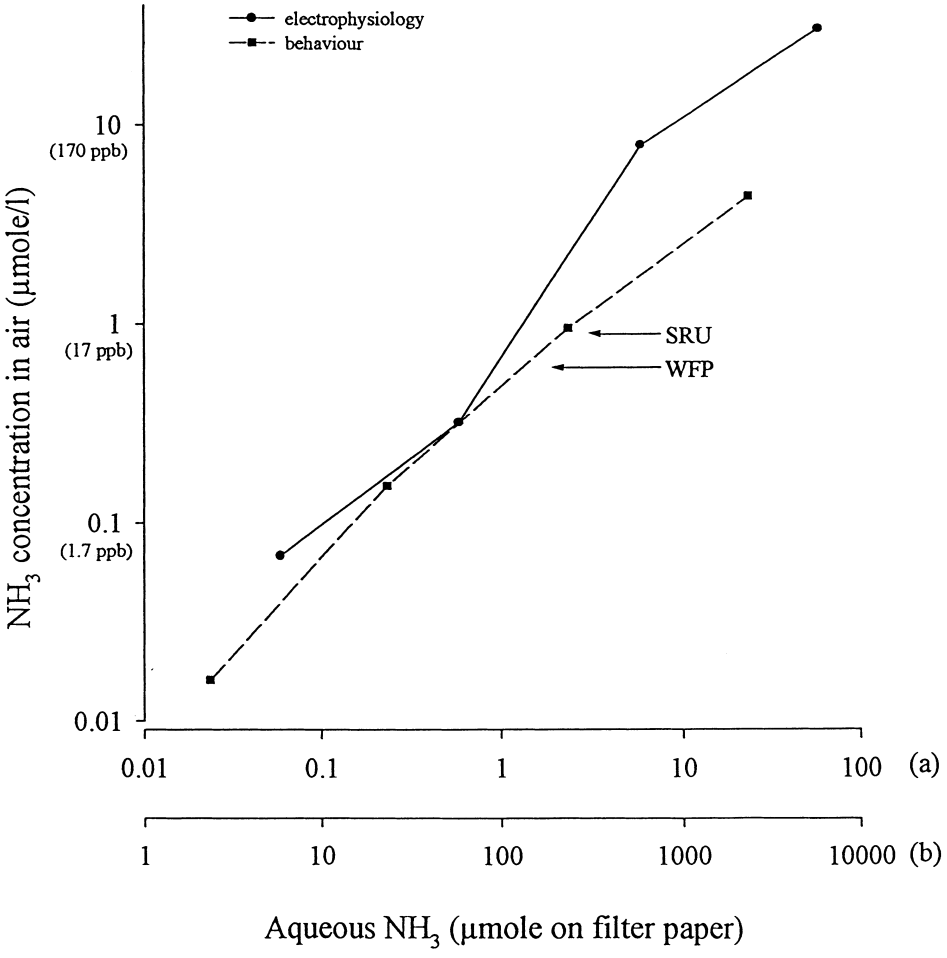

Fig. 9 Plots of the release rate of NH_3 into the stimulus-air ($\mu mol \cdot l^{-1}$) reaching sensory organs in electrophysiology set-up and nymphs on the servosphere from different amounts of aqueous NH_3 (µmol) applied to filter paper in 5-ml stimulus cartridges and 1-l gas-wash bottles, respectively. The release rate was measured over four increasing log doses for both the electrophysiology and behavioural experiments using the Tecator flow-injection and gas-diffusion technique (cf. Materials and 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 two or three 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

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 μmol 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 and 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 and 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 and 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 (2–17 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 and 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 and 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.

Sensory physiology of grooved-peg sensilla

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 × 2 μm diam. 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 diameter 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 and Müller 1976), and on other haematophagous arthropods such as mosquitoes (McIver 1982; Cribb and Jones 1995) and on the Haller's organ of ticks (Hess and 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 subpopulations. The first sub-type, GP1, with three spontaneously firing cells has a NH_3-excited receptor with a large amplitude spike. This receptor is also sensitive to other amines but at a sensitivity ten 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 bigger amplitude spike in this sensillum responds to short-chain fatty-acids and to host odour extracts. Bernard (1974) has also reported differences in response profiles 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, and 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 and Guerin 1994b; Haggart and Davis 1980;) and mosquitoes (Kellogg 1970), and fatty-acid sensitive receptor cells have been reported from grooved-peg sensilla of ticks (Steullet and 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 depolarisable receptors.

Significance of ammonia for triatomines

Ammonia has been identified in effluvia from vertebrates such as in saliva, eccrine sweat, and urine (Lentner 1981; Albone 1984). 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 and Fukami 1991), the flour mite *Acarus siro* (Levinson et al. 1991a), and fruit flies (Bateman and Morton 1981; Mazor et al. 1987; Robacker and 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 and Guerin 1994) as well as for the flour mite *Acarus siro* (Levinson et al. 1991b), the cockroach (Roth and Cohen 1973; Sakuma and Fukami 1991), and boll weevils (Mitchell et al. 1975). A series of nitrogen-containing volatiles isolated in vapours over faecal-papers from *Triatoma* spp. proved unattractive (Cruz-López and 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 (J. Taneja, P.M. Guerin, unpubl. obs.). 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 and 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 and 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 and 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, 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 and 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.

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