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

J. Taneja · P. M. Guerin

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 *Triatominae (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 SAMII 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 around 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:

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

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:

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

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

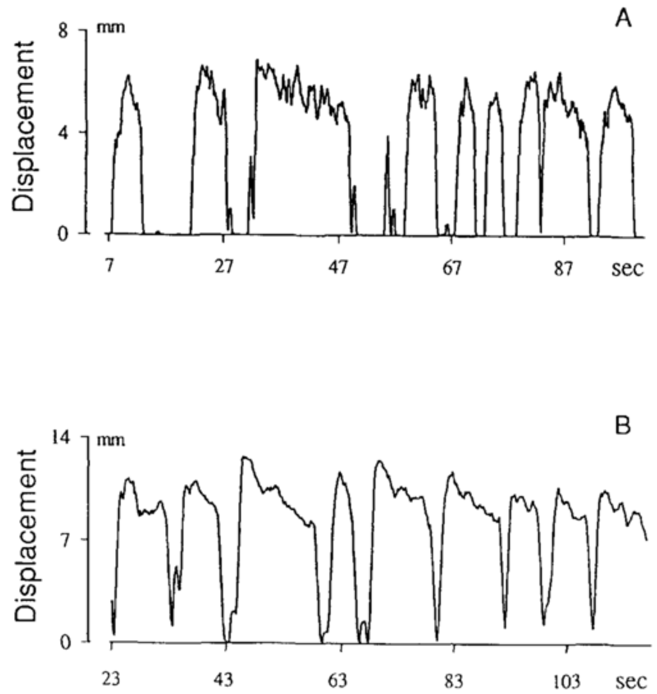

Fig. 1 Displacement of two *Triatoma* (**A** and **B**) as sampled every 0.1 s during a 90 s walk on the servosphere. Triatomines walk in bouts which start at speeds higher than the mean, and decelerate thereafter. These bouts are separated by stops or periods at much lower speed (< 5 mm s^{-1}), both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

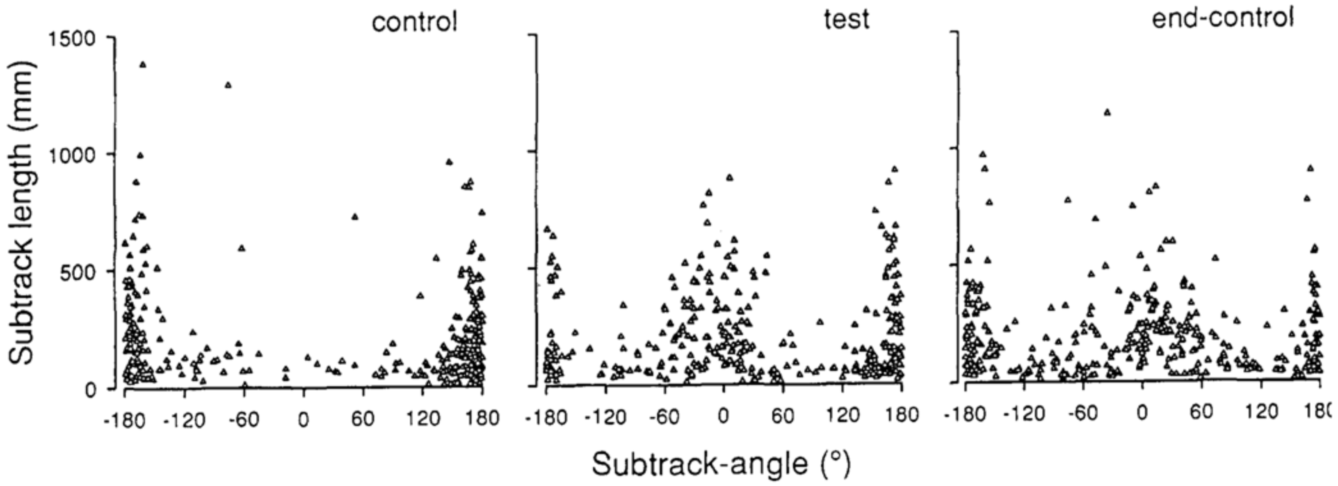

Fig. 2 Scatter graphs of subtrack lengths plotted against subtrack-angles for the successive control, test, and end-control periods of experiments to determine *Triatoma*'s response to rabbit urine-odour ($n = 22$). The bugs walked essentially downwind during the initial control period in the clean air-stream. 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

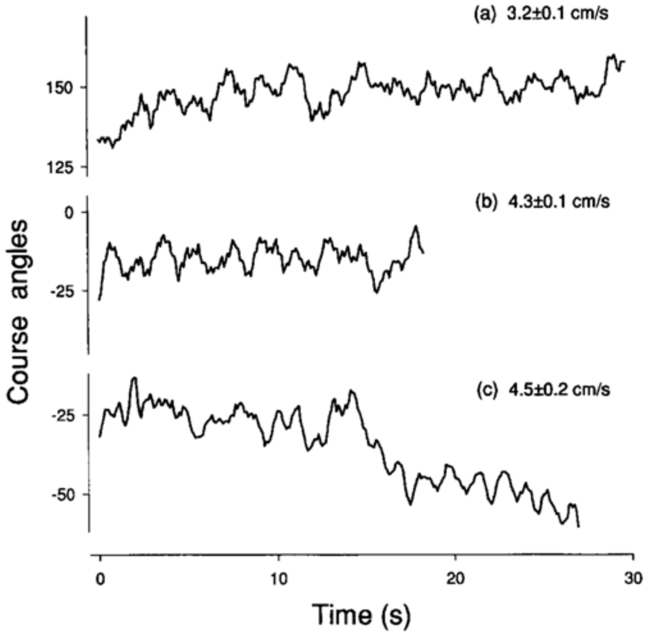

Fig. 3 Course angles with respect to wind (0°) of consecutive 0.1 s vectors of subtracks made by 3 different *Triatoma* during control (**a**), test (**b**) and end-control (**c**) periods of experiments with rabbit urine-odour. These detailed representations of the angles described by the bugs, after 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

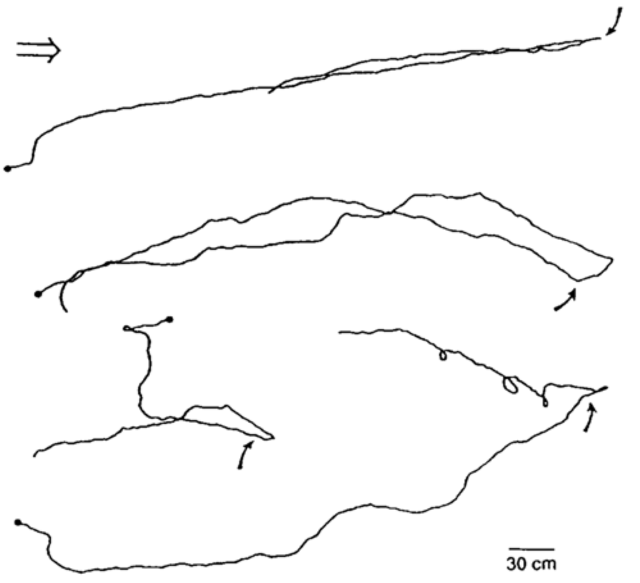

Fig. 4 Tracks made by four *Triatoma* on the servosphere depicting their responses to the odour of rabbit urine. The tracks started (●) with the bugs walking downwind (open arrow top left) in a pure air-stream (0.1 m s^{-1}). After odour of rabbit urine was added (bold arrows), the bugs turned to walk upwind. Stops made by the bugs in their tracks, including those made to turn upwind (see Fig. 6), 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.

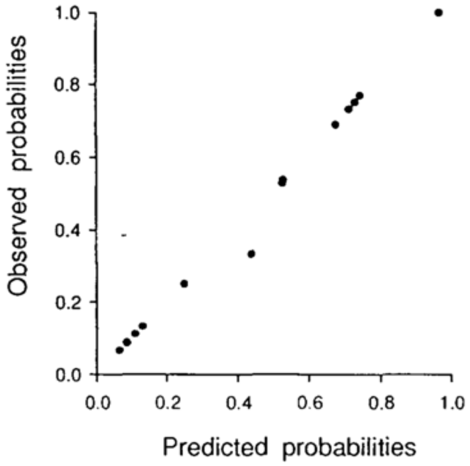

Fig. 5 Probabilities of upwind walks (P_{sb}) by 14 *Triatoma* responding to mouse-odour, as predicted by the binary response model of the logistic procedure in SAS (see text) plotted against the observed values for the same bugs. The high correspondence of the observed to predicted values clearly demonstrates 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*^{a}	3.88 ± 0.42*^{a}
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*

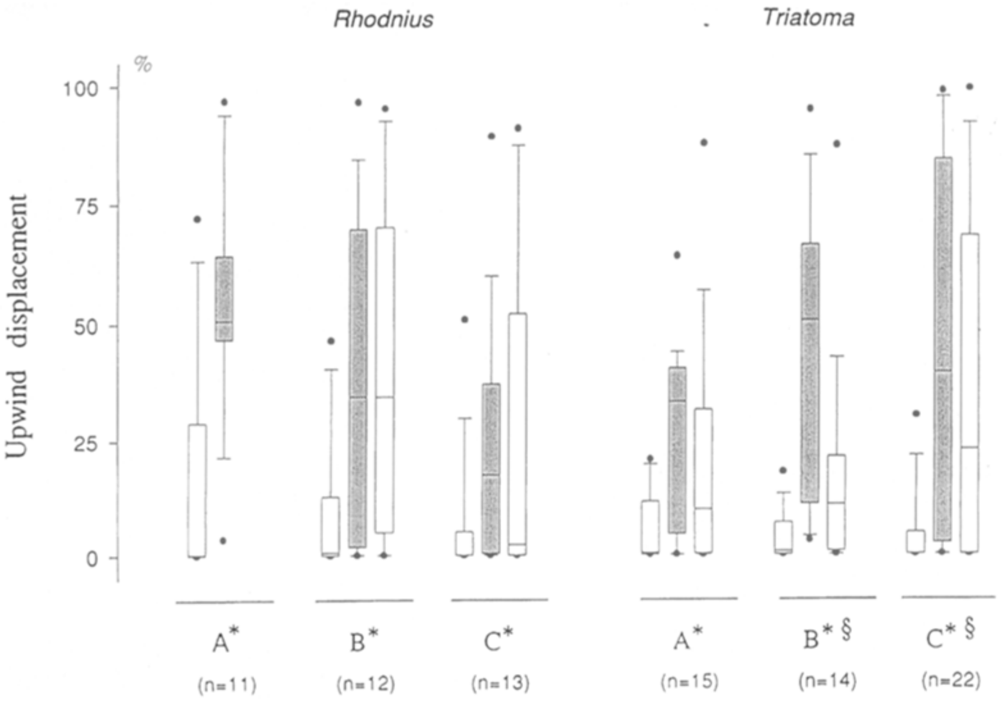

Fig. 6 Box plots of the upwind displacement (60° either side of due upwind) as % of the total for the period made by *Rhodnius* (left) and *Triatoma* (right) in response to 0.6% CO_2 (**A**), mouse-odour (**B**) and rabbit urine-odour (**C**). Responses to each treatment are divided into test period (*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

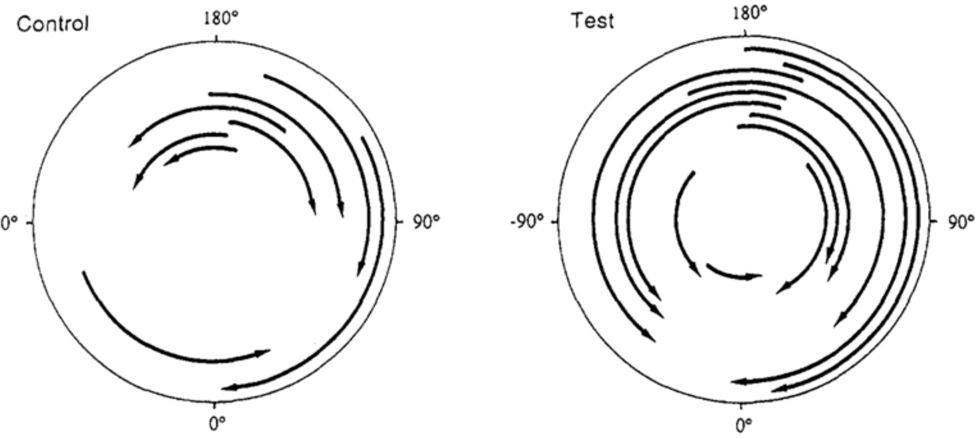

Fig. 7. Arcs representing the first upwind turns made by 11 *Triatoma* in response to rabbit urine-odour (test) as compared to the biggest upwind turns of at least 30° made anywhere during the preceding control period. *Triatoma* walks predominantly downwind in pure air. Each arc represents the size, 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 ($33 \pm 1.48\ \mathrm{mm\,s^{-1}}$ for *Rhodnius* and $48 \pm 1.68\ \mathrm{mm\,s^{-1}}$ 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 information (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 vertrebrates, 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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