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Attraction of the tropical bont tick, *Amblyomma variegatum*, to human breath and to the breath components acetone, NO and CO₂

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Abstract Ticks are of medical and veterinary importance and employ several cues in search of a host. Olfaction is one modality by which ticks locate a blood-meal and breath is the major vent of gaseous and volatile metabolites from the host that could contribute to this search. We studied the responses of a hunter tick, *Amblyomma variegatum*, to diluted human breath and five of its components (acetone, CO₂, NO, isoprene and NH₃) while walking in an air stream on a locomotion compensator. Diluted breath elicited the greatest responses of all treatments in terms of time to onset of upwind walk, attraction, speed and local search behaviour after stimulus off. Acetone, NO and CO₂ also attracted, but with a reduced speed in the case of acetone and NO. Neither isoprene nor NH₃ induced any response. Our study indicates that breath was the most adequate stimulus tested. It also attracted two other ixodid tick species, *Rhipicephalus sanguineus* and *Ixodes ricinus*, as well as the argasid tick, *Ornithodoros moubata*. It appears that the evolution of resource tracking in ticks included sensory and behavioural adaptations for recognition and orientation to host metabolites that are regularly expelled in breath.

Introduction

Exhaled breath is a blend of small molecules rich in CO₂, the end-product of aerobic metabolism. Due to the proximity of blood to the alveolar space, other gases, H₂O, ketone bodies and small, mostly apolar, volatiles also diffuse continuously across the pulmonary membrane. As such, breath provides a constant source of products that may contribute to host-finding by ectoparasites. In view of the mixture of compounds that breath contains, it is likely that the olfactory system of haematophagous arthropods has evolved to recognise this

natural blend. However, except for studies on mosquitoes (Healy and Copland 1995) and tsetse flies (Warnes 1990), little is known of the effect of this major host product in the absence of other modalities. Ticks have been associated with vertebrates since the late Palaeozoic and surpass all other arthropods in the number and variety of diseases which they transmit to domestic animals (Obenchain and Galun 1982). One could expect these non-flying ectoparasites, which need to detect hosts in the vicinity, to have developed adaptations that permit them to respond to breath.

Olfaction is an important sensory modality employed by ticks to locate vertebrate hosts (Guerin et al. 2000), where odour perception is limited to fewer than 100 chemoreceptors on each foreleg (Hess and Vlimant 1986). The responses of half of these receptors in the hunter tick chosen for this study, *Amblyomma variegatum* (Acari: Ixodidae), have been characterised. These include sensory units responding to the breath components CO₂, H₂S and NH₃ (Steullet and Guerin 1992a, b, 1994). Five breath compounds were presented to adult ticks and their behavioural responses compared with their response to diluted breath. Four of these (CO₂, NH₃, acetone and isoprene) were selected based on their differing functionality and relatively high concentrations. NO was chosen in view of its effect on oxygen uptake in hypoxic conditions (Beall et al. 2001).

Materials and methods

We employed a locomotion compensator (servosphere, Kramer 1976; see Table 1) to quantify the walking responses of adult ticks (McMahon et al. 2001). Attraction is expressed as the increase in displacement in an upwind cone 60° either side of upwind in the test compared to the control period. Latency is defined as the time (in seconds) taken by a tick to start walking in the upwind cone after onset of a test stimulus; ticks that did not enter this cone were assigned a latency value of 60 s. Simultaneous video recordings also allowed us to record local searches that occur when the tick describes small circles or abruptly turns downwind within 10 s after removal of the test stimulus from the air stream (McMahon et al. 2001). They are never observed in controls. The statistical sig-

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nificance of attraction, latency, percentage change in speed and percentage change in turning angle for a given treatment was determined using the Wilcoxon signed rank test (two-tailed) whereas inter-treatment differences of these responses were assessed using the Wilcoxon-Mann-Whitney test (two-tailed). Local search behaviours between treatments were compared using the Fisher exact test (two-tailed). Probabilities for the above tests were calculated using S-Plus (V3.3 release 1, 1995; StatSci, Seattle, USA) on a PC. The effect of increasing doses on latency and attraction was estimated using the Jonckheere trend test (after Siegel and Castellan 1988), whose test statistic (J) was calculated manually.

Computer-controlled solenoid valves introduced charcoal-filtered air (150 ml min^{-1}) from control or test gas-wash bottles (500 ml) into the stimulus delivery tube (3.5 l min^{-1}) for consecutive 1 min control, test and end-control periods. Prior to presentation of diluted breath to the tick, air from the control gas-wash flask with 20 ml distilled water was introduced into the air stream to preclude a shift in humidity during the test period with breath. The same solenoid valves were used to deliver NO and CO₂ from pressurised gas cylinders to the stimulus delivery air stream (charcoal-filtered air served as control, see Table 2 for the details of the odours delivered). No shift in either temperature or humidity was observed between the control and test periods for any treatment ($<0.1 \text{ RH}\%$, $<0.1^\circ\text{C}$).

Results

Blowing diluted breath (1,500 ppm CO₂ above ambient) over the ticks induced strong attraction (median 48%,

Table 1 Details of locomotion compensator and stimulus delivery

Component	Dimensions
Sphere diameter	500 mm
Vectors sampled	$>0.1 \text{ mm}$ every 0.2 s. From these, the displacement in a given direction, speed and turning angle were computed
Stimulus delivery tube	25 mm \times 15 mm positioned 35 mm from the sphere apex
Air flow at the sphere apex	0.18 m sec^{-1}
Radius of walk about the apex	1 cm
Temperature, RH	23–25°C, 70 RH%

Table 2 Details of test stimuli delivered

Treatment	Source (after 15 min equilibration)	Control	Concentration at sphere apex	Proportion of compound presented in diluted (30 \times) human breath
Diluted human breath	Freshly exhaled	Distilled water	1,500 ppb CO ₂ ^a	
CO ₂	20,000 ppm in O ₂	Charcoal-filtered air	60–1,500 ppb ^a	4, 17, 30 and 100%
NO	1 ppm in N ₂	Charcoal-filtered air	2.2 ppb ^b	450% (Leone et al. 1994)
Acetone	250 ng in 0.1 ml of paraffin oil on filter paper	0.1 ml paraffin oil on filter paper	7 ppb ^c	30% (Skrupskii 1995)
Isoprene	10, 100 ng in 0.1 ml of paraffin oil on filter paper	0.1 ml paraffin oil on filter paper	2.3, 23 ppb ^c	38 and 380% (Mendis et al. 1994)
NH ₃	0.0025, 0.25, 25% NH ₄ OH in 0.4 ml of distilled water on filter paper	0.4 ml distilled water on filter paper	0.04–400 ppb ^d	6, 600 and 60,000% (Norwood et al. 1992)

^a Concentration of CO₂ above ambient measured by an IR gas analyser ($\pm 2 \text{ ppm}$ resolution)

^b 2.2 ppb NO (the lowest dose deliverable) estimated by dilution in the main air stream

$n=16$, $P<0.0001$, Wilcoxon signed rank test; Table 3, Fig. 1a) with a short latency (median 6 s; Table 3). Diluted breath also modified the gait of the ticks in the air stream. In controls, the walk of ticks alternates between an eight-legged walk of constant speed and angular velocity, and a laboured six-legged walk at low speed (Fig. 2) and increased angular velocity. Diluted breath, however, induced long bouts of the eight-legged walk at increased ($>50\%$) speed in half of the animals tested (Figs. 1a and 2). When the valve delivering breath was shut, half of the ticks exhibited a local search in response to withdrawal of breath from the air (Table 3; $P=0.0009$; Fisher exact test).

Acetone caused the strongest responses among the breath components tested, in terms of attraction (median 35%, $n=17$, $P<0.0001$, Wilcoxon signed rank test), short latency (median 8 s) and decreased speed (median -26% , $n=17$, $P<0.0001$, Wilcoxon signed rank test; Table 3; Figs. 1b and 2). The injection of NO into the air stream elicited weaker attraction (median 5%, $n=21$, $P=0.0215$, Wilcoxon signed rank test) and a longer latency (median 10 s) but a similar reduction in speed to that induced by acetone (median -13% , $n=21$, $P<0.0001$, Wilcoxon signed rank test; Table 3; Figs. 1c and 2). These speed reductions were associated with the induction of the six-legged walk and increased angular velocity (median $+11\%$, $P=0.0079$ for acetone and $+8\%$, $P=0.0032$ for NO; Wilcoxon signed rank test). CO₂ attracted *A. variegatum* on the servosphere at both 60 ppm (median 16%, $n=19$, $P=0.0181$, Wilcoxon signed rank test; Fig. 1d) and at 250 ppm above ambient (median 6%, $n=27$, $P=0.0055$, Wilcoxon signed rank test). Higher concentrations (450 ppm and 1,500 ppm above ambient) of CO₂ tended to decrease any upwind displacement by the ticks ($J=3.389$, $P=0.0004$) and increase the response latency ($J=2.236$, $P=0.0185$, Jonckheere trend test; Table 3). This contrasts with the attraction observed towards diluted breath containing 1,500 ppm

^c Acetone and isoprene concentrations estimated by assuming complete volatilisation of both compounds in the gas-wash bottle

^d NH₃ concentration determined by Taneja and Guerin (1997) using the Tecator colorimetric method

Fig. 1 Sample tracks described by *Amblyomma variegatum* adults on the locomotion compensator in response to breath and three of its constituents: **a** human breath containing 1,500 ppm CO₂ above ambient after dilution in the stimulus air stream, **b** acetone (approx. 0.7 ppb), **c** NO (2 ppb) and **d** CO₂ (60 ppm above ambient). The tracks started (*bold circle*) with the ticks walking downwind (*open arrow*) in the control period; the *bold arrows* on the tracks indicate stimulus on and off; *bars beneath each track* represent a displacement of 20 cm. Note the shorter displacement elicited by acetone and NO compared with breath and CO₂, and the local search behaviour induced by withdrawal of breath from the air stream

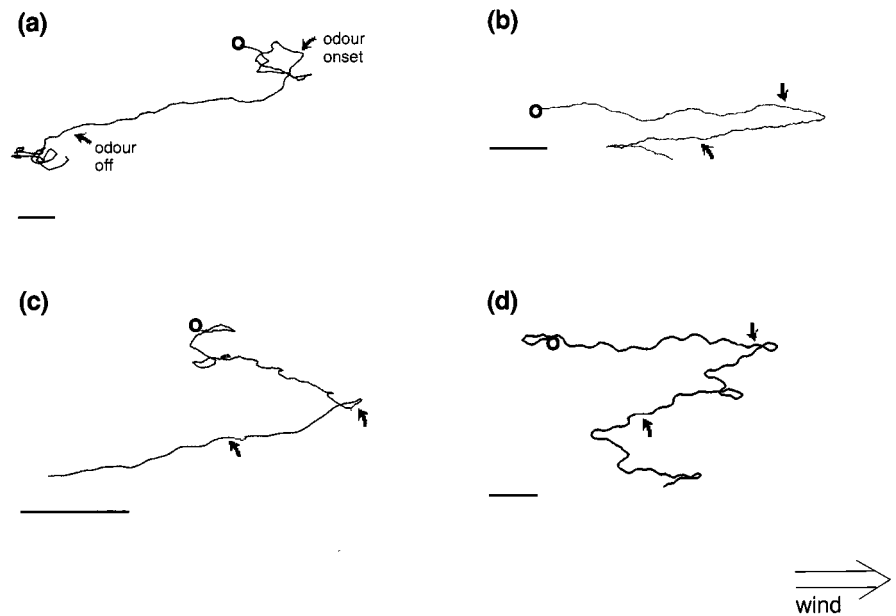


Table 3 Responses (latency, attraction, speed and local search behaviour, see text) of *Amblyomma variegatum* adults on the locomotion compensator to diluted breath and three of its constituents; acetone, NO and CO₂. The significance of attraction and change in speed for a treatment was calculated using the Wilcoxon signed rank test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Inter-treatment com-

parisons of latency, attraction and walking speed were made with the Wilcoxon-Mann-Whitney test, whereas comparisons of local search frequencies were carried out using the Fisher exact test. *Treatments sharing the same letter* for a given behaviour are not significantly different ($P > 0.05$)

Treatment	Ticks tested (<i>n</i>)	Latency		Attraction		Speed		Local search	
		Median time to enter upwind cone in test (s)		Median % difference in displacement		Median % change in speed		Proportion of ticks showing responses to stimulus removal	
Diluted human breath (1,500 ppm CO ₂)	16	6	a	+48***	a	5	d	9/16	a
Acetone (approx. 7 ppb)	17	8	b	+35***	ab	-26***	ab	2/17	b
NO (2 ppb)	21	10	bd	+5*	c	-13***	bc	0/21	b
CO ₂ (60 ppm)	19	12	abc	+16*	bc	-2	d	1/19	b
CO ₂ (250 ppm)	22	24	cd	+6**	c	-3	d	0/22	b
CO ₂ (450 ppm)	27	31	d	-14	d	7	d	1/27	b
CO ₂ (1,500 ppm)	21	34	cd	-13	d	12	d	0/21	b
Acetone + CO ₂ (approx. 7 ppb) (60 ppm)	17	7	ab	+39***	ab	-30***	a	3/17	b
NO + CO ₂ (2 ppb) (60 ppm)	16	16	cd	+8*	c	4	d	0/16	b
NO + CO ₂ (2 ppb) (1,500 ppm)	10	19	cd	-26*	d	2	d	0/16	b

Concentrations of CO₂, NO and acetone represent levels above ambient at the servosphere apex

CO₂ above ambient (Table 3), confirming that other breath components interact with CO₂ at the concentration it occurs in breath. No CO₂ concentration tested evoked a significant change in speed (Table 3; Fig. 2). However, the phenomenon of increased speed with an eight-legged walk was occasionally observed (5% of ticks) in response to the higher concentrations of CO₂ tested, as observed with diluted breath. Removal of acetone, NO or CO₂ did not induce local search behaviour to the extent induced by removal of breath (Table 3). Neither isoprene (approx. 2.3 and approx. 23 ppb; $n=12$ and 16, respectively) nor ammonia (0.04, 4 and 400 ppb, $n=12$, 16 and 10, respectively) elicited any change in behaviour.

Limited tests with binary mixtures failed to show any synergism or inhibition. Responses to acetone (approx. 7 ppb) were unchanged by the admixture of CO₂ (60 ppm above ambient; Table 3), and responses to two concentrations of CO₂ (60 ppm and 1,500 ppm above ambient) were unchanged upon admixture of NO (2.2 ppb; Table 3).

Discussion

We have demonstrated that diluted breath is an olfactory attractant for *A. variegatum* in the absence of other sensory modalities. None of the components or mixtures

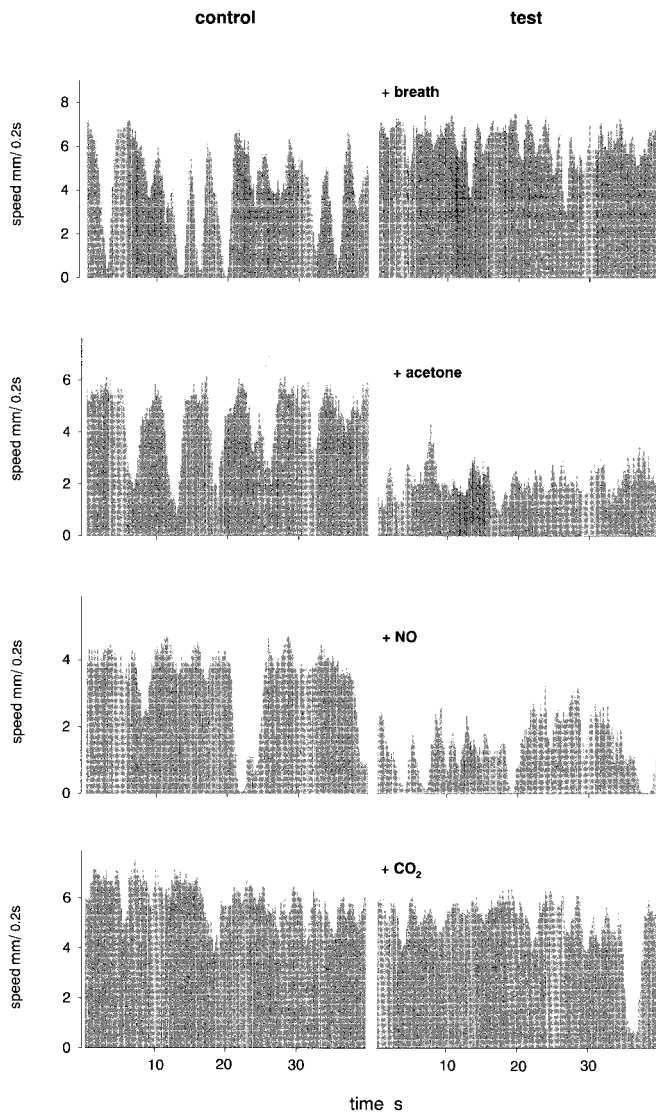


Fig. 2 The walking speed of the four ticks represented in Fig. 1 during the control and test periods in response to breath and three of its constituents on the locomotion compensator. Displacements of the ticks were sampled every 0.2 s and a running mean over five vectors was applied at 40 s intervals for graphical representation. Note the increased walking speed in the presence of breath (observed in half of the ticks presented with this stimulus), the contrastingly lower speed in response to acetone and NO, and the unchanged speed in the presence of CO₂

tested could match the response to breath in terms of latency, attraction and frequency of local search. Moreover, breath induced a smooth upwind walk to the source. It certainly appears that *A. variegatum* is better adapted to respond to the mixture that constitutes breath rather than to the single breath compounds tested. Nevertheless, individual breath components significantly affect tick behaviour. Acetone induced the greatest attraction after breath, yet also induced the largest reduction in speed and a higher angular velocity. These strong effects on behaviour, as well as the fact that exhalation is the primary excretory exit for this compound, suggest that it

is a key component. Acetone attracts tsetse and stable flies and, intriguingly, also induces an increase in angular velocity among these flying insects (Paynter and Brady 1993; Schofield and Brady 1997). NO was a weak attractant for *A. variegatum* yet significantly modified the walking speed of ticks on the servosphere. This small molecule has multiple physiological roles including insect olfactory processing (Müller and Hildebrandt 1995) and vasodilation in vertebrates (Ignarro et al. 1987). While NO elicited the same locomotor response in *A. variegatum* as acetone, its contribution to the response towards diluted breath is not clear. Admixture of NO to CO₂ (albeit at ratios much higher than those occurring in human breath) did not influence the response to CO₂. Moreover, unlike acetone or CO₂, the primary source of NO in vertebrates may be the reduction of sweat nitrate (Weller et al. 1996), where release from the sum of skin parts may exceed that of breath. It is therefore possible that NO is more important as a component of whole host odour than as a component of breath. The CO₂ concentration (45,000 ppm) of mammalian exhalations is constant (Lindstedt and Thomas 1994), representing a level some 100 times above ambient. For this reason, CO₂ can provide an important infochemical for most, if not all, haematophagous arthropods. In this study, however, CO₂ induced a relatively weak upwind displacement, only attracting *A. variegatum* at levels less than 450 ppm above ambient. Steullet and Guerin (1992a) have demonstrated the existence of a CO₂ receptor on the foreleg of *A. variegatum* that is sensitive to shifts in this range. Nevertheless, the lack of attraction to higher CO₂ levels is somewhat surprising in view of the success achieved with blocks of dry ice in trapping adults in the field (Barré et al. 1997). It may be that on the servosphere ticks were restricted to an air stream where the CO₂ concentration was roughly equal throughout, whereas in the field they have the freedom to orient upwind along the appropriate dose in the stimulus plume.

As breath is a complex mixture of several gases and more than a hundred volatiles (Krotoszynski et al. 1977), we cannot claim to have identified either the most important components or their interactions. Small apolar hydrocarbons predominate among the organic molecules present in breath due to a low partition coefficient that enables them to diffuse preferentially into the alveolar space. However, our results do not seem to suggest an olfactory role for such compounds. Isoprene failed to induce any change in the behaviour of *A. variegatum*. Furthermore, control gas-wash bottles containing paraffin as the solvent had no effect on the walking ticks even though the headspace in such bottles contained nanogramme quantities of several short- and long-chain hydrocarbons (C. McMahon and P.M. Guerin, unpublished). We also could not find activity for NH₃ despite the presence of sensory units for this product in *A. variegatum* (Steullet and Guerin 1994). NH₃ has been reported to attract triatomine bugs (Taneja and Guerin 1997) and to elicit leg-waving behaviour in the ixodid tick *Rhipicephalus sanguineus* (Haggart and Davis 1980). It

may be that presentation of NH₃ with other chemicals is necessary in order to elicit a behavioural response in *A. variegatum*, as shown by Geier et al. (1999) for mosquitoes.

Based on the modifications of *A. variegatum* behaviour reported here, we conclude that diluted breath is a strong attractant and represents the most adequate stimulus we have tested. Human breath tested under the same conditions also attracts two ixodid ambush tick species, *Ixodes ricinus* and *Rhipicephalus appendiculatus*, and the argasid tick, *Ornithodoros moubata* (C. McMahon and P.M. Guerin, unpublished). The evolution of resource tracking in this group of ectoparasites thus appears to have included sensory and behavioural adaptations for recognition and orientation to host metabolites that are regularly exhaled. Nevertheless, what we have recorded may not represent the maximal response of ticks to breath. Certain components, such as acetone, vary considerably with the state of the host (Skrupskii 1995). In addition, odours from other body parts may further modify the effects of breath.

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