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Attractive and repellent host odours guide ticks to their respective feeding sites

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Summary. We have studied on-host behaviour of adults of the brown ear tick (*Rhipicephalus appendiculatus* Neumann, 1901) and the red-legged tick (*R. evertsi* Neumann, 1897), which prefer to feed mainly inside the ears and the anal regions of bovids respectively. Both species were found to be relatively successful in orienting toward and locating their respective feeding sites from different parts of the host body. Our observations suggested the operation of both avoidance (closer to the feeding site of the other) and attraction (closer to its own feeding site) responses of the ticks. In the laboratory, odour trapped from cattle ears attracted *R. appendiculatus* but repelled *R. evertsi*, whereas that from the anal region had an opposite effect. This odour-based 'push-pull' pair of stimuli may largely account for efficient orientation behaviour of the two tick species to their respective feeding sites. We propose that such concurrent deployment of repulsive and attractive cues may be quite widespread among arthropods and related organisms that specialise on specific hosts or microenvironments in the performance of their biological functions.

Key words. *Rhipicephalus appendiculatus* – *R. evertsi* – feeding sites – cattle – repellent and attractive odours – push pull

Introduction

Blood-feeding arthropods have evolved a variety of relationships with their mammalian hosts. Wide variations occur in host specificity, duration and multiplicity of contacts, and in host-location behaviour (Gibson & Torr 1999). On host, related species may also demonstrate predilection for feeding at different sites. Although the signals used by some blood-feeders to locate their preferred hosts have been a subject of considerable research, only casual attention has been given to feeding site location behaviour of relevant arthropods. In this study, we report on-host behaviour of adults of two sympatric tick species, the brown ear tick (*Rhipicephalus appendiculatus* Neumann, 1901) and the red-legged tick (*R. evertsi* Neumann, 1897), which prefer to feed mainly inside the ears and the anal regions of bovids respectively (Walker 1974).

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Material and methods

All procedures requiring experimental animals were approved by ICIPE's Institutional Animal Care and Use Committee and were performed in compliance with guidelines published by Kenya Veterinary Association and Kenya Laboratory Animal Technician Association.

Ticks

The two tick species used (*R. appendiculatus* and *R. evertsi*) were obtained from colonies at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. Rearing conditions and management were as described previously (Bailey 1960; Irvin & Brocklesby 1970).

On-host observation studies

On-host behaviour studies used one of two Friesian steers (body wt. 217 – 470 kg), with no prior exposure to ticks, held in a cratch facility at ICIPE. The responses of ticks and their navigation patterns were monitored from six different body locations (Fig. 1a and b) of Friesian steers representing varying distance from preferred feeding sites and possible areas of alightment by the ticks from their questing positions on the vegetation (Browning 1976). One tick at a time of mixed age and either sex was introduced at one of six sites and observed for up to 8 hours. *R. appendiculatus* were observed from 0700 hr to 1800 hr and *R. evertsi*, which is active at night, between 1800 and 0600 hr. All observations were made under dry weather with day temperatures in the range 24-28 ºC and night temperatures in the range 20-24 ºC. Each observation was replicated twenty times.

Odour trapping

These were carried out using adsorbent sachets (4 × 2.5 cm) made up of stainless mesh-wire (250 mesh) containing either activated charcoal (0.5 g, 0.2 µg mesh; Chromopack, Middelberg, The Netherlands) or reverse-phase C_{18}–bonded silica (0.2g, 16-40 µm size; Sigma Aldrich Chemicals, Dorset, UK) similar to the prototype described previously (Gikonyo *et al*. 2002). The sachets were held on the inner side of ear pinna or the anal region with rubber bands for 12 hr. The adsorbent from each sachet was transferred into a Pasteur pipette and eluted with redistilled dichloromethane (4ml, > 99.9 %). Eluents from 60 trapping cycles were pooled, concentrated and stored at –20 ºC until used for bioassays. For bioassays, aliquots (2 mg) of the concentrate was taken up in dichloromethane (25 ml) to give ~80 ppm solution.

2-choice climbing assay

A set up consisting of an aluminium base (15 × 7 × 1.5 cm) with a pair of aluminium rods (26 cm l × 0.7 cm d) 7 cm apart covered

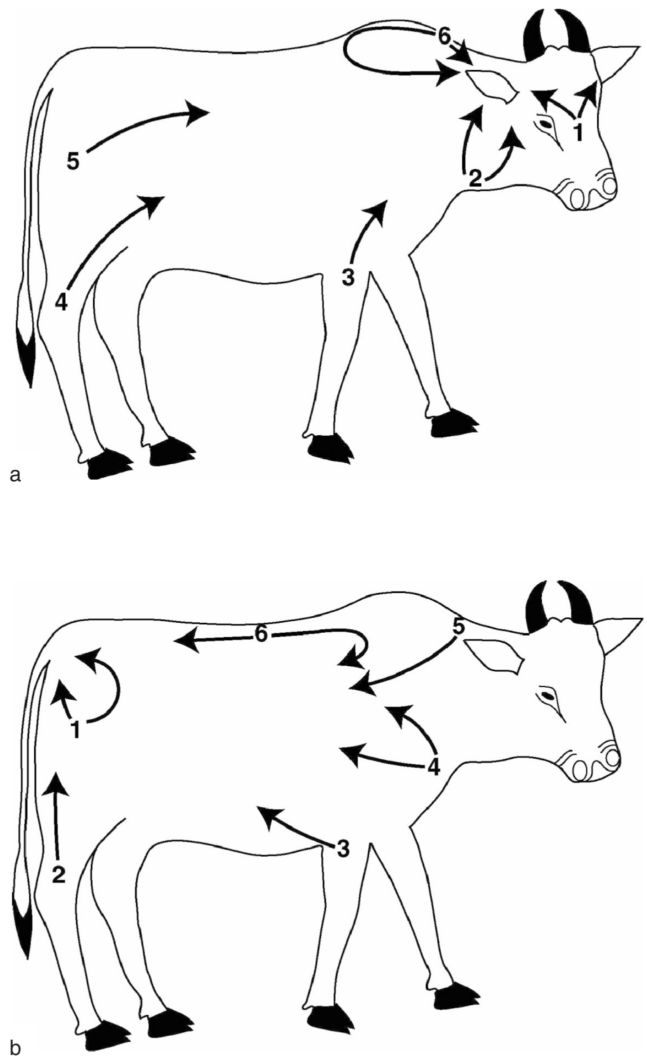

Fig. 1 General orientations of *R. appendiculatus* (a) and *R. evertsi* (b) that initiated movements from different release points (1-6)

with glass tubes (0.8 cm d) was used (Fig. 2). A strip of filter paper (Whatmann N0. 7, 2 cm wide) was stapled to form a collar around the upper parts of each tube to provide the source of test odours. One collar on the pair of tubes was treated with test odour solution and the other with the solvent (dichloromethane) to serve as control. After the solvent was allowed to evaporate (10min), these tubes were shielded with wider tubes (4.5 cm d) from 3 cm above the aluminium base to facilitate relatively uniform vertical gradients of the test odours along the 3.7 am gap between two tubes. Wet cotton wool plugs on the top of these tubes ensured relatively high humidity (>75 %) within the columns. Ticks of mixed age and sex (5) were placed at the centre of the aluminium base and observed for 60 minutes. The apparatus was placed in a tray with shallow water, which prevented the dispersal of test ticks from the base. Initial comparison of the responses of the ticks in the set up with and without residual dichloromethane on one and both sides, showed no bias for either side and no effects of the residual solvent. Each assay was replicated 12 times. The number of ticks that climbed on treated and control columns were counted. Mean % attraction or repellency (–ve attraction) was calculated using the formula $N_t–N_c/N_t+N_c \times 100$, where Nt and Nc represent the number of ticks that climbed the treated and control glass tubes, respectively.

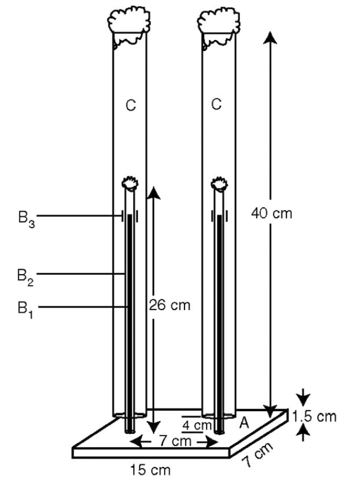

Fig. 2 Tick climbing bioassay apparatus (placed in a tray with shallow water, not shown): A, aluminium base; B_1, aluminium rod (26 cm l × 0.7 cm d); B_2, 0.8 cm d glass tube; B_3, filter paper collar; C, 4.5 cm d glass tube

Statistical analyses

On host data were analysed by analysis of variance (ANOVA) using the general linear model (GLM) procedure for SAS for PC (SAS Institute, 1999 – 2000), after log (n + 1) transformation. The means were compared by Students-Newman-Keuls test (Sokal & Rohlf 1995) at $P = 0.05$. 2-choice assay data were analysed by Student's *t*-test.

Results and discussion

On-host observations of the ticks showed a typical sequence of behaviour involving: (i) a stationary phase at the release point, which was accompanied by outstretching of legs and adoption of a posture suggestive of scanning activity; (ii) random, seemingly exploratory movements; (iii) a clear directional movement resulting from bouts of strides and halts, interspersed with mis-turns and readjustments; and (iv) gradual arrestment closer to the feeding site followed by attachment. Interestingly, following initial random movements most of the respondents at each release point oriented toward their respective feeding sites (Fig. 1a and b), although, during the observation period, some appeared subsequently to lose their way. The proportion of ticks at different sites that initiated movement (respondents), the average time from placement on the steer to initial movements (reaction time), and initial speed were noted. The number of ticks that reached their feeding sites and the time between release and arrival were also recorded (Table 1a and b).

The results show relatively high rates of successful orientation of the ticks to their respective feeding sites and suggest the mediation of specific stimuli in the process. We first hypothesized that gradients of volatile odours from these sites may provide the appropriate orientation signals. Although this would account for lower responses (proportion of respondents, their reaction time and initial speed)

Table 1 On host responses of ticks released at different body sites of Friesian steers

Body locations	% Respondents	Reaction time (hr)	Initial speed (cm/min)	% Reaching feeding site	Time taken to reach feeding site (hr)
(a) *R. appendiculatus*					
1. Forehead	100	0.13 ± 0.03[a,b]	2.00 ± 0.49[a,b]	95	3.74 ± 0.66[a,b]
2. Dewlap	80	0.58 ± 0.22[b,c]	1.02 ± 0.25[b,c,d]	40	3.78 ± 0.95[a,b]
3. Fore leg (upper part)	45	0.84 ± 0.40[c]	0.93 ± 0.31[c,d]	15	(2.28 ± 0.56)
4. Rear leg (upper part)	50	0.72 ± 0.25[c]	0.80 ± 0.13[d]	20	(5.21 ± 1.41)
5. Escutcheon	85	0.06 ± 0.02[a]	2.12 ± 0.42[a]	65	5.15 ± 0.59[a]
6. Shoulder	85	0.24 ± 0.10[a,b]	1.96 ± 0.43[a,b]	80	3.21 ± 0.52[a,b]
(b) *R. evertsi*					
1. Escutcheon	100	0.03 ± 0.01[a]	2.77 ± 0.21[a]	85	3.31 ± 0.53[c]
2. Rear leg (upper part)	100	0.09 ± 0.06[a]	1.79 ± 0.28[b,c]	90	5.71 ± 0.76[b,c]
3. Fore leg (upper part)	80	0.78 ± 0.39[b]	1.06 ± 0.13[c,d]	35	5.17 ± 1.02[b,c]
4. Dewlap	55	1.64 ± 0.66[c]	0.95 ± 0.22[d]	20	(9.24 ± 0.86)
5. Shoulder	70	0.08 ± 0.07[a]	3.04 ± 0.24[a]	60	6.51 ± 0.81[a,b]
6. Back	100	0.08 ± 0.06[a]	2.09 ± 0.40[b]	75	5.41 ± 0.71[b,c]

For a given column, means with the same superscript are not significantly different from one another at P = 0.05 (Students-Newman-Keuls test). Figures in parentheses in the last columns were not included in the statistical comparison because of the relatively low number of the ticks that arrived at the feeding sites

of ticks deposited on locations further away from the sites (upper rear and fore legs for *R. appendiculatus*; upper fore leg and dewlap for *R. evertsi*), the behaviour of each species closer to the feeding site of the other suggested an additional effect (Table 1a and b). For *R. appendiculatus*, this is reflected in a relatively large proportion of the ticks that took off from the upper rear region within a significantly short time (0.06 ± 0.02 hr from escutcheon, compared to 0.72 ± 0.25 hr and 0.84 ± 0.40 hr from the upper rear and fore legs, respectively) at relatively high speed (2.12 ± 0.42 cm/min) comparable to conspecifics placed on the forehead close to the ears (2.00 ± 0.49 cm/min). Likewise, the reaction time and initial speed of *R. evertsi* deposited at the shoulder closer to the ear (0.08 ± 0.07 hr and 3.04 ± 0.24 cm/min, respectively) were not significantly different from those deposited at the escutcheon near the anal region (0.03 ± 0.01 hr and 2.77 ± 0.21 cm/min, respectively). Significantly, ticks of both species that were placed closer to the feeding site of other moved away and none were seen to navigate into the area during the observation period, indicating that local stimuli like temperature and/or humidity were unlikely to be primarily responsible and that the ticks were probably exhibiting an avoidance response at the sites. Accordingly, we modified our hypothesis to include the possibility of concurrent operation of both repellent and attractant effects ('push' and 'pull') in on-host orientation behaviours of the two tick species.

To verify this, we studied the responses of the ticks in the laboratory to odour collections from the ear and anal region of the steers, respectively. The bioassay design exploited the well-known predisposition of the ticks to climb up and aggregate on grass stems to await passing hosts (Browning

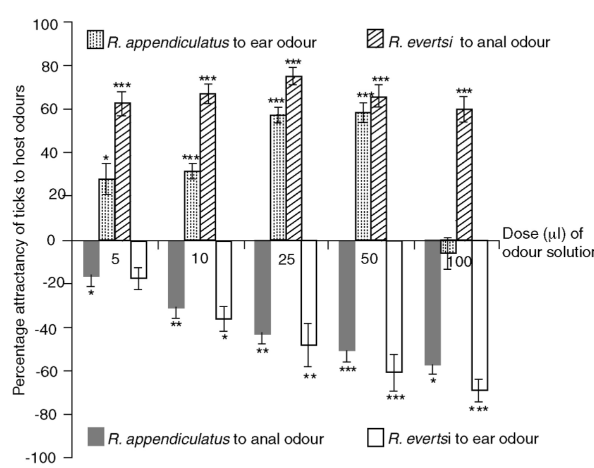

Fig. 3 Mean percentage attractancy or repellency of ear and anal volatiles to *R. appendiculatus* and *R. evertsi* in climbing bioassays (*, ** and *** indicate statistical significance at P = 0.05, 0.01 and 0.001, respectively)

1976; Chiera 1985). A choice of two glass covered rods, one with a vertical concentration gradient of the test odour and the other with clean air was offered to groups of ticks. *R. appendiculatus* was attracted to the ear volatiles but repelled by anal volatiles (Fig. 3). On the other hand, *R. evertsi* was repelled by the ear volatiles, but attracted to the anal volatiles. Thus, the odour collections from the two sites have opposite effects on the two tick species and support our hypothesis on the operation of both repellent and attractant effects in the feeding site location behaviours of these ticks.

However, at their respective feeding sites, other signals may also be involved in site selection process. Our observations show that closer to their respective feeding sites, the ticks are gradually arrested before finally attaching for feeding, suggesting the mediation of non-volatile and perhaps specific biophysical stimuli at these sites. Further studies on the physical and chemical characteristics of these sties are needed to elucidate the factors associated with their selection by the two tick species for feeding.

The concept of integrated use of the forces repulsion (or deterrence) and attraction (or stimulation), i.e. 'push-pull', was previously proposed as an efficient and sustainable way of diverting insect pests from a desired crop to a trap crop (Miller & Cowles 1990). Recently, it was effectively deployed in reducing damage by stem-borers to maize (*Zea mays* L.) in smallholder farms in Africa (Khan *et al*. 2001). The present study represents the first demonstration of the exploitation of the tactic in nature, and suggests that the phenomenon may be quite widespread among arthropods and related organisms, particularly where specialisation occurs in their interactions with their hosts or environmental niches in functions such as selection and location of hosts, feeding and oviposition.

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