

Walking by *Ixodes ricinus* ticks: intrinsic and extrinsic factors determine the attraction of moisture or host odour

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Summary

Spontaneous walking by *Ixodes ricinus* nymphs in the horizontal plane was investigated within choice arenas to test whether these ticks move preferentially up a humidity gradient, and whether they are attracted towards host odour, specifically volatile substances from the back of a dog's ear. Responses by ticks with high and low fat contents and in different states of hydration were compared. Ticks with higher energy reserves were more likely to walk horizontally over short distances. Only if

they were slightly dehydrated were they more likely to walk towards fully saturated air than drier air, and only if the atmosphere was sufficiently wet were they likely to walk towards odour secreted by host skin. It seems that, under certain circumstances, ticks of this ambushing species will move short distances towards host odour.

Key words: tick, *Ixodes ricinus*, walking, humidity gradient, host kairomone.

Introduction

Although ticks are parasites that depend entirely on their vertebrate hosts for their liquid food, Ixodid species spend >90% of each generation off the hosts, out of reach of this energy source. Blood meals are restricted to one per life stage (larva, nymph and adult), during which a wide variety of pathogens may be transmitted. Contact between ticks and hosts is achieved by active hunting (periodic rapid forays from sheltered sites) by a few genera (e.g. *Hyalomma*, *Amblyomma*), by waiting in nests or lairs (e.g. *Ixodes hexagonus* Leach, the hedgehog tick), or by ambushing a passing animal from a vantage point on the vegetation (e.g. *I. ricinus* L.). In the latter case, host questing behaviour carries the penalty of increased rates of water loss so that these ticks must return to the moist litter layer periodically where they can reabsorb water from atmospheres above a certain humidity level (Knulle and Rudolph, 1982; Lees, 1946; Macleod, 1935). Thus locomotion is principally in the vertical plane, while horizontal displacement is conventionally thought to be very limited. Recent work by Perret et al. (Perret et al., 2003), however, revealed that *I. ricinus* nymphs walk far greater distances than was previously imagined. Continuous automated observations of ticks constrained within vertical channels revealed walking up and down the channels over distances of several metres, most often after periods of quiescence, which was interpreted as the equivalent of horizontal displacement. Most walking occurred soon after the onset of darkness, and ticks walked further after quiescence when subjected to increasing moisture stress. In field experiments, *I. scapularis* Say nymphs dispersed

average distances of 2–3 m, and adults >5 m, over a period of several weeks, apparently by their own locomotion (Carroll and Schmidtman, 1996).

Many terrestrial invertebrates move up humidity gradients towards moisture, for obvious reasons, and a range of Ixodid tick species respond to certain host-produced substances (kairomones). Carbon dioxide, for example, stimulates some tick species to move, sometimes towards the source, e.g. *Amblyomma variegatum* Fabricius, *A. hebraeum* Koch (Anderson et al., 1998; Barré et al., 1997; McMahon and Guerin, 2002; Norval et al., 1989), although not *Ixodes* spp. to any great extent (Lavender and Oliver, 1996; Schulze et al., 1997). Eruptions of volatile rumen metabolites from ungulates also attract *Amblyomma* and *Ixodes* species (Donzé et al., 2004). In contrast, substances from glands on the legs of deer and the dorsal surface of dogs' ears elicit an arrestant response so that ticks (*Dermacentor variabilis* Say, *Amblyomma americanum* L. and *I. scapularis*) aggregate in places associated with host presence (Carroll, 2002; Carroll et al., 1995; Carroll et al., 1996).

In this study we set out to answer the following questions arising from these observations: to what extent do *I. ricinus* ticks walk spontaneously in the *horizontal* plane, and do they do this to seek the two resources that are crucial for their survival, water vapour and/or hosts? Furthermore, do they move at random, or do humidity gradients and/or volatile host odours direct their walking? We also investigated whether ticks behaved differently depending on the level of their energy (fat) reserves and state of hydration, as both these factors affect the

tick's critical equilibrium humidity, the atmospheric humidity at which water efflux and influx are balanced (Lees, 1946).

Materials and methods

Tick collection and maintenance

Unfed *I. ricinus* nymphs were collected on 12th May 2004 from the Clarendon Estate in Wiltshire, southern England (~1°43'W, 51°33'N) by conventional blanket-dragging. In the laboratory they were stored in 2×8 cm tubes closed with gauze, held over saturated magnesium sulphate solution in closed vessels to maintain an atmosphere of 90% relative humidity (RH) (Winston and Bates, 1960). Three groups of ticks were exposed to different conditions. Group 1, high-fat, fully hydrated: ticks were kept in the dark within a refrigerator at 4°C. Group 2, high-fat, semi-dehydrated: as group 1, but the day before use 100 ticks were exposed to 50% RH at 24°C for 4 h, followed by 80% RH at 4°C for 8 h overnight and finally 80% RH at 24°C for 1 h before being introduced to the arena. Group 3, low-fat, fully hydrated: immediately after collection, ticks were held at room temperature (~22°C), exposed to the natural light:dark regime for May–June, over a 4-week period in order to induce a lower fat content (Randolph and Storey, 1999), before being returned to the refrigerator until use from July onwards.

To verify that the fat content of the high-fat and low-fat groups of ticks did indeed differ, samples of 40 ticks from each of groups 1 and 3 were subjected to the standard method of lipid content estimation (Randolph and Storey, 1999). Briefly, the ticks were dried, individually weighed, immersed in three changes of chloroform for 24 h each, re-dried and re-weighed to the nearest 0.1 µg.

Experimental design

Four choice arenas each consisted of two transparent polystyrene boxes (17.5 cm×12 cm×5 cm) with tight-fitting lids, joined by an acetate tunnel (3.8 cm diameter, 28 cm long) sealed into holes cut in one side of each box. Humidity was measured with a Squirrel data logger (Grant Instruments Ltd, Cambridge, UK), using a probe placed within different parts of the arena. Placing a small dish of water in one box and leaving the other box empty produced a realistic even humidity gradient of 97–67% RH within 1 h, changing to 100–79.5% after 24 h; At the ambient temperature within the experimental room (20°C), this is equivalent to a saturation deficit (SD) of 5.65–3.51 mmHg at the dry end [$SD=(1-RH/100)4.9463e^{0.0621T}$ where RH is percentage relative humidity and T is temperature (°C) (Randolph and Storey, 1999)]; this is similar to midday summer conditions measured 30 cm above ground level in woodland (S. Randolph, unpublished observations). Host odour was collected by rubbing the back of a dog's ear thoroughly with a 4 cm×4 cm piece of absorbent medical gauze that was then sealed in a polythene bag and chilled until used within 24 h. The gauze was placed in one box of the arena at the same time as the ticks were introduced, after which the apparatus was sealed for the duration of each experiment.

For each replicate, a set of 20 nymphs was placed into a 1 cm×3 cm clear plastic tube with both ends closed by gauze held in place by elastic bands. A length of cotton thread was attached to each piece of gauze. These tubes were returned to the humidity chambers within the refrigerator for several days before use. To start an experimental run, one tick-laden tube was slipped into the centre of each arena tunnel with the cotton threads passing out through the closed box lids. The arena was then left undisturbed for 1 h, after which the threads were pulled very gently to remove the gauzes from each end of the tube. The gauzes, with any ticks on them, were left close to the tube. Following Perret et al.'s observations (Perret et al., 2003) that most ticks started to walk after the onset of darkness, for the first hour after the ticks were free to move the lights were left on, but then switched off, leaving only a red safety light to allow observations. The positions of the ticks (whether in the tunnel or the box) were scored at 1, 2 and 24 h post-release. More frequent and prolonged (up to 48 h) pilot observations had revealed that well over half the ticks that left the introduction tube had moved to their final position within the first couple of hours; ticks were not observed to move to and fro along the arena, and the pattern of the distribution of ticks within each arena did not change beyond 24 h.

Each of the following conditions (a-d; not in alphabetical order, match those in Figs 1 and 2) was replicated four times simultaneously for each of the group 1 (high-fat) and group 3 (low-fat) ticks by using the four arenas; two arenas were set at right angles to the other two, and the test conditions were arranged at opposite ends within each orientation. (a) Humidity gradient (see above) with no host odour, to test the hypothesis that *I. ricinus* nymphs move up a humidity gradient. (c) Host odour gradient in a uniformly dry atmosphere (67% RH, 5.65 mmHg SD), and (d) host odour gradient in a uniformly wet atmosphere (100% RH, zero SD), to test the hypothesis that *I. ricinus* nymphs respond to host kairomones. (b) Humidity gradient plus host odour gradient, with the host odour placed at the dry end, to test whether *I. ricinus* nymphs move towards host odour despite the risk of desiccation or *vice versa*. Group 2 (semi-dehydrated high-fat) ticks were exposed only to condition a, using 25 nymphs per replicate.

Statistical analysis

The response of each tick was treated as independent because nymphal *I. ricinus* ticks are not known to interact in such a way as to influence the onset of each other's walking activity, and ticks did not aggregate in clusters that would indicate the operation of assembly pheromones or con-specific arrestment stimuli (Grenacher et al., 2001; Guerin et al., 2000; Sonenshine, 2004). Furthermore, within each of the combinations of environmental and physiological conditions, all four replicates could be combined because there was no significant difference between replicates in the distribution of ticks between each half of each arena (binary logistic regression analysis: $0.925 > P < 0.078$), apart from the group 1 high-fat ticks in condition c above when the percentage of

nymphs counted in the odour half varied from 25 to 83% ($P=0.001$).

Two distinct responses were examined by binary logistic regression, using the GENMOP procedure in SAS, within each of two analyses. Analysis A (no odour, condition a above): taking fat content as a categorical predictor, response 1 – the probability that ticks moved at all, response 2 – the probability of moving towards high humidity. Analysis B (odour at one end, conditions b, c and d above): taking fat content and moisture conditions as categorical predictors, response 1 – the probability that ticks moved at all, response 2 – the probability of moving towards the odour. Where appropriate, simple χ^2 tests were also performed.

Results

Fat contents

The fat content of the group 3 ticks (0.0039 ± 0.0021 mg, mean \pm s.d.) was lower than that of the group 1 ticks (0.0047 ± 0.0027 mg). The difference in absolute fat content was not statistically significant, but was not markedly less than the difference between samples of ticks collected from a site in the same region of the UK in May and June 2000 (0.0011 mg difference) (Randolph et al., 2002). Fat content is positively correlated with tick size as measured by its fat-free (reduced) dry mass. When corrected for size (fat/reduced dry mass), the group 3 ticks did indeed have a significantly lower mean fat content than group 1 ticks (Student's t -test=2.033, $P=0.039$, d.f.=74).

Walking activity (response 1)

Between 40 and 100% of each set of 20 fully hydrated ticks (groups 1 and 3) walked out of the introduction tube within 24 h. A higher proportion of high-fat than low-fat ticks had walked by 24 h, and statistically this difference was almost significant in the absence of odour (Fig. 1A) (83.75% vs 71.25%, $\chi^2=3.51$, $P=0.06$), and strongly significant with the larger sample sizes in the presence of odour (Fig. 1B–D) (74.17% vs 63.75%, $\chi^2=6.08$, $P=0.014$). There was no effect of moisture conditions and no interaction between moisture and fat levels. (The data are presented in Fig. 1 as means per replicate with standard deviations, rather than overall percentages based on individual ticks, to show the modest degree of variation between replicates.) Amongst the four sets of 25 high-fat semi-dehydrated ticks (group 2), on average 84% (range 72–92) walked out of the introduction tube within 24 h.

Amongst the fully hydrated ticks, the difference in activity between high-fat and low-fat groups typically (but not invariably – see Fig. 1C) started within 1 h of the ticks' release. As time passed, more ticks were counted in the boxes while the numbers within the connecting tunnel stayed more constant; by 24 h the ratio of ticks in the boxes and tunnel (2:1 for both high- and low-fat ticks) did not reflect the fourfold greater surface area of the boxes. This indicates that up to one-third of the walking ticks stopped in the tunnel before reaching the boxes and did not move up and down the arena stopping at random.

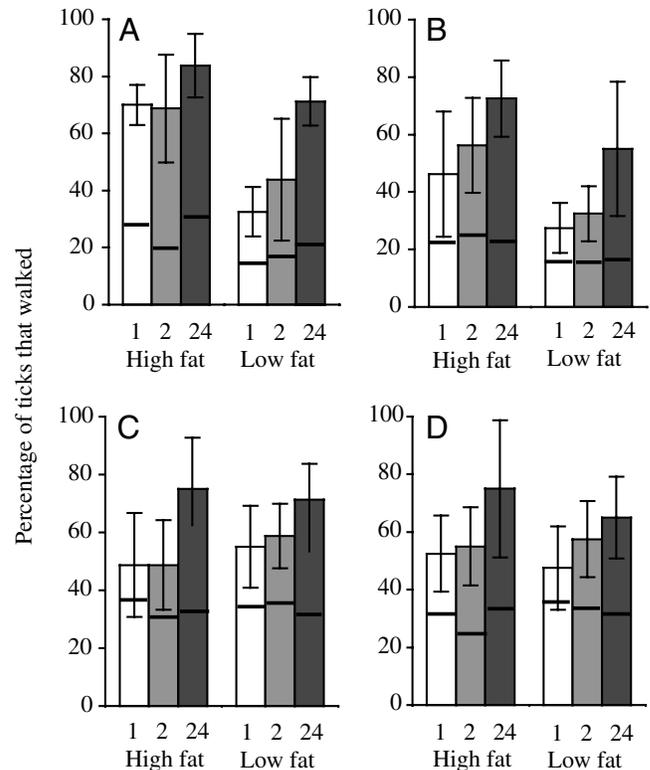


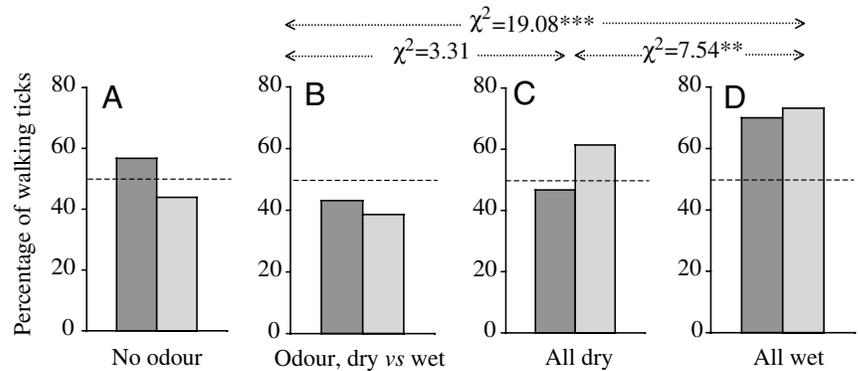
Fig. 1. The percentage of high- and low-fat ticks that walked out of the introduction tube after 1 (white bars), 2 (light grey bars) and 24 h (dark grey bars). Each column shows the mean (\pm s.d.) over the four replicates under each of four experimental conditions: (A) the wet–dry gradient with no odour; (B) the wet–dry gradient with odour at the dry end; (C) uniform dry conditions with odour at one end; and (D) uniform wet conditions with odour at one end. The horizontal lines across each column show the proportion of ticks in the connecting tunnel (lower half) and the end boxes (upper half).

Direction of movement (response 2)

In the absence of odour, there was no significant difference in the proportion of the fully hydrated ticks (groups 1 and 3) that moved towards the dry or wet end of the arena, and no effect of fat content on the direction of movement (Fig. 2A) ($\chi^2=2.03$, $P=0.15$). There was no effect of moisture conditions or fat levels on the proportion of ticks that walked all the way to the boxes (59–72%) rather than stopping in the connecting tube. Of the 84 semi-dehydrated high-fat (group 2) nymphs that walked when exposed to the same humidity gradient, a significantly greater proportion (52=62%) had moved towards the wet end (with 45 reaching the box) than had moved towards the dry end (32, with 26 to the box) ($\chi^2=4.76$, $P<0.05$).

The ticks' response to odour was significantly affected by moisture conditions (Fig. 2B–D). Only in the uniformly wet arena did a greater proportion of ticks walk towards the odour source, and this was true of both high-fat (70%) and low-fat (73%) ticks (Fig. 2D). As fully hydrated ticks showed no strong preference for high humidity, or for host odour in dry conditions, the lack of a significant directional response towards odour despite the risk of desiccation (Fig. 2B) is not

Fig. 2. The percentage of high-fat (dark stipple) and low-fat (light stipple) ticks, of those that walked out of the introduction tube after 24 h, counted over all four replicates within one half of the arena (box plus connecting tunnel). (A) Ticks in the wet half of the arena (no odour). (B–D) Ticks in the odour half of the arena, where B has a wet–dry gradient with odour at the dry end, C has uniform dry conditions with odour at one end, and D has uniform wet conditions with odour at one end. χ^2 values show comparisons between moisture conditions. The broken horizontal lines indicate an equal distribution of ticks between each half of the arenas.



surprising [$\chi^2=3.18$ (not significant), for combined high-fat and low-fat ticks]. Whatever the moisture conditions and fat levels, a higher proportion of the ticks that walked towards the odour went all the way to the box (70–82%), rather than stopping in the connecting tube, than those that walked in the opposite direction (50–69%) ($\chi^2=4.20$, $P<0.05$).

Discussion

Walking is costly in terms of energy, and all that we know of *I. ricinus* ticks suggests that they limit such activity. In the automated observations by Perret et al. (Perret et al., 2003) of long-distance walking by *I. ricinus* nymphs constrained within vertical channels, walking occupied only 6.6% of the 10 days of continuous observations. Within the 24 h periods of observations of spontaneous walking by ticks in the horizontal plane reported here, 27% of ticks did not walk at all, consistent with observations that periods of quiescence or questing may persist for over 20 h, even several days (Lees and Milne, 1951; Perret et al., 2003). Of those that did walk, 30–50% did not walk beyond the 14 cm length of one half of the connecting tube of the arena, although any deviation from the straight line would have added distance. This is less than the median vertical displacement (both up and down) of 43 cm (max 9.7 m) after periods of quiescence and 17 cm (max 2.9 m) after questing, with the former positively correlated with SD (Perret et al., 2003). Our ticks were probably not fully quiescent as defined by Perret et al. (immobile for more than 2 h within 2 cm of the wet base of the vertical tracks) when released from the introduction tubes. Nor were they subjected to the extreme atmospheric dryness (9.3 mmHg SD) that was associated with the longest vertical distances. The positive geotropism shown by *I. ricinus* at SD >4.4 mmHg (Macleod, 1935), however, introduces an additional driving force in the vertical plane not present in our horizontal arrangement. It is not known how far ticks walked within the boxes, but prolonged walking round and round was not seen during continuous pilot observations. Limited dispersal also applies to *I. scapularis*: in a field experiment, the majority of nymphs were captured 2–3 m from a release point within 2 or 3 weeks, with a very few reaching 5–6 m by weeks 3–4 (Carroll and Schmidtman, 1996).

Under what circumstances, therefore, do *I. ricinus* ticks

undertake horizontal displacement, as opposed to moving simply in the vertical plane between questing and re-hydration sites in the field? The simple laboratory arena did not take account of the vegetation structure in natural field conditions, where ticks must traverse surfaces that are at many different angles (including vertical) in order to move even short horizontal distances. Nevertheless, the results presented here suggest that ticks of this ambushing species (as opposed to ‘hunting’ species) do not walk about entirely at random, responding to stimuli thus encountered by chance, but rather are stimulated to walk, and to choose their direction, by both intrinsic and extrinsic factors. If questing ticks persistently fail to contact hosts, conserving any remaining energy is clearly an essential part of their sit-and-wait strategy, but ticks that do not move may be caught in a downward spiral towards starvation. Our results indicate that ticks with lower energy reserves were indeed less likely to walk, yet overall 66% of even the low-fat nymphs (with energy levels similar to field ticks in June, at least 4 months after the start of the questing season in southern England) did move horizontally over short distances. Intrinsic moisture conditions of ticks also exerted an influence. Whereas a mild degree of dehydration did not stimulate greater walking activity (comparing like with like, high-fat ticks in the absence of host odour), it did increase the probability of ticks moving up a humidity gradient. Biologically, this is as would be expected.

Furthermore, under conditions of uniformly high humidity both high-fat and low-fat ticks walked towards a source of host odour. At present, we can only speculate as to why high humidity promoted this response. The odour, consisting of unidentified volatile compounds secreted from the back of a dog’s ear, might have been expected to saturate the whole arena over the 24 h period of observation (Waladde and Rice, 1982). Possibly a high atmospheric vapour content impeded the dissemination of the molecules thereby creating a stronger gradient, or alternatively aided the detection of the molecules by the tick’s olfactory sensilla within the Haller’s organ (Guerin et al., 2000; Leonovich, 2004). This accords with recent observations that *I. ricinus* is attracted to volatile rumen metabolites (Donzé et al., 2004), which would reach ticks within a naturally moist air stream. Alternatively, *I. ricinus* might be more responsive to host odour when the moisture stress of walking is less, which is consistent with Perret et al.’s

observation (Perret et al., 2003) that most ticks start to walk after a period of quiescence in moist conditions near the base of the vertical channels and with the onset of darkness, i.e. at night, although this effect was not seen under the cruder light conditions used here (Fig. 1). Whatever the precise cause of these humidity effects, these results show that under certain circumstances secretions from dog skin can act as a kairomone (a host-produced substance that stimulates tick appetite behaviour), but do so by attracting ticks to move towards the source, rather than merely acting as an arrestant on contact as shown for other tick species (Carroll, 2002). The fact that extrinsic moisture conditions had no effect on locomotory activity *per se* (both the probability of walking and the probability of going as far as the box when in the odour half of the arena) shows that the accumulation of ticks in the odour half of the arena in high humidity was not due to increased random walking and therefore increased chance of encountering the host odour followed by akinesis. Odour from dog's skin appeared to be acting as an attractant for *I. ricinus*, as indicated by laboratory results for *I. persulcatus* and *I. scapularis* using a T-shaped olfactometer (Dobrotvorsky et al., 1999). In a field experiment, when *I. scapularis* adults were released centrally within a 2 m diameter circle of upright wooden skewers, within 2 weeks they accumulated on skewers anointed with substances rubbed from the external glands on the legs of white-tailed deer to a significantly greater extent than on control skewers (Carroll et al., 1996). Deer secretions could have been acting as an attractant, but these observations do not exclude the possibility that the adult *I. scapularis*, which can walk >4 m within 2 weeks (Carroll and Schmidtman, 1996), ascended both types of skewers but only stayed (i.e. were arrested) on the anointed skewers. Given the low effectiveness of CO₂ traps in attracting *Ixodes* spp. in the field (Schulze et al., 1997), it is possible that host secretions are more important than exhaled breath in stimulating these ticks to walk.

In conclusion, the results presented here indicate that nymphal *I. ricinus* with higher energy reserves are more likely to walk horizontally, but only if slightly dehydrated are they more likely to walk towards fully saturated air than drier air, and only if the atmosphere is sufficiently wet are they likely to walk towards odour secreted by host skin. It seems that, under certain circumstances, ticks of this ambushing species will move short distances towards odours associated with their hosts.

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