

IXODID TICKS AVOID CONTACT WITH LIQUID WATER

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Summary

Larvae of the cattle tick *Boophilus microplus* and all life stages of the European sheep tick *Ixodes ricinus* avoid walking on a wet membrane surface surrounding a dry patch. Of 170 reactions made at a border with liquid water by 22 *B. microplus* larvae, 40 % consisted of immediate turns to the opposite side to bring all the legs back onto a dry patch, 41 % were walks along the border, during which the ticks maintained contact with both the dry and wet zones, and 19 % were returns to the dry patch after a short excursion onto the wet surround. Since contact with one front leg tip was sufficient to cause return reactions from the wet surface in most of the border contacts, the water

receptor(s) that enable ticks to perceive the wet surface are probably located in terminal pore sensilla on the first-leg tarsi. Observations on the return reactions of ticks with different groups of chemosensilla masked confirmed this. Ticks have an ambiguous relationship with water: they appear to avoid direct contact with it, but they need a high humidity to compensate for any deficit in body water.

Key words: tick, *Boophilus microplus*, *Ixodes ricinus*, water reception, hygroreception, water balance, desiccation, contact chemoreception, behaviour.

Introduction

Ticks belong to the early land arthropods and are all obligate feeders on vertebrate blood. Depending on the type of host association, ticks can be divided into two groups: endophilic species, which live in the nests or burrows of their hosts and so can feed regularly, and exophilic species, which live in exposed habitats (forest, savannah or meadow) actively hunting or ambushing their hosts. The latter are well equipped to perceive chemical and physical stimuli. The highly developed first pair of legs, serving as functional antennae, carry the dorsally placed Haller's organ bearing numerous sensilla. These sensilla house chemoreceptive cells responding to volatiles from hosts and congeners (Diehl et al., 1991; Steullet and Guerin, 1992a,b, 1994a,b; Price et al., 1994). The ventral side of the legs, especially the tip of the tarsus and the palps, carries contact chemosensilla with a terminal pore (Elizarov, 1963; Waladde, 1978; Hess and Vlimant, 1986).

For exophilic species, periods of questing and exposure to dehydration often alternate with periods of rehydration to recompense their body water deficit (Lees, 1948; Knülle and Rudolph, 1982), and this behavioural pattern can repeat itself many times during the life cycle. Information about ambient humidity is therefore important for ticks to regulate their water balance (Lees, 1946, 1948). No recordings have been made from hygroreceptors in ticks, but there is morphological evidence that hygroreceptors similar to those of insects (Altner et al., 1977) are present in Haller's organ sensilla (Waladde and Rice, 1982; Hess and Vlimant, 1986).

To conserve water, quiescent ticks have a pattern of regular discontinuous ventilation, a state that is replaced by rapid ventilation and an increased metabolic rate during active uptake of water in dehydrated individuals placed at high humidity (Fielden and Lighton, 1996). Furthermore, ticks will actually orientate towards zones of sufficiently high humidity (Lees, 1948; Hair et al., 1975), as do other terrestrial arthropods such as isopods (Sorensen and Bell, 1986). This was confirmed in a recent detailed study on the European tick *Ixodes ricinus* by Kahl and Alidousti (1998), who showed that adults and nymphs with a water deficit will approach a water drop, although they avoid contact with the liquid; the same phenomenon was observed for *Boophilus microplus* larvae (Krijgsman, 1937). In the high humidity near the drop, the ticks can recompense any water deficit by active absorption of water vapour using a hyperosmotic salivary gland secretion around the hypostome (Gaede and Knülle, 1997). Some authors suggest that the absorption of such 'distilled' water, instead of drinking ground water in which pathogens circulate, helps the tick to avoid infection (Kahl and Alidousti, 1998). In addition, ticks do not attach to feed on wet surfaces on the host such as the lips and mouth cavity, nose, eyes and anus, where attachment is highly problematic because of the degree of disturbance to the host. To test whether this avoidance behaviour is due to the wetness of the surface, we studied how different life stages of tick species behaved on a dry patch surrounded by a water-saturated surface. We found that the ticks returned systematically to the

dry surface each time they encountered the wet surround with their front legs.

Materials and methods

Ticks

Boophilus microplus (Canestrini), the cattle tick, was obtained from Novartis Research, St Aubin, FR, Switzerland, where they were reared on cattle. Tick larvae were held in an environmental cabinet under long-day conditions of 10h:10h, 28 °C, 85 % relative humidity separated by 2h ramps representing dawn and dusk. Larvae of *B. microplus* used in experiments were 2–10 weeks old. To dehydrate ticks, 6-week-old *B. microplus* larvae were held for more than 24 h at 25–35 % relative humidity (Schuntner and Tatchell, 1970).

Nymphs and adult *Ixodes ricinus* L. were captured in the wild during spring and summer near Neuchâtel and held at 14 °C/95 % relative humidity in the dark. Larvae of the same species were obtained from a laboratory culture. All instars of *I. ricinus* were kept at ambient temperature and daylight at 95±5 % relative humidity in a closed container.

Bioassay

Tests with individual ticks were designed to quantify their behaviour at the border between a dry patch and a wet surround. A polycarbonate Millipore membrane (Isopore HTTP, 10 µm thick), with a zone treated with silicone glue and stretched over a water surface, was used to create a clear border separating dry and wet surfaces. This hydrophilic membrane soaks up liquid water through 0.4 µm diameter pores perpendicular to the surface. The presence of water on the matt membrane surface was demonstrated first by lightly sweeping the surface with a dry brush, which induced a shiny water film, and then by showing that a glass capillary (160 µm diameter, 1 µl micropipette) brought into contact with the membrane took up approximately 0.5 µl of water within 30 s.

One part of the membrane was rendered impermeable to water by covering the side facing the water with silicone glue (Wacker Elastosil N10, München, Germany). The outline of the patch was marked with a graphic pen (0.1 mm) and left to dry for more than 12 h. The diameter of this circular dry patch was adapted to the life stage of the tick, 10 mm for *B. microplus* and *I. ricinus* larvae and 20 mm for *I. ricinus* nymphs and adults. In addition, a concentric surround (2.5 mm wide for *B. microplus* and *I. ricinus* larvae, and 5 mm wide for *I. ricinus* nymphs and adults) was marked to delimit the experimental area. To study the alternative scenario, i.e. the reaction of the tick to encountering a dry patch when coming from a wet surface, an experiment with a dry ring on the membrane was made using *B. microplus* larvae. This arena consisted of three zones, the wet central patch (10 mm diameter), a dry ring (5 mm wide) surrounding it and a wet surround (2.5 mm wide).

The membrane, supported by a fine plastic grid (1 mm), was stretched over a cylindrical polystyrene reservoir (50 mm diameter, 20 mm high) glued to a glass sheet 8 mm thick. The membrane was held with adhesive tape between the bevelled

wall of the reservoir and a second plastic cylinder (53 mm diameter, 50 mm high), forming an upper chamber surrounding the experimental surface (Fig. 1). The reservoir was filled with demineralised water, and the bioassay system was placed on a warm plate. By heating the reservoir, the temperature at the membrane surface was maintained at 32±2 °C. Water evaporating during the experiment through untreated sections of the membrane created a layer of high humidity (99–100 % relative humidity) 1 mm above the surface. Because of the temperature drop towards the air in the test chamber (22 °C), the relative humidity 1 mm above the membrane at the centre of the dry patch varied from 65 to 95 %. Water evaporating through the membrane was replaced using a plastic syringe (50 ml) connected *via* silicone tubing to the reservoir (Fig. 1).

In a control experiment in which the patch and the surround were both dry, a membrane with a central silicone patch was fixed onto a glass plate and heated to 32±2 °C, as above. High humidity (80–95 % relative humidity) in a Perspex chamber (30 cm×15 cm×23 cm; width × depth × height) surrounding the heating plate was ensured by lining the walls of the chamber and the surface of the plate beside the membrane with water-soaked filter paper.

Temperature was measured using a digital thermoprobe (BAT12, Sontek Inc., Clifton, NJ, USA) and humidity using a hygrometer (Hygro-AirII, Schiltknecht Messtechnik, Gossau, ZH, Switzerland).

Recording of tracks

To analyse the details of tracks, the experimental surface was filmed full-screen (field of view 36–42 mm in the diagonal) from above using a black-and-white video camera (Panasonic WV-BP310) equipped with an Optem zoom (no. 65 No 19 91 91, Optem International, Fairport, NY, USA; working distance 45 cm) or with the camera connected to a

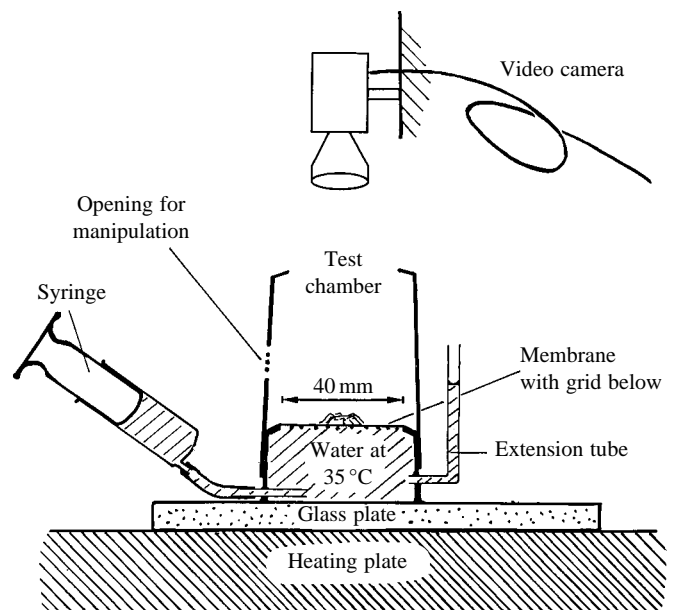


Fig. 1. Bioassay system with test chamber, heating plate and video camera (for details, see text).

Zeiss operating microscope (working distance 25 cm) to observe with accuracy the position of the tick. Cold light made the tick clearly visible as a dark silhouette on the homogeneous light background of the membrane. Recordings were made on an S-VHS video recorder (Panasonic AG7350) for playback, and the video signal was fed simultaneously through a frame grabber (PC Vision Plus, Imaging Technology Inc., Woburn, MA, USA) into a video-tracking and motion-analysis software system (EthoVision, former VTMS, V1.01 to V1.6, Noldus Technology b.v., Wageningen, NL) running on a personal computer (486DXII, 66 MHz). The coordinates of the centre of the animal's body, for tracking analysis purposes termed the 'centre of gravity', were determined using a spatial resolution of 254 pixels \times 254 pixels, corresponding to 0.096–0.198 mm in the x direction and 0.063–0.132 mm in the y direction, depending on the diameter of the test arena. Sampling the position of the tick at approximately double its step frequency (2.94 samples s^{-1}) gave the most accurate representation of the track and served to smooth the primary zigzag of the gait. To estimate accurately the distance moved by the larvae and their velocity, every second coordinate was sufficient. *I. ricinus* nymphs and adults walked faster, so every coordinate was used. Small movements, with a vector length of less than 0.5 mm, recorded when the tick slowed down or stopped were accommodated by summing them until the resultant vector exceeded 0.5 mm. However, such adjustments never contributed to more than 5% of the track length since ticks walked steadily.

Experimental procedure

A naive tick, already moving in the holding container (above), was transferred using a fine-haired brush through a hole in the wall of the plastic cylinder surrounding the experimental surface. The trial started as soon as the tick was released close to the middle of the experimental arena and ended when the centre of gravity of the tick passed the outer circle delimiting the test arena.

Coordinates of 20–26 error-free recordings per experimental condition were analysed, and the duration on the dry patch, the length of the whole track and the velocity on the dry patch were calculated for each individual. The medians of these values for each test situation were compared using the Mann–Whitney U -test. Encounters made by *B. microplus* larvae with the wet surround with at least one front leg were counted from the video tape.

Masking sensory organs of *Ixodes ricinus* nymphs

For this experiment, we used only nymphs that had reacted upon first contact at the border of a dry 20 mm diameter patch with a wet surround. Haller's organ alone, Haller's organ and the tips of the palps or the ventral first-leg tarsus and the tips of the palps of these ticks were covered with a small droplet of synthetic resin glue (Konstruvit, Geistlich, Wolhusen, LU, Switzerland) to mask the sensilla. The animals were allowed at least 10 min to adapt to the new situation before being tested. Border reactions of masked *I. ricinus* nymphs upon their first

contact with the wet surface were characterised as (1) straight walks off the dry patch, (2) leaving the dry patch after stopping at the border, and (3) returning at the border to the dry patch. Only track data for animals in which the masking glue was still in place after the trial were analysed. Ticks that succeeded in removing the synthetic covering of the tarsal sensilla were used as controls to assess whether masking altered their behaviour. Comparisons of the border reactions of the ticks before and after masking, and among different groups of ticks, were made using Fisher's exact test.

Results

Hydrated ticks, i.e. *B. microplus* larvae and all life-stages of *I. ricinus*, walking on a dry membrane surface showed a strong response to encountering a wet surround (shown for *B. microplus* in Fig. 2A,B). *B. microplus* larvae coming from the dry patch met the border of the wet surface and put at least one

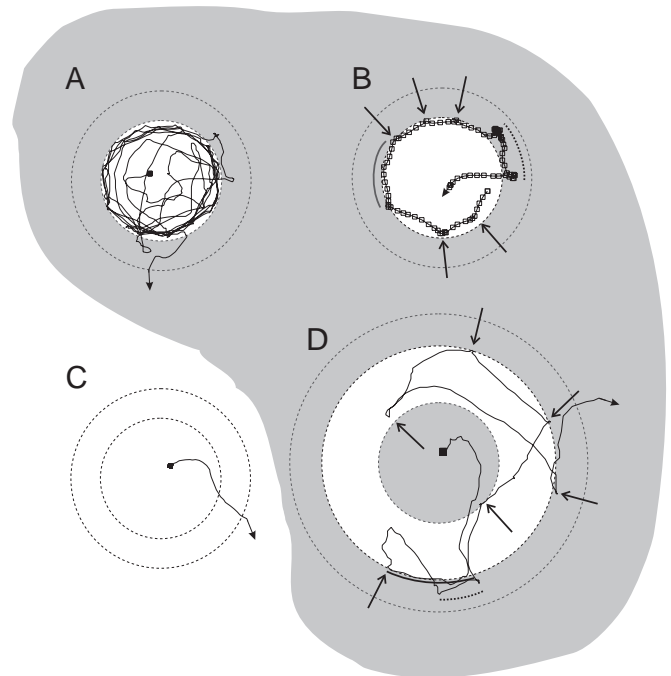


Fig. 2. Examples of tracks described by *Boophilus microplus* larvae on a dry patch with a wet (A,B) and dry (C) surround and a dry ring with wet surrounds (D). The tick was released at the black square, and the recording terminated when the tick passed the outer circle. (A) All encounters with the border in this recording, except the last, led to turns back to the dry surface (duration of recording 232 s). (B) A section (79 s) of the recording shown in A demonstrates that brief turns at the border (arrows), walks astride the border (bold arc) and short excursions onto the wet surface (dotted line) contribute to the response. (C) Walks made by larvae on a completely dry surface (control) are rather short (10 s). (D) Ticks coming from a wet surface stay on a dry ring surrounded by a wet surface (77 s). The symbols are as in B. The dry patch in A–C is 10 mm in diameter, surrounded by a 2.5 mm ring; the wet patch in D is 10 mm in diameter, the dry ring is 5 mm wide with a 2.5 mm wide wet surround; wet surfaces are shaded; drawings are to scale.

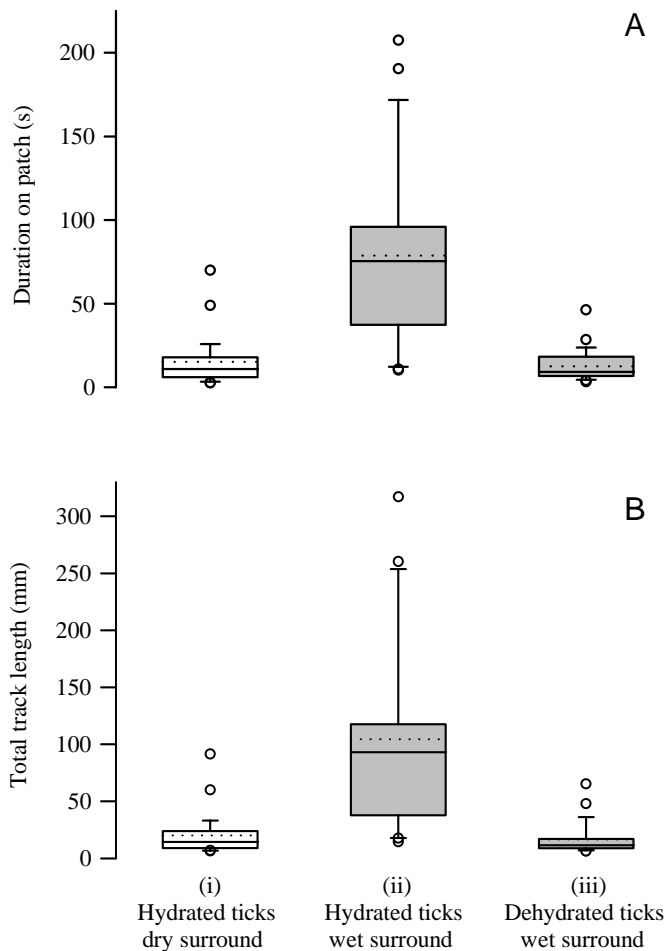


Fig. 3. Time spent (A) and total length of tracks (B) of hydrated *Boophilus microplus* larvae on a dry patch with a dry (i) and a wet (ii) surround. Data are also given for dehydrated larvae on a dry patch with a wet surround (iii). The limits of the boxes indicate the twenty-fifth and seventy-fifth percentiles, the solid line in the box is the median, the dotted line is the mean, the capped bars indicate the tenth and the ninetieth percentiles, and data points outside these limits are plotted as circles; groups were significantly different (Mann–Whitney *U*-test, $P \leq 0.0001$), except for the behaviour patterns of the hydrated ticks on the dry surround and the dehydrated ticks on the wet surround ($P \geq 0.7$); $N=25$ for dry, $N=22$ for wet, $N=22$ for wet dehydrated.

front-leg tarsus down on the wet surround. From 170 border contacts made by 22 tick larvae, the following return reactions were observed. (1) The simplest reaction (40%) was an immediate turn to the opposite side after unilateral contact by one first leg with the wet surface, which brought all the legs of the tick back onto the dry patch. (2) In 41% of border contacts, the tick rotated partially, so that the unilateral contact with the wet surface was maintained; such ticks walked for some time astride the border and returned to the dry patch later. (3) In 19% of border contacts, the tick left the dry patch for the wet surround but returned to the dry patch after a short excursion there (Fig. 2B). All three types of return reaction brought the tick larvae with all legs back onto the dry surface. Rather

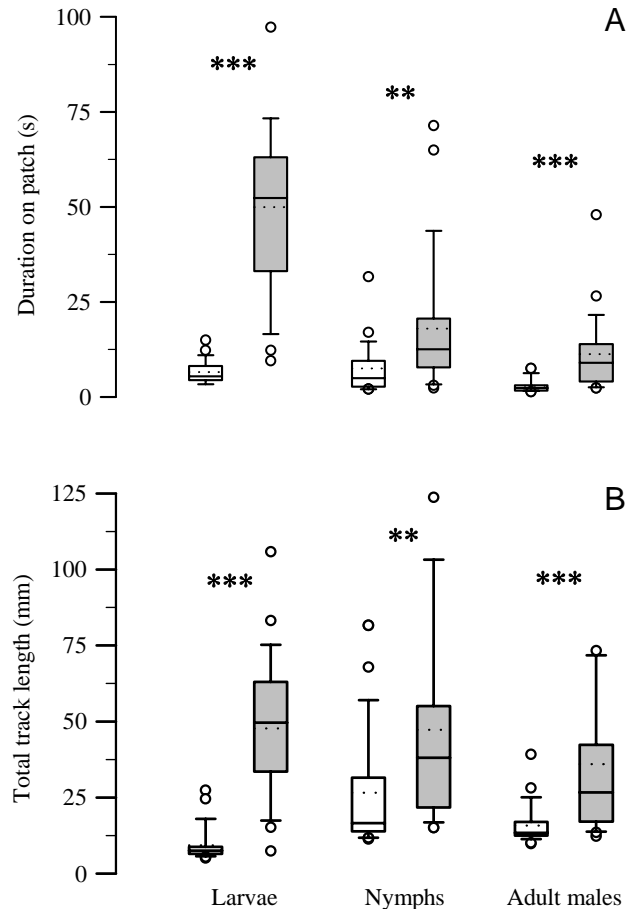


Fig. 4. Time spent (A) and total track length (B) of all stages of hydrated *Ixodes ricinus* on a dry patch with a dry surround (open boxes) and on a dry patch with a wet surround (shaded boxes). The patch is 10 mm in diameter with a 2.5 mm wide surround for larvae and 20 mm in diameter with a 5 mm wide surround for nymphs and adult males. Comparisons were made between the responses to dry and wet surrounds for each stage: ** $P \leq 0.001$; *** $P \leq 0.0001$, Mann–Whitney *U*-test. Box plot limits are as in Fig. 3.

straight walks on the dry patch alternated with distinct turns at the border, and a complete circle was made with between 7–8 border contacts, interrupted by periods of walking astride the border (Fig. 2B). With time, the border reaction ceased, i.e. *B. microplus* larvae came to accept walking on the wet surround and finally left the experimental arena. In contrast with these border responses, *B. microplus* larvae walking from a dry patch to a surround that was dry (control) walked straight off (for an example, see Fig. 2C). The number (n) of returns at the border determined the time (t) a tick larva spent on the dry patch, and these parameters are correlated ($t=11.9+1.4n$, $r^2=0.9$, $P \leq 0.0001$ for slope, *F*-test, $N=22$). Consequently, the overall track lengths on the dry patch with the wet surround were approximately seven times longer (median 93 mm, range 14–317 mm, $N=22$, Fig. 3Aii,Bii) than the track lengths of the uniformly dry controls with a silicone-treated patch (median 14 mm, range 6–91 mm, $N=25$, Mann–Whitney *U*-test, $P \leq 0.0001$, Fig. 3Ai,Bi). Dehydrated *B. microplus* larvae



Fig. 5. The larval stage of the cattle tick *Boophilus microplus* bears contact chemosensilla on the tips of the legs (black arrow) and on the mouthparts (white arrow). This front view is taken from a video recording in which the sensilla on the palps are protruded.

behave like hydrated larvae on a dry surround in that they walked off the dry patch, ignoring the wet border (Fig. 3Aiii,Biii).

The tracks of hydrated instars recorded on the central wet patch surrounded by a dry ring confirmed that it was the wet surface that was avoided. After walking onto the dry ring, *B. microplus* larvae stayed there by returning every time the wet surfaces on either side were encountered (Fig. 2D). The phenomenon of avoidance of a wet surface was also shown by all instars of hydrated *I. ricinus*: larvae, nymphs and adult males spent more time and made longer walks on a dry patch with a wet surround than on a dry silicone-treated patch with a dry surround (Fig. 4).

The behaviour of the three groups of *I. ricinus* with masked sensilla at the border with the wet surface was significantly different from their behaviour before masking (Fisher's exact test, $P \leq 0.001$). Approximately half of each group of ticks walked straight off (Fisher's exact test, $P \geq 0.6$, Table 1). The highest proportion of returns to the dry patch occurred when

only Haller's organ was masked. Covering both the palps and Haller's organ with glue did not reduce the number of ticks recognising the border compared with masking Haller's organ alone. When both the palps and the ventral tarsus were masked, ten of the individuals that walked off showed a different reaction at the border compared with those with a masked Haller's organ in that they stopped before walking onto the wet surround (Fisher's exact test, $P \leq 0.01$). Of 17 ticks that succeeded in removing the glue from the ventral or dorsal leg tips, 14 reacted to the border, i.e. their reaction was not significantly different from their response before masking (Fisher's exact test, $P \geq 0.2$; Table 1).

Discussion

Ixodid ticks of different life stages avoided walking onto a wet surface surrounding a dry patch. The border response following the encounter with a wet zone consisted of one of three reactions: immediate turns to the opposite side to bring all their legs back onto the dry patch; walks for some time along the border, during which the ticks maintained contact with both the wet and dry zones; and returns to the dry patch after a short excursion onto the wet surround. The shape of the patch ensured that the ticks were forced into repeated contact with the water. Changes in the behavioural pattern of the ticks with time allowed them to limit their time on the dry patch (T. Kröber and P. M. Guerin, unpublished), such that the ticks eventually accepted walking onto the wet surface and finally left the experimental arena. Insect and mites reacting at the borders of a resource display the same response pattern as the returns demonstrated here by ticks to avoid water (Strand and Vinson, 1982; Casas, 1988; Waage, 1978).

When a tick encountered the border between the dry and the wet surface, it was confronted with liquid water on the membrane and a high relative humidity in the air immediately above it. Since contact with one front-leg tip was sufficient to cause a strong return reaction away from the wet surface for 40% of the border contacts, the sensory organs that permit the ticks to perceive this are probably located on the first-leg tarsi.

Table 1. Behavioural reaction of masked *Ixodes ricinus* nymphs after the first contact with a wet surround at the border of a circular dry patch (diameter 20 mm)

Masked region	Total <i>N</i>	Number walking straight off onto wet surround	Number stopping at border followed by walking off onto wet surround	Number returning at border to dry patch
Haller's organ	24	14	1	9
Haller's organ and palps	20	13	4	3
Ventral tarsal tips and palps	24	10	10	4
Ticks that removed the glue from their tarsi	17	3	2	12

Haller's organ, the ventral tarsal tips of the first-leg tarsi and the mouthparts (palpal organs) were covered with resin glue.

In all three cases, half the ticks left the dry patch and walked onto the wet surround (Fisher's exact test, $P \geq 0.001$ compared with the behaviour before the masking). The quality of the border reaction changed when, instead of Haller's organ, the contact chemosensilla of the first-leg tarsi and palps were masked (Fisher's exact test, $P \leq 0.01$), i.e. instead of returning at the border, the ticks stopped briefly and then walked off the dry patch.

Gustatory terminal-pore sensilla orientated ventrally at the tip of the tarsi in all life stages of the ixodid ticks studied here (Hess and Vlimant, 1986; Thonney, 1987) may house water-receptor cells. Such sensilla also occur in groups of 8–12 on the palps (Jorgensen, 1984, Waladde, 1976, 1977; Fig. 5). Masking both the ventral tarsal sensilla and the palps resulted in a return by only 20% of the ticks at the border. Masking the palps in addition to Haller's organ had no additional effect on behaviour at the border compared with that resulting from the masking of Haller's organ alone. This suggests that sensory organs located outside Haller's organ and the palpal organ are involved in the perception of liquid water.

Pore-tipped hairs on the ventral tarsal surface of another arachnid, the sand scorpion *Paruroctonus mesaenis*, enable it to turn and to locate sources of liquid water (Gaffin et al., 1992). The use of similar types of hairs on tick tarsi to avoid liquid water may also have survival value. During their quiescent overwintering period at temperate and northern latitudes, ticks need to stay away from water in the litter zone to avoid inoculative freezing induced by contact with ice crystals (Dautel and Knülle, 1997). Indeed, throughout their period of activity on the ground, even dehydrated ticks need to avoid contact with liquid water because they may be susceptible to infection by circulating pathogens (Kahl and Alidousti, 1998).

There is evidence from the masking experiments that Haller's organ may be involved in the perception of zones of high humidity. In addition to receptors for liquid water, arthropods also have receptors that can detect water vapour, such as the moist and dry receptors occurring in various sensilla on the antennae of insects (Altner and Prillinger, 1980) and the legs of arachnids (Tichy and Loftus, 1996). During its regular foraging for water, the sand scorpion uses a combination of receptors sensitive to both liquid water and water vapour (Gaffin et al., 1992). Ultrastructural studies suggest that, in addition to olfactory receptors, hygroreceptors may also occur in Haller's organ sensilla on the dorsal aspect of the first-leg tarsi in ticks (Hess and Vlimant, 1986). Half the ticks that normally recognised the border between the dry and wet surfaces walked straight onto the wet surround when their Haller's organ was covered with glue. In contrast, 40% of the ticks with ventral tarsal and palpal sensilla masked, i.e. with Haller's organ still exposed, stopped before walking onto the wet surround and occasionally waved their front legs in the air in a manner reminiscent of normal questing for air-borne stimuli by ticks. This involvement of Haller's organ in the perception of water vapour may have accounted for the avoidance of zones of high humidity by *I. ricinus* nymphs (Lees, 1948) and the orientation to water droplets shown by dehydrated *I. ricinus* (Kahl and Alidousti, 1998).

The water balance state of the ticks strongly influenced the border response: dehydrated ticks did not avoid the wet surface. Lees (1948) also observed that the strong avoidance of high humidity in unfed *I. ricinus* females disappeared with progressive desiccation. The ambiguous relationship of ticks with water, i.e. their need for water vapour to maintain their

water balance while actually avoiding contact with liquid water, may be of value for survival.

Dehydrated *I. ricinus* approach water droplets to recover their body water deficit, but do not contact them (Kahl and Alidousti, 1998). In any case, ticks show an affinity for hydrophobic surfaces such as the cuticle of congeners when aggregating (Wilkinson, 1953), the sebum-covered pelage of hosts and the waxy cuticle of plants that act as ambush sites. Water may occur on the host in the form of sweat containing salts (Shirreffs and Maughan, 1997; Lentner, 1984) and, when saturated salt solutions were presented below the membrane used here, the border reactions shown by the ticks disappeared (T. Kröber and P. M. Guerin, unpublished observations).

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