

IMPRIMATUR POUR LA THESE

**Wasservermeidung und Verhaltensreaktionen auf
Stimuli der Wirtsoberfläche bei Schildzecken
(Ixodidae)**

de M. Thomas KROEBER

UNIVERSITE DE NEUCHATEL

FACULTE DES SCIENCES

La Faculté des sciences de l'Université de
Neuchâtel sur le rapport des membres du jury,

MM. P. Guerin (directeur de thèse),
P.-A. Diehl, J. Bouvier (St-Aubin FR) et
W. Haas (Erlangen D)

autorise l'impression de la présente thèse.

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IXODID TICKS AVOID CONTACT WITH LIQUID WATER

T. KRÖBER AND P. M. GUERIN*

Institute of Zoology, University of Neuchâtel, Rue Emile Argand 11, Ch-2007, Neuchâtel, Switzerland

*e-mail: patrick.guerin@zool.unine.ch

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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, hygrometry, 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 hygrometry receptors in ticks, but there is morphological evidence that hygrometry receptors 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, Sontortek 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

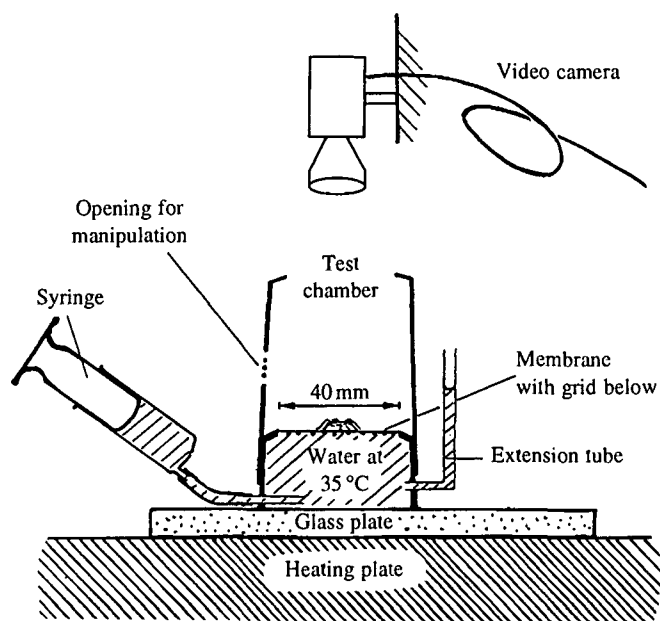


Fig. 1. Bioassay system with test chamber, heating plate and video camera (for details, see text).

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, 66MHz). 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

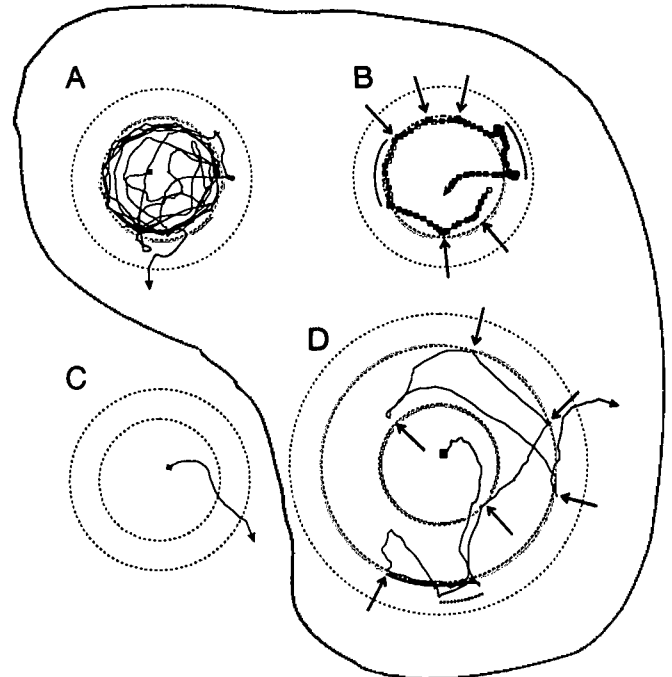


Fig. 2. Examples of tracks described by *Boophilus microplus* larvae on a dry patch with a wet (A,B) and dry (C) surround and a dry ring with wet surrounds (D). The tick was released at the black square, and the recording terminated when the tick passed the outer circle. (A) All encounters with the border 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.

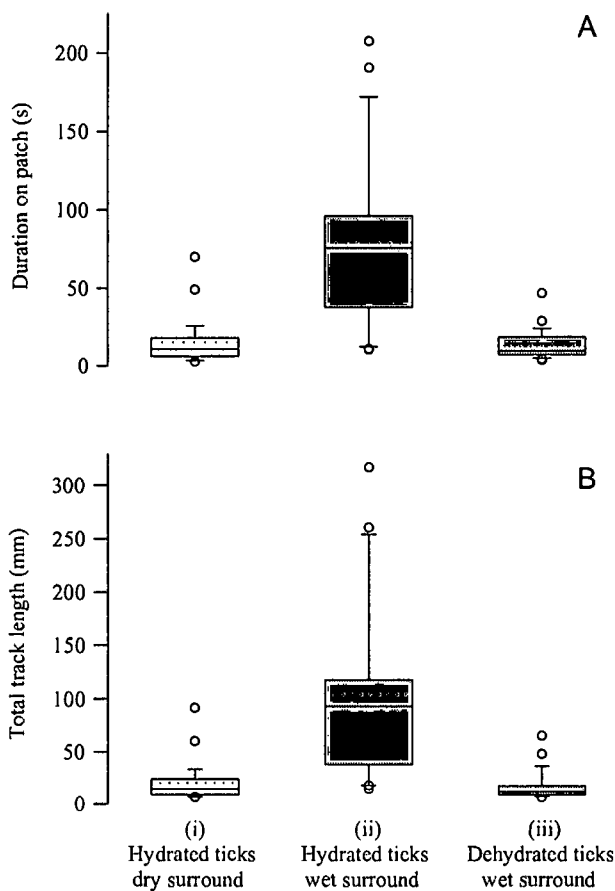


Fig. 3. Time spent (A) and total length of tracks (B) of hydrated *Boophilus microplus* larvae on a dry patch with a dry (i) and a wet (ii) surround. Data are also given for dehydrated larvae on a dry patch with a wet surround (iii). The limits of the boxes indicate the twenty-fifth and seventy-fifth 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

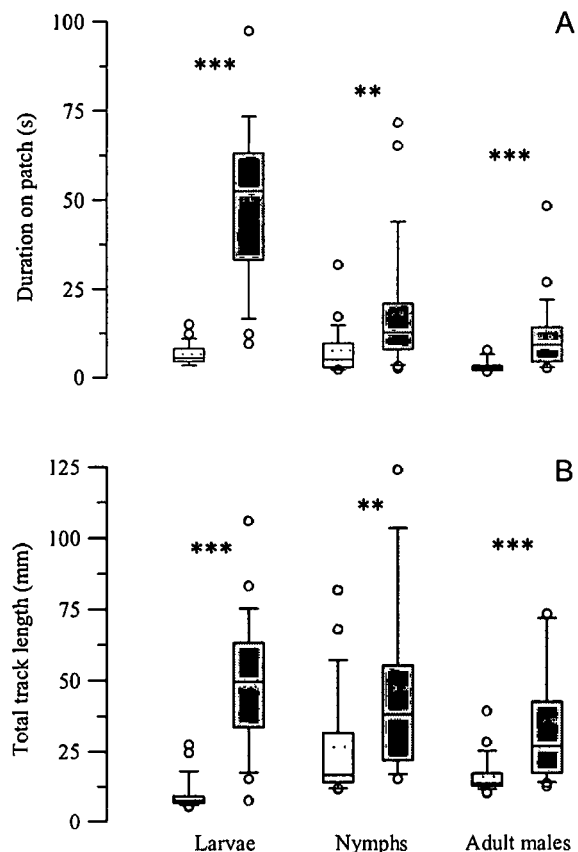


Fig. 4. Time spent (A) and total track length (B) of all stages of hydrated *Ixodes ricinus* on a dry patch with a dry surround (open boxes) and on a dry patch with a wet surround (shaded boxes). The patch is 10 mm in diameter with a 2.5 mm wide surround for larvae and 20 mm in diameter with a 5 mm wide 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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The Dynamics of an Avoidance Behavior by Ixodid Ticks to Liquid Water

T. Kröber^{1,2} and P. M. Guerin¹

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Life stages of different tick species avoid walking on a wet surface surrounding a dry patch by systematically returning to the dry each time they contact the wet surface beyond the border with the tip of a first leg tarsus. Sequential analysis of the border behaviors shows that repetitive contact with the water increases the probability of walks astride the border. Ticks accept this unilateral contact with the water for longer intervals and eventually walk on to the wet surface after a combination of a short patch walk followed by a border walk which is longer than the foregoing ones. Staying time on a small circular patch is shorter than on a large one, arising probably from faster adaptation of peripheral receptors following a higher frequency of border contacts. However, an equal number of border reactions on patches of different sizes and shapes suggests that a 'counter' in the CNS may also influence dry patch departure.

KEY WORDS: tick; *Boophilus microplus*; *Ixodes ricinus*; *Amblyomma variegatum*; give-up time; avoidance; adaptation; sequential analysis; water.

INTRODUCTION

Foraging within a restricted area is advantageous for animals when a resource occurs in discrete patches, but limiting the residence time on such a patch is important when prolonged presence decreases an animal's chance for survival. The hymenopteran parasite *Nemeritis canescens* foraging for hosts leaves a resource patch when the encounters between oviposition sites

¹Institute of Zoology, University of Neuchâtel, Ch-2007 Neuchâtel, Switzerland.

²To whom correspondence should be addressed; e-mail: Thomas.Kroeber@zool.unine.ch.

exceeds a certain 'give up time' (Waage, 1979). This change in behavior is linked to a shift in search bout lengths, as reported for another parasitoid, *Leptopilina clavipes* (Hemerik *et al.*, 1993). Staying on a patch can be an arrestment due to a stimulus from the resource, as for the parasitic wasps *Cardiochiles nigriceps* (Strand and Vinson, 1982) and *Nemeritis canescens* (Waage, 1978), but can also be induced by the presence of a repulsive stimulus in the surroundings (*sensu* phototaxis, reviewed by Bell and Tobin, 1982). In both cases, residence time on a patch is a major factor determining eventual departure as accounted for by Charnov's marginal value theorem (Charnov, 1976; for summary see Pyke, 1984), which predicts that the optimum staying time is when the marginal fitness gain drops to the maximum average that can be achieved there.

We have shown that larvae of the cattle tick, *Boophilus microplus* (Canestrini), and all life stages of the European sheep tick, *Ixodes ricinus* (L.), avoid walking on a water-saturated membrane surface surrounding dry patches of the same membrane (Kröber and Guerin, 1999).

Kahl and Alidousti (1998) showed that dehydrated *I. ricinus* adults and nymphs will approach a water drop to recover their body water deficit, but avoid contact with the liquid. The same phenomenon was observed for *Boophilus microplus* larvae (Krijgsman, 1937). This ambiguous relationship of ticks toward water, i.e., their need for water vapor while actually avoiding contact with liquid water, is of significance for these acarids. Off the host, absorbing water instead of drinking groundwater in which pathogens circulate permits the ticks to avoid infection (Kahl and Alidousti, 1998). On the host, ticks do not attach to feed on wet surfaces such as lips and mouth cavity, nose, eyes, and anus where attachment is highly problematic due to the degree of disturbance to the host.

Here we describe how the life stages of the different tick species returned systematically to the dry patch each time they contacted the wet surface beyond it with the tips of their front leg tarsi.

The fortuitous opportunity afforded by our ability to monitor the ticks' behaviors on the confines of the dry patch permitted us to investigate how the allocation of time to different reactions at the borders with the wet surface affected the tick's behavior. Sequential analysis of these reactions allowed us to follow shifts in behaviors which eventually led to dry patch departure.

MATERIAL AND METHODS

Ticks

B. microplus, the cattle tick, and larvae of the tropical bont tick, *Amblyomma variegatum* Fabricius, were obtained from laboratory cultures

at Novartis Animal Health Inc., St. Aubin, FR, Switzerland, and kept in an environmental cabinet (Kröber and Guerin, 1999). Male *B. microplus* were collected between the 13th and 19th day of development on a steer, i.e., between molt and dropoff of females (Falk-Variant *et al.*, 1994). They were transferred to glass tubes, and were either used for experiments within 6 h or held for up to 6 days attached to the ears of New Zealand White rabbits (de Bruyne and Guerin, 1994). Such males were removed 30 min before an experiment. Nymphs and larvae of *I. ricinus* were obtained from a laboratory culture. All instars of *I. ricinus* were kept at ambient temperature and daylight conditions at $95 \pm 5\%$ r.h. in a closed container.

Steer Hair Extract

Hair (66 g) was shaved off of a young Simmental steer from an area 50×60 cm on one body flank and collected in a 1000-ml round-bottom flask. The hair was extracted in three successive 20-min steps, first with 250 ml and then twice with 100 ml dichlormethane (CH_2Cl_2 , Merck, extra pure grade). The extracts were combined, filtered (Macherey & Nagel glass fiber filter MN GF-2, $0.5\text{-}\mu\text{m}$ pores, Düren, GFR), concentrated by rotoevaporation to 130 ml and stored in a freezer at -80°C . The amount of material of low volatility per unit volume was estimated by evaporating 1 ml of extract on a glass slide and weighing after 30 min at room temperature. The amount of material used in the host-simulating bioassay (below) is henceforth indicated as the 'low volatile mass' (LVM) of the extract.

Bioassay

Tests with individual ticks were designed to quantify behavioral changes over time. The time ticks spent at different behaviors on the dry surface permitted investigation of the mechanism of the border reaction and departure from the dry patch. To record behavioral responses of ticks at the border separating dry and wet surfaces, a water-permeable membrane was used (Kröber and Guerin, 1999). In this study we employed a Baudruche membrane[®] (Joseph Long Inc., Belleville, NJ). The membrane was washed prior to use with acetone and hexane (both extra pure grade, Prochemie, Avenches, FR, Switzerland) and dried for more than 12 h. The membrane has a fibrous structure which soaks by capillarity when stretched over water. The presence of water on the matte membrane surface was rendered evident by lightly sweeping the surface with a dry brush, which induced a shiny water film on the membrane, and a glass capillary ($160\ \mu\text{m}$ diameter, $1\ \mu\text{l}$ pipette) brought into contact with the membrane took up about $0.1\ \mu\text{l}$ water within 3 min.

Dry patches of different shapes and sizes were employed to study different aspects of the ticks' behaviors at the border. They were created by covering a portion of the Baudruche membrane with between 50 and 100 $\mu\text{g}/\text{cm}^2$ steer hair extract dissolved in CH_2Cl_2 and applied with a glass micropipette. After such treatments the glass capillary no longer sucked water from the surface. The edges of the dry patch and the untreated surround were marked with a graphic pen (0.1 mm) on the underside of the membrane. The same amount of solvent alone was applied to the corresponding area of the controls and allowed to evaporate for 15 min before experiments.

Membranes were placed over a cylindrical reservoir (50 mm diameter, 20 mm high) filled with saline (0.9% NaCl, Merck p.a. grade in deionized water) and glued to an 8-mm-thick glass sheet on a warm plate. The membrane was stretched by fixing it between the beveled wall of the reservoir and a second plastic cylinder (53 \times 50 mm, diameter \times height), the latter cylinder forming an upper chamber surrounding the experimental surface. Liquid evaporating during the experiment through the membrane was replaced by a plastic syringe (50 ml) connected via silicone tubing to the reservoir. An open glass capillary connected to the reservoir served to release pressure from beneath the membrane. By heating the reservoir, the temperature was maintained at $32 \pm 2^\circ\text{C}$ at the membrane surface and the humidity in the chamber was at $95 \pm 5\%$ r.h., measured at a distance of 10–25 mm over the membrane. In a control experiment where the patch and the surround were both dry, a membrane with the central treated patch was fixed on a glass plate and heated to $32 \pm 2^\circ\text{C}$, as above. High humidity (80–95% r.h.) in a Perspex chamber (30 \times 15 \times 25 cm, width \times depth \times height) surrounding the heating plate was assured by lining the walls of the chamber and the surface of the plate beside the membrane with water-soaked filter paper. Temperatures were measured with a digital thermoprobe (BAT 12, Sortek Inc., Clifton, NJ) and humidity with a hygrometer (Hygro-Air II, Schiltknecht Messtechnik, Gossau, ZH, Switzerland).

Track Recording

A naive tick, already moving in the holding container (above), was transferred with a fine marten 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 dry patch and ended when the center of the tick's body (for track analysis purposes termed the 'center of gravity') passed the outer circle of the experimental arena 2.5 mm away or left the viewing field of the camera in the case of the triangular patch (see below). The staying time was monitored with a stopwatch and

the number of border contacts was counted for each animal. First-leg waving ('questing': Lees, 1948) during short stops and slow walks, where the animal raised one or both front legs in the air instead of walking with them on the surface, occasionally caused the duration of the walks concerned (see definitions below) to be up to five times longer. This led to exclusion from detailed track analysis of 2 of 14 trials on the 10-mm patch with *B. microplus* larvae.

The experimental surface was filmed full-screen (view field diagonal 36–42 mm) from above using a video camera (Canon Ci-20 PR or PCO XC-77 RR-CE) either equipped with a Optem zoom (No. 65 19 91 91, Optem International, Fairport NY, working distance 45 cm) or the camera connected to a Zeiss operational microscope (working distance 25 cm) in order to observe with accuracy the position of the tips of the first leg pair on a Sony Trinitron video monitor. Cold light made the tick clearly visible as a dark silhouette on a homogenous light background. This was created by placing a white plastic sheet at the base of the reservoir. Recordings were made on an S-VHS video recorder (Panasonic AG 7350) for playback.

In order to analyze details of tracks, the video signal from a time-coded tape (time-code generator ProGamma, Inter University Expertise Centre, Groningen, NL) was fed into a video tracking and motion analysis software system (Etho Vision, former VTMAS, V1.01 to V1.4, Noldus Technology b.v., Wageningen, NL) running on a PC (486 DX II, 66 MHz) via a frame grabber (PC Vision Plus, Imaging Technology Inc., Woburn, MA). The coordinates of the animal's center of gravity were determined using a spatial resolution of 254×254 pixels corresponding to 0.132 mm in the *X* and 0.072 mm in the *Y* direction. *B. microplus* larvae walk with a mean of 1.7 steps/s (Kröber and Guerin, 1999), a step being defined as all the movements of one front leg from raising it until it is put down again. Sampling the animal's position at about double its step frequency, track vectors of 2.94 samples/s, gave the most accurate representation of the track in terms of smoothing the primary wigwag of the gait and correct representation of turn angles. At this sampling rate, change of walking direction with a one-step-turn (see below) at the border was split over turn angles of 2–3 track vectors. The total distance moved by a tick larva was calculated from the sum of track vectors between the start of the trial and the intersection of its track with the outer limit of the experimental arena.

Analysis of the Border Reactions

The behavioral reactions of *B. microplus* larvae at the border with the wet surround were studied in detail on a 10-mm circular patch, and to show

that the mechanism of the behavior at the border was independent of patch size, the analysis was repeated using data obtained from a 25-mm patch. The strength of the return at the border with the wet surface allowed detailed categorization of behavioral elements. For this, the time-coded videotapes were inspected frame by frame and the behaviors were recorded using behavioral analysis software (The Observer and Tape Analysis Module V.3.0, Noldus Technology b.v., Wageningen, NL).

To describe a tick's location, three different zones (patch, wet surround, and border) were defined. When the tick had all its legs on the dry surface it was considered to be walking on the patch zone (Fig. 1A). When all of the tick's legs were in contact with the wet surface it was considered to occur on the wet surround (Fig. 1E, except 11). When the tick walked along the border in contact with both the wet and dry surfaces with its legs it was considered to occur on the border zone (Fig. 1D, 5–9). Each time a tick coming from the patch or wet surround zones made contact with the border with one of its leg tips, the subsequent border reaction was recorded (Fig. 1B, C, D, F: 1, 2, 5, and 12).

One type of border reaction was the one-step-turn (OST) during which the tick touched the border with one front leg tip and immediately turned away to continue walking on the dry patch (Fig. 1B: 1). Although the tick was in contact with the border during a one-step-turn, the animal was recorded as occurring on the patch because of the punctual nature of the contact. During the second type of border reaction, the multi-step-turn (MST, Fig. 1C), the animal walked for several steps astride the borderline. Such ticks undertook a body axis correction during forward movement along a continuous curve which brought them back to the dry surface. A multi-step-turn can be regarded as a special case of a border walk (below) during which the animal turned exclusively in one direction, i.e., the turn angles between the vectors (see below) of the recorded track section were all of a given sign, but never zero (Fig. 1B: 2–4). The third type of border reaction was the border walk, where the tick walked for two or more consecutive steps with at least one leg astride the borderline (Fig. 1D: 5–9). During such border walks the ticks walked with a slight zigzag track such that the turn angles of track sections had positive and negative signs as well as zero values. Sometimes the tick changed walking direction by turning 180°, but kept contact with both the dry and the wet zone. The fourth type of border reaction occurred when a tick returned to the patch after an excursion onto the wet surround (Fig. 1E). In such cases, after contact with the dry patch (12) it crossed the border in several steps (12, 13) and was recorded on the patch again after the last leg had lost contact with the border or the surround (14).

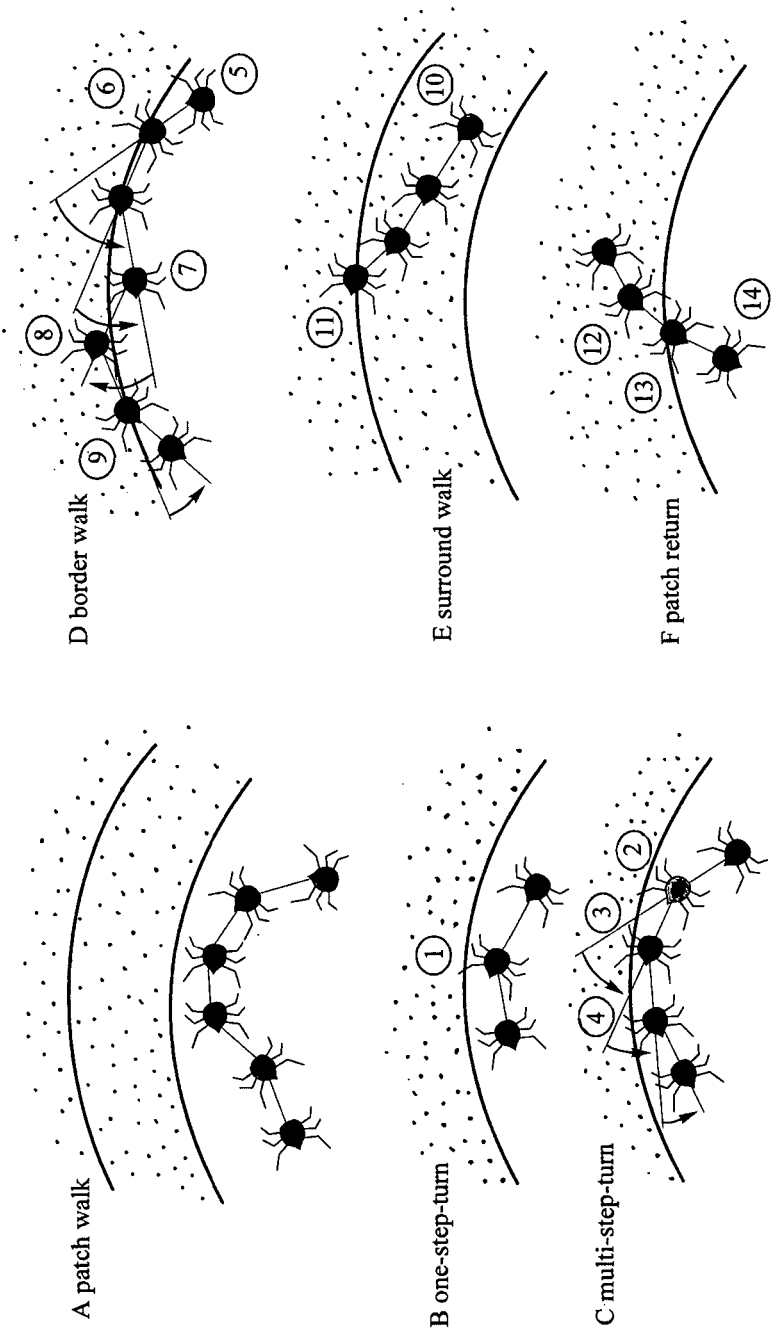


Fig. 1. Representation of the different portions of tracks which were quantified. (A) A patch walk; (B) a one-step-turn (1); (C) a multi-step-turn (2-4); (D) a border walk (5-9); (E) a wet surround walk (10-11); (F) a patch return (12-14). The trial ended (E) when the tick's center of gravity passed the outer circle of the experimental arena (11). Shaded area represents the wet surround.

Analysis of Changes in Behavior with Time

In order to study how the length of time the tick was walking (experimental time) influenced the duration of patch walks and border walks, and how the duration of these walks affected decisions at the border, the tracks were split into patch walks and border walks. Patch walks were further subdivided into two subcategories: (1.1) the start of the track, i.e., between the release point and the first encounter with the border of the dry patch, and (1.2) sections between successive border contacts during which the tick walked on the patch. Border walks were further categorized according to the tick's decision after such walks: (2.1) normal border walks prior to the tick's return to the patch, (2.2) border walks preceding a transition to the surround, and (2.3) the last border walk in a trial.

Patch walks between successive border contacts (1.2) and pooled border walks were examined for monotonic changes with time. In case of a significant correlation (Spearman) a linear regression model was calculated using logarithmic-transformed data. The independence of decisions made by the ticks upon arrival at the border from experimental time and duration of the previous patch walk was tested using a linear logistic regression model for binary response data by the method of maximum likelihood. This model estimated the probability of border walk occurrence versus that of one-step-turns or multi-step-turns. The durations of border walks were noted to investigate whether decisions after border walks, as categorized above, were influenced by border walk duration. We looked at the independence of the border walk duration from the duration of the previous patch walk, and also examined the second-order dependence of the decision following a border walk by looking at the duration of the penultimate behavior, i.e., the foregoing patch walk.

The fact that the ticks changed their walking direction, clockwise or counterclockwise, on the circular patch made it possible to analyze whether previous and prolonged unilateral contact with the wet surface during the previous border walk had any influence on behaviors once the opposite leg was brought into contact with the wet surface. Each border contact made by such ticks on the 10-mm-diameter patch was noted with respect to its clockwise or counterclockwise displacement along the border. Assuming that only peripheral adaptation and habituation influences behavioral changes over time at the border, then a reset of the system should have been visible each time the animal changed its walking direction with respect to the border. To analyze this, the clock was reset to zero at the change of walking direction so that periods of walking in a given direction started with the first border contact with a given front leg in a given direction and ended with the last border departure in that direction. Nearly normally distributed data were obtained by excluding each first border walk within a series of walks in the same

direction. The influence of both time scales, i.e., the time walking in a given direction and experimental time, on the probability of border walks versus one-step-turns plus multi-step-turns was examined using multiple linear logistic regression, and the effect of each process on the duration of border walks was estimated by multiple regression analysis.

Logarithmic transformation of all time scales and durations achieved almost normal distributed data and permitted application of linear statistical analyses. Statistics of all experiments were calculated with SAS (V6.08, 1989) and PStat (V2.15, 1992), both running on a VAX computer or S-Plus (V3.3 release 1, 1995, StatSci) on a PC. Probabilities for Mann–Whitney U -test (P_U), Wilcoxon test (P_W), Kruskal–Wallis test (P_{KW}), or F -test (P_F) are given.

Turns at the Border of a Triangular Patch

Since ticks arrived, on average, at higher angles to the border on a triangular patch than on the circular patches, this was used to investigate the nature of movements made by *B. microplus* larvae upon contact with water at the border of a dry triangular surface with 15-mm equilateral sides treated with steer hair extract. Fourteen walks were recorded on the triangular patch and for each border contact the type of turn and direction with respect to the side of the leg which first contacted the wet surface was recorded. Comparison of the videotape with the track printout permitted precise location of border contacts. Random samples of 24 *one-step-turns*, 26 *multi-step-turns*, and 26 *border walks* when the tick returned to the patch were examined. In addition, we measured all 11 *turns to the arena* from the patch border not induced by larval arrivals at the vertices of the triangle. The size of the angles of arrival and departure from the border were measured by manually fitting a mean vector over a period 2 s preceding and 2 s following a border contact. These angles were compared with the Mann–Whitney U -test (unpaired) and the Wilcoxon test (paired).

Effects of Unilateral Masking of First Leg Tarsi

In this experiment either the left or the right first-leg tarsus of *B. microplus* males was covered with a small droplet of synthetic resin glue (Konstruvit®, Geistlich, Wolhusen, LU, Switzerland) and the animals were allowed at least 10 min to adapt to the new situation. Some animals succeeded in removing the glue just like a glove and had to be treated a second time. Ten left- and right-leg-masked animals were allowed to walk on a 12-mm dry patch covered with steer hair extract with an untreated 4-mm-wide concentric surround. The behavior of the ticks at the border was analyzed using

the video tracking system (above) and the direction of turns at the border in the clockwise (right turns) and counterclockwise (left turns) directions was noted.

RESULTS

Responses to the Wet Surface

In all trials larvae and males of *B. microplus*, larvae of *A. variegatum*, and larvae and nymphs of *I. ricinus* spent most of the time walking. All ticks showed a strong response at the border of the dry patch with water (Fig. 2A, Fig. 3). During 12 trials with *B. microplus* larvae recorded on circular dry patches of different sizes and 14 trials on a triangular patch, a similar median number of border contacts per trial with a wet surround were observed (241 border contacts on the 10-mm patch, 212 on the 25-mm patch, and 204 on the triangular patch, $P_{KW} \geq .3$; Table I). Control tracks on the completely dry or wet membrane were rather simple as larvae mostly walked straight out of the experimental arena (Fig. 2C).

Overall, track lengths recorded for *B. microplus* larvae on the 10-mm dry patch surrounded by a wet surface were some 12 times longer (median 153, 41–243 mm) than on the dry controls (median 13, 7–25 mm, $P_U \leq .01$), and tracks recorded on a 25-mm dry patch with a wet surround were 2.5 times longer (median 412, range 19–1619 mm) compared to those on the 10-mm dry patch with a wet surround.

It is evident that it is the return at the border which contributed to the greater time spent by ticks on the dry patch surrounded by a wet surface. The underlying mechanisms of this strong response to water and the time sequence of the decisions undertaken at the border were analyzed in detail for *B. microplus* larvae.

Angular Aspects of Border Reactions

On the triangular patch, ticks that left the border at a low angle crossed the dry surface with a rather straight walk and hit the border of the opposite side at a high angle, resulting in more frequent multi-step-turns (30% of the border contacts) and fewer one-step-turns (15%; Table I). One-step-turns occurred when the tick approached the border at a relatively low angle ($<25^\circ$) and only one front leg contacted the wet surface causing the tick to turn immediately, bringing all legs back onto the treated patch (Fig. 4). Multi-step-turns occurred when the angle of arrival at the border was high

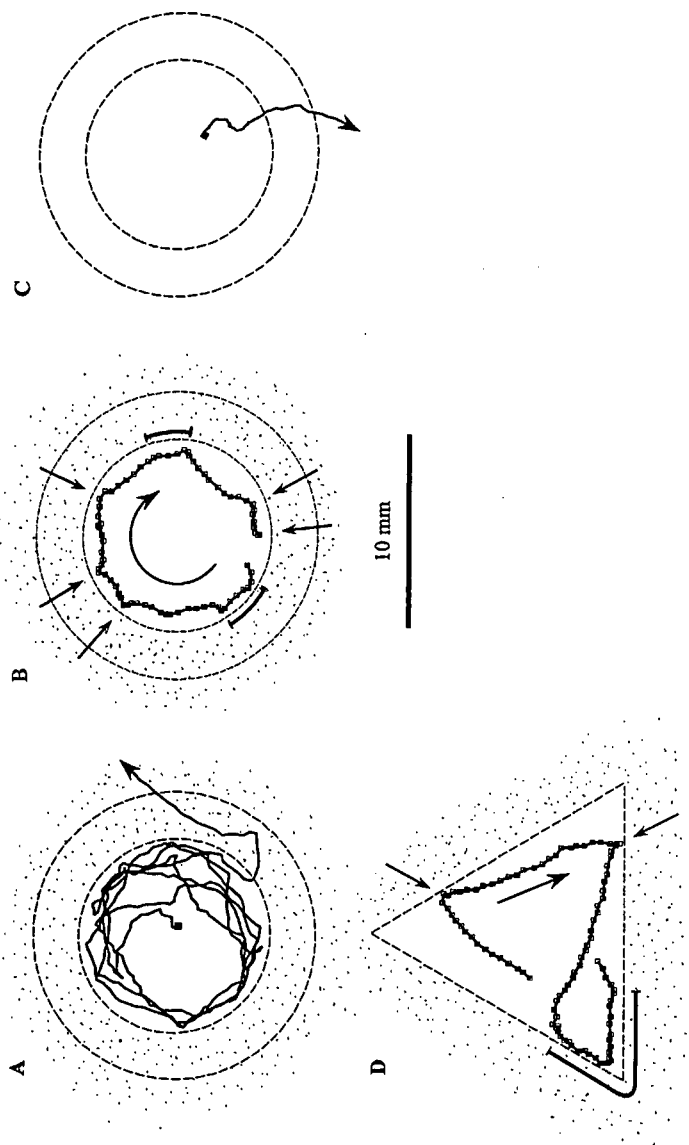


Fig. 2. Tracks described by *Boophilus microplus* larvae on circular and triangular dry patches (inner unshaded zones in A, B, and D) surrounded by a wet surface and on the corresponding dry control (C). The tick was released at the black square; the walk in (A) lasted 130 s. Sections of records on the circular (B, 14 s) and triangular (C, 30 s) patches demonstrate that brief *one-step-turns* at the border (arrows) or walks astride the border (bold rings) are used to maintain contact with the dry surface. Border walks (bold line in D) sometimes guided the tick larvae around the angles of the equilateral triangle. Walks on the complete dry membrane were short (20 s in C).

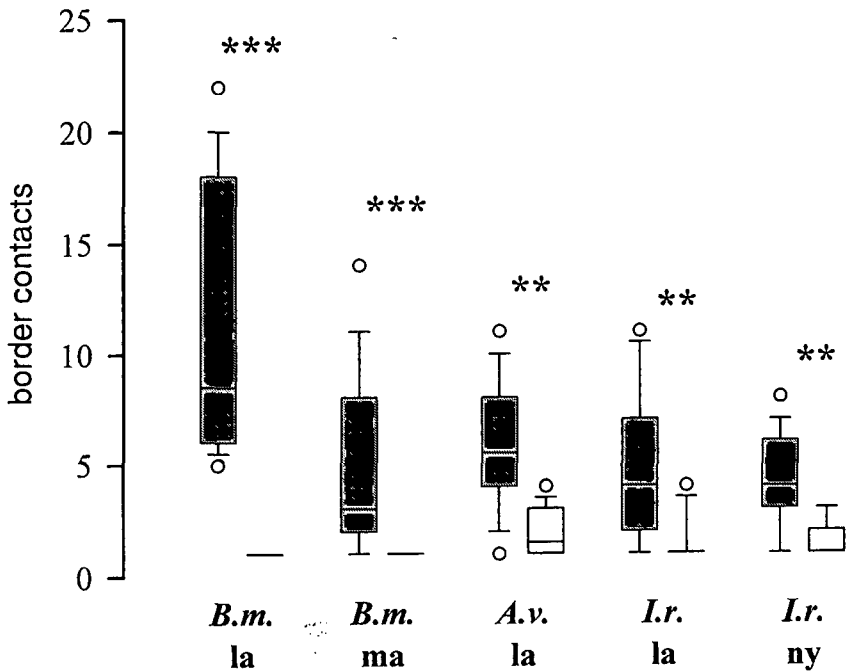


Fig. 3. Number of border contacts made by different life stages of three hard tick species on a dry patch (12 mm diameter) treated with steer hair extract on a wet surround. Shaded boxes, dry patch; blank boxes, wet control patch. B.m. la: *Boophilus microplus* larvae; B.m. ma: *B. microplus* males; A.v. la: *Amblyomma variegatum* larvae; I.r. la: *Ixodes ricinus* larvae; I.r. ny: *I. ricinus* nymphs. The limits of the boxes indicate the 25th and 75th percentiles, the solid line in the box is the 50th percentile, capped bars indicate the 10th and the 90th percentiles, and data points outside these limits are plotted as circles. Comparisons between wet and dry patches on a wet surround were made for each species with the Mann-Whitney *U*-test, *** $P \leq .001$, ** $P \leq .01$; $n = 10$ for each box.

(>50°). Once the tick put one front leg on the border or on the wet surface outside, it started to turn back toward the dry surface (turn angles of the same sign) such that several steps along the border were necessary to undertake the large shift in body axis. Border walks occurred following any angle of arrival at the border. In this case the tick larva walked for some time astride the border before turning back onto the dry surface (hatched box, Fig. 4). The angle of departure from the border was always low (OST 20.5°, MST 31.5°, and border walks 17.0°), regardless of the angle of arrival (median OST 26.5°, MST 56.5°, and border walks 73°; Fig. 4, example track Fig. 2D). Even though the tick arrived often at a high angle at the border of the triangular patch, it returned as often to the dry patch with an OST or MST as on the circular one (Table 1).

Table I. Frequencies of Walks in Different Zones and Number of Border Contact Types Made by *B. microplus* Larvae at the Border of Dry Patches of Different Sizes and Shapes Surrounded by a Water-Covered Surface, and in One Case by a Dry Surface (Control)

	10-mm circular patch												2.5-mm circular patch:				1.5-mm equilateral triangular patch: Surround wet			
	Surround wet						Surround dry						Surround wet				Surround wet			
	n	Median	Range	Sign. ^a	n	Median	Range	Sign. ^b	n	Median	Range	Sign. ^a	n	Median	Range	Sign. ^a	n	Median	Range	Sign. ^a
Patch walk	225	12	(2-22)	a	12	1	(1)	***	140	12	(3-26)	a	174	12	(2-31)	a				
Border contact	241	22	(5-37)	b	12	1	(1)	***	212	17.5	(7-40)	b	204	15	(2-37)	b				
One-step-turn	99	6	(1-22)	c	0	0	—	***	67	4.5	(1-14)	c	29	1.5	(0-7)	c				
Multi-step-turn	4	0	(0-1)	n.t.	0	0	—	n.t.	25	1.5	(0-5)	n.t.	65	2	(0-14)	n.t.				
Border walk	138	11.5	(2-21)	d	12	1	(1)	***	120	12	(3-26)	d	129	9	(2-17)	d				
Patch return	10	0	(0-3)	n.t.	0	0	—	n.s.	5	0	(0-2)	n.t.	10	0.5	(0-3)	n.t.				
Arena walk	22	1	(1-4)	n.t.	12	1	(1)	n.s.	17	1	(1-3)	n.t.	14	1.5	(1-4)	n.t.				
Tracks analyzed	12				12				12				14							

^aFor a given behavior, medians between trials on different patch types followed by the same letter are not different, $P_{Kw} \geq .3$, $P_U \geq .3$; n.t., not tested. Total number, median, and the range are given.

^bComparisons between wet and dry surround for the 10-mm patch: *** $P_U \leq .001$, ** $P_U \leq .01$; n.s., not significant; n.t., not tested.

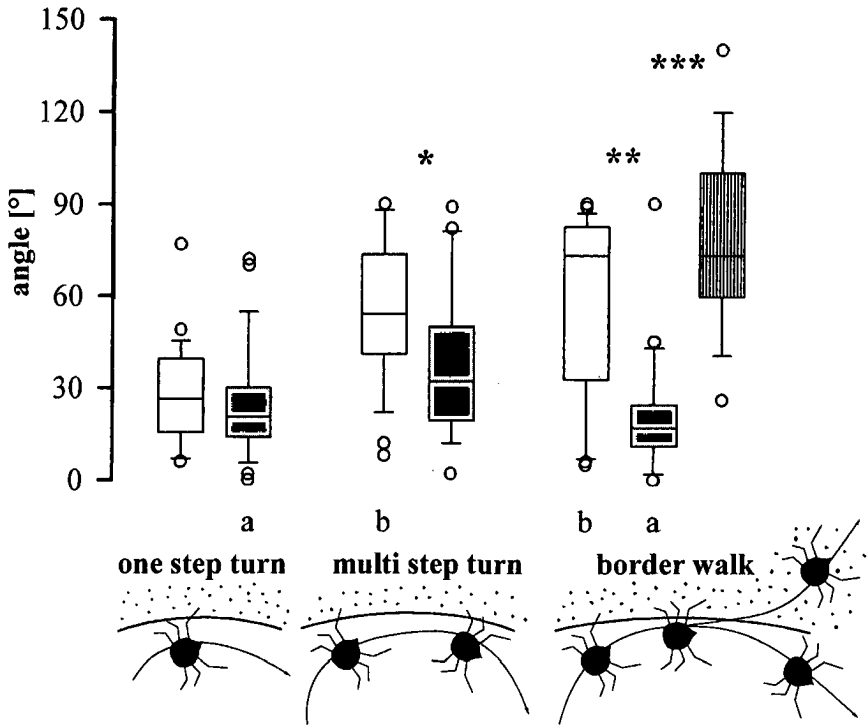


Fig. 4. Box plots of angles of arrival (blank) and departure (shaded) by *B. microplus* larvae at the border of a dry triangular patch created with a steer hair extract. The sketch under each pair of boxes shows a typical example. For box plot details see legend to Fig. 3. Data are from 24 one-step-turns, 26 multi-step-turns, 26 border walks, and 11 turns toward the wet surround (vertical stripes). Angles of arrival and departure for the different border behaviors are significantly different, $P_U \leq .05$, except those with the same letter, $P_U \geq .3$; significant differences between angles of arrival and departure within a behavioral category are marked: * $P_W \leq .05$, ** $P_W \leq .001$, *** $P_W \leq .0001$; $n = 14$ larvae.

On the circular patch, the ticks more often encountered the border at a low angle and returned mostly to the dry surface with one-step-turns. Consequently, the angles of departure were small and the ticks soon encountered the border again at a low angle of arrival. Multi-step-turns occurred only exceptionally on the circular patch (Table I), such that patch walks with rather straight sections of constant duration on the dry surface alternated with turns at the border, and a full circle was made with between 7 and 8 border contacts (Figs. 2B, Fig. 4). This pattern was interrupted by periods walking astride the border.

The reason ticks left the border at a low angle became clear in observations on one-step-turns by *B. microplus* males arriving at the border of a

12-mm patch. The leg first placed on the border of the wet arena dictated the turning direction of the animal's body at the border. Males put one front leg tip outside the dry patch, lifted this leg off the wet surround almost immediately, and displaced it so as to regain contact with the dry surface. During this process, the tip of the front leg was employed to touch the substrate at one or a series of points in its arc of propagation until it finally came down again on the dry surface. In the next step, the tick shifted its body axis sufficiently to accommodate this first leg at its normal walking angle to the body on the dry surface. The tick then continued to walk straight. This behavior of males was similar to that observed for *B. microplus* larvae on the 10-mm patch.

Angles made by ticks moving away from the border to the wet surround were always high. This was in response to loss of contact to the dry surface by the second leg, which, until then, had been on the dry patch. On the triangular patch the subsequent swing by the tick (median 73°) to the wet side was more than twice the size of any returns onto the dry surface from the border (median 22° , $P_U \leq .00001$, Fig. 4).

Behaviors at the Patch Border Changed with Time

Analysis of the succession of behavioral events following a border contact on a 10-mm patch revealed that 43% of the encounters with the border by *B. microplus* larvae coming from the 10-mm patch resulted in one-step-turns (99 of 241 border contacts; Fig. 5). Only four multi-step-turns were observed (see below). Following the remaining 57% of border contacts, the larvae walked astride the border, i.e., in unilateral contact for some time with the water outside the patch. In 116 of 138 border walks the ticks returned to the patch. Only 9% left for the wet surrounding and in nearly half of these cases the ticks returned sharply to the dry patch after a short excursion on the wet surface (Fig. 5). At the beginning of the trial, patch walks were separated mostly by one-step-turns (Fig. 6) and had a median duration of 2.8 s (0.4–27.2 s, $n = 225$) on the 10-mm patch and 9.2 s (0.6–100.8 s, $n = 193$) on the 25-mm patch. These were nearly constant throughout the trials (10-mm patch $r^2 = .005$, $P_F = .3$; 25-mm patch $r^2 = .001$, $P_F = .6$). Later the tick larvae accepted unilateral contact with the wet surface and walked astride the border (Fig. 6). Following this transition, the probability of border walks compared to the occurrence of one-step-turns and multi-step-turns rose with time. (Fig. 7A, B).

The decisions made at the border were influenced by the experimental time and only moderately by the duration of patch walks, as shown by analysis of events on the 25-mm patch with *B. microplus* larvae. Multiple logistic regression including experimental time and patch walk duration revealed

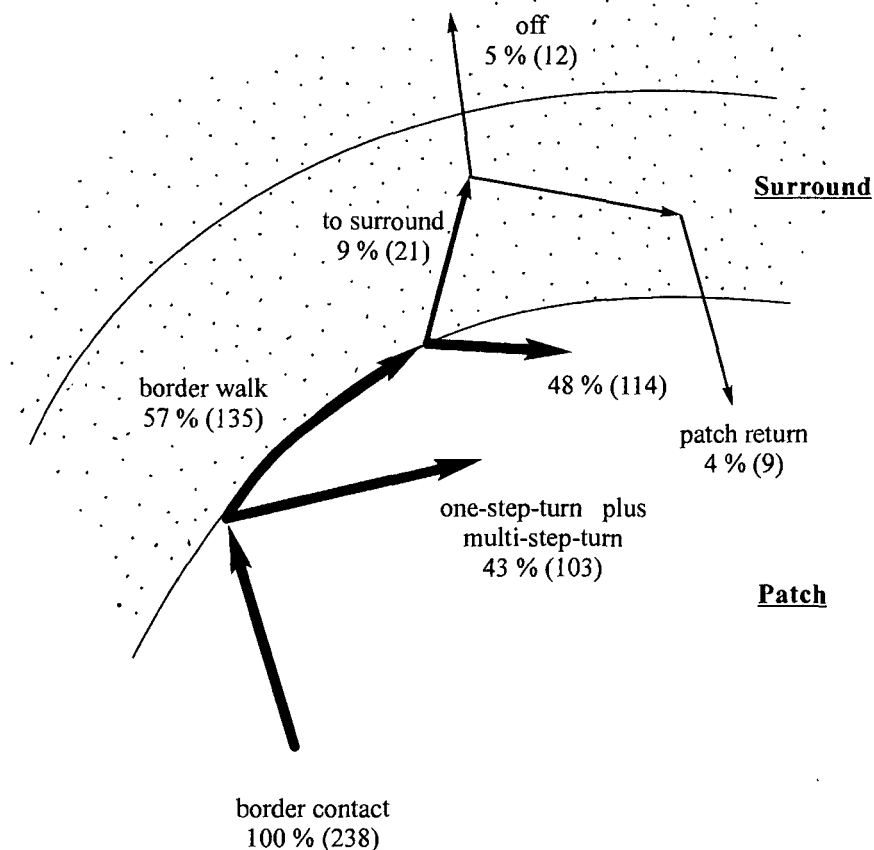


Fig. 5. Succession plot of the prevalence of different decisions made by *B. microplus* larvae at the border of a dry circular patch (10-mm diameter, wet surround shaded). Pooled data from tracks of 12 ticks which made 241 encounters with the border while walking on the membrane. After a border walk only 9% of the larvae left the dry patch, and half of these returned to the dry surface. Values indicated are proportions of the total (actual number of events in parentheses).

that both parameters had inverse effects on the probability of border walks. With time, ticks accepted to stay in unilateral contact to the wet surface for a longer time during border walks and this especially after short patch walks (Fig. 7A). The multiple logistic regression model from data on the 10-mm patch revealed only a small influence of the time walking in a given direction on the probability of border walks (Fig. 7B) compared to the experimental time. The effect of the experimental time was clearly stronger on the 10-mm patch compared to the 25-mm patch (confidence intervals of the slope for experimental time of 0.80 ± 0.23 [\pm SE] on the 10-mm patch and of 0.37 ± 0.17

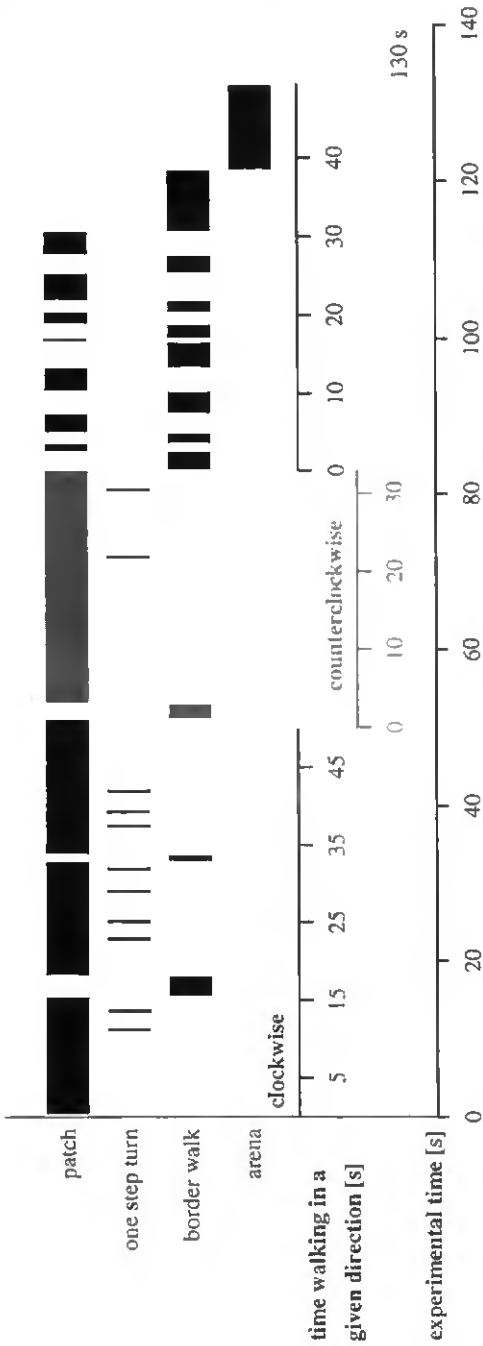


Fig. 6. Bar plot of the time course of different behaviors observed when a *B. microphilus* larvae walked on a circular, dry, 10-mm-diameter patch with a wet surround. Note the shift from one-step-turns to border walks on the dry patch and the tendency for longer border walks with time. Periods walking clockwise around the patch are black and counterclockwise periods are gray. A second time scale, reset to zero for each bout in a given direction, is provided above the experimental time scale (for further explanation see text). The original track is given in Fig. 2A.

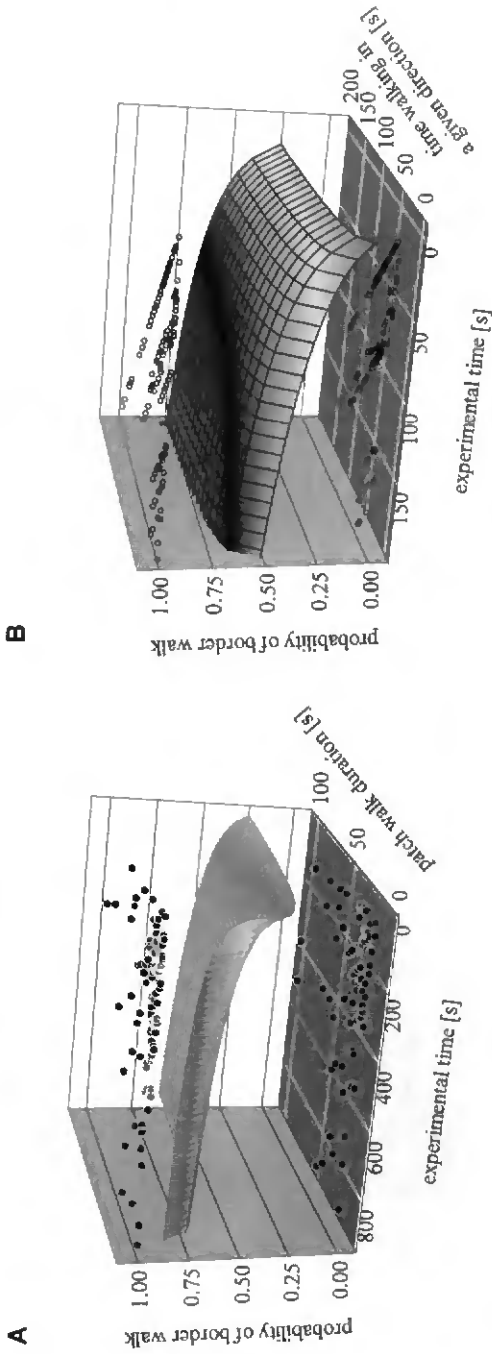


Fig. 7. Time dependence of different types of walks and decisions made at the border of a circular dry patch with a wet surround by *B. microplus* larvae. The probability of border walks is dependent on three parameters. (A) On a 25-mm patch it decreases with longer foregoing patch walks and increases with experimental time; $-2 \log$ likelihood 251.4, patch walk duration $P_{\chi^2} \leq .1$, experimental time $P_{\chi^2} \leq .01$; the dots in the '0-plane' are decisions from 76 one-step-turns + multi-step-turns and the dots in the '1-plane' are 117 border walks. (B) The probability that border walks increases with experimental time and time walking in a given direction, $-2 \log$ likelihood 254.2, experimental time $P_{\chi^2} \leq .0008$, time walking in a given direction $P_{\chi^2} \leq .19$. decisions from 104 one-step-turns and multi-step-turns, and 138 border walks on a 10-mm patch are plotted as circles.

on the 25-mm patch did not overlap). The border walk duration grew faster with experimental time on 10-mm patch than on the 25-mm patch and was different for both patch sizes (regression of 136 border walks on the 10-mm patch, slope = .46, $r^2 = .2$, $P_F < .0001$; and 114 border walks on the 25-mm patch, slope = .24, $r^2 = .06$, $P_F < .001$; and are different, $P \leq .07$ [method from Hald, 1967]).

The influence of the time walking in a given direction on duration of border walks was minor compared to the experimental time (total $R^2 = .22$, $P_F \leq .001$; experimental time $r_{part}^2 = .20$, $P_F \leq .001$; time walking in given direction $r_{part}^2 = .01$, $P_F \leq .22$; $n = 138$ border walks on the 10-mm patch).

Separation of border walks into subclasses showed that the median duration of the last border walk, i.e., before a larva finally left the patch, was significantly longer than the median of those which occurred when the larva returned to the patch (Table II). This was also true for the triangular patch ($P_{KW} \geq .4$, $n = 12, 12$, and 11 , respectively; Table II). With the exception of the last border walk, the duration of border walks on the 10-mm patch was not influenced by the duration of the previous patch walk (Spearman correlation coefficient: $-.007$, $n = 109$, $P > .94$). The decision at the end of the final border walk on the 25-mm patch was dependent (Second-order dependence) on the duration of the previous patch walk, i.e., final abandonment of the dry patch was linked to significantly shorter preceding patch walks. The median duration of 102 patch walks preceding normal border walks was 8.9 s (1–85 s) compared to a duration of 3.3 s (0.8–100.8 s) for the 12 patch walks preceding the last border walk ($P_U \leq .001$).

Behavior of Unilaterally Masked *B. microplus* Males

The tracks of males with one masked first-leg tarsus appeared more tortuous in trials on both the wet and dry membranes compared to those of unmasked individuals. Some individuals showed a remarkable wigwag path on the wet control membrane, where they walked continuously, whereas on the dry membrane they tended to probe with the mouthparts. Turns at the border of the patch were induced only upon contact with the wet surface via the unmasked tarsus, resulting in left-masked individuals preferring counterclockwise (6 of 9) and right-masked ones making mostly clockwise tracks (6 of 8, $P \leq .004$, exact Fischer test). This occurred because the tick turned toward the masked-leg side following contact by the unmasked front leg with the wet surface. Because of the circular patch, the animal soon recontacted the wet surface and a new correction occurred.

Table II. Durations of Border Walks Made by *B. microplus* Larvae at the Border of Dry Patches of Different Sizes and Shapes Surrounded by Water^a

Type ^b	10-mm circular patch			25-mm circular patch			15-mm equilateral triangular patch					
	Median	Range	n	Sign.	Median	Range	n	Sign.	Median	Range	n	Sign.
Normal border walk	2.8	(0.6–22.3)	114	AB	3.7	(0.4–26.8)	104	C	2.7	(0.7–20.2)	120	D
Transition to arena	7.9	(3.9–13.8)	10	A	7.4	(5.0–12.2)	4	n.t.	7.9	(9.9–13.8)	8	n.t.
Last border walk	5.8	(3.2–17.3)	12	Ba	8	(3.1–16.6)	12	Ca	5.5	(1.5–16.7)	11	Da

^aMedians on the same patch followed by the same capital letter are different ($P_U \leq .02$). Medians for the same border walk type on the different patches followed by the same lowercase letter are not different ($P_{Kw} \geq .4$, $P_U = 1$); n.t., not tested.

^bBorder walks were classified either as *normal* when the larvae returned to the dry patch without waving the first legs in the air, as the *last border walk* during a trial, and as *transition-to-arena* walks when they preceded an excursion to the arena. Data from 12, 12, and 14 larvae, respectively, for the 10-mm, 25-mm, and triangular patches.

DISCUSSION

The Border Response

The response at the border of a wet surface reported here for larvae and male *Boophilus microplus*, larvae of *Amblyomma variegatum*, and for all life stages of *Ixodes ricinus* (Kröber and Guerin, 1999) would suggest that the underlying mechanism of the response to the wet surface is a general phenomenon in Ixodid ticks. Other experiments (Kröber and Guerin, 1999) have shown that rendering the patch dry with silicone glue instead of steer hair extract induces the same border response. This and the fact that the steer hair extract demonstrated no border effect in the dry controls shows that it was the contrast between the dry and wet surfaces which caused the border response.

At first, contact with the wet surface via one front-leg tarsus was sufficient to cause the tick to turn enough to the opposite side so as to regain contact with the dry patch, i.e., the one-step-turns. During one-step-turns, the ticks shifted the body axis only as much as necessary to avoid the wet surface in the next step, and the change of direction comprised some 48° ($7-116^\circ$), i.e., the sum of angles of arrival and departure. This border reaction influenced by the water beyond the dry patch can be classified as a negative chemotropotactic response (Kennedy, 1978; Frankel and Gunn, 1940). Under natural conditions *B. microplus* larvae climb down in the morning from the top of the dew-covered grass stems which form their ambush points (Wilkinson, 1954). Here small angles of departure upon encountering water permit them to travel through the field of droplets, whereas high angles would cause the tick to walk in circles. Our observations on male *Boophilus* showed that although the leg which contacted the water was dropped to the substrate in successive points in the arc of propagation, it was only brought resolutely down when the dry substrate was encountered again. This strongly suggests that the first-leg tarsi are equipped with sensory receptors to perceive the wet surface. Unilateral masking of sensilla on the tarsus caused a loss of the avoidance response following border contact by the masked leg, i.e., a right-masked animal could only use its left front leg to detect the border of the dry patch and compensated on contact with the wet surface with a turn to the right.

When the tick arrived at the border of the dry surface at a high angle, i.e., one in which it could not regain complete contact with the dry surface in a one-step-turn, it either turned sharply with a multi-step-turn to bring both front legs back to the dry surface or walked for some time astride the border. After recontacting the border a number of times, the ticks arriving there eventually only rotated so much that unilateral contact with the dry surface was not lost, i.e., it undertook border walks. The tick stages studied here generally

walk relatively slowly, therefore they can react instantaneously to encounters with a stimulus (allothetic cue; Kennedy, 1978) and overshoots are rare. When overshoots on the wet surface did occur, they were followed by nearly 180° loops. During such excursions onto the wet surface, ticks probably regain the dry surface using ideothetic cues, as in walking beetles and isopods (Havukkala and Kennedy, 1984; Sorensen and Bell, 1986). Return reactions occur upon encountering an unfavorable stimulus, earlier termed 'phobotaxis' or 'reflex action' (reviewed by Bell and Tobin, 1982), as at the border of a humid zone provided for dry-acclimatized *Tenebrio molitor* (Gunn and Pielou, 1940) and at the border between zones at different temperatures for *I. ricinus* (Lees, 1948).

Eventual Acceptance of the Wet Surface

Only 4% of the initial border contacts led to acceptance of the wet surface. However, the probability that contact with the wet surface was followed by a continuous walk astride the border rose with the time the tick spent walking on the membrane surface, but was reduced after longer patch walks. Furthermore, the duration of border walks increased over time, and the border walk preceding any excursion onto the wet surrounding was always clearly longer. With time, the ticks accepted unilateral sensory input from the wet surface for a longer interval and eventually walked on the wet surface after a combination of a short patch walk followed by a border walk which was longer than the foregoing ones, independent of patch size or shape. In such cases, contact by the second front leg (until then on the dry surface) with the water led the tick distinctly away from the dry surface due to the underlying behavioral reflex of turning in the direction opposite to the side from which the water is freshly perceived.

Different neuronal processes may be involved in accepting to walk on the wet surface. During periods of walking in a given direction with continuous exposure of sensillae on one leg tip to the water, peripheral adaptation (Coro *et al.*, 1998; Wood *et al.*, 1997) could contribute to the decision to leave the dry patch. Furthermore, patch walks were shorter on the 10-mm patch than on the 25-mm patch, so that the frequency of contacts to water during one-step-turns and multi-step-turns was higher on the smaller one. This resulted in a staying time on the 10-mm patch half that on the 25-mm one. Shorter patch walks (0–10 s) would strongly decrease the degree of disadaptation (Thompson and Spencer, 1966), and indeed as experimental time extended, the probability of a border walk rose following such short patch walks.

It is noteworthy that *B. microplus* larvae made the same number of border contacts on patches of different sizes and shapes and that the last

border walks were of the same duration. In addition, we found that the time walking in a given direction affected only to a minor degree the shift from one-step-turns to border walks and the increase in border walk duration, i.e., the system was not reset to zero after a change of direction. Both findings support the existence of a type of counter in the central nervous system determining when the avoidance reaction is to be given up. The waning response to the border with the wet surface implicating adaptation of peripheral receptors combined with CNS processes allows the tick to delimit the number of returns and ultimately escape.

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Wasservermeidung und Verhaltensreaktionen auf Stimuli der Wirtsoberfläche bei Schildzecken (Ixodidae)

Thomas Kröber (1999) Doktorarbeit, Institut für Zoologie, Universität Neuchâtel

Zusammenfassung

Stimuli von der Wirtsoberfläche spielen eine bedeutende Rolle beim Verbleiben (Arrestment) und Festsetzen von Schildzecken auf ihren Wirtstieren. Der Einfluss dieser Stimuli bei der Wirts-Parasit-Beziehung wurde in dieser Arbeit auf verschiedenen Ebenen untersucht.

- (1) Die Wahrnehmungsfähigkeit für Hautlipide wurde mit elektrophysiologischen Methoden bei Kontaktchemosensillen von *Boophilus microplus*-Weibchen nachgewiesen. Chemorezeptorzellen von 2 Palpensensillen mit terminaler Pore werden durch Rinderhaarlipide stimuliert. Oberflächenlipide der Wirtstiere spielen vermutlich bei der Wirtserkennung von Schildzecken eine wichtige Rolle.
- (2) Bei den gleichen Palpensensillen wird eine Rezeptorzelle von Kalium-haltigen Salzen in Abhängigkeit von der Dosis stimuliert. K^+ -Ionen stimulieren darüber hinaus Rezeptorzellen der Kontaktchemosensillen an den Tarsenspitzen weiterer Lebensstadien prostriater und metastriater Zecken wie *Ixodes ricinus* und *Amblyomma variegatum*. Demgegenüber hatten Natrium-haltige Salze keinen oder nur einen geringen Effekt. Ein höheres K^+/Na^+ Verhältnis zeichnet den Schweiß von Herbivoren im Vergleich zu Karnivoren aus. Die Gegenwart von hohen K^+ -Konzentrationen könnte ein Signal zur Erkennung von Herbivoren darstellen, zu denen die Mehrzahl der Wirte der Schildzecken zählen.
- (3) Larven von *B. microplus* und Nymphen von *Rhipicephalus sanguineus* aggregieren bevorzugt auf dem Sektor eines Filterpapiers, der mit einer definierten Menge Rinderhaarlipiden behandelt wurde. Vermutlich ist diese Arrestment-Wirkung von Oberflächenlipiden der Wirtstiere ein bei Schildzecken verbreitetes Phänomen.
- (4) Durch Fraktionierung mittels Festphasenextraktion wurden die Rinderhaarlipide in zehn Fraktionen unterschiedlicher Polarität aufgetrennt. Mindestens zwei mittelpolare Fraktionen verursachen Arrestment-Aktivität bei *B. microplus* Larven.
- (5) Ein Extrakt der Rinderhaarlipide spielt eine wichtige Rolle bei der erfolgreichen Fixierung auf *in vitro* Fütterungsmembranen bei 37°C. Die Hautlipide steigerten die Fixierungsrate von *A. hebraeum* Nymphen auf solchen Membranen um bis zu 30%. Der Einfluss der Rinderhaarlipide ist mit der Wirksamkeit weiterer bedeutender Fixierungsfaktoren vergleichbar wie z.B. mechanischer Stimuli (z.B. Haare und Membrantextur), Phagostimuli im Fütterungsmedium unter der Membran und Aggregations-Fixierungs-Pheromonen. Durch die Kombination von Stimuli verschiedener Modalitäten (Tast-, Geschmacks- und Geruchssinn) können hohe Fixierungsraten *in vitro* erzielt werden.

Zusammenfassung

- (6) In einem weiteren Teil der Arbeit ist die Vermeidung von flüssigem Wasser bei Schildzecken beschrieben. Schildzecken suchen in der Natur Zonen mit hoher Luftfeuchte auf, um ihr Wasserdefizit auszugleichen. Hydratierte Individuen vermeiden jedoch Kontakt mit flüssigem Wasser.
- (7) Bei einem Laborversuch mit hydratierten Larven und Männchen von *B. microplus*, Larven von *A. hebraeum* und allen Stadien von *I. ricinus*, die auf einer trockenen Inselfläche inmitten einer nassen Membranfläche laufen, wenden diese unverzüglich am Rande der nassen Fläche um.
- (8) Da Kontakt mit einer Tarsenspitze des ersten Laufbeinpaares hinreichend ist, um die Umkehrreaktion auszulösen, sind wahrscheinlich Kontaktchemosensillen an den Tarsenspitzen für die Wasser-Wahrnehmung verantwortlich. Verhaltensbeobachtungen von Zeckenlarven, bei denen potentielle Hygrorezeptoren an den Tarsen oder Palpen mit Klebstoff maskiert wurden, bestätigen diese Hypothese. Diese Zecken reagierten nicht bei Kontakt mit der nassen Membranfläche.
- (9) Sequentielle Analyse des Verhaltens der Zecken am Rande der nassen Fläche zeigt, dass wiederholter Kontakt mit dem Wasser die Wahrscheinlichkeit erhöht, dass die Zecke der Randlinie folgt. Mit voranschreitender Zeit akzeptieren die Zecken den einseitigen Kontakt mit Wasser für längere Perioden. Sie tolerieren die nasse Umgebung schliesslich nach einer Kombination aus einer kurzen Wegstrecke auf der trockenen Fläche und einem Randlauf, der deutlich länger ist, als die vorhergehenden.
- (10) Offensichtlich tragen kürzere Wegstrecken und die hohe Frequenz der Randberührungen auf einer kleinen Kreisfläche zu Adaptationsprozessen bei, infolgedessen die Zecken eine kleinere Kreisfläche schneller verlassen als eine grössere. Dass unabhängig von der Form und Grösse der trockenen Fläche diese nach der gleichen Zahl von Randberührungen verlassen wird, deutet auf die Existenz eines ‚zentralnervösen Zählmechanismus‘ hin, der die Toleranz der nassen Fläche beeinflusst.
- (11) Die ambivalente Beziehung der Schildzecken zu Wasser – sie vermeiden den Kontakt mit flüssigem Wasser und benötigen andererseits Zonen mit hoher Luftfeuchte, um ihr Körperwasserdefizit auszugleichen – steigert wahrscheinlich die Überlebenswahrscheinlichkeit von Schildzecken sowohl in der parasitischen als auch in der nicht parasitischen Lebensphase.
- (12) Schildzecken bevorzugen hydrophobe Oberflächen. Die Kutikula von Artgenossen, die sebum-beschichtete Oberfläche des Wirtsfells und vielleicht auch die Wachskutikula der Pflanzen enthalten Signalstoffe, die den Schildzecken geeignete Plätze zur Aggregation bzw. Sexualpartner, Nahrungsquellen, und unter Umständen auch Erfolg versprechende Aufflaurpunkte signalisieren.

Contact chemostimuli affecting aggregation, attachment and water avoidance by Ixodid ticks

Thomas Kröber (1999) PhD Thesis, Institute of Zoology, University of Neuchâtel

Summary

Host surface stimuli affect arrestment and attachment by ixodid ticks. The effect of these stimuli in the host relationships of ticks is developed at different levels in this thesis.

- (1) The perception of skin lipids via contact chemosensilla was demonstrated for female *Boophilus microplus* using electrophysiological methods. Chemoreceptors in two terminal pore sensilla of the palps respond to steer hair lipids. Skin lipids from the host may play a role in host recognition and host attachment by ticks.
- (2) A receptor cell in the palp sensilla also showed a dose response reaction when stimulated with salts containing potassium ions. K^+ -ions also stimulated a receptor cell in contact chemosensilla on the tips of the tarsi in different life-stages of other pro- and metastriate tick species such as *Ixodes ricinus* and *Amblyomma variegatum*. Na^+ -containing salts had none or only a very small effect. As a higher K^+/Na^+ ratio distinguishes the sweat of herbivores from carnivores, the presence of high levels of K^+ could serve in recognition of herbivores that constitute the majority of hosts for ixodid ticks.
- (3) Larvae of *B. microplus* and nymphs of *Rhipicephalus sanguineus* aggregate on a sector of a filter paper disk treated with a defined dose of bovine hair lipid extract. This arrestment effect of lipids from the host surface is probably a response common to ixodid ticks.
- (4) Solid phase extraction was used to separate the steer hair extract into 10 fractions of different polarity, and at least two fractions of medium polarity were found to cause arrestment of *B. microplus* on a treated sector of a filter paper disk.
- (5) The bovine hair lipid extract plays an important role for successful attachment *in vitro* by ticks to an artificial membrane at 37°C. Skin lipids increased the attachment rate of *A. hebraeum* nymphs on such membranes by up to 30%. The effect of the steer hair lipids was about as strong as other attachment stimuli such as mechanical (hair and membrane texture), phagostimuli in the feeding medium under the membrane, and aggregation-attachment pheromones. Combining stimuli of different modalities (mechanical, taste, and smell) led to a higher *in vitro* attachment rate.
- (6) In another part of the thesis, avoidance of liquid water by ixodid ticks is described. In nature, ixodid ticks orientate towards zones of high humidity in order to compensate their body water deficit although they avoid contact with liquid water.

Summary

- (7) In a laboratory assay, hydrated larvae and males of *B. microplus*, larvae of *A. hebraeum* and all life-stages of *I. ricinus* running on a dry patch surrounded by a wet surface turn on encountering the wet surface.
- (8) Since contact with the tip of the tarsus of the first pair of legs is sufficient to initiate the border reaction on such a patch, it is concluded that contact chemosensilla on these appendages are responsible for water perception. Behavioural observations on ticks with terminal sensilla of the first leg tarsi covered with glue confirmed this hypothesis as the ticks failed to respond to the wet surface surrounding the dry patch.
- (9) Sequential analysis of the border behaviours showed that repetitive contact with the water increased the probability of walks astride the border with the wet surround. With time ticks accepted unilateral contact with the water for longer intervals, and eventually walked on to the wet surface after a combination of a short patch walk followed by a border walk that was longer than the foregoing ones.
- (10) Staying time on a small circular dry patch with a wet surround was shorter than on a large one, arising probably from faster adaptation of peripheral receptors following a higher frequency of border contacts on the small one. However, the equal number of border reactions observed on dry patches of different sizes and shapes suggests that a 'counter' in the CNS may also influence dry patch departure.
- (11) 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 during their parasitic phase as well as in the off-host phase.
- (12) Ticks show an affinity for hydrophobic surfaces such as the cuticle of conspecifics, the sebum-covered pelage of hosts, and the waxy cuticle of plants. Such loci, respectively, signal suitable aggregation sites or sexual partners, feeding sources and useful ambush sites.

Réactions comportementales des tiques Ixodidae au contact de l'eau et aux stimuli gustatifs provenant de la peau de l'hôte

Thomas Kröber (1999) Thèse, Institut de Zoologie, Université de Neuchâtel

Résumé

Les stimuli gustatifs provenant de la peau de l'hôte ont une influence sur l'agrégation et l'attachement des tiques Ixodidae. L'effet des ces stimuli dans les relations hôte-parasite a été démontré à différents niveaux dans cette thèse.

- (1) La perception des lipides de la peau de bovin par les sensilles gustatives des femelles de *Boophilus microplus* a été testée en électrophysiologie. Deux cellules réceptrices dans deux sensilles des palpes ont été stimulées par les lipides de la peau de l'hôte. Ces derniers jouent probablement un rôle dans la reconnaissance et l'attachement sur les hôtes chez les Ixodidae.
- (2) La cellule réceptrice stimulée par les lipides de la peau semble être également activée par des sels contenant des ions K^+ . Ces mêmes ions stimulent aussi une cellule réceptrice des sensilles sur les extrémités des tarsi de différents stades parmi les Pro- et Metastriata comme *Ixodes ricinus* et *Amblyomma variegatum*. Par contre, des sels contenant des ions Na^+ n'ont aucun ou seulement un faible effet sur ces sensilles. Le rapport K^+/Na^+ de la sueur des herbivores est élevé par rapport aux carnivores. Une concentration élevée des ions K^+ pourrait alors représenter un signal qui permettrait de reconnaître les herbivores; c'est chez ces derniers que l'on trouve la majorité des hôtes des tiques Ixodidae.
- (3) Les larves de *B. microplus*, et les nymphes des *Rhipicephalus sanguineus* s'agrègent de préférence sur le secteur d'un papier-filtre traité avec une dose définie d'un extrait des lipides de poils de bovin. Cet effet d'arrêt des lipides de surface est probablement un phénomène répandu chez les Ixodidae.
- (4) Extraction sur phase solide a été utilisée pour séparer l'extrait des poils de bovin dans 10 fractions de polarités différentes. Au moins deux fractions avec une polarité moyenne provoquent une réaction d'arrêt chez les larves de *B. microplus* sur des disques en papier-filtre.
- (5) L'extrait des lipides de la peau de bovin joue un rôle important pour la fixation des tiques d'Ixodidae sur les membranes d'alimentation *in vitro* à 37°C. Le taux de fixation des nymphes *Amblyomma hebraeum* pouvait augmenter jusqu'à 30% par application des lipides de la peau sur de telles membranes. L'influence de ces lipides est du même ordre que d'autres facteurs de fixation importants, comme les stimuli mécaniques (p. ex. poils et texture de la membrane), les phagostimuli dissous dans le milieu sous la membrane, et les phéromones d'agrégation-attachement. En combinant des stimuli de différentes modalités (sens mécanique, goût, odeur), on peut obtenir des taux de fixation importants pour des élevages *in vitro*.

Résumé

- (6) Une autre partie de la thèse décrit le comportement visant à éviter l'eau liquide chez les tiques Ixodidae. Dans la nature les tiques Ixodidae recherchent des zones avec une humidité élevée, pour compenser leur déficit en eau. Pourtant les individus hydratés évitent le contact avec l'eau liquide.
- (7) Dans un biotest en laboratoire, des tiques bien hydratées, larves et mâles de *B. microplus*, larves de *A. hebraeum* et tous les stades de *I. ricinus*, qui marchent sur une surface sèche entourée d'une surface de membrane mouillée, retournent immédiatement sur la surface sèche dès qu'il y a un contact avec la bordure mouillée.
- (8) Le contact de l'extrémité des tarsi de la première paire de pattes avec l'eau est suffisant pour provoquer la réaction d'évitement. On peut en conclure que ce sont probablement des sensilles de contact présentes à l'extrémité des tarsi qui sont responsables de la perception de l'eau. Des expériences réalisées en masquant les sensilles à l'extrémité des premiers tarsi avec de la colle confirment cette hypothèse. Les tiques ne répondent plus au contact avec la surface mouillée entourant la surface séchée.
- (9) L'analyse séquentielle du comportement des tiques au bord de la surface mouillée montre que le contact répétitif avec celle-ci augmente la probabilité du déplacement le long de cette même bordure. Avec le temps écoulé, les tiques acceptent le contact unilatéral avec l'eau pour des périodes de plus en plus longues. Après une combinaison d'un parcours court sur la surface sèche et une marche clairement plus longue que les précédentes sur la bordure, elles finissent par marcher sur la surface mouillée.
- (10) Les temps passés par les tiques sur un petit cercle sec étaient nettement plus courts que sur un cercle plus grand. Une fréquence élevée des contacts avec la bordure sur la petite surface entraîne probablement des adaptations plus rapides au niveau des organes sensoriels de la périphérie. Sur une surface de plus petit diamètre, les contacts avec la bordure sont fréquents et les tiques quittent donc plus rapidement un petit cercle qu'un grand. Par contre la surface sèche sera abandonnée indépendamment de sa forme et de sa taille après le même nombre de contacts : cela renforce l'hypothèse de l'existence d'un 'compteur du système nerveux central', qui influence la tolérance pour la surface mouillée.
- (11) Les tiques Ixodidae ont une relation ambiguë avec l'eau - elles recherchent des zones avec une humidité élevée, pour maintenir leur balance hydrique, et elles évitent le contact avec l'eau liquide. Cette relation augmente vraisemblablement leur probabilité de survie aussi bien dans la phase parasitaire que dans la phase non parasitaire.
- (12) En tout cas, les tiques Ixodidae préfèrent des surfaces hydrophobes comme la cuticule des congénères, la surface des hôtes couverte de sébum, et également la surface des plantes couverte par des cires. Ces surfaces lipidiques représentent des signaux chimiques qui indiquent respectivement des endroits pour s'agréger et pour trouver des partenaires sexuels, des sources d'alimentation convenables, et aussi des endroits pour guetter des hôtes.