Published in Journal of Insect Behavior 13, issue 3, 397-420, 2000
which should be used for any reference to this work

The Dynamics of an Avoidance Behavior by Ixodid Ticks to Liquid Water

T. Kröber[1,2] and P. M. Guerin[1]

[1]Institute of Zoology, University of Neuchâtel, Ch-2007 Neuchâtel, Switzerland.
[2]To whom correspondence should be adressed; e-mail: Thomas.Kroeber@zool.unine.ch.

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 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* phobotaxis, 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 ± 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-μm 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 μm diameter, 1 μl pipette) brought into contact with the membrane took up about 0.1 μl 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 μg/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 × 50 mm, diameter × 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 $32 \pm 2^{\circ}$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 $32 \pm 2^{\circ}$C, as above. High humidity (80–95% r.h.) in a Perspex chamber (30 × 15 × 25 cm, width × depth × 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, Sensortek 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).

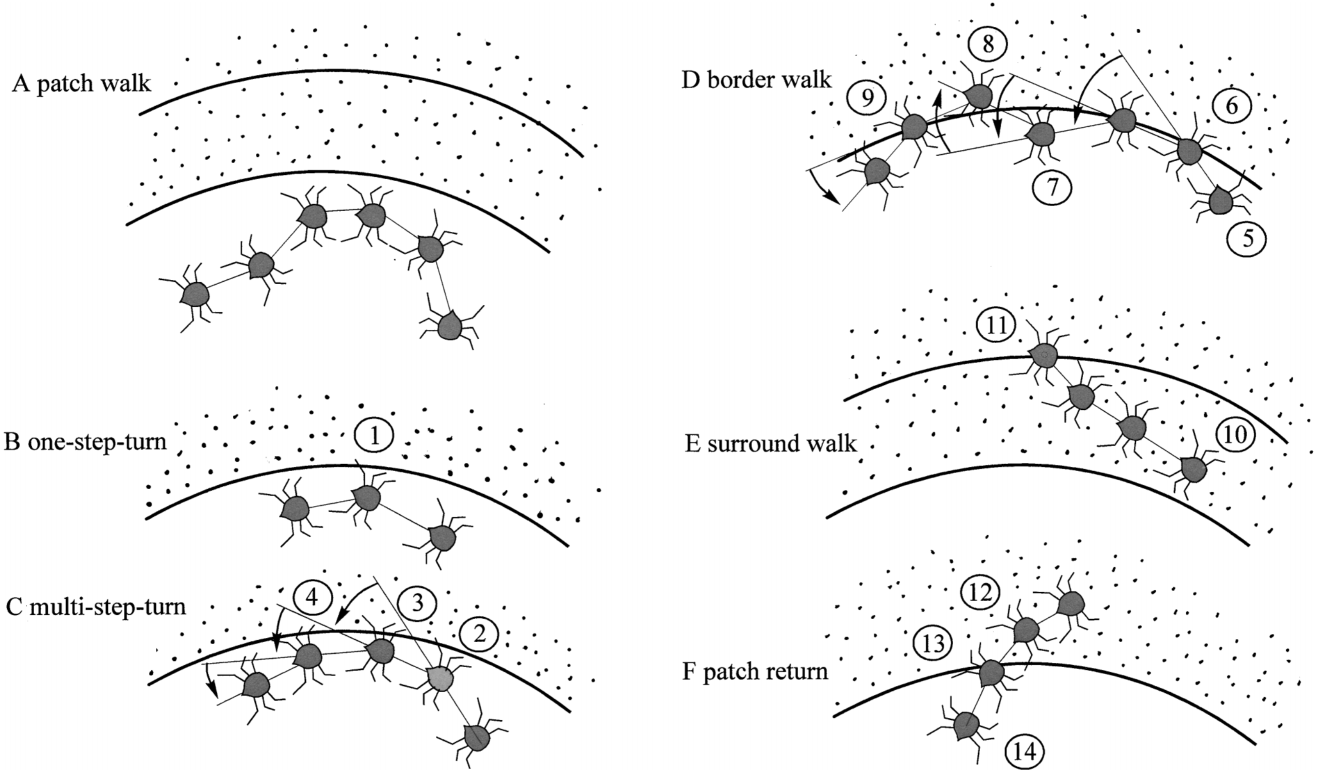

Fig. 1. Representation of the different portions of tracks which were quantified. (A) A patch walk; (B) a one-step-turn (1); (C) a multi-step-turn (2–4); (D) a border walk (5–9); (E) a wet surround walk (10–11); (F) a patch return (12–14). The trial ended (E) when the tick's center of gravity passed 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, $P_{\mathrm{KW}} \geq .3$; 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°) 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

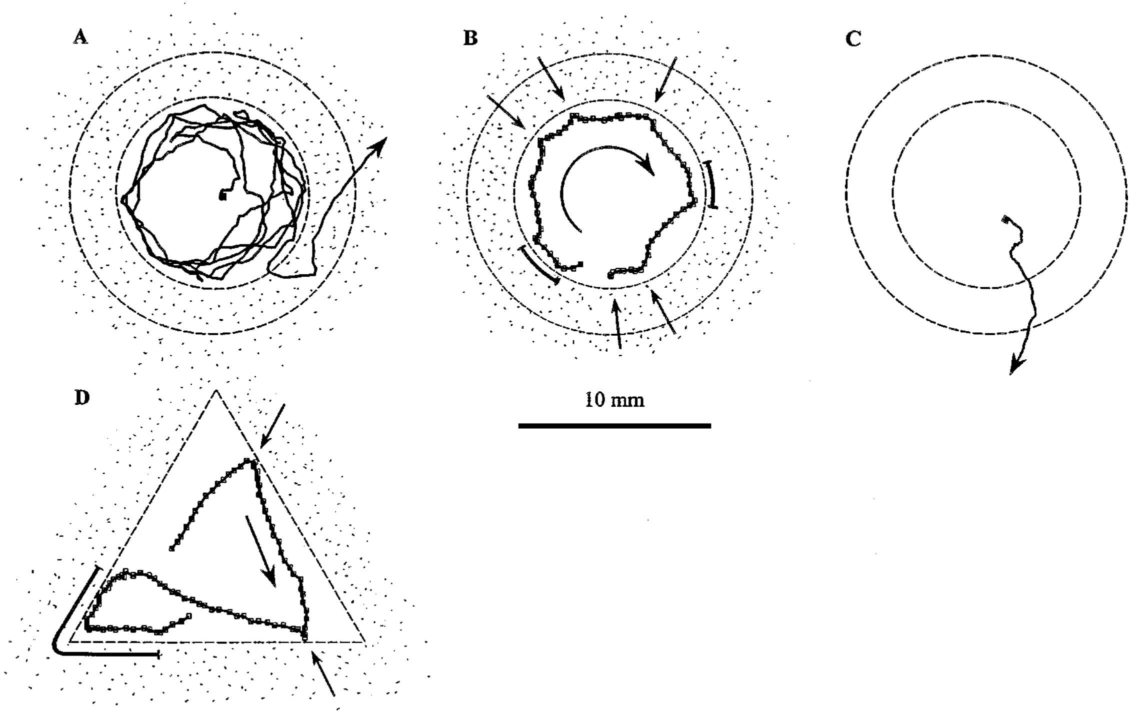

Fig. 2. Tracks described by *Boophilus microplus* larvae on circular and triangular dry patches (inner unshaded zones in A, B, and D) surrounded by a wet surface and on the corresponding dry control (C). The tick was released at the black square; the walk in (A) lasted 130 s. Sections of records on the 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).

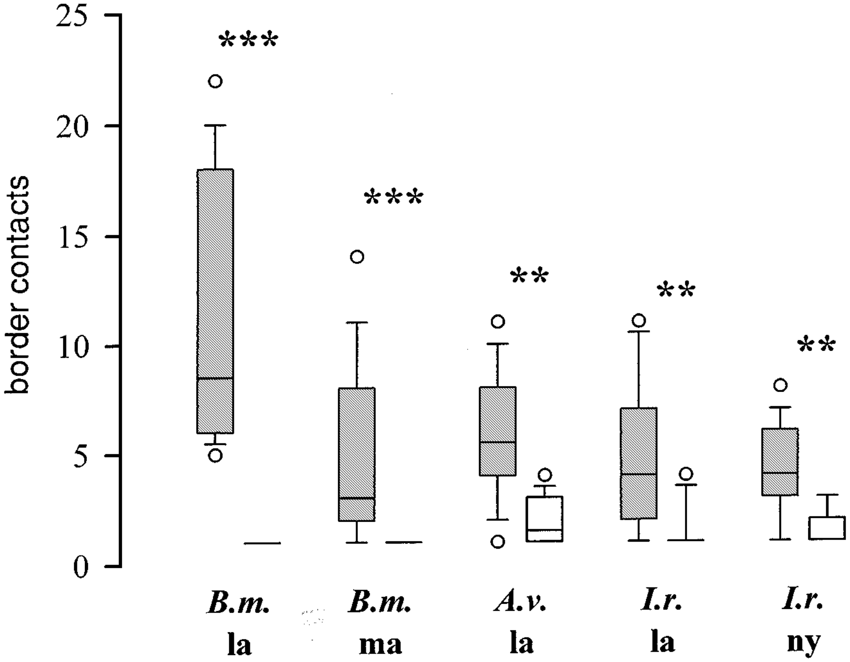

Fig. 3. Number of border contacts made by different life stages of three hard tick species on a dry patch (12 mm diameter) treated with steer hair extract on a wet surround. Shaded boxes, dry patch; blank boxes, wet control patch. B.m. la: *Boophilus microplus* larvae; B.m. ma: *B. microplus* males; 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 I).

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								25-mm circular patch: Surround wet				15-mm equilateral triangular patch: Surround wet			
	Surround wet				Surround dry											
	n	Median	Range	Sign.[a]	*n*	Median	Range	Sign.[b]	*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)	n.t.
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			

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

[b] Comparisons 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.

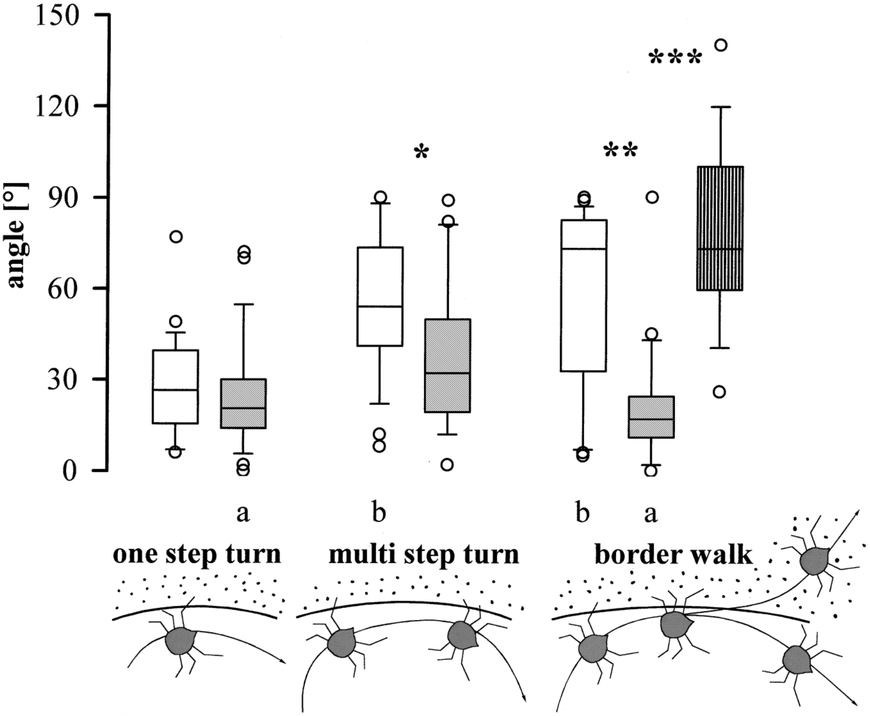

Fig. 4. Box plots of angles of arrival (blank) and departure (shaded) by *B. microplus* larvae at the border of a dry triangular patch created with a steer hair extract. The sketch under each pair of boxes shows a typical example. For box plot details see legend to Fig. 3. Data are from 24 one-step-turns, 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

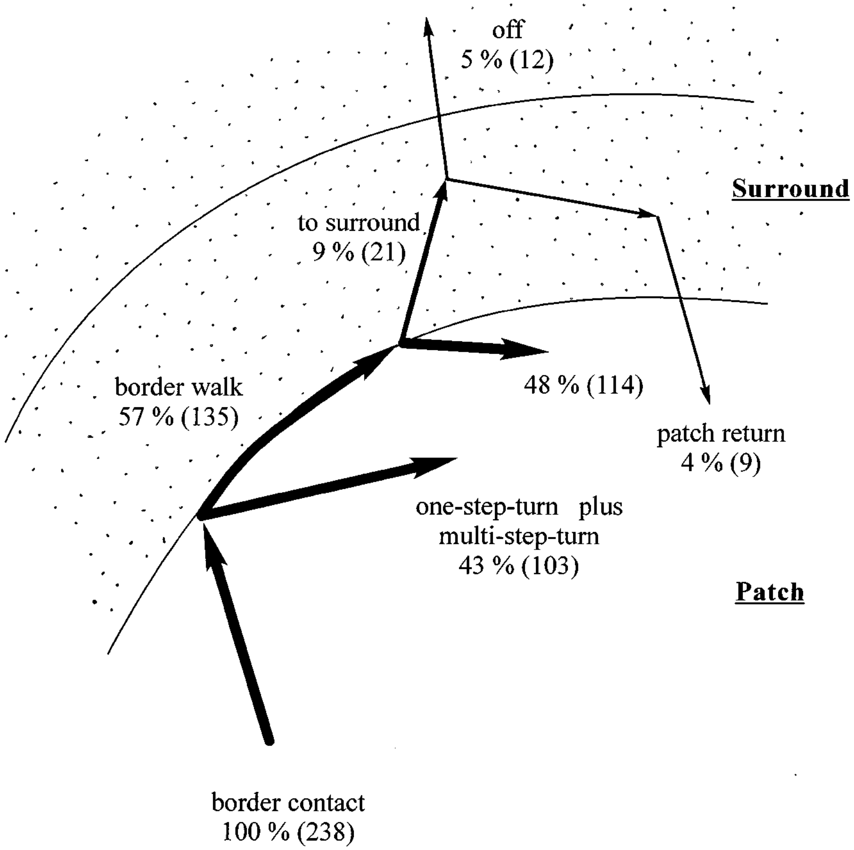

Fig. 5. Succession plot of the prevalence of different decisions made by *B. microplus* larvae at the border of a dry circular patch (10-mm diameter, wet surround shaded). Pooled data from tracks of 12 ticks which made 241 encounters with the border while walking on the membrane. After a border walk 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 [±SE] on the 10-mm patch and of 0.37 ± 0.17

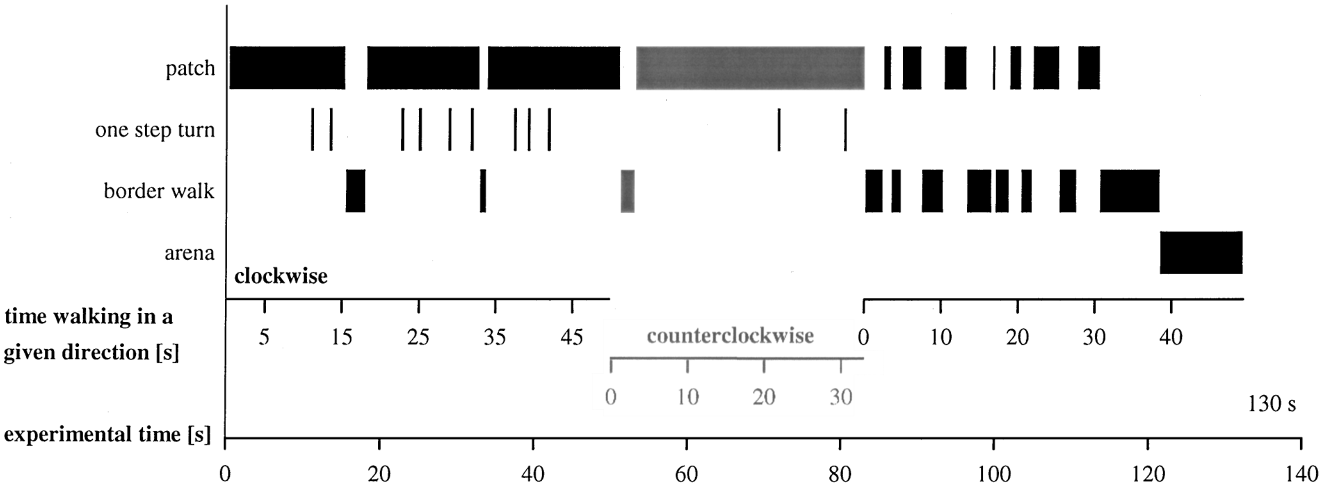

Fig. 6. Bar plot of the time course of different behaviors observed when a *B. microplus* larvae walked on a circular, dry, 10-mm-diameter patch with a wet surround. Note the shift from one-step-turns to border walks on the dry patch and the tendency for longer border walks with time. Periods walking 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 explantation see text). The original track is given in Fig. 2A.

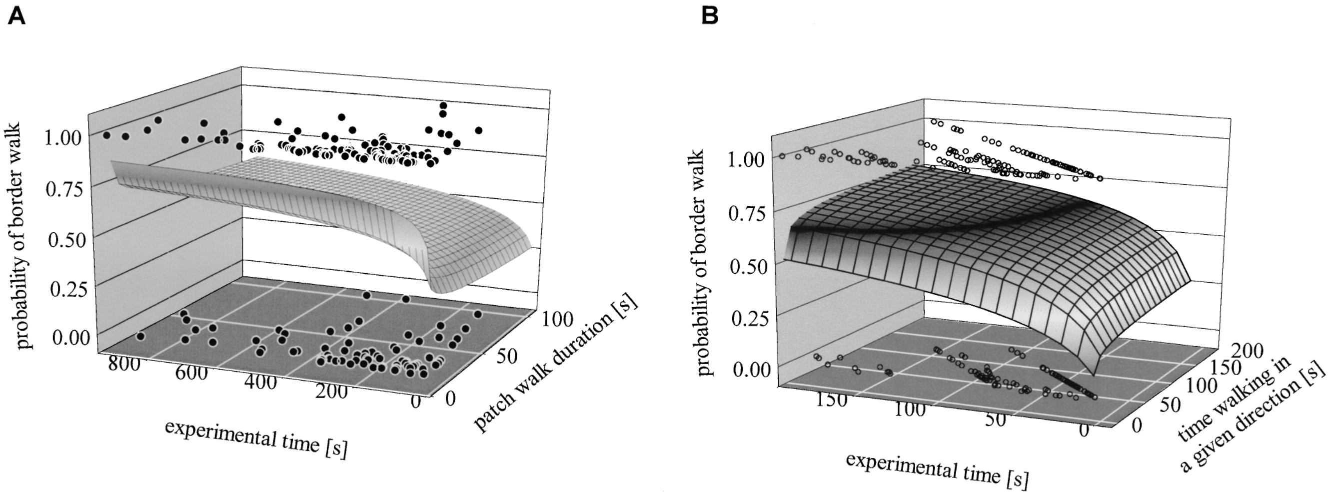

Fig. 7. Time dependence of different types of walks and decisions made at the border of a circular dry patch with a wet surround by *B. microplus* larvae. The probability of border walks is dependent on three parameters. (A) On a 25-mm patch it decreases with longer foregoing patch walks and increases 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 $r^2_{\text{part}} = .20$, $P_F \leq .001$; time walking in given direction $r^2_{\text{part}} = .01$, $P_F \leq .22; n = 138$ 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 ($P_{\text{KW}} \geq .4, n = 12, 12$, 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 counterxclockwise (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]

	10-mm circular patch				25-mm circular patch				15-mm equilateral triangular patch			
Type[b]	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

[a] Medians 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 ($P_{\mathrm{KW}} \geq .4$, $P_U = 1$); n.t., not tested.

[b] Border 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°), 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.

ACKNOWLEDGMENTS

Special thanks to Prof. Peter Allan Diehl for valuable comments on the manuscript. We also thank J. Moret for help with various aspects of the statistics and Dr. J. Jonczy and M. Rohrer of Novartis Animal Health SA, St. Aubin (Switzerland) for the regular supply of ticks. We wish to thank the Foundation Research 3R (Refine, Reduce, Replace), CH-3110 Münsingen, the Roche Research Foundation, and the Swiss Office for Education and Science for financing this work. This article is part of the Ph.D. thesis of Thomas Kröber at the University of Neuchâtel.

REFERENCES

Bell, W. J., and Tobin, T. R. (1982). Chemo-orientation. *Biol. Rev.* **57:** 219–260.

Charnov, E. L. (1976). Optimal foraging, the marginal value theorem. *Theor. popul. Biol.* **9:** 129–136.

Coro, F., Perez, M., Mora, E., Boada, D., Conner, W. E., Sanderford, M. V., Avila, H., Schuppe, H., and Burrows, M. (1998). Receptor cell habituation in the A(1) auditory receptor of four noctuoid moths. *J. Exp. Biol.* **201:** 2879–2890.

de Bruyne, M., and Guerin, P. M. (1994). Isolation of 2,6-dichlorophenol from the cattle tick *Boophilus microplus*. Receptor cell responses but no evidence for a behavioral response. *J. Insect Physiol.* **40:** 143–154.

Falk-Vairant, J., Guerin, P. M., de Bruyne, M., and Rohrer, M. (1994). Some observations on mating and fertilization in the cattle tick *Boophilus microplus*. *Med. Vet. Entomol.* **8:** 101–103.

Frankel, G. S., and Gunn, D. L. (1940). *The Orientation of Animals. Kineses, Taxes and Compass Reactions*, Oxford University Press, Oxford.

Gunn, D. L., and Pielou, D. P. (1940). The humidity behavior of the mealworm beetle, *Tenebrio molitor* L. III. The mechanism of the reaction. *J. Exp. Biol.* **17:** 307–316.

Hald, A. (1967). *Statistical Theory with Engineering Applications*, 7th ed., Wiley, New York.

Havukkala, I., and Kennedy, J. S. (1984). A programme of self-steered turns as a humidity response in *Tenebrio*, and the problem of categorizing spatial manoeuvres. *Physiol. Entomol.* **9:** 157–164.

Hemerik, L., Driessen, G., and Haccou, P. (1993). Effects of intra-patch experiences on patch time, search time and searching efficiency of the parasitoid *Leptopilina clavipes*. *J. Anim. Ecol.* **62:** 33–44.

Kahl, O., and Alidousti, I. (1998). Bodies of liquid water as a source of water gain for *Ixodes ricinus* ticks (Acari, Ixodidae). *Exp. Appl. Acarol.* **21:** 731–746.

Kennedy, J. S. (1978). The concepts of olfactory 'arrestment' and attraction. *Physiol. Entomol.* **3:** 91–98.

Krijgsman, B. J. (1937). Reizphysiologische Untersuchungen an blutsaugenden Arthropoden im Zusammenhang mit ihrer Nahrungswahl. III. Teil: Versuche mit Zecken. *Arch. Neerl. Zool.* **2:** 401–413.

Kröber, T., and Guerin, P. (1999). Ixodid ticks avoid contact with liquid water. *J. Exp. Biol.* **202:** 1877–1883.

Lees, A. D. (1948). The sensory physiology of the sheep tick, *Ixodes ricinus*. *J. Exp. Biol.* **25:** 145–207.

Pyke, G. H. (1984). Optimal foraging theory: A critical review. *Annu. Rev. Ecol. Syst.* **15:** 523–575.

Sorensen, K., and Bell, W. J. (1986). Orientation responses of an isopod to temporal changes in relative humidity: Simulation of a "humid patch" in a "dry habitat." *J. Insect Physiol.* **32:** 51–57.

Strand, M. R., and Vinson, S. B. (1982). Behavioral response of the parasitoid *Cardiochiles nigriceps* to a kairomone. *Entomol. Exp. Appl.* **31:** 308–315.

Thompson, R. F., and Spencer, W. A. (1966). Habituation: A model phenomenon for the study of neuronal substrates of behaviour. *Psychol. Rev.* **73:** 16–43.

Waage, J. K. (1978). Arrestment responses of the parasitoid, *Nemeritis canescens*, to a contact chemical produced by its host, *Plodia interpunctella*. *Physiol. Entomol.* **3:** 135–146.

Waage, J. K. (1979). Foraging for patchily-distribute hosts by the parasitoid, *Nemeritis canescens*. *J. Anim. Ecol.* **48:** 353–371.

Wilkinson, P. R. (1954). Observations on the sensory physiology and behaviour of larvae of the cattle tick, *Boophilus microplus* (Can.) (Ixodidae). *Aust. J. Zool.* **1:** 345–357.

Wood, E. R., Wiel, D. E., and Weeks, J. C. (1997). Neural correlates of habituation of the proleg withdrawal reflex in larvae of the hawk moth, *Manduca sexta*. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **180:** 639–657.