# On the visual perception of the eyes in adult *Hyalomma truncatum* ticks (Acari: Ixodidae)

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(Received 5 June 1996; accepted 30 March 1997)

ABSTRACT

Adult *Hyalomma truncatum* ticks with uncovered and foil-covered eyes were exposed to an upright-positioned rectangle as a target giving a luminance contrast ratio of 5:1 at a sun-simulating radiation. The transmission rate of the foil was less than 0.003%. Significantly ($p \leq 0.05$) more locomotorally active ticks with uncovered eyes (36.6%) responded to the target than ticks with foil-covered eyes (7.3%). When the rectangle was illuminated by monochromatic light at wavelengths ranging between 420 and 648 nm, the target induced a positive scototaxis in ticks with uncovered eyes regardless of the wavelength range. In contrast, ticks with covered eyes did not exhibit a positive scototaxis at wavelength ranges of 553–585 and 608–648 nm and very few ticks responded only to other wavelength ranges. The results indicate that the eyes are the only or at least the most essential sense organs in the visual system of adult *H. truncatum* ticks.

**Key words:** Ticks, *Hyalomma truncatum*, visual signals, perception, eyes.

## INTRODUCTION

All the ixodid and argasid ticks studied have been found to react to light regardless of the presence or absence of eyes, as has already been summarized in detail (Koch, 1989; Bergermann, 1996). Hence, it is justified to state that the eyes are not the only photoreceptors of ticks. However, whether the eyes of ixodid ticks, which are paired and located on the lateral margins of the scutum, are actually capable of perceiving visual signals has not definitely been proven experimentally. The functional assignment of the eyes as light-sense organs is so far based on the histological and ultrastructural organization when considering the eyes in adult ticks of *Amblyomma americanum* (Phillis and Cromroy, 1977), *Hyalomma asiaticum* (Leonovich, 1979), *Hyalomma dromedarii* (El Shoura, 1988) and *Rhipicephalus evertsi mimeticus* (Gothe *et al*., 1990) as well as on electroretinogram (ERG) measurements in *Dermacentor variabilis* (Carroll and Pickens, 1987), *H. dromedarii* (Carroll and Pickens, 1987; Kaltenrieder, 1989; Kaltenrieder *et al*., 1989) and *Amblyomma variegatum* (Kaltenrieder, 1989; Kaltenrieder *et al*., 1989). The morphological studies

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0168-8162 

revealed a rather simple structural organization; the eyes consist of a transparent lens with numerous pore canals as an outer part and a cluster of unipolar neutrons below the lens communicating with the optic nerve. Electrophysiological investigations resulted in notably weak ERG signals which indicated a maximum spectral sensitivity in the blue range ($\approx$470 nm) for *H. dromedarii* and *A. variegatum* and in the green range ($\approx$510 nm) for *D. variabilis*. However, optical radiation and visual signals are important behavioural-relevant stimuli for ticks, which are capable of perceiving differences in light intensity as well as the direction of light and responding to optical radiation of a sun-simulating spectral range as well as to monochromatic light. This visual performance has been demonstrated in unfed adult ticks of *Hyalomma truncatum* in particular, which are very resistant to harsh climatic conditions and live mostly in semi-desert, steppe and savanna (Wilson *et al*., 1993). The tick is widely distributed in Africa, actively seeks its host and is of veterinary and medical importance because of its marked vector competence and capability to induce toxicoses (Werder, 1992).

Previous studies have revealed that unfed adult ticks of *H. truncatum* respond phototactically positive to horizontally (Leuterer and Gothe, 1991) and vertically (Werder and Gothe, 1993) incident optical radiation of a sun-simulating wavelength spectrum as well as to various monochromatic light ranges even at an irradiance analogous to a full moon night (Leuterer and Gothe, 1991). Furthermore, it has been demonstrated that adult ticks exhibit a positive scototaxis to targets of different shape, size, luminance contrast ratio and elevation angle. The number of responding ticks increased with the target size, but targets in the shape of an upright-positioned, 312 $cm^2$ rectangle at an elevation angle of 13$^\circ$ giving a luminance contrast ratio of 5:1 were the most attractive and were significantly preferred over objects of other shape, elevation angle and luminance contrast ratio (Kopp and Gothe, 1995a). This behaviour indicates that adult ticks are able to sort, select and process visual signals, but they are probably not endowed with a true colour vision (Kopp and Gothe, 1995b). Adult ticks of *H. truncatum* are therefore particularly appropriate for behavioural experiments with regard to the functional relevance of the eyes as light-sense organs. Accordingly, the aim of this study was to investigate whether the perception of visual signals is to be attributed to the eyes in male and female ticks of *H. truncatum*.

## MATERIALS AND METHODS

Unfed male and female *H. truncatum* ticks between 4 and 11 weeks old were tested. They originated from South Africa and had been reared in the laboratory since 1990 on cattle. During off-host phases, all stages of the ticks were kept at 27$^\circ$C and 80% relative humidity (RH). Investigations into the perception of visual signals by the eyes were carried out in the same test arenas as described previously (Kopp and Gothe, 1995a,b). Unfed male and female ticks, with and without cover of the outer lens including the orbita of both eyes by a foil impervious to light, were exposed to an upright-positioned, 312 $cm^2$ rectangle at a sun-simulating radiation and monochromatic light of various wavelength ranges. This target had been previously proven to be the most attractive object as significantly more unfed adult *H. truncatum* ticks responded scototactically positive to it than to targets of other shapes and sizes (Kopp and Gothe, 1995a).

*Investigations into the perception of the target illuminated by a sun-simulating spectral range*

The test arena and the light source (a 1000 W xenon lamp, XBO 1000 W/HS/AFR, Starna, Pfungstadt, FRG) used in this experiment were described by Kopp and Gothe (1995a). Briefly, the investigations were carried out in a test arena ($120 \times 120 \times 60$ cm) which was constructed of cardboard and covered with white self-adhesive plastic sheeting (Alkor, Munich, FRG). In the centre of the ceiling an aperture of $10 \times 10$ cm was cut for the entrance of the optical radiation (a sun-simulating spectral range of 190–2600 nm). The light intensity corresponded to $4.70 \times 10^3$ mW $cm^{-2}$ and was measured by a precision radiometer (Type IL 1700, International Light, Massachusetts, USA). The rectangle used as the target was cut from a 1 mm thick plastic plate, whose front side was covered with dark grey plastic sheeting. The luminance contrast ratio of the target against the white wall of the test arena was determined by an exposure meter (Lunasix 3, Gossen, Erlangen, FRG). The target was firmly fixed in the middle of a wall at floor level of the arena giving a luminance contrast ratio of 5:1 and was offered to the test ticks.

At first, male and female ticks, whose eyes including the orbita were covered, approximately 2–3 h before starting the test, with a graphite foil of $300 \times 300$ μm (Plano, article G 3347, Marburg, FRG) and impervious to light, were tested. The transmission rate of the foil was measured by a spectrometer (Perkin Elmers, UV/VIS/NIR Spectrometer, Lamada 19, Überlingen, FRG) and was less than 0.003%. Before being tested the ticks were kept at 27°C and 80% RH. Thereafter, the ticks were transferred into the test arena and exposed to the target. After a 2 min exposure period, the graphite foils were immediately removed carefully with tweezers and the ticks were transferred to an incubator at 27°C and 80% RH and kept there for 48 h. Thereafter, the ticks were exposed to the target in the test arena again. As a control, the target was presented to ticks whose dorsum was provided with two equal-sized foils, but leaving the outer lens and the orbita uncovered.

All the tests were carried out in the arena at 65–75% RH, 27–31°C and a $CO_2$ concentration of 0.04–0.09 vol%. Sixty male and 60 female ticks were used per test or for each control experiment. Three ticks of the same sex were exposed to daylight in a glass tube, enclosed by a paper plug, for approximately 15 min. Thereafter, they were activated in a water bath of 31°C for 3 min and by human breath for 3 s and released onto the floor in the centre of the arena at a distance of 56 cm to the target. Immediately thereafter, the door of the arena was closed and a digital stop watch was started. During the following 2 min observation, the time that elapsed until the ticks started moving was registered. The number of exposed as well as locomotive ticks which reached the target was recorded. The number of ticks which remained motionless or did not touch the walls of the arena was recorded. After exposure of 15 ticks, the target was rotated clockwise. After testing 60 male or female ticks, the inner walls of the arena and the target were cleaned with 70% ethanol.

*Investigations into the perception of the target irradiated by monochromatic light*

The test arena used in this experiment was described by Kopp and Gothe (1995b). Briefly, the investigations were carried out in a cardboard test arena of $64 \times 64 \times 40$ cm which was coated inside with a pearl slide screen to increase the reflectance of entering radiation. The monochromatic radiation of different wavelength ranges was generated by an illumination device using a 450 W xenon lamp. Wide-band, horizontally emitted radiation in the sun-simulating spectral range of 190–2600 nm was adjusted parallel by a moveable collimator and focused on the entrance slit of a monochromator (Macam, Livingston, Scotland). The monochromator splits the radiation into its spectral components, enabling the selection of individual wavelength ranges between 300 and 801 nm by rotation of the optical grating. Wavelength ranges of 420–481, 509–560, 553–585 and 608–648 nm, representing blue, green, yellow and red light, respectively, were selected. The narrow-band radiation passing through the exit slit of the monochromator was concentrated by a condensor and was then projected onto the target in the arena. The intensity of the irradiance of each wavelength range at the exposure site of the ticks and directly in front of the centre of the target was adjusted by regulation of the lamp current to $3.07 \times 10^{-5}$ mW cm$^{-2}$ and $2.61 \times 10^{-5}$ mW cm$^{-2}$, respectively and was controlled with the precision radiometer. The spectral bandwidth of the four wavelength ranges was determined by turning the grating of a second analogous monochromator, whose entrance slit was attached to the exit slit of the monochromator used in the experiment and by measuring the remaining transmission of radiation with the radiometer.

All the tests were carried out in the arena at 65–75% RH, 27–31°C and a $CO_2$ concentration of 0.04–0.09 vol%. At each wavelength range 60 male and 60 female adult ticks, whose eyes including the orbita were covered by the graphite foil approximately 2–3 h before starting the test, were exposed to a black, upright-positioned and 312 cm$^2$ rectangle placed in the middle of a side wall at floor level under a luminance contrast ratio of 2:1. Immediately after the 2 min exposure period, the graphite foils were removed and the test ticks were kept at 27°C and 80% RH for 48 h in the incubator. Thereafter, the ticks were exposed to the target in the test arena again. The test ticks were released in groups of three in the centre of the test arena at a distance of 32 cm to the target and were observed for 2 min.

The results of the experiments were analysed by the $\chi^2$ test with a significance level of 0.05. On the assumption that all moving ticks spread randomly along the four walls of the arena, the probability of a target contact was calculated as the ratio of the width of the target to the length of the walls of the arena. The actual contact rates were compared with the expected value. If the observed ratio exceeded the stochastical one, a positive scototaxis was concluded.

## RESULTS

The male and female ticks did not differ significantly in any of the experiments and were therefore pooled.

Under a sun-simulating radiation and a luminance contrast ratio of 5:1 the target elicited higher contact rates than the randomly expected ones, irrespective of whether the eyes of the ticks were covered with the graphite foil or not. Therefore, the target induced a positive scototaxis in both ticks with covered and uncovered eyes. However, significantly more ticks with uncovered eyes (ticks after removal of the graphite foil as well as control ticks) responded scototactically positive than ticks whose eyes were blocked by the foil (Table 1). Furthermore, significantly more ticks with covered eyes migrated to the walls of the test arena with uncovered eyes. With regard to the other evaluation criteria there were no significant differences between the ticks with and without cover of their eyes.

The investigations into target perception under monochromatic radiation giving a luminance contrast ratio of 2:1 revealed that, when illuminated by the wavelength ranges selected in this experiment, the target elicited higher contact rates in ticks with uncovered eyes than the randomly expected ones (Table 2). Therefore, the target induced a positive scototaxis in ticks with uncovered eyes

TABLE 1

Positive scototaxis (%) of exposed ticks, locomotive ticks and ticks reaching a wall of the test arena revealed by exposure to an upright-positioned, 312 $cm^2$ rectangle under sun-simulating radiation at a luminance contrast ratio of 5:1

| | Exposed ticks | Locomotive ticks | Ticks reaching a wall at the test arena |
|---|---|---|---|
| Test ticks with foil-covered eyes | 6.6* | 7.3* | 14.3* |
| Test ticks after removal of the foils | 34.2** | 36.6** | 63.1** |
| Control ticks carrying foils outside the area of the eyes | 33.3** | 34.5** | 53.3** |

Different symbols indicate that the differences are statistically significant ($p \leq 0.05$), the symbols for the statistical evaluation compare the results within a column only.

regardless of the wavelength range. However, the ticks with covered eyes exhibited a positive scototaxis exclusively in the wavelength ranges of 420–481 and 509–560 nm, since the actual contact rates exceeded the stochastical ones only in these wavelength ranges, even though the differences were slight.

The comparison of the results of all the tests revealed significant differences (Table 2). The percentages of scototactically positive responding ticks with uncovered eyes under sun-simulating radiation and blue monochromatic light (420–481 nm) were significantly higher than the rates of positive scototaxis under monochromatic illumination at the wavelength ranges of 553–585 and 608–648 nm. In addition, significantly more ticks started moving within 1 s or 10 s under sun-simulating radiation than under monochromatic wavelength ranges of 553–585 and 608–648 nm, irrespective of whether the eyes were covered or not.

## DISCUSSION

These investigations clearly confirm previous studies (Kopp and Gothe, 1995a,b) which recorded that adult *H. truncatum* ticks exhibited a positive scototaxis to targets irradiated by a sun-simulating spectral range of 190–2600 nm or by visible light of different monochromatic wavelength ranges at the same irradiation level. However, considering the percentages of scototatically positive responding ticks of the locomotorally active ticks, it has to be stated that, at 36.6%, distinctly and significantly more ticks with uncovered eyes permanently occupied the upright-positioned, 312 $cm^2$ rectangle under the illumination of a sun-simulating spectral range giving a luminance contrast ratio of 5:1 than ticks with foil-covered eyes, of which only 7.5% migrated to the

TABLE 2

Target contact and locomotory activity within 1 and 10 s of ticks with and without covered eyes (%) exposed to an upright-positioned, 312 $cm^2$ rectangle under sun-simulating and monochromatic radiation at luminance contrast ratios of 5:1 and 2:1, respectively

| | Target contact | | Locomotory activity within 1 s | | Locomotory activity within 10 s | |
|---|---|---|---|---|---|---|
| Wavelength (nm) | Eyes uncovered | Eyes covered | Eyes uncovered | Eyes covered | Eyes uncovered | Eyes covered |
| 190–2600 | 34.2A | 6.6A | 26.6A | 39.1A | 40.0A | 58.3A |
| 420–481 | 33.3Aa | 9.2Aa | 13.3Aa | 25.8Aa | 30.0Aa | 66.6Aa |
| 509–560 | 23.3Aab | 7.5Aa | 15.0Aa | 23.3Aab | 36.6Aa | 56.6Aa |
| 553–585 | 10.8Bb | 2.5Aa | 2.5Bb | 5.8Bc | 17.5Ba | 29.2Bb |
| 608–648 | 15.0Bb | 3.3Aa | 5.8Bab | 10.0Bbc | 15.8Ba | 25.8Bb |

The letters for the statistical evaluation compare the results within a column only. Upper case letters compare the sun-simulating with the monochromatic light, and the lower case letters compare the monochromatic wavelength ranges. Different letters indicate that the differences are statistically significant ($p \leq 0.05$).

target and remained there. Consequently, it seems justified to conclude that visual signals are perceived and passed on by the eyes. This means that the eyes of adult *H. truncatum* may be classified by function as light-sense organs. On the assumption that moving ticks spread randomly along the walls of the test arena and calculating the probability of the expected target contact, it is, however, indicated that ticks with covered eyes also responded scototactically positive. The observed ratio of 7.3%, exceeded the stochastical one, even though the difference is very slight. The assessment of positive scototaxis is correct even when the actual rates of target-occupying ticks are only slightly higher than the stochastical ratios. However, the exceeding, by 4.7%, of the expected value (2.6%) does not convincingly exclude a still random distribution of moving ticks with covered eyes along the walls of the test arena.

Assuming that a positive scototaxis was actually exhibited by adult *H. truncatum* whose eyes were covered by foils leaving a transmission of less than 0.003% for visible light only, it may be argued that either visual signals are perceived by the eyes even at extremely low light intensities or the ticks are provided with extraretinal photoreceptors. As has already been summarized in detail, all the eyeless ticks studied have been found to be responsive to light (Koch, 1989; Bergermann, 1996). Accordingly, eyeless ticks have to be equipped with extraretinal photoreceptors which possibly also exist in eye-bearing ixodid ticks. Whether extraretinal photoreceptors actually exist in eye-bearing ixodid ticks has still to be clarified and has been discussed only for *H. dromedarii* (Kaltenrieder, 1989; Kaltenrieder, *et al*., 1989). It was stated that the extraretinal photoreceptors are localized along the outer rim of the scutum/alloscutum and that the responses considered as positive phototaxis were not impaired by painting black paint over this region, the eyes and/or the dorsal surface of the ticks, resulting in a transmission rate of 1% for visible light. However, the mode of measuring the transmission rate has not been reported. Since orientations towards a light stimulus were possible even with the eyes, extraretinal photoreceptors and/or the dorsal surface occluded, it was suggested that either the paint did not completely block the light, additional extraretinal photoreceptors exist or scattered light enters through the cuticle eliciting a phototactically positive response. When the eyes and the extraretinal photoreceptors were painted over, the responses judged as positive phototaxis were not reduced but required an increase of the threshold irradiance only. In conclusion, it has been stated therefore that apart from the eyes no other very sensitive photoreceptor plays a role in the visual system of *H. dromedarii* (Kaltenrieder, 1989; Kaltenrieder *et al*., 1989). Thus, the existence of extra-retinal photoreceptors in *H. dromedarii* ticks is not definitely proven.

The classification of eyes by function as light-sense organs in *H. truncatum* is in correspondence with ERG measurements from outside the margin of the eyes as well as with behavioural bioassays concerning other metastriate tick species. Thus, for *D. variabilis* and *H. dromedarii* it has been shown that flashes of monochromatic light produce ERG pulses. The plots of the ERG responses of both species were unimodal, with *D. variabilis* peaking at 510 nm and *H. dromedarii* at 450–470 nm. The ERG signals, however, were notably weak (Carroll and Pickens, 1987). The spectral sensitivity maximum in the ERG at 470 nm was later confirmed for *H. dromedarii* and was stated also for *A. variegatum* (Kaltenrieder, 1989; Kaltenrieder *et al*., 1989), without, however, reporting where the recording electrode was inserted.

The function of the eyes as light-sense organs is also indicated by behavioural experiments with *H. asiaticum* revealing that ticks with destroyed eyes did not respond to a host from a distant position, but exhibited a host-seeking activity when they were at close range to a host. Accordingly, it has been concluded that long- and short-distance orientation occurs visually and olfactorily, respectively (Kupressova *et al*., 1977). This conclusion was supported by behavioural studies in the field as *H. asiaticum* ticks whose eyes were covered with a black paint did not recognize a host from 2 m distance. In contrast to ticks with uncovered eyes running straight up to the host, ticks with covered eyes wandered around without any orientation, abruptly changed direction and reached the host by accident only (Leonvich, 1986).

The importance of eyes in *H. truncatum* as receptors of visual signals is supported by the responses of the ticks with uncovered eyes to the same target but illuminated by monochromatic light at wavelength ranges of 420–481 (blue), 509–560 (green), 553–585 (yellow) and 608–648 nm (red) at a luminance contrast ratio of 2:1. The percentages of ticks occupying the target under monochromatic radiation of 420–481 and 509–560 nm did not differ significantly from those when the ticks were exposed to the target irradiated by the sun-simulating spectral range at a luminance contrast ratio of 5:1. In contrast, the ticks with covered eyes did not exhibit a positive scototaxis under yellow and red monochromatic light and very few ticks responded scototactically positive at the other wave-length ranges. Consequently, it appears justified to conclude that the eyes in adult *H. truncatum* ticks are the only or at least the most essential sense organs in the visual system.

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