



# Blood-seeking horseflies prefer vessel-imitating temperature gradients on host-mimicking targets: Experimental corroboration of a new explanation of the visual unattractiveness of zebras to tabanids



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## ABSTRACT

Several hypotheses tried to explain the advantages of zebra stripes. According to the most recent explanation, since the borderlines of sunlit white and black stripes can hamper thermal vessel detection by blood-seeking female horseflies, striped host animals are unattractive to these parasites which prefer hosts with a homogeneous coat, on which the temperature gradients above blood vessels can be detected more easily. This hypothesis has been tested in a field experiment with horseflies walking on a grey barrel with thin black stripes which were slightly warmer than their grey surroundings in sunshine, while in shade both areas had practically the same temperature. To eliminate the multiple (optical and thermal) cues of this test target, we repeated this experiment with improved test surfaces: we attracted horseflies by water- or host-imitating homogeneous black test surfaces, beneath which a heatable wire ran. When heated, this invisible and mechanically impalpable wire imitated thermally the slightly warmer subsurface blood vessels, otherwise it was thermally imperceptible. We measured the times spent by landed and walking horseflies on the test surface parts with and without underlying heated or unheated wire. We found that walking female and male horseflies had no preference for any (wired or wireless) area of the water-imitating horizontal plane test surface on the ground, independent of the temperature (heated or unheated) of the underlying wire. These horseflies looked for water, rather than a host. On the other hand, in the case of host-imitating test surfaces, female horseflies preferred the thin surface regions above the wire only if it was heated and thus warmer than its surroundings. This behaviour can be explained exclusively with the higher temperature of the wire given the lack of other sensorial cues. Our results prove the thermal vessel recognition of female horseflies and support the idea that sunlit zebra stripes impede the thermal detection of a host's vessels by blood-seeking horseflies, the consequence of which is the visual (non-thermal) unattractiveness of zebras to horseflies.

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## 1. Introduction

Several explanations have been published about the possible functions of zebra stripes. Since they are thoroughly reviewed, discussed and criticized in the book of Caro (2016), we only list them here with the relevant references: (i) apparent size increase (Cott, 1966; Cloudsley-Thompson, 1984; Vaughan, 1986; Caro, 2016), (ii) decreased visibility in twilight (Galton, 1851; Kipling, 1908; Cott, 1966; Cloudsley-Thompson, 1984; McLeod, 1987; Morris, 1990), (iii) dazzling the eyes of predators with moving stripes (Cott, 1957; Kruuk, 1972; Eltringham, 1979; Morris, 1990; How and Zanker, 2014), (iv) optical camouflage from predators (Wallace,

1867; Darwin, 1871; Wallace, 1879; Poulton, 1890; Thayer, 1909; Marler and Hamilton, 1968; Caro, 2009; Melin et al., 2016), (v) intraspecific communication by individual stripe patterns (Cloudsley-Thompson, 1984; Kingdon, 1984; Becker and Ginsberg, 1990; Morris, 1990; Prothero and Schoch, 2003), (vi) warning colouration (Poulton, 1890; Caro, 2016), (vii) visual defence against blood-sucking tsetse flies and tabanids carrying pathogens of serious diseases (Harris, 1930; Vale, 1974; Waage, 1981; Jordan, 1986; Foil, 1989; Estes, 1992; Gibson, 1992; Egri et al., 2012a; Blahó et al., 2013; Caro et al., 2019; Horváth et al., 2019a; How et al., 2020), (viii) visual signalling of fitness (Ruxton, 2002), (ix) cooling by convective air eddies above sunlit stripes (Cloudsley-Thompson, 1984; Kingdon, 1984; Morris, 1990; Louw, 1993; Cobb and Cobb, 2019). The experimentally best proven advantage of heterogeneous (e.g. striped or spotted) coat

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patterns is the visual unattractiveness to biting tsetse flies and horseflies (Vale, 1974; Waage, 1981; Gibson, 1992; Blahó et al., 2012, 2013; Egri et al., 2012a; Caro et al., 2014, 2019; Horváth et al., 2014, 2018; Caro and Stankowich, 2015; Caro, 2016; Melin et al., 2016; Kojima et al., 2019; How et al., 2020; Pereszlényi et al., 2021).

Recently, a new explanation was proposed for the unattractiveness of striped host animals to blood-seeking female tabanids (Takács et al., 2022): a female horsefly alighted on a host has to find a blood vessel (Fig. 1). Vessel detection may be performed with thermoreception on the basis of vessel-induced gradients of the surface temperature  $T$ . In sunshine, these  $T$  gradients of the skin above the slightly warmer blood vessels are difficult to distinguish from the  $T$  gradients induced by the hairs at the borderlines of warmer black and cooler white stripes. Therefore, the borderlines of sunlit white and black stripes can hamper successful thermal vessel detection. This may also be an important reason why female horseflies avoid striped (or spotted) host animals and prefer hosts with homogeneous coats, on which the  $T$  gradients above blood vessels can be detected more easily.

Takács et al. (2022) tested this hypothesis in a field experiment with tabanids walking on a host-imitating grey test target (barrel) with vessel-mimicking vertical and horizontal thin black stripes which were slightly warmer than their grey surroundings in sunshine, while under shady conditions both black and grey areas had practically the same  $T$ . They found that horseflies spent more time walking on thin black stripes than surrounding grey areas

as expected by chance, but only if the test target was sunlit. This was because the black stripes were warmer than the surrounding grey areas in the sun, but not in the shade. All these are consistent with the horseflies' experimentally proven attraction to warmer targets (Horváth et al., 2010, 2019b; Blahó et al., 2012, 2013; Krcmar et al., 2014; Otártics et al., 2019), because they can escape from warmer substrates more easily to avoid the parasite-repellent reactions of the attacked host (Horváth et al., 2020a). The often false blood vessel localizations at the numerous black-white borderlines of the zebra coat, the subsequent painful bites with unsuccessful blood-sucking attempts and the parasite-repellent reactions of the host significantly increase the chance that horseflies cannot escape from the host's responses and are swatted by them. To reduce this risk, an appropriate behaviour is the avoidance of hosts with striped (or any heterogeneous) coat pattern.

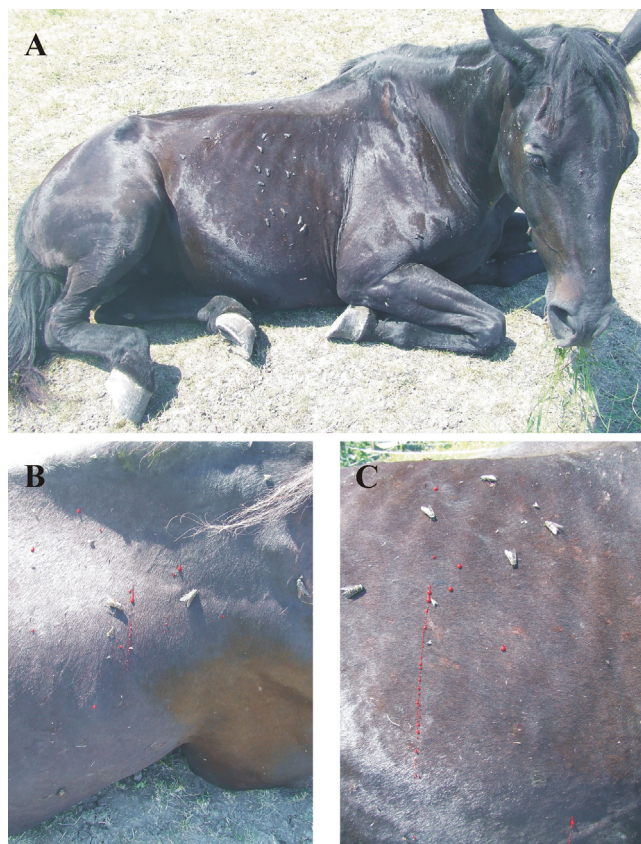
The sunlit black-striped grey barrel used as a test target in the field experiment of Takács et al. (2022) offered both optical and thermal cues for host-seeking female tabanids: the black stripes were visually distinguishable from their grey surroundings, because the former reflected light with less intensity  $I$  and a larger degree of polarisation  $d$  than the latter; furthermore the stripes had higher temperatures  $T$  than the grey areas. Thus, without an appropriate control experiment, the finding that tabanids preferred the sunlit black stripes over sunlit grey areas could be explained as follows: tabanids preferred the black stripes due to their (i) smaller  $I$ , and/or (ii) larger  $d$ , and/or (iii) higher  $T$  compared to the brighter, less polarised and cooler grey areas. In a control experiment with the same black-striped grey barrel in the shade (when the stripes and their grey surroundings had practically the same  $T$ ) the tabanid preference for the shady stripes disappeared. From this Takács et al. (2022) concluded that since the used test target demonstrated only thermal effects, their results can be explained by this thermal difference.

In spite of this control experiment of Takács et al. (2022), a more convincing method would have been to present only thermal cues, when the blood vessel imitating surface regions can be discerned only on the basis of their higher  $T$ , while their optical cues ( $I$  and  $d$ ) are the same as those of their surroundings. To eliminate the multiple cues of the black-striped grey barrel used by Takács et al. (2022), we repeated their experiment with improved test targets: we attracted male and female horseflies by water- or host-imitating homogeneous black test surfaces, beneath which ran an electrically heatable wire. The invisible and mechanically impalpable wire thermally imitated the slightly warmer subsurface blood vessels when heated, otherwise it was thermally imperceptible. We measured the times spent by landed and walking tabanids on the test surface parts with and without underlying wires when the latter were heated and unheated. We wanted to ascertain whether horseflies prefer the thin surface regions above the wire only if it is heated and thus warmer than the surroundings. If the wired surface parts were preferred to the unwired ones only in the case of heated wire, this preference could be explained exclusively with its higher temperature given the lack of other sensorial (optical/visual or tactile) cues. This would unambiguously prove the thermal vessel recognition of (female) tabanids and support the idea that sunlit zebra stripes (or dark-bright spots) impede the thermal detection of a host's vessels by blood-seeking horseflies.

## 2. Materials and methods

### 2.1. Ethics declarations

In our field experiments we studied the behaviour of horseflies (Tabanidae) landing on our test surfaces. Since these parasites are



**Fig. 1.** A tabanid-attacked horse. (A) A dark brown horse attacked by numerous female horseflies (*Tabanus bovinus*) in a field near the Hungarian village of Szokolya. (B, C) The flies have bitten blood vessels, from which the blood has dripped intensely. Photographs were taken by Gábor Horváth in July 2019, and are being published, with the permission of the horse's owner, Csaba Viski, Szokolya, Hungary.

non-protected insects in Hungary, no approval from an ethical committee was necessary. The owner (Csaba Viski, Szokolya, Hungary) of a horsefly-attacked horse permitted us to take the photographs presented in Fig. 1. We confirm the followings: (i) for our studies no institutional permission, licence or approval were necessary. (ii) No animals (horseflies, horses) were killed specifically for the purpose of these studies.

## 2.2. Field experiments

Our field experiments were performed from 3 July to 10 August 2022 on a Hungarian horse farm in Szokolya (47° 52' N, 19° 00' E), where horseflies (Tabanidae) were abundant. On the days of experiments (Supplementary Tables S1–S3) the weather was warm and sunny (25–40 °C) with sporadic cumulus clouds on some afternoons. In these experiments we investigated the behaviour of tabanid flies which had landed on a sunlit shiny (smooth) black horizontal or tilted (60° from the horizontal) plane test surface, and on a sunlit shiny (smooth) black vertical cylindrical target (Fig. 2A,D,G and 3A,D). An electrically heatable wire ran below the smooth black test surfaces. The tilted and vertical test targets imitated dark host animals (mainly ungulates) of female tabanids, and the heated wire mimicked the slightly higher temperature of the skin over blood vessels of hosts. On the other hand, the horizontal test surface imitated a water surface for water-seeking polarotactic male and female tabanids. The aim of these experiments was to reveal whether horseflies landing and crawling on the test surfaces (i) can or cannot detect the subsurface heated wire on the basis of the temperature gradients above the warmer wires imitating blood vessels being warmer than the surrounding tissues of a host animal, and (ii) do or do not prefer the thin surface

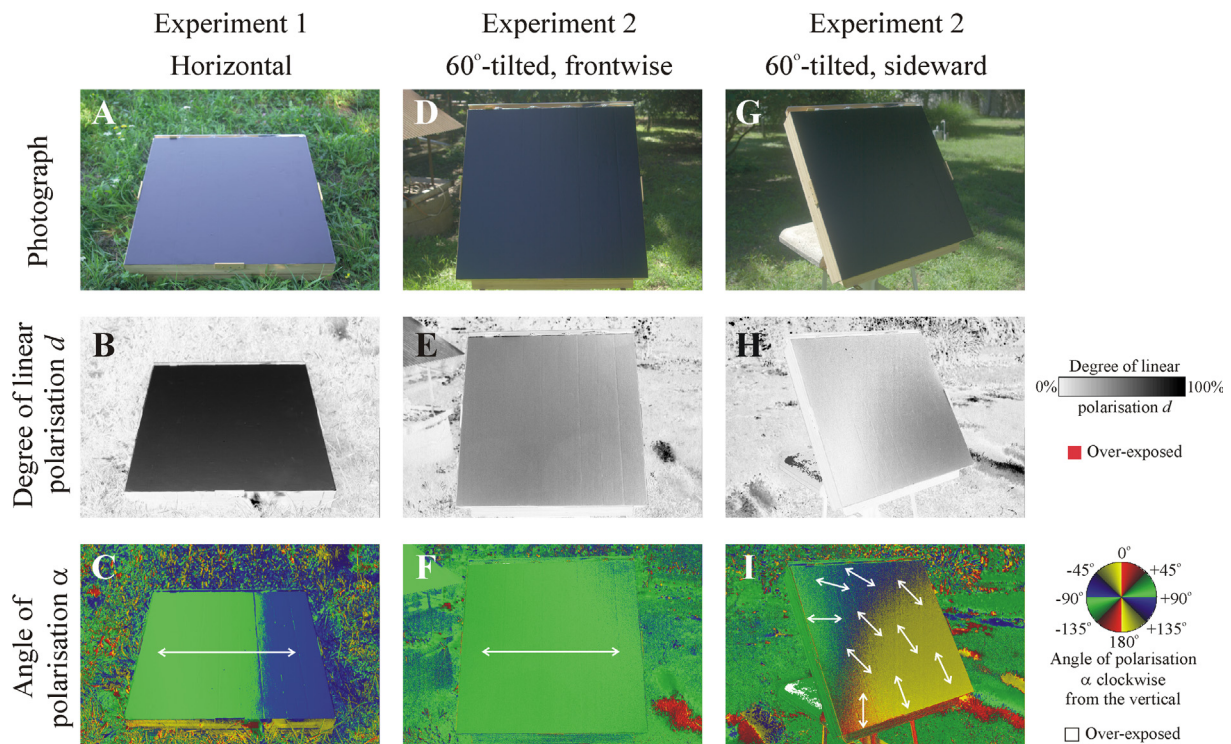
areas above the heated wire. Note that tabanids can sense the surface temperature only when they contact a target after landing.

The experimenter was sitting on a chair during the experiment at a distance of 2 m from the test surface and continuously observed the sunlit test target. They wore white clothes and a white hat to minimize their visual attractiveness to tabanids (Horváth et al., 2010).

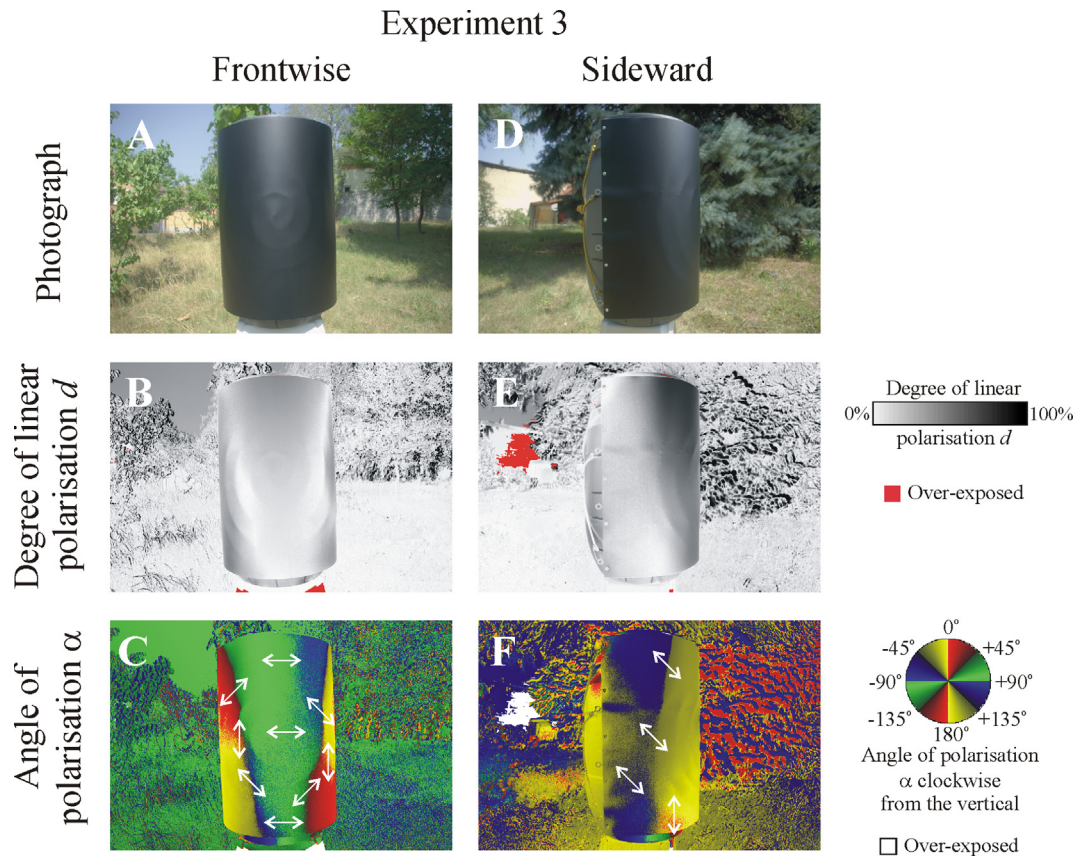
The horseflies alighting and walking on the test surface were observed by the same person from a distance of 2 m. An alighted horsefly showed two distinct behavioural elements prior to flying away: (i) staying in a place for a while, or (ii) walking around with some stops. During walking it frequently swept the substrate with its two forelegs to test the surface characteristics.

Our goal was to measure the times horseflies spent walking on the wireless and wired areas of the test surface. The observer concentrated only on walking tabanids monitoring the substrate. Using two stop-watches, the observer measured the times spent by walking tabanids above areas with underlying wires (with the first stop-watch held in the right hand) and above regions without underlying wires (with the second stop-watch held in the left hand) of the test surface. When a horsefly alighted on the wired/wireless area and started to walk, the right/left stop-watch was started and was stopped when it flew away, or walked onto a wireless/wired area. This procedure was repeated throughout the experimental days from morning (9:00 h = local summer time = Universal Time Coordinated + 2 h = UTC + 2 h) to afternoon (17:00 h = UTC + 2 h). If after landing a horsefly stayed in place prior to flying away, its staying period was not registered with the stop-watch, since this reaction was irrelevant with respect to seeking something (e.g. vessel).

Since we used dry (i.e. non-sticky) targets, the same horsefly might have alighted several times on the test surface. This pseu-



**Fig. 2.** Polarisation characteristics of our plane test surfaces. Reflection polarisation patterns of the plane test surface used in field experiment 1 (A–C) and 2 (D–I) measured with imaging polarimetry in the green (550 ± 40 nm) spectral range when the optical axis of the polarimeter was 56° [ $\approx 0_{\text{Brewster}} = \arctan(n = 1.5) = 56.3^\circ = \text{Brewster angle}$ ] from the vertical (A–C), and horizontal (D–I). In the patterns of the angle of polarisation  $\alpha$  (measured clockwise from the vertical) double-headed arrows show the local direction of polarisation (C, F, I). In A–C and D–F, the observer/polarimeter faced toward the solar meridian, while in case of G–I the sunlight came from the upper left corner of the picture.



**Fig. 3.** Polarisation characteristics of our cylindrical test surface. Reflection polarisation patterns of the vertical cylindrical test target used in field experiment 3 measured with imaging polarimetry in the green ( $550 \pm 40$  nm) spectral range when the optical axis of the polarimeter was horizontal. In the patterns of the angle of polarisation  $\alpha$  (measured clockwise from the vertical) double-headed arrows show the local direction of polarisation (C, F). In A–C, the sunlight came from behind the observer/polarimeter, while in case of D–F it came from the upper right corner of the picture.

doreplication could have been avoided only with sticky test targets, on which a landed horsefly could not have walked and flown away, thus the time the fly spent on the target could not have been measured.

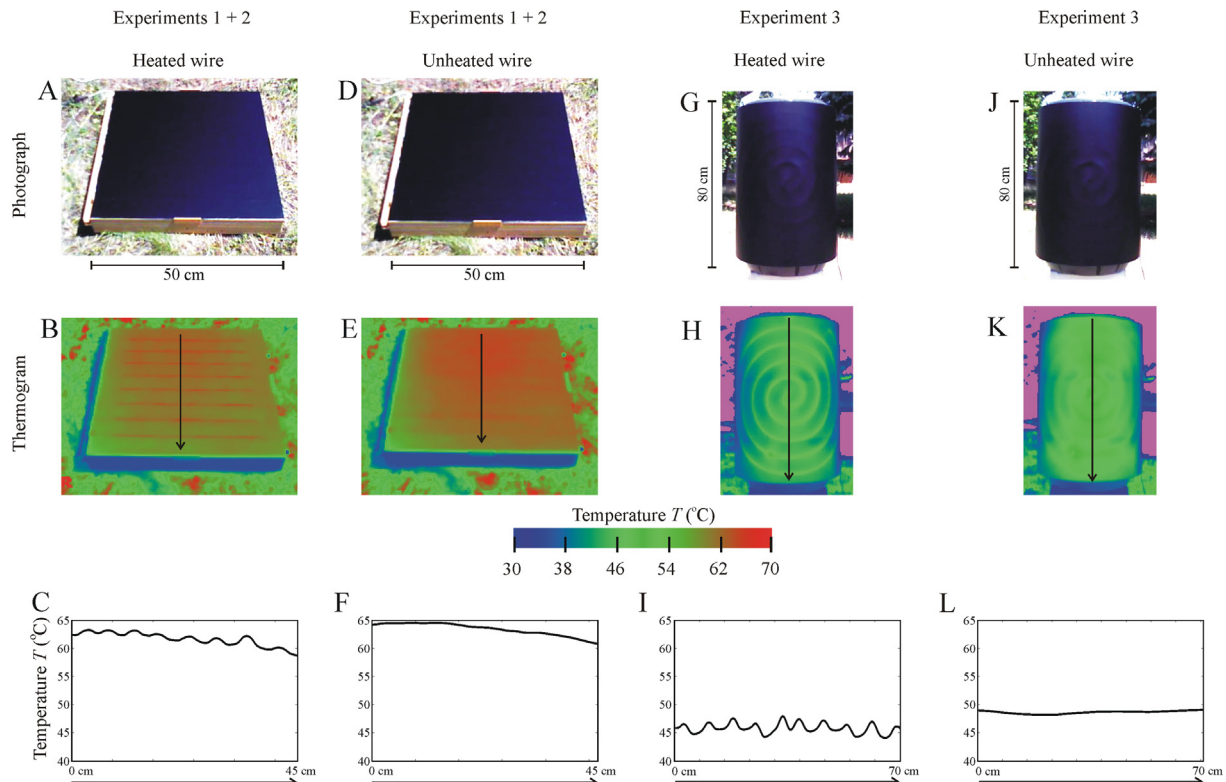
The identification of the species of horseflies alighted on the test surfaces was impossible. However, it was obvious that they were tabanids (Diptera: Tabanidae). In previous field experiments (Herczeg et al., 2014, 2015) the following tabanid species were found to occur at the same study site: *Tabanus tergstinus*, *Tabanus bromius*, *Tabanus bovinus*, *Tabanus autumnalis*, *Atylotus fulvus*, *Atylotus loewianus*, *Atylotus rusticus*, *Haematopota italica*. In female tabanids the eyes are separated by a frontal stripe, while in males the eyes are normally touching (Chvala et al., 1972). These characteristics of the tabanid eyes could be differentiated in our experiments, in which only female tabanids landed on the 60°-tilted plane and the vertical cylindrical test surfaces, while the horizontal plane test surface on the ground attracted both female and male tabanids that sought water (Horváth et al., 2014).

Our host-imitating artificial test targets differed from the real host animals (mainly ungulates) of tabanids. In spite of this, we did not measure the spectral reflectance of our test surfaces and did not compare it to that of real hosts, because such a comparison was unnecessary. The only relevant goal of our field experiments was to imitate the temperature gradients of the host's skin above blood vessels by the heated wire below the black test surfaces. According to the thermograms presented in Fig. 4 of this work (see also Figs. 3 and 5 of Horváth et al., 2018, and Fig. 4 of Takács et al., 2022), this imitation was appropriate.

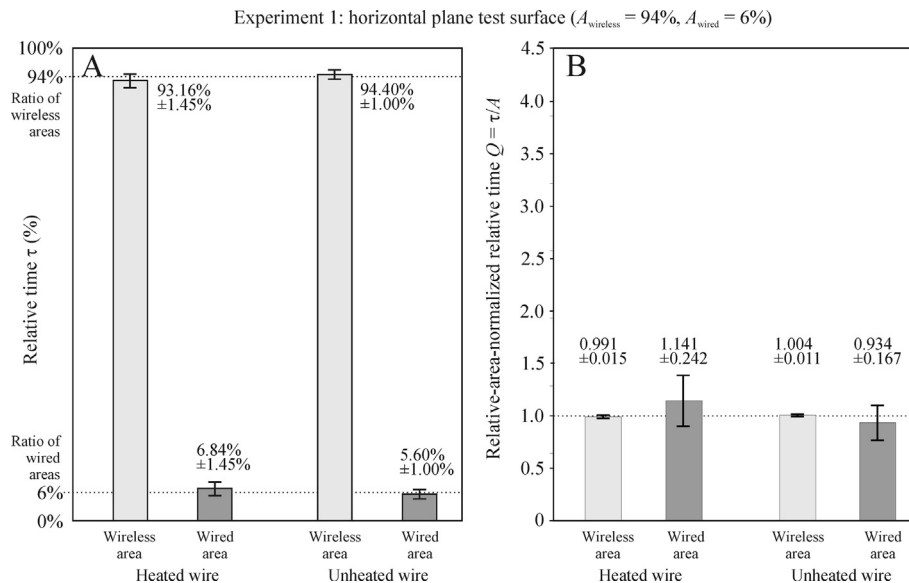
### 2.2.1. Experiments with plane test surfaces

The first type of test surface was composed of a smooth (shiny) black plastic sheet ( $50 \text{ cm} \times 50 \text{ cm} \times 0.5 \text{ mm}$ ) fixed on a light yellow wooden frame ( $50 \text{ cm} \times 50 \text{ cm} \times 10 \text{ cm}$ ) that was tiltable from 0° (horizontal) to 90° (vertical) relative to the horizontal. Below the black plastic sheet a resistance wire (diameter = 1 mm) meandered in eight parallel straight channels (width = 5 mm, length = 40 cm) between paperboard bands (thickness = 5 mm, width = 5.4 cm, length = 50 cm). The distance between the neighbouring wire channels was 5.5 cm. The black test surface was a smooth plane, and the underlying meandering wire was neither mechanically, nor visually, nor olfactorially sensible by the alighted tabanids. The plastic sheet had a very shallow ( $<0.01 \text{ mm}$ ) indentation above the wire channel, which enabled to observe whether a landed tabanid is or is not above the wire. The wire was electrically heatable, it was supplied by a 12 Volt rechargeable lead acid battery (UPS Power MC38-12, 12 V, 38 Ah/10 H). The temperature of the wire was controlled by the Joule heat formed in it due to the conducted electric current, the amperage of which was changeable by a manual potentiometer. The switch-on state of the electric current, that is the heating of the wire, was indicated by the lighting of a LED.

In experiment 1, with a horizontal plane test surface (from 9:00 to 17:00 h = local summer time = UTC + 2 h on 3, 8, 14, 21, 28 July and 2, 8 August 2022) the continuously sunlit surface was laid on the ground (Fig. 2A). In this case, it optically modelled a horizontally polarising water surface for water-seeking polarotactic male and female horseflies (Horváth et al., 2014). On a given experimental day, the electric supply of the wire below the test surface was



**Fig. 4.** Photos and thermograms of our plane and cylindrical test surfaces. Photograph and thermogram of the plane test surface with heated (A–C) and unheated (D–F) wire used in field experiments 1 and 2, and the vertical cylindrical test target with heated (G–I) and unheated (J–L) wire used in field experiment 3. The surface temperature  $T$  ( $^{\circ}\text{C}$ ) along the arrows in the thermograms is shown in C, F, I, L.



**Fig. 5.** Results of experiment 1. (A) Mean  $\pm$  S.D. (%) of the relative times  $\tau_{\text{wireless}} = t_{\text{wireless}}/(t_{\text{wireless}} + t_{\text{wired}})$  and  $\tau_{\text{wired}} = t_{\text{wired}}/(t_{\text{wireless}} + t_{\text{wired}})$  spent by tabanids walking on the wireless ( $t_{\text{wireless}}$ ) and wired ( $t_{\text{wired}}$ ) areas of the horizontal plane test surface with underlying heated and unheated wire in field experiment 1. Relative area  $A_{\text{wireless}} = 94\%$  of the test surface was wireless, and relative area  $A_{\text{wired}} = 6\%$  was underlined by wire. (B) Mean  $\pm$  S.D. of the relative-area-normalized relative times  $Q = \tau_{\text{wireless}}/A_{\text{wireless}}$  and  $\tau_{\text{wired}}/A_{\text{wired}}$  for heated and unheated wire in field experiment 1 (numerical data is provided in Supplementary Table S1, statistics in Table 1 and Supplementary Table S4).

switched on and off hourly. Thus, the wire was periodically (hourly) heated or unheated.

In experiment 2, with a  $60^{\circ}$ -tilted plane test surface (from 9:00 to 17:00 h = local summer time = UTC + 2 h on 4, 10, 17, 23, 30 July and 3, 9 August 2022) the wooden frame of the continuously sunlit

tilted ( $60^{\circ}$  from the horizontal) test surface was placed on a white tetrapodal plastic stool (height = 46 cm) (Fig. 2D,G). In this case it optically modelled a dark host animal for host-seeking female horseflies (Horváth et al., 2014). The azimuth direction (i.e. the horizontal component of the normal vector) of the tilted test sur-

face was turned hourly in such a way that its surface was illuminated by direct sunlight. On a given experimental day, the wire was heated or not heated hourly as in experiment 1.

In experiments 1 and 2, the surface area of the quadratic plane test surface with side length  $a = 50$  cm is  $A_{\text{test}} = a^2$ , and below it ran eight straight channels with width  $w = 0.5$  cm, each filled by a wire with length  $b = 40$  cm. The surface area of the eight straight wired channels is  $A_{\text{wired}} = 8bw$ . The proportion of the area of the eight wired channels relative to the whole area of the quadratic test surface is  $Q_{\text{wired}} = A_{\text{wired}}/A_{\text{test}} = 8bw/a^2 = 0.064 \approx 6\%$ . The proportion of the area of the surface parts without underlying wire relative to the whole test surface is  $Q_{\text{wireless}} = (A_{\text{test}} - A_{\text{wired}})/A_{\text{test}} = (a^2 - 8bw)/a^2 = 1 - Q_{\text{wired}} = 0.936 \approx 94\%$ . Hence, 94% of the plane test surface was wireless, and 6% was underlaid by wire (imitating warm blood vessels when heated).

### 2.2.2. Experiment with a vertical cylindrical test surface

The second type of test surface was composed of an outer smooth (shiny) black plastic sheet (100 cm  $\times$  70 cm  $\times$  0.5 mm) underlaid by another plastic sheet (100 cm  $\times$  70 cm  $\times$  1 mm), both fixed on a vertical plastic barrel (height  $h = 80$  cm, radius  $r = 22$  cm). Between the two plastic sheets a resistance wire (diameter = 1 mm, length = 520 cm) ran in a spiral form within a circular area with a diameter of 60 cm in such a way that within this circle there were five coaxial semicircles including the circular perimeter. The radial distance between two neighbouring semicircles was  $60/9 = 6.7$  cm. The wire spiralling beneath the cylindrical outer black plastic sheet was neither mechanically, nor visually, nor olfactorially sensible by the landed tabanids. The outer black plastic sheet had a very slight (height < 0.01 mm, width = 5 mm) bulge above the wire, which enabled observation of whether an alighted horsefly was or was not above the wire. The wire was heatable as in the experiments 1 and 2.

In experiment 3 (from 9:00 to 17:00 h = local summer time = UTC + 2 h on 6, 11, 18, 26, 31 July and 5, 10 August 2022), the continuously sunlit vertical cylindrical black target was placed on a white tetrapodal plastic stool (height = 46 cm) (Fig. 3A,D). In this case, the test surface optically modelled a dark host animal for host-seeking female horseflies (Horváth et al., 2014). This test target was turned hourly in such a way that its surface was illuminated by direct sunlight. On a given experimental day, the spiralling wire was heated or unheated hourly as in experiments 1 and 2.

The surface area of the vertical cylindrical test surface with height  $h = 70$  cm and width  $v = 100$  cm is  $A_{\text{test}} = hv$ , and below it ran a spiralling wire with a length of  $L = 520$  cm. Although the wire diameter was only 1 mm, the width of the slight bulge above the wire was  $d = 5$  mm. Thus, the effective surface area of the test surface underlaid by the wire is  $A_{\text{wired}} = Ld$ . The proportion of the wired area relative to the whole area of the cylindrical surface is  $Q_{\text{wired}} = A_{\text{wired}}/A_{\text{test}} = Ld/hv = 0.037 \approx 4\%$ . The proportion of the area of the surface part without underlying wire relative to the whole surface is  $Q_{\text{wireless}} = (A_{\text{test}} - A_{\text{wired}})/A_{\text{test}} = (hv - Ld)/hv = 1 - Q_{\text{wired}} = 0.963 \approx 96\%$ . Hence, 96% of the vertical cylindrical test surface was wireless, and 4% was underlaid by wire (imitating a warm blood vessel when heated).

### 2.3. Imaging polarimetry

Tabanids are polarisation-sensitive insects (Horváth et al., 2008; Meglic et al., 2019): (i) during host finding, blood-seeking females are attracted to linearly polarised light, independent of the direction of polarisation (Horváth et al., 2014), while (ii) males and females seek water by means of the horizontal polarisation of water-reflected light (Horváth et al., 2008). Thus, we measured the reflection polarisation characteristics of the test surfaces (Figs. 2

and 3) used in our field experiments with imaging polarimetry in the red ( $650 \pm 40$  nm), green ( $550 \pm 40$  nm) and blue ( $450 \pm 40$  nm) parts of the spectrum (Horváth and Varjú 1997, 2004).

### 2.4. Thermography

The temperature distributions (thermograms) of the sunlit horizontal and 60°-tilted plane test surfaces (Fig. 2) and the sunlit vertical cylindrical test target (Fig. 3) with heated and unheated wire used in the field experiments were measured with a thermocamera (FLIR E8-XT, nominal precision =  $\pm 2$  °C for ambient temperature 10–35 °C) under a clear sky in sunshine. These thermograms serve as a demonstration of the typical temperature patterns of our test surfaces under sunny conditions when host-seeking female tabanids are active. The surface temperature  $T$  along a straight line on the test surfaces was determined with our custom-developed software.

### 2.5. Statistics

In our field experiments, we compared ratios of the total time horseflies spent above the heated or unheated wire with the surface ratio of the test surface without underlying wire. After calculating the mean and standard deviation of the measured time ratios, we divided these time ratios with the corresponding area of the surface with or without underlying wire to obtain relative time values, independent of the wired or wireless parts of the test surface. Using a single-factor ANOVA test for the sunlit test surfaces with heated and unheated wires, we compared the relative times for the wired and wireless areas. We chose a significance level of  $P = 0.05$ . For statistical analyses, we used the statistical function package of Microsoft Excel 2022.

## 3. Results

### 3.1. Reflection polarisation characteristics of test surfaces

When the plane test surface was horizontal in experiment 1 (Fig. 2A), it reflected horizontally polarised light (Fig. 2C) with maximal degrees of linear polarisation  $87\% \leq d \leq 95\%$  (Fig. 2B) at and near the Brewster angle  $\theta_{\text{Brewster}} = \arctan(n = 1.5) = 56.3^\circ$  from the vertical, where  $n = 1.5$  is the refractive index of the plastic sheet covering the plane test target. In experiment 2, the 60°-tilted plane test surface, seen from frontwise (Fig. 2D), reflected horizontally polarised light (Fig. 2F) with medium degrees of polarisation  $45\% \leq d \leq 58\%$  (Fig. 2E). On the other hand, the tilted test surface, seen from the side (Fig. 2G), reflected moderate degrees of polarisation  $23\% \leq d \leq 55\%$  (Fig. 2H) with oblique angles of polarisation  $-50^\circ \leq \alpha \leq -40^\circ$  clockwise from the vertical (Fig. 2I). Note, however, that since the reflection-polarisation characteristics of the plane test surface depend on the direction of view and the illumination conditions, Fig. 2 represents only some typical polarisation patterns measured by imaging polarimetry in the green (550 nm) part of the spectrum. In the red (650 nm) and blue (450 nm) spectral ranges, the reflection polarisation patterns were very similar to those in the green range due to the colourless (black) feature of the test surface.

For the interpretation of the reactions of polarotactic horseflies observed in experiments 1 and 2 (see Section 3.3), the most relevant polarisation difference between the horizontal and the 60° tilted test surfaces was that the horizontal test surface always reflected horizontally polarised light, while the tilted surface did not always reflect horizontally, but also obliquely or vertically polarised light, depending on the viewing direction. Considering

the polarisation characteristics, the horizontal test surface mimicked water surface (Horváth and Varjú, 1997; Horváth et al., 2008; Horváth, 2014), while the tilted test surface imitated a host animal for horseflies (Horváth et al., 2014).

Depending on the angle of reflection, the vertical cylindrical test target used in experiment 3 (Fig. 3A,D) reflected light with degrees of polarisation  $0^\circ \leq d \leq 95^\circ$  (Fig. 3B,E) and horizontal ( $\alpha \approx \pm 90^\circ$ ), oblique ( $5^\circ < \alpha < 85^\circ$ ,  $95^\circ < \alpha < 175^\circ$ ,  $-85^\circ < \alpha < -5^\circ$ ,  $-175^\circ < \alpha < -95^\circ$ ) or vertical ( $\alpha \approx 0^\circ$  or  $180^\circ$ ) angles of polarisation (Fig. 3C,F). The direction of polarisation of light reflected from a surface is always perpendicular to the plane of reflection determined by the dominating incident ray of sun- and/or skylight, the point observed and the observer's viewing direction (optical axis of the polarimeter). Hence, both  $d$  and  $\alpha$  varied in wide ranges, as is typical for the host animals of horseflies (Horváth et al., 2014).

An important feature of all three test targets is that the wire underlying the black plastic sheet covering is practically imperceptible in the polarisation patterns (Fig. 2A,D,G; 3A,D), while it is slightly perceptible in the photograph of the targets (Fig. 2B,C,E,F, H,I; 3B,C,E,F).

### 3.2. Thermograms of test surfaces

Fig. 4 shows the photographs and thermograms of the sunlit test targets and the surface temperature  $T$  ( $^\circ\text{C}$ ) along the arrows in the thermograms at  $35^\circ\text{C}$  air temperature used in field experiments 1–3. When the wires were heated, the wired areas of the test surfaces were approximately 2–3  $^\circ\text{C}$  warmer than the unwired areas (Fig. 4B,C,H,I). When the wires were unheated, there were practically no  $T$ -differences between the wired and unwired areas (Fig. 4E,F,K,L). The reason for the tiny ( $0.5^\circ\text{C}$ ) change in  $T$  above the areas underlaid by unheated wires (Fig. 4E,F,K,L) was that the local  $T$  depended on the reflection angle of sunlight reflected from the slightly curved surface of the shallow/slight indentations/bulges of the black plastic sheet above wires. The temperature distribution of the test targets plays an important role in the interpretation of time periods that tabanids spent walking on the test surfaces (see section 3.3).

### 3.3. Time periods walking horseflies spent on test targets with heated and unheated wires

In experiment 1, both female and male horseflies landed on the horizontal plane test surface laid on the ground. When the underlying wire was unheated, horseflies walking on the test surface spent  $\tau_{\text{wired,unheated}} = 5.60 \pm 1.00\%$  and  $\tau_{\text{wireless,unheated}} = 94.40 \pm 1.00\%$  of their total time on the wired and wireless areas, respectively (Fig. 5A, Supplementary Table S1). Since the ratio  $\tau_{\text{wired,unheated}} = 5.6\%$  is approximately the same as the surface ratio  $A_{\text{wired}} = 6\%$  of the wired areas, furthermore  $\tau_{\text{wireless,unheated}} = 94.4\%$  approximately equals to  $A_{\text{wireless}} = 94\%$  of the wired areas, we conclude that the walking horseflies preferred neither the wired nor the wireless areas of the test surface when the wire was unheated.

When the underlying wire was heated, horseflies walking on the horizontal test surface spent  $\tau_{\text{wired,heated}} = 6.84 \pm 1.45\%$  and  $\tau_{\text{wireless,heated}} = 93.16 \pm 1.45\%$  of their total time on the wired and wireless areas, respectively (Fig. 5A, Supplementary Table S1). Since the ratio  $\tau_{\text{wired,heated}} = 6.84\%$  is approximately the same as the surface ratio  $A_{\text{wired}} = 6\%$  of the wired areas, furthermore  $\tau_{\text{wireless,heated}} = 93.16\%$  nearly equals to  $A_{\text{wireless}} = 94\%$  of the wired areas, we conclude again that the walking horseflies preferred neither the wired nor the wireless areas of the horizontal test surface when the wire was heated.

On the basis of the above, our final conclusion is that the walking (female and male) horseflies had no preference for any (wired or wireless) area of the horizontal plane test surface, independent

of the temperature (heated or unheated) of the underlying wire. This conclusion is corroborated by Fig. 5B presenting the mean  $\pm$  standard deviation of the relative-area-normalized relative times  $Q_{\text{wireless}} = \tau_{\text{wireless}}/A_{\text{wireless}}$  and  $Q_{\text{wired}} = \tau_{\text{wired}}/A_{\text{wired}}$ . As shown in Table 1 (see also Supplementary Table S4), when the wire was heated, then  $Q_{\text{wired,heated}} = 1.141 \pm 0.242$  on the wired areas was not statistically significantly different from  $Q_{\text{wireless,heated}} = 0.991 \pm 0.015$  on the wireless areas. Similarly, when the wire was unheated, then there was no statistically significant difference between  $Q_{\text{wired,unheated}} = 0.934 \pm 0.167$  and  $Q_{\text{wireless,unheated}} = 1.004 \pm 0.011$  at significance level of  $P = 0.05$  (Table 1, Supplementary Table S4).

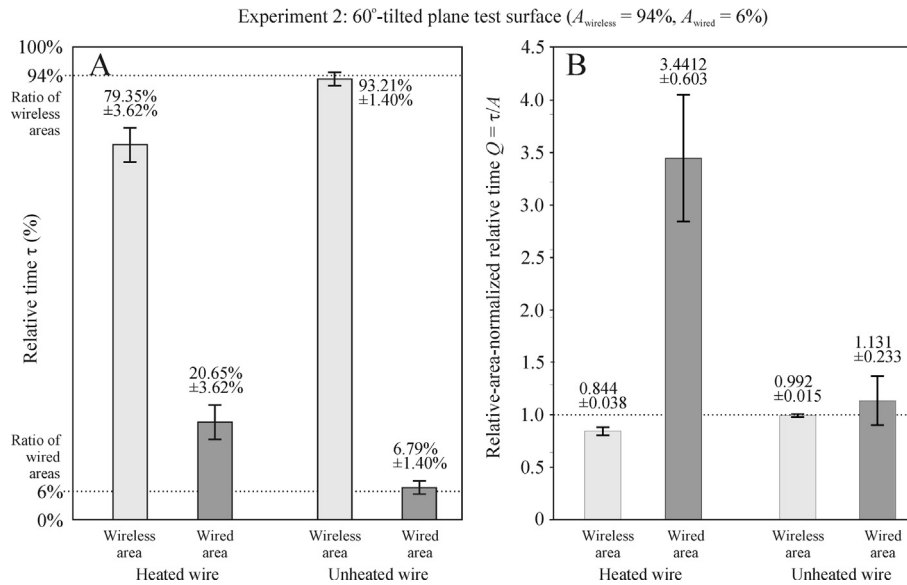
In experiment 2, only females landed on the  $60^\circ$ -tilted plane test surface. When the underlying wire was unheated, females walking on this test surface spent  $\tau_{\text{wired,unheated}} = 6.79 \pm 1.40\%$  and  $\tau_{\text{wireless,unheated}} = 93.21 \pm 1.40\%$  of their total time on the wired and wireless areas, respectively (Fig. 6A, Supplementary Table S2). Since the ratio  $\tau_{\text{wired,unheated}} = 6.79\%$  is approximately the same as the surface ratio  $A_{\text{wired}} = 6\%$  of the wired areas, and furthermore  $\tau_{\text{wireless,unheated}} = 93.21\%$  is nearly equal to  $A_{\text{wireless}} = 94\%$  of the wired areas, we conclude that the walking tabanids preferred neither the wired nor the wireless areas of the  $60^\circ$  tilted plane test surface when the wire was unheated.

When the underlying wire was heated, the average time ratio that walking female tabanids spent on the wired areas was  $\tau_{\text{wired,heated}} = 20.65 \pm 3.62\%$  (Fig. 6A, Supplementary Table S2) which is approximately 3.4 times more time than the surface ratio  $A_{\text{wired}} = 6\%$  of the wired areas. From this, we conclude that horseflies walking on the  $60^\circ$  tilted plane test surface preferred surface regions above the heated wire to regions without underlying wire. This conclusion is confirmed by Fig. 6B showing the mean  $\pm$  standard deviation of the relative-area-normalized relative times  $Q_{\text{wireless}}$  and  $Q_{\text{wired}}$ . As shown in Table 2 (see also Supplementary Table S5), when the wire was heated, then  $Q_{\text{wired,heated}} = 3.4412 \pm 0.603$  on the wired areas was statistically significantly higher than  $Q_{\text{wireless,heated}} = 0.844 \pm 0.038$  on the wireless areas. However, when the wire was unheated, then there was no significant difference between  $Q_{\text{wired,unheated}} = 1.131 \pm 0.233$  and  $Q_{\text{wireless,unheated}} = 0.992 \pm 0.015$  at a significance level of  $P = 0.01$  (Table 2, Supplementary Table S5).

**Table 1**

Tukey-Kramer post-hoc test for differences between the relative-area-normalized relative times  $\tau_{\text{wireless}}/A_{\text{wireless}}$  and  $\tau_{\text{wired}}/A_{\text{wired}}$ , where  $\tau_{\text{wireless}} = t_{\text{wireless}}/(t_{\text{wireless}} + t_{\text{wired}})$  and  $\tau_{\text{wired}} = t_{\text{wired}}/(t_{\text{wireless}} + t_{\text{wired}})$  are the relative times spent by tabanids walking on the wireless ( $t_{\text{wireless}}$ ) and wired ( $t_{\text{wired}}$ ) areas of the horizontal plane test surface in field experiment 1, where  $A_{\text{wireless}} = 94\%$  is the relative area of the test surface without underlying wire, and  $A_{\text{wired}} = 6\%$  is the relative area of the surface with underlying wire (see also Supplementary Table S4).  $Q_{\text{wireless}} = (A_{\text{test}} - A_{\text{wired}})/A_{\text{test}}$  is the proportion of the area of the surface parts without underlying wire relative to the whole test surface, and  $Q_{\text{wired}} = A_{\text{wired}}/A_{\text{test}}$  is the proportion of the area of the wired area relative to the whole area of the test surface.

Comparison	Absolute mean difference	Critical value	Significance
$Q_{\text{wireless}}$ with heated wire vs $Q_{\text{wired}}$ with heated wire	0.150	0.217	Not significant
$Q_{\text{wireless}}$ with heated wire vs $Q_{\text{wireless}}$ with unheated wire	0.013	0.217	Not significant
$Q_{\text{wireless}}$ with heated wire vs $Q_{\text{wired}}$ with unheated wire	0.057	0.217	Not significant
$Q_{\text{wired}}$ with heated wire vs $Q_{\text{wireless}}$ with unheated wire	0.136	0.217	Not significant
$Q_{\text{wired}}$ with heated wire vs $Q_{\text{wired}}$ with unheated wire	0.207	0.217	Not significant
$Q_{\text{wireless}}$ with unheated wire vs $Q_{\text{wired}}$ with unheated wire	0.071	0.217	Not significant



**Fig. 6.** Results of experiment 2. (A) Mean ± S.D. (%) of the relative times  $\tau_{\text{wireless}} = t_{\text{wireless}}/(t_{\text{wireless}} + t_{\text{wired}})$  and  $\tau_{\text{wired}} = t_{\text{wired}}/(t_{\text{wireless}} + t_{\text{wired}})$  spent by tabanids walking on the wireless ( $t_{\text{wireless}}$ ) and wired ( $t_{\text{wired}}$ ) areas of the 60° tilted plane test surface with underlying heated and unheated wire in field experiment 2. Relative area  $A_{\text{wireless}} = 94\%$  of the test surface was wireless, and relative area  $A_{\text{wired}} = 6\%$  was underlaid by wire. (B) Mean ± S.D. of the relative-area-normalized relative times  $Q = \tau_{\text{wireless}}/A_{\text{wireless}}$  and  $\tau_{\text{wired}}/A_{\text{wired}}$  for heated and unheated wire in field experiment 2 (numerical data is provided in Supplementary Table S2, statistics in Table 2 and Supplementary Table S5).

**Table 2**

Tukey-Kramer post-hoc test for differences between the relative-area-normalized relative times  $\tau_{\text{wireless}}/A_{\text{wireless}}$  and  $\tau_{\text{wired}}/A_{\text{wired}}$ , where  $\tau_{\text{wireless}} = t_{\text{wireless}}/(t_{\text{wireless}} + t_{\text{wired}})$  and  $\tau_{\text{wired}} = t_{\text{wired}}/(t_{\text{wireless}} + t_{\text{wired}})$  are the relative times spent by tabanids walking on the wireless ( $t_{\text{wireless}}$ ) and wired ( $t_{\text{wired}}$ ) areas of the 60° tilted plane test surface in field experiment 2, where  $A_{\text{wireless}} = 94\%$  is the relative area of the test surface without underlying wire, and  $A_{\text{wired}} = 6\%$  is the relative area of the surface with underlying wire (see also Supplementary Table S5).  $Q_{\text{wireless}} = (A_{\text{test}} - A_{\text{wired}})/A_{\text{test}}$  is the proportion of the area of the surface parts without underlying wire relative to the whole test surface, and  $Q_{\text{wired}} = A_{\text{wired}}/A_{\text{test}}$  is the proportion of the area of the wired area relative to the whole area of the test surface.

Comparison	Absolute mean difference	Critical value	Significance
$Q_{\text{wireless}}$ with heated wire vs $Q_{\text{wired}}$ with heated wire	2.597	0.477	Significant
$Q_{\text{wireless}}$ with heated wire vs $Q_{\text{wireless}}$ with unheated wire	0.147	0.477	Not significant
$Q_{\text{wireless}}$ with heated wire vs $Q_{\text{wired}}$ with unheated wire	0.287	0.477	Not significant
$Q_{\text{wired}}$ with heated wire vs $Q_{\text{wireless}}$ with unheated wire	2.450	0.477	Significant
$Q_{\text{wired}}$ with heated wire vs $Q_{\text{wired}}$ with unheated wire	2.310	0.477	Significant
$Q_{\text{wireless}}$ with unheated wire vs $Q_{\text{wired}}$ with unheated wire	0.140	0.477	Not significant

In experiment 3, again only female horseflies landed on the vertical cylindrical test target. When the underlying wire was unheated, females walking on this target spent  $\tau_{\text{wired,unheated}} = 4.56 \pm 0.49\%$  and  $\tau_{\text{wireless,unheated}} = 95.44 \pm 0.49\%$  of their total time on the wired and wireless areas, respectively (Fig. 7A, Supplementary Table S3). Since the surface percentages  $A_{\text{wired}} = 4\%$  and  $A_{\text{wireless}} = 96\%$  of the wired and wireless areas are approximately the same as  $\tau_{\text{wired,unheated}} = 4.56\%$  and  $\tau_{\text{wireless,unheated}} = 95.44\%$ , we conclude that the walking tabanids preferred neither the wired nor the wireless areas of the cylindrical test target when the wire was unheated.

When the underlying wire was heated, the average time ratio that walking female tabanids spent on the wired areas was  $\tau_{\text{wired,heated}} = 16.77 \pm 2.45\%$  (Fig. 7A, Supplementary Table S3) which is approximately 4.2 times more time than the surface ratio  $A_{\text{wired}} = 4\%$

of the wired areas. From this, we conclude again that walking tabanids preferred surface areas above the heated wire to areas without underlying wire. This conclusion is supported by Fig. 7B and Table 3 (see also Supplementary Table S6): when the wire was heated, then  $Q_{\text{wired,heated}} = 4.193 \pm 0.613$  on the wired areas was statistically significantly higher than  $Q_{\text{wireless,heated}} = 0.867 \pm 0.026$  on the wireless areas. However, when the wire was unheated, then there was no significant difference between  $Q_{\text{wired,unheated}} = 1.139 \pm 0.122$  and  $Q_{\text{wireless,unheated}} = 0.994 \pm 0.005$  at a significance level of  $P = 0.01$  (Table 3, Supplementary Table S6).

The above statistical tests have also shown that the presence of a wire alone did not affect the behaviour of the horseflies in any of the experiments, since there was no statistically significant difference between  $Q_{\text{wired,unheated}}$  and  $Q_{\text{wireless,unheated}}$ . This result proves that the wires beneath the shiny black smooth plastic test surface were mechanically impalpable for the walking horseflies.

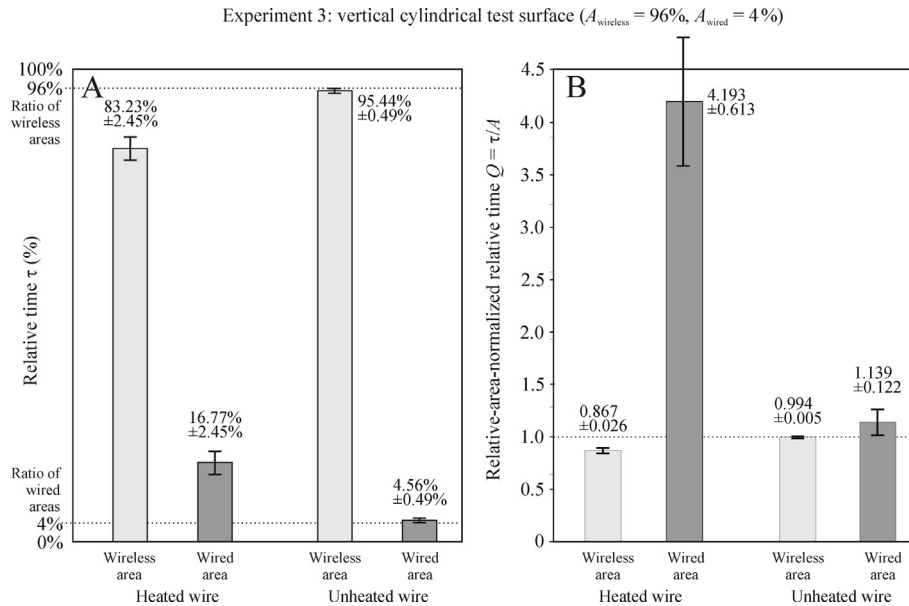
#### 4. Discussion

The landing of tabanids on black objects/surfaces depends on their sex and the tilt angle  $\delta$  of the surface, measured from the horizontal (Horváth et al., 2020b): The landing frequency of male and female tabanids is highest for horizontal ( $\delta = 0^\circ$ ) surfaces and is minimal for  $\delta = 75^\circ$ . The landing frequency of males decreases in a monotonous way to zero with increasing  $\delta$ . Female landing has a primary maximum and minimum at  $\delta = 0^\circ$  and  $\delta = 75^\circ$ , respectively, and a secondary peak at  $\delta = 90^\circ$ . Both sexes are strongly attracted to and land on nearly horizontal ( $0^\circ \leq \delta \leq 15^\circ$ ) surfaces, and vertical surfaces are also very attractive but only for females.

Female horseflies generally do not alight on host animals wearing black-white striped fur to seek for blood vessels (Horváth et al., 2010, 2014, 2019a; Blahó et al., 2012, 2013; Egri et al., 2012a; Caro et al., 2014, 2019; Caro, 2016). The reason for this avoidance is rather enigmatic. On thicker black/white stripes there are only vessel-induced temperature gradients on the skin surface below the hair layer, thus tabanids could detect vessels with thermoreception as easily as on hosts with homogeneous coloured coats.

The optical and/or olfactorial cues of the host animals of horseflies attract these parasites (Foil, 1989; Krcmar, 2005; Krcmar et al.,





**Fig. 7.** Results of experiment 3. (A) Mean  $\pm$  S.D. (%) of the relative times  $\tau_{\text{wireless}} = t_{\text{wireless}}/(t_{\text{wireless}} + t_{\text{wired}})$  and  $\tau_{\text{wired}} = t_{\text{wired}}/(t_{\text{wireless}} + t_{\text{wired}})$  spent by tabanids walking on the wireless ( $t_{\text{wireless}}$ ) and wired ( $t_{\text{wired}}$ ) areas of the vertical cylindrical test surface with underlying heated and unheated wire in field experiment 3. Relative area  $A_{\text{wireless}} = 96\%$  of the test surface was wireless, and relative area  $A_{\text{wired}} = 4\%$  was underlaid by wire. (B) Mean  $\pm$  S.D. of the relative-area-normalized relative times  $\bar{Q} = \tau_{\text{wireless}}/A_{\text{wireless}}$  and  $\tau_{\text{wired}}/A_{\text{wired}}$  for heated and unheated wire in field experiment 3 (numerical data is provided in Supplementary Table S3, statistics in Table 3 and Supplementary Table S6).

**Table 3**

Tukey-Kramer post-hoc test for differences between the relative-area-normalized relative times  $\tau_{\text{wireless}}/A_{\text{wireless}}$  and  $\tau_{\text{wired}}/A_{\text{wired}}$ , where  $\tau_{\text{wireless}} = t_{\text{wireless}}/(t_{\text{wireless}} + t_{\text{wired}})$  and  $\tau_{\text{wired}} = t_{\text{wired}}/(t_{\text{wireless}} + t_{\text{wired}})$  are the relative times spent by tabanids walking on the wireless ( $t_{\text{wireless}}$ ) and wired ( $t_{\text{wired}}$ ) areas of the vertical cylindrical test surface in field experiment 3, where  $A_{\text{wireless}} = 96\%$  is the relative area of the surface without underlying wire, and  $A_{\text{wired}} = 4\%$  is the relative area of the surface with underlying wire (see also Supplementary Table S6).  $Q_{\text{wireless}} = (A_{\text{test}} - A_{\text{wired}})/A_{\text{test}}$  is the proportion of the area of the surface parts without underlying wire relative to the whole test surface, and  $Q_{\text{wired}} = A_{\text{wired}}/A_{\text{test}}$  is the proportion of the area of the wired area relative to the whole area of the test surface.

Comparison	Absolute mean difference	Critical value	Significance
$Q_{\text{wireless}}$ with heated wire vs $Q_{\text{wired}}$ with heated wire	3.326	0.461	Significant
$Q_{\text{wireless}}$ with heated wire vs $Q_{\text{wireless}}$ with unheated wire	0.127	0.461	Not significant
$Q_{\text{wireless}}$ with heated wire vs $Q_{\text{wired}}$ with unheated wire	0.272	0.461	Not significant
$Q_{\text{wired}}$ with heated wire vs $Q_{\text{wireless}}$ with unheated wire	3.199	0.461	Significant
$Q_{\text{wired}}$ with heated wire vs $Q_{\text{wired}}$ with unheated wire	3.054	0.461	Significant
$Q_{\text{wireless}}$ with unheated wire vs $Q_{\text{wired}}$ with unheated wire	0.145	0.461	Not significant

2005, 2014; Lehane, 2005; Krcmar and Maric, 2006; Mihok and Mulye, 2010; Mihok and Lange, 2012; Baldacchino et al., 2013, 2014; Mihok and Carlson, 2021). After alighting on the host, tabanid behaviour is also governed by the host's body temperature (Thorsteinson, 1958; Bracken and Thorsteinson, 1965; Lehane, 2005; Horváth et al., 2019b, 2020a). In our field experiments 2 and 3, we showed that blood-seeking female horseflies, alighting on test targets optically imitating hosts, prefer the elongated thin surface areas above wires only, if the wires are heated and imitate warmer blood vessels. This finding experimentally confirms the hypothesis of Takács et al. (2022), who proposed that the  $T$ -gradients of the skin above the slightly warmer blood vessels can be confused with the  $T$ -gradients of the skin at the borderlines of cooler white and warmer black zebra stripes.

As demonstrated by thermograms of horses, the skin overlying major blood vessels is warmer than skin areas further from these vessels (Soroko and Davies-Morel, 2016; Soroko and Howell, 2018; Horváth et al., 2020a; Takács et al., 2022). In our field experiments 2 and 3, blood-seeking female tabanids were attracted to the elongated thin warmer surface areas above heated wires mimicking the warmer skin surface above blood vessels. Sunlit black skin stripes/spots are warmer than white ones, but this difference in temperature  $T$  between black and white regions disappears in shade (Horváth et al., 2018; Pereszlényi et al., 2021). Takács et al. (2022) proposed that this  $T$  difference/gradient at the borderline of dark and bright pelage regions of zebras (or other host animals of tabanids with heterogeneous fur patterns) can confuse the thermal perception of blood vessels by blood-seeking female horseflies, the consequence of which is the visual unattractiveness of zebras to tabanids: at the borderlines of sunlit white and black zebra stripes similar  $T$ -gradients occur on a skin surface without an underlying vessel. The vessel-seeking horseflies deceived by these  $T$ -gradients bite the skin to suck blood not only at sites above vessels, but also at the black-white borderlines. Such an unsuccessful blood-sucking attempt evokes the host's parasite-repelling reactions that are dangerous for biting horseflies. These borderline-elicited false vessel locations, the painful bitings and the host's fly-repellent reactions increase the risk that the biting tabanid is swatted by the host. According to Takács et al. (2022), the avoidance of striped (and spotted) hosts is an adaptive/adequate evolutionary strategy to reduce this risk.

Takács et al. (2022) also pointed out that since in shade the white and black zebra stripes have the same temperature (Cena and Clark, 1973; Benesch and Hilsberg, 2003; Benesch and Hilsberg-Merz, 2006; Horváth et al., 2018, 2019b; Cobb and Cobb, 2019),  $T$  gradients do not occur at the white-black borderlines, and therefore horseflies can detect blood vessels by thermoreception on striped (spotted) hosts as easily as on homogeneous ones. Nevertheless, female tabanids attack hosts predominantly in sunshine. The main reason for this is that they prefer to land on warmer hosts (Horváth et al., 2019b), because they can escape more easily from warmer surfaces (Horváth et al., 2020a).

In our field experiments 2 and 3, female horseflies alighted on the host-imitating black test surfaces. During walking, they preferred the elongated thin warmer surface areas above heated wires to the colder surrounding areas exclusively if the wires were heated, i.e. warmer than the ambient temperature. If the wires were unheated, and thus there was practically no temperature difference between the wired and wireless surface areas, this preference disappeared. This latter finding excludes the possibility that the walking tabanids might prefer the surface regions above wires due to their shallow (<0.01 mm) indentation (in experiment 2) or slight (<0.01 mm) bulge (in experiment 3). Note that there were neither mechanical nor optical cue differences between the wired and wireless surface areas. Therefore, the wires were perceptible only by thermoreception, if they were heated. Consequently, in experiments 2 and 3 the female horseflies did not prefer the heated wired surface areas due to these indentations/bulges, which made it possible for the observer to visually detect the wired regions.

Takács et al. (2022) hypothesised that the borderlines between black and white stripes create temperature gradients across zebras' coats – with black fur retaining more heat in sunlight – that mimic the heat output of blood vessels near the surface of the skin, which horseflies detect by thermoreception. To test this, they conducted a field experiment to explore how horseflies reacted to a zebra model formed of a vertical grey barrel with thin (0.5 cm) black stripes. The barrel was orientated so that the stripes were either sunlit or shady, in order to introduce temperature gradients across the surface. In sunlight, the black stripes were approximately 1.5–2 °C warmer than the grey background, while in shade there was no temperature difference between stripes and grey areas. Female horseflies spent proportionally 2.8 times more time walking (19.6% of time) on sunlit black stripes, even though black stripes made up only 7% of the total surface ratio. In comparison, horseflies spent 6.4% of the time on stripes on the shaded side of the barrel where there was no temperature gradient. These findings appeared to confirm that female horseflies are attracted to warmer areas, likely confusing the barrel stripes for blood vessels, according to the authors. Takács et al. (2022) propose that the confusion caused by the borders between warmer black stripes and cooler white stripes will lead horseflies to bite repeatedly but unsuccessfully in the search for blood vessels, and that this action may trigger a reaction from the host animal – such as a tail swish, for example – that poses a risk to the fly. Therefore, avoiding striped animals may be a sensible evolutionary strategy that horseflies have developed.

In our experiments 2 and 3, warm blood vessels were imitated by heated wires below the smooth black test surface. The warmer surface areas above heated wires corresponded to the sunlit warmer black stripes on the grey barrel in the experiment of Takács et al. (2022). The important methodological improvement of our experiments 2 and 3 was that only temperature gradients could occur on our homogeneous black test targets and there was no optical (intensity and polarisation) contrast between the wired and wireless regions. In both experiments, we obtained very similar results to Takács et al. (2022): female horseflies spent proportionally 3.4 times (experiment 2) and 4.2 times (experiment 3) more time walking above heated wires, even though wired surface areas made up only 6% (experiment 2) and 4% (experiment 3) of the total surface ratio. In comparison, horseflies spent only 1.13–1.14 times (experiments 2 and 3) the relative-area-normalized relative time above unheated wires where there was no temperature gradient.

Tabanids are polarotactic insects, that is they are attracted to linearly polarised light (Horváth et al., 2014). The higher the degree of linear polarisation  $d$ , the stronger this attraction (Kriska et al., 2009). They possess two different kinds of polarotaxis: (i) both females and males are attracted to horizontally polarised light, if such light stimulates the ventral side of their compound eyes (Horváth et al., 2008). By this optical cue they detect the water sur-

face (Horváth, 2014). Both sexes need water to drink, bath (for body cooling), and find each other to copulate near water. Furthermore, females find drinking host animals (to suck blood for egg development) at water bodies, and lay their eggs onto leaves of water plants or in mud (Horváth et al., 2014). (ii) Female tabanids find their host animals partly by the degree of polarisation  $d$  of host-reflected light, independent of the angle/direction of polarisation (Egri et al., 2012b; Horváth et al., 2017).

In experiment 1, both female and male tabanids landed and walked on the smooth black horizontal plane test surface reflecting horizontally polarised light. During walking, they had no preference for the surface areas above heated wires imitating warm blood vessels. This finding can be interpreted in such a way that in this case the landing tabanids thought the plane test surface was water on the basis of the horizontally polarised surface-reflected light. After alighting, they tested the surface quality with their sense organs. Since they assumed that they are on a water surface, neither sex sought blood vessels (males do not suck blood, females suck blood only from host animals). Since they did not seek blood vessels – by means of temperature gradients of the surface – they paid no attention to the warmer surface areas above heated wires.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2022.10.001>.

## References

- Baldacchino, F., Carrier, J., Porciani, A., Buatois, B., Dormont, L., Jay-Robert, P., 2013. Behavioural and electrophysiological responses of females of two species of tabanid to volatiles in urine of different mammals. *Med. Vet. Entomol.* 27, 77–85.
- Baldacchino, F., Manon, S., Puech, L., Buatois, B., Dormont, L., Jay-Robert, P., 2014. Olfactory and behavioural responses of tabanids to octenol, phenols and aged horse urine. *Med. Vet. Entomol.* 28, 201–209.
- Becker, C.D., Ginsberg, J.R., 1990. Mother-infant behaviour of wild Grevy's zebra: adaptations for survival in semi-desert East Africa. *Anim. Behav.* 40, 1111–1118.
- Benesch, A.R., Hilsberg, S., 2003. Infrarot-thermographische Untersuchungen der Oberflächentemperatur bei Zebras. *Zool. Garten NF* 2, 74–82.
- Benesch, A.R., Hilsberg-Merz, S., 2006. Oberflächentemperaturen bei Zebrastreifen. *Natur Mus.* 136, 49–56.
- Blahó, M., Egri, Á., Báhidzski, L., Kriska, G., Hegedüs, R., Åkesson, S., Horváth, G., 2012. Spottier targets are less attractive to tabanid flies: on the tabanid-repellency of spotty fur patterns. *PLoS One* 7, e41138.
- Blahó, M., Egri, Á., Száz, D., Kriska, G., Åkesson, S., Horváth, G., 2013. Stripes disrupt odour attractiveness to biting horseflies: Battle between ammonia, CO<sub>2</sub>, and colour pattern for dominance in the sensory systems of host-seeking tabanids. *Physiol. Behav.* 119, 168–174.
- Bracken, G.K., Thorsteinson, A.J., 1965. The orientation behaviour of horse flies and deer flies (Tabanidae: Diptera). IV. The influence of some physical modifications of visual decoys on orientation of horse flies. *Entomol. Exp. Appl.* 8, 314–318.

- Caro, T., 2009. Contrasting coloration in terrestrial mammals. *Phil. Trans. Roy. Soc. B* 364, 537–548.
- Caro, T., 2016. *Zebra Stripes*. University of Chicago Press, Chicago, USA.
- Caro, T., Stankowich, T., 2015. Concordance on zebra stripes: a comment on Larison et al., 2015. *Roy. Soc. Open Sci.* 2, 150323. <https://doi.org/10.1098/rsos.150323>.
- Caro, T., Izzo, A., Reiner, R.C., Walker, H., Stankowich, T., 2014. The function of zebra stripes. *Nature Commun.* 5, 226–228. <https://doi.org/10.1038/ncomms4535>.
- Caro, T., Argueta, Y., Briolat, E.S., Bruggink, J., Kasprowsky, M., Lake, J., Mitchell, M.J., Richardson, S., How, M., 2019. Benefits of zebra stripes: Behaviour of tabanid flies around zebras and horses. *PLoS One* 14, e0210831.
- Cena, K., Clark, J.A., 1973. Thermographic measurements of the surface temperatures of animals. *J. Mammal.* 54, 1003–1007.
- Chvala, M., Lyneborg, L., Moucha, J., 1972. The Horse Flies of Europe (Diptera, Tabanidae). Entomological Society of Copenhagen, Copenhagen, Denmark.
- Cloudsley-Thompson, J.L., 1984. How the zebra got his stripes – new solutions to an old problem. *Biologist* 31, 226–228.
- Cobb, A., Cobb, S., 2019. Do zebra stripes influence thermoregulation? *J. Nat. Hist.* 53, 863–879.
- Cott, H.B., 1957. *Adaptive Colouration in Animals*. John Dickens, Northampton, UK.
- Cott, H.B., 1966. *Colouration in Animals*. Methuen, London, UK.
- Darwin, C.R., 1871. The Descent of Man, and Selection in Relation to Sex vol. 2, 302.
- Egri, Á., Blahó, M., Kriska, G., Farkas, R., Gyurkovszky, M., Ákesson, S., Horváth, G., 2012a. Polarotactic tabanids find striped patterns with brightness and/or polarization modulation least attractive: an advantage of zebra stripes. *J. Exp. Biol.* 215, 736–745.
- Egri, Á., Blahó, M., Sándor, A., Kriska, G., Gyurkovszky, M., Farkas, R., Horváth, G., 2012b. New kind of polarotaxis governed by degree of polarization: attraction of tabanid flies to differently polarizing host animals and water surfaces. *Naturwissenschaften* 99, 407–416. <https://doi.org/10.1007/s00114-012-0916-2>.
- Eitringham, S.K., 1979. *The Ecology and Conservation of Large African Mammal*. Macmillan, Basingstoke, UK.
- Estes, R.D., 1992. *The Behavior Guide to African Mammals Including Hoofed Mammals, Carnivores*. University of California Press, Los Angeles, USA, Primates.
- Foil, L.D., 1989. Tabanids as vectors of disease agents. *Parasitol. Today* 5, 88–96.
- Galton, F., 1851. *South Africa*. Minerva Library, New York, USA.
- Gibson, G., 1992. Do tsetse-flies 'see' zebras? A field study of the visual response of tsetse to striped targets. *Physiol. Entomol.* 17, 141–147.
- Harris, R.H.T.P., 1930. Report on the Bionomics of the Tsetse Fly. Provincial Administration of Natal, Pietermaritzburg, South Africa.
- Herczeg, T., Blahó, M., Száz, D., Kriska, G., Gyurkovszky, M., Farkas, R., Horváth, G., 2014. Seasonality and daily activity of male and female tabanid flies monitored in a Hungarian hill-country pasture by new polarization traps and traditional canopy traps. *Parasitol. Res.* 113, 4251–4260.
- Herczeg, T., Száz, D., Blahó, M., Barta, A., Gyurkovszky, M., Farkas, R., Horváth, G., 2015. The effect of weather variables on the flight activity of horseflies (Diptera: Tabanidae) in the continental climate of Hungary. *Parasitol. Res.* 114, 1087–1097.
- Horváth, G., 2014. Chapter 16. Polarization patterns of freshwater bodies with biological implications. In: Horváth, G. (Ed.), *Polarized Light and Polarization Vision in Animal Sciences*. Springer, Heidelberg, Germany, pp. 333–344.
- Horváth, G., Majer, J., Horváth, L., Szivák, I., Kriska, G., 2008. Ventral polarization vision in tabanids: horseflies and deerflies (Diptera: Tabanidae) are attracted to horizontally polarized light. *Naturwissenschaften* 95, 1093–1100.
- Horváth, G., Blahó, M., Kriska, G., Hegedüs, R., Geric, B., Farkas, R., Ákesson, S., 2010. An unexpected advantage of whiteness in horses: the most horsefly-proof horse has a depolarizing white coat. *Proc. Roy. Soc. B* 277, 1643–1650.
- Horváth, G., Egri, Á., Blahó, M., 2014. Chapter 22. Linearly polarized light as a guiding cue for water detection and host finding in tabanid flies. In: Horváth, G. (Ed.), *Polarized Light and Polarization Vision in Animal Sciences*. Springer, Heidelberg, Germany, pp. 525–559.
- Horváth, G., Szörényi, T., Pereszlényi, Á., Geric, B., Hegedüs, R., Barta, A., Ákesson, S., 2017. Why do horseflies need polarization vision for host detection? Polarization helps tabanid flies to select sunlit dark host animals from the dark patches of the visual environment. *Roy. Soc. Open Sci.* 4. <https://doi.org/10.1098/rsos.170735>.
- Horváth, G., Pereszlényi, Á., Száz, D., Barta, A., Jánosi, I.M., Geric, B., Ákesson, S., 2018. Experimental evidence that stripes do not cool zebras. *Sci. Rep.* 8, 9351. <https://doi.org/10.1038/s41598-018-27637-1>.
- Horváth, G., Pereszlényi, Á., Ákesson, S., Kriska, G., 2019a. Striped bodypainting protects against horseflies. *Roy. Soc. Open Sci.* 6. <https://doi.org/10.1098/rsos.181325>.
- Horváth, G., Pereszlényi, Á., Tóth, T., Polgár, S., Jánosi, I.M., 2019b. Attractiveness of thermally different, uniformly black targets to horseflies: *Tabanus tergustinus* prefers sunlit warm shiny dark targets. *Roy. Soc. Open Sci.* 6. <https://doi.org/10.1098/rsos.191119>.
- Horváth, G., Varjú, D., 1997. Polarization pattern of freshwater habitats recorded by video polarimetry in red, green and blue spectral ranges and its relevance for water detection by aquatic insects. *J. Exp. Biol.* 200, 1155–1163.
- Horváth, G., Varjú, D., 2004. Polarized Light in Animal Vision – Polarization Patterns in Nature. Springer, Heidelberg, Germany.
- Horváth, G., Pereszlényi, Á., Egri, Á., Tóth, T., Jánosi, I.M., 2020a. Why do biting horseflies prefer warmer hosts? Tabanids can escape easier from warmer targets. *PLoS One* 15, e0233038.
- Horváth, G., Pereszlényi, Á., Egri, Á., Fritz, B., Guttmann, M., Lemmer, U., Gomard, G., Kriska, G., 2020b. Horsefly reactions to black surfaces: attractiveness to male and female tabanids versus surface tilt angle and temperature. *Parasitol. Res.* 119, 2399–2409. <https://doi.org/10.1007/s00436-020-06702-7>.
- How, M.J., Zanker, J.M., 2014. Motion camouflage induced by zebra stripes. *Zool.* 117, 163–170.
- How, M.J., Gonzales, D., Irwin, A., Caro, T., 2020. Zebra stripes, tabanid biting flies and the aperture effect. *Proc. Roy. Soc. B* 287, 20201521. <https://doi.org/10.1098/rspb.2020.1521>.
- Jordan, A.M., 1986. *Trypanosomiasis Control and African Rural Development*. Longman, New York, USA.
- Kingdon, J., 1984. The zebra's stripes: an aid to group cohesion. In: MacDonald, D. (Ed.), 1984. *The Encyclopaedia of Mammals*, Equinox, Oxford, UK, pp. 486–487.
- Kipling, R., 1908. *Just So Stories*. Macmillan, London, UK.
- Kojima, T., Oishi, K., Matsubara, Y., Uchiyama, Y., Fukushima, Y., Aoki, N., Sato, S., Masuda, T., Ueda, J., Hirooka, H., Kino, K., 2019. Cows painted with zebra stripes can avoid fly biting. *PLoS One* 14, e0223447.
- Krcmar, S., 2005. Response of horse flies (Diptera, Tabanidae) to different olfactory attractants. *Biol. Bratislava* 60, 611–613.
- Krcmar, S., Maric, S., 2006. Analysis of the feeding sites for some horseflies (Diptera, Tabanidae) on a human in Croatia. *Colleg. Antropol.* 30, 901–904.
- Krcmar, S., Merdic, E., Kopl, M., 2005. Diurnal periodicity in the biting activity of horsefly species in the Kopački rit Nature Park, Croatia (Diptera: Tabanidae). *Entomol. General.* 28, 139–146.
- Krcmar, S., Radolj, V., Lajos, P., Lukacevic, I., 2014. Efficiency of colored modified box traps for sampling tabanids. *Parasite* 21, 67. <https://doi.org/10.1051/parasite/2014068>.
- Kriska, G., Bernáth, B., Farkas, R., Horváth, G., 2009. Degrees of polarization of reflected light eliciting polarotaxis in dragonflies (Odonata), mayflies (Ephemeroptera) and tabanid flies (Tabanidae). *J. Ins. Physiol.* 55, 1167–1173.
- Kruuk, H., 1972. *The Spotted Hyena*. University of Chicago Press, Chicago, USA.
- Lehane, M.J., 2005. *The Biology of Blood-sucking in Insects*. Cambridge University Press, Cambridge, UK.
- Louw, G.N., 1993. *Physiological Animal Ecology*. Longman, New York, USA.
- Marler, P., Hamilton, W.J., 1968. *Mechanisms of Animal Behaviour*. Wiley, New York, USA.
- McLeod, D.N.K., 1987. Zebra stripes. *New Sci.* 115, 68.
- Meglic, A., Ilic, M., Piriš, P., Skorjanc, A., Wehling, M.F., Kreft, M., Belusic, G., 2019. Horsefly object-directed polarotaxis is mediated by a stochastically distributed ommatidial subtype in the ventral retina. *PNAS* 116, 21843–21853.
- Melin, A.D., Kline, D.W., Hiramatsu, C., Caro, T., 2016. Zebra stripes through the eyes of their predators, zebras, and humans. *PLoS One* 11, e0145679.
- Mihok, S., Carlson, D.A., 2021. New materials for improving catches of horseflies (Diptera: Tabanidae) in Nzi traps. *Med. Vet. Entomol.* 35, 580–594.
- Mihok, S., Lange, K., 2012. Synergism between ammonia and phenols for *Hybomitra* tabanids in northern and temperate Canada. *Med. Vet. Entomol.* 26, 282–290.
- Mihok, S., Mulye, H., 2010. Responses of tabanids to Nzi traps baited with octenol, cow urine and phenols in Canada. *Med. Vet. Entomol.* 24, 266–272.
- Morris, D., 1990. *Animal Watching. A Field Guide to Animal Behaviour*, Jonathan Cape, London, UK.
- Otártics, M.Z., Altbäcker, V., Solymosi, K., Mátics, R., Romvári, R., Farkas, S., 2019. Efficacy of H-traps is affected by exposure to sunshine. *Natura Croatica* 28, 257–269.
- Pereszlényi, Á., Száz, D., Jánosi, I.M., Horváth, G., 2021. A new argument against cooling by convective air eddies formed above sunlit zebra stripes. *Sci. Rep.* 11, 15797. <https://doi.org/10.1038/s41598-021-95105-4>.
- Poulton, E.B., 1890. *The Colours of Animals*. Kegan Paul Trench, Trubner, London, UK.
- Prothero, D.R., Schoch, R.M., 2003. *Horns, Tusks, and Flippers: The Evolution of Hoofed Mammals*. Johns Hopkins University Press, Baltimore, USA.
- Ruxton, G.D., 2002. The possible fitness benefits of striped coat coloration for zebra. *Mam. Rev.* 32, 237–244.
- Soroko, M., Davies-Morel, M.C.G., 2016. *Equine Thermography in Practice*. CABI, Wallingford, UK.
- Soroko, M., Howell, K., 2018. Infrared thermography: current applications in equine medicine. *J. Equ. Vet. Sci.* 60, 90–96.
- Takács, P., Száz, D., Vincze, M., Slíz-Balogh, J., Horváth, G., 2022. Sunlit zebra stripes may confuse the thermal perception of blood vessels causing the visual unattractiveness of zebras to horseflies. *Sci. Rep.* 12, 10871. <https://doi.org/10.1038/s41598-022-14619-7>.
- Thayer, A.H., 1909. *Concealing Coloration in the Animal Kingdom*. Macmillan, New York, USA.
- Thorsteinson, A.J., 1958. The orientation behaviour of horse flies and deer flies (Tabanidae: Diptera). I. The attractance of heat to tabanids. *Entomol. Exp. Appl.* 1, 191–196.
- Vale, G.A., 1974. The response of tsetse flies (Diptera, Glossinidae) to mobile and stationary baits. *Bull. Entomol. Res.* 64, 545–588.
- Vaughan, T.A., 1986. *Mammalogy*. North Arizona University, Flagstaff, USA.
- Waage, J.K., 1981. How the zebra got its stripes – biting flies as selective agents in the evolution of zebra coloration. *J. Entomol. Soc. South Africa* 44, 351–358.
- Wallace, A.R., 1867. Mimicry and other protective resemblances among animals. *Westminster Foreign Quart. Rev.* 32, 1–43.
- Wallace, A.R., 1879. The protective colours of animals. *Science* 2, 128–137.