

New kind of polarotaxis governed by degree of polarization: attraction of tabanid flies to differently polarizing host animals and water surfaces

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Abstract Aquatic insects find their habitat from a remote distance by means of horizontal polarization of light reflected from the water surface. This kind of positive polarotaxis is governed by the horizontal direction of polarization (E-vector). Tabanid flies also detect water by this kind of polarotaxis. The host choice of blood-sucking female tabanids is partly governed by the linear polarization of light reflected from the host's coat. Since the coat-reflected light is not always horizontally polarized,

host finding by female tabanids may be different from the established horizontal E-vector polarotaxis. To reveal the optical cue of the former polarotaxis, we performed choice experiments in the field with tabanid flies using aerial and ground-based visual targets with different degrees and directions of polarization. We observed a new kind of polarotaxis being governed by the degree of polarization rather than the E-vector direction of reflected light. We show here that female and male tabanids use polarotaxis governed by the horizontal E-vector to find water, while polarotaxis based on the degree of polarization serves host finding by female tabanids. As a practical by-product of our studies, we explain the enigmatic attractiveness of shiny black spheres used in canopy traps to catch tabanids.

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Introduction

The orientated motion of animals toward a spatial source governed by a particular cue is called taxis. One of the most well-known taxes is phototaxis (Menzel 1979; Jékely 2009). In the early 1980s, Schwind (1983, 1984, 1985a,b) discovered the phenomenon of polarotaxis: He found that the water bug *Notonecta glauca* is attracted to horizontally polarized light if this optical cue stimulates its ventral eye region. The function of this positive polarotaxis is to help in the detection of aquatic habitats by means of the horizontally polarized light reflected from the water surface. Later, this kind of polarotaxis governed by the horizontal polarization of reflected light has been found in many other aquatic insect species (Schwind 1989,

1991, 1995, 1999; Wildermuth 1998; Horváth and Varjú 2004; Csabai et al. 2006; Kriska et al. 2006, 2007, 2008, 2009; Horváth et al. 2007, 2008, 2010a,b, 2011; Lerner et al. 2008; Horváth and Kriska 2008).

Tabanid flies lay their eggs onto water plants or into mud (Majer 1987; Lehane 2005); thus, they have to find water, a process that is also based on polarotaxis (Horváth et al. 2008; Kriska et al. 2009). Females of many tabanid species have to find a host animal to obtain a blood meal that ensures egg development. The host choice of these tabanids is partly governed by the linear polarization of light reflected from the host's coat (Horváth et al. 2010b; Egri et al. 2012). This polarization-based, i.e. polarotactic, behaviour of female tabanids, however, should be different from the polarotaxis governed by the horizontal E-vector direction because the coat-reflected light is not always horizontally polarized (Horváth et al. 2010b; Egri et al. 2012).

What is the exact optical cue of polarotaxis serving the host choice in tabanids? To answer this question, we performed choice experiments in the field with tabanid flies. We used various aerial and ground-based visual targets with different degrees of polarization (the percentage of the totally linearly polarized component oscillating in the plane of the direction of polarization) and directions of polarization, and counted the tabanids trapped by these test surfaces. We report here on a new kind of polarotaxis based on the degree of polarization. We show that both female and male tabanids use polarotaxis governed by the horizontal direction of polarization to find water, while the polarotaxis based on the degree of polarization serves host finding by female tabanids. As a practical by-product, we reveal the reason for the attractiveness of black spheres used in canopy traps to catch tabanid flies.

Materials and methods

Experiment 1 was performed between 1 August and 14 September 2010 on a Hungarian horse farm at Szokolya (47°52' N, 19°00' E). To study the influence of surface roughness of black spherical visual targets to the attractiveness of canopy traps for tabanids, two equal traps were used, the pyramidal canopy of which was made of a common white tulle. In one of these traps, the visual target was a matte black sphere, while it was a shiny black sphere in the other trap (Fig. 1; Fig. S1a of “Electronic supplementary material”). The diameter of both spheres was 50 cm. The shiny sphere was a common beach ball sprayed with black paint. The matte sphere was a beach ball covered with matte black felt. The spheres were hung 1 m above the ground level with a string in such a way that their equator was at the height of the lowermost margin of the tulle canopy. The two traps were set up 20 m apart from each other near a large (50×50 m) paddock where horses were kept. These horses

attracted numerous tabanids. Both traps were simultaneously either in the sun or in the shade. The tabanids attracted to the trap landed on the visual target, and after leaving the target they became entrapped by the canopy and a translucent plastic container at its top (Fig. S1c, d of “Electronic supplementary material”). We periodically counted the number of tabanids caught by the traps. After each counting, the visual targets were reversed under the two canopies. The captured specimens were stored in 70 % ethyl alcohol for later taxonomical identification.

This experiment was repeated between 19 June and 18 September 2011 on the same horse farm (Fig. S1a of “Electronic supplementary material”). The aim of this repetition was to double the duration of the field test from 6 to 12 weeks because the original 6 weeks in 2010 seemed to be not long enough.

Experiment 2 was conducted between 19 June and 18 September 2011 on the same horse farm as in experiment 1. The aim of this experiment was to investigate the influence of the shape of the visual target of the canopy trap on its attractiveness to tabanids. Both canopy traps were identical and the same as used in experiment 1. In one of the traps, the visual target was a matte black cylinder, while it was a shiny black cylinder in the other trap (Fig. 2; Fig. S1b of “Electronic supplementary material”). The diameter and the height of both cylinders were both 40 cm. The shiny cylinder was a pail made of smooth black plastic. The matte cylinder was a pail covered with matte black felt. The cylinders were fixed to the vertical metal rod of the canopy trap 1 m above the ground level in such a way that their equator was at the height of the lowermost margin of the tulle canopy. The two traps were set up 20 m apart from each other near a large (50×50 m) paddock where horses were kept. This paddock was 200 m apart from the one at which the other two canopy traps used in experiment 1 were set up. Other details of this experiment were the same as in experiment 1.

Experiment 3 was carried out between 22 June and 1 September 2011 on a field near the horse farm of experiments 1 and 2. The aim was to test the role of the direction of polarization in the attractiveness of horizontal and vertical homogeneous dark grey targets to polarotactic tabanids. We used six test surfaces made of a wooden board (43×43×2 cm) painted matte white and covered with a linearly polarizing sheet (43×43 cm, P-W-44, Schneider, Bad-Kreuznach, Germany). The polarizing sheets were fixed with tiny nails on the white substrate. Along a line, 5 m apart from each other, three test surfaces were fixed vertically 1 m above the ground between vertical metal rods stuck into the ground. The other three test surfaces were laid horizontally on the ground along a line 5 m apart from each other (Fig. S2 of “Electronic supplementary material”). The transmission direction of the vertical polarizers V-S₁, V-S₂ and V-S₃ was vertical, tilted at 30° and horizontal, respectively. Seen perpendicular to the line passing through the

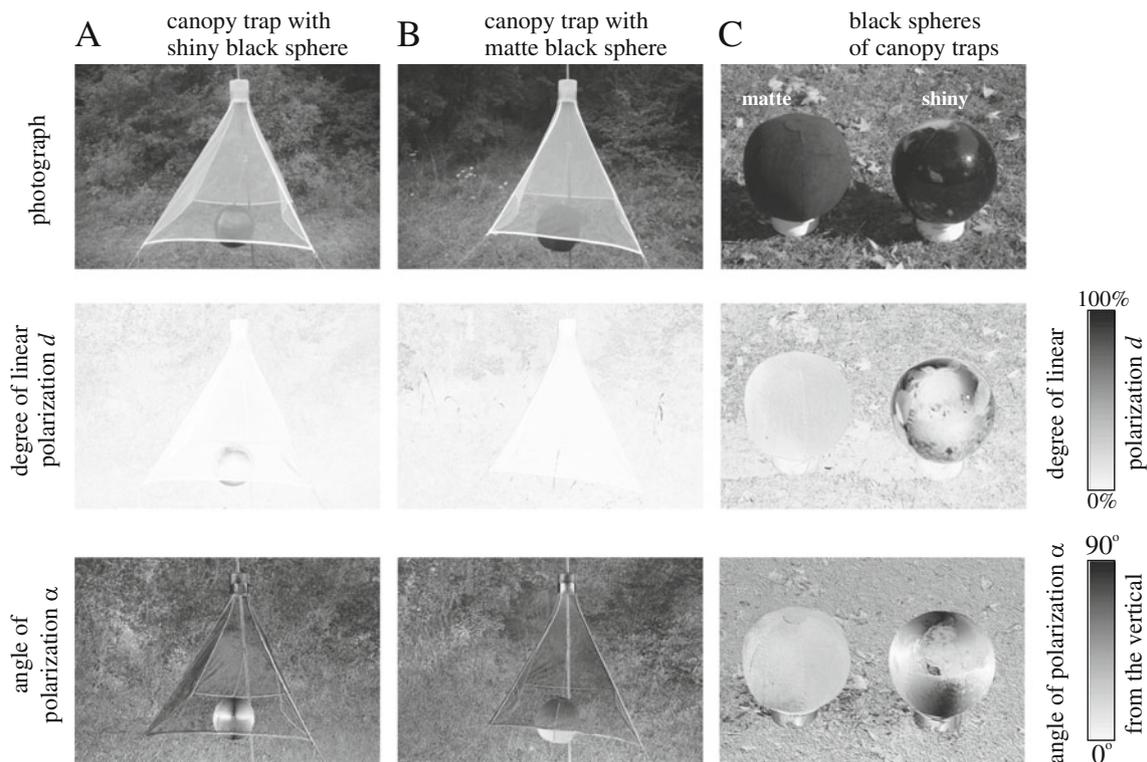


Fig. 1 Photograph and patterns of the degree of polarization d and angle of polarization α of light reflected from the canopy traps and their visual targets (matte and shiny black spheres) used in experiment 1 measured by imaging polarimetry in the blue (450 nm) spectral range

under shady (a, b) and sunlit (c) conditions. The elevation angle of the polarimeter's optical axis was -15° (a, b) and -45° (c) from the horizon

centre of the horizontal polarizers H-S₁, H-S₂ and H-S₃, their transmission direction was vertical, tilted at 45° and horizontal, respectively. The horizontal distance between the lines of the horizontal and vertical test surfaces was 1 m. All six test surfaces were covered with transparent insect monitoring glue (BabolnaBio). We counted and removed the tabanids trapped by these sticky test surfaces periodically when all other captured insects were also removed, the order of the test surfaces was randomized and the insect monitoring glue was refreshed. The brightness, colour (dark grey) and degree of polarization d ($\approx 100\%$) of the test surfaces were the same, but the direction of polarization was different due to the different transmission directions of the polarizing sheets. Species identification of tabanids collected from the sticky surfaces was impossible because their body was damaged seriously. They were, however, unambiguously tabanid flies (Diptera: Tabanidae), and their sexes could be determined on the basis of the anatomical characteristics of their head as observed with a magnifier lens ($\times 10$): in males, the left and right compound eyes contact dorsally, whereas they do not contact in females. To determine the species occurring in the region at the time of this

experiment, in a parallel experiment lasting from 22 June till 1 September 2011 at the same study site, we captured tabanids with a trap made of a rectangular black plastic tray (50×50 cm) filled with transparent salad oil that was proven to be an efficient tabanid trap (Horváth et al. 2008).

Experiment 4 was done from 2 July to 5 September 2010 in a wetland field near Dinnyés, a Hungarian village ($47^\circ 8' 44''$ N, $18^\circ 34' 12''$ E). Since there was a cattle farm at Elzamajor 1 km from the experiment site, a huge number of tabanid flies occurred around the experimental area. As shiny black test surfaces, we used four spheres (beach balls with a diameter of 41 cm sprayed with black paint) and four horizontal plastic boards (1×1 m) grouped into the following four pairs (Fig. S3 of “Electronic supplementary material”): (I) board and sphere on the ground, (II) board and sphere in the air 75 cm above the ground level, (III) board in the air 75 cm above the ground and sphere on the ground and (IV) board on the ground and sphere in the air 75 cm above the ground. The horizontal distance between the members of each test surface pair was 1 m, while the pairs were 3 m apart from each other along a straight line. The spheres were fixed with a string to a

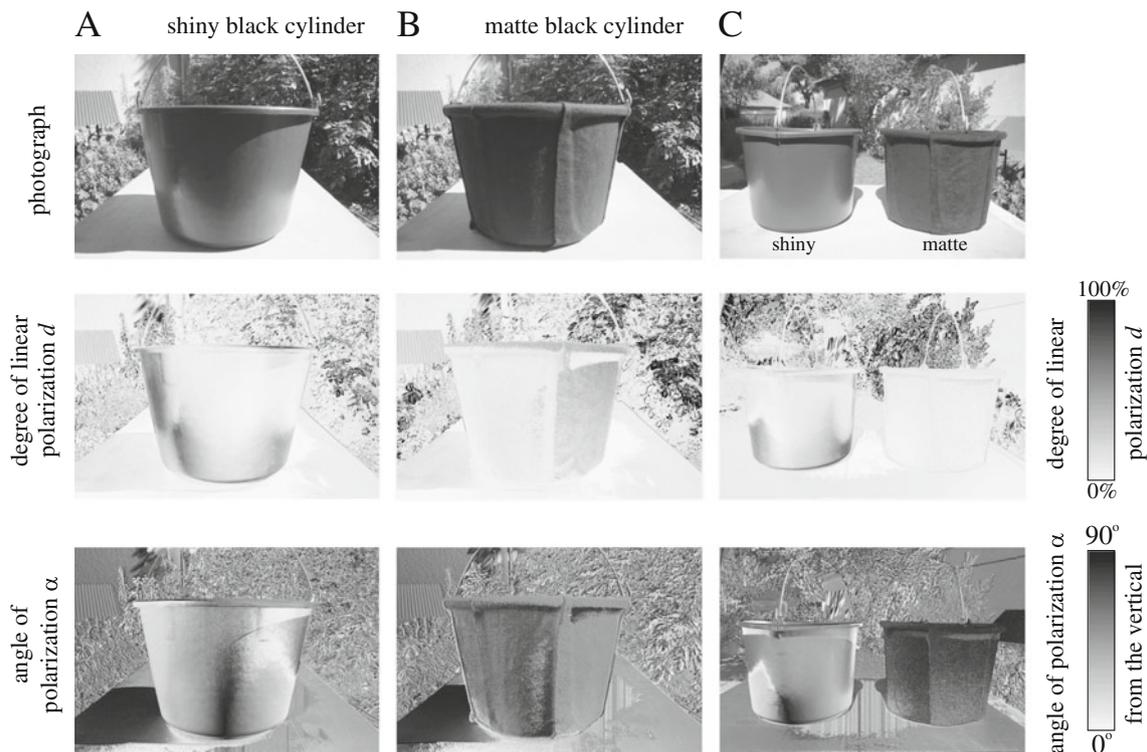


Fig. 2 Photograph and patterns of the degree of polarization d and angle of polarization α of light reflected from the visual targets (matte and shiny black cylinders) used in experiment 2 measured by imaging polarimetry in the blue (450 nm) spectral range under sunlit conditions.

The elevation angle of the polarimeter's optical axis was -10° from the horizon. In **a** and **b**, the targets were illuminated by sunlight from the right side, while in **c** the sun shone from the back of the observer (polarimeter)

vertical metal rod stuck into the ground. The height (0 or 75 cm) of the spheres was controlled by the length of this string. The aerial plastic boards were fixed horizontally to four vertical metal rods at 75 cm in height, while the boards on the ground were fixed to the grassy soil by L-shaped metal hooks stuck into the ground. All eight shiny black test surfaces were covered by insect monitoring glue (BabolnaBio). The tabanids trapped by each sphere and plastic board were counted and removed with all captured insects periodically, the glue was refreshed and the order of the test surface pairs was randomly changed. The sex of trapped tabanids was determined, but their species identification was impossible because of the same reason as cited in experiment 3.

Numbers of replications and days of experiments: In experiments 3 and 4, sticky visual targets with different reflection–polarization characteristics captured tabanids, the carcasses of which were counted and removed periodically, then the order of the test surfaces was randomly changed. Since the trapped tabanids and all other insects were removed, the newly arrived tabanids were not influenced by the view of insect carcasses. Thus,

the altered situation after each tabanid counting represented a new replication of the experiment. In experiment 3, the number R of replications during a test period composed of number of days D were $R=24$ and $D=72$. In experiment 4, these numbers were $R=36$ and $D=66$. These numbers of replications were large enough to detect statistically evaluable differences in the numbers of trapped tabanids.

The reflection–polarization characteristics of the test surfaces used in experiments 1–4 were measured by imaging polarimetry in the red (650 ± 40 nm=wave-length of maximal sensitivity \pm half bandwidth of the CCD detectors of the polarimeter), green (550 ± 40 nm) and blue (450 ± 40 nm) parts of the spectrum. The method of imaging polarimetry has been described in detail by Horváth and Varjú (1997, 2004). Here we present only the polarization patterns measured in the blue part of the spectrum. Practically, the same patterns were obtained in the red and green spectral ranges because the test surfaces were colourless (white, grey or black).

Statistical analyses (ANOVA, Tukey multiple-comparison tests and binomial χ^2 test) were performed with the use of the program Statistica 7.0.

Results

Reflection–polarization characteristics of test surfaces

Figure 1 shows the reflection–polarization characteristics of the canopy traps and their visual targets (matte and shiny black spheres) used in experiment 1 as measured by imaging polarimetry in the blue (450 nm) spectral range under shady and sunlit conditions. Since the components of the traps were colourless (white, grey or black), quite similar patterns were obtained in the green (550 nm) and red (650 nm) parts of the spectrum. The white tulle of the canopy reflected weakly polarized light ($d < 10\%$), the matte black sphere reflected moderately polarized light ($d < 30\%$), while the shiny black sphere reflected highly polarized light ($d \leq 100\%$), depending on the angle of reflection. The high degrees of polarization d of light reflected from the shiny black sphere were also visible through the canopy tulle (Fig. 1a). Under shady conditions, the direction of polarization of canopy-reflected light was nearly horizontal. If the canopy was sunlit, it reflected light usually with oblique directions of polarization relative to the horizontal, depending on the direction of view with respect to the sun. The same was true for the matte black sphere. On the other hand, the direction of polarization of light reflected from the shiny black sphere was always tangential, that is, parallel to its circular contour. Thus, the E-vector of reflected light was approximately horizontal (displayed by bright green and blue colours in the patterns of the angle of polarization α) on the top and bottom of the shiny black sphere; it was nearly vertical (displayed by bright red and yellow colours in the α -patterns) at the left and right sides of the sphere, while it was tilted (displayed by dark colours in the α -patterns) at other regions of the sphere. This tangential E-vector distribution of the shiny black sphere was also visible through the white tulle of the canopy (Fig. 1a).

Figure 2 displays the reflection–polarization patterns of the visual targets (matte and shiny black cylinders) used in experiment 2 and measured by imaging polarimetry in the blue (450 nm) spectral range under sunlit conditions. The matte black cylinder reflected weakly ($d < 10\%$) or moderately ($d < 30\%$) polarized light if it was shady or sunlit, and the degree of polarization d of reflected light depended on the angle of reflection θ . Depending on θ , the shiny black cylinder reflected weakly ($d < 10\%$), moderately ($d < 30\%$) or highly ($d \leq 100\%$) polarized light, and d was maximal ($d \approx 100\%$) at the Brewster angle ($\theta_{\text{Brewster}} = \arctan n = 56.3^\circ$ from the normal vector of the reflecting surface, where $n = 1.5$ is the refractive index of the

plastic material of the cylinder). Depending on the direction of view with respect to the sun and on the orientation of the local normal vector of the cylinder surface, both the matte and the shiny black cylinders reflected light with different directions of polarization changing from vertical through oblique to horizontal. Practically, the same reflection–polarization characteristics of the matte and shiny black cylinders were obtained for the green and red spectral ranges.

In experiment 3, the vertical test surfaces V-S₋, V-S| and V-S/ with horizontal, vertical and oblique transmission direction of their linear polarizers had homogeneous dark grey colour and reflected almost totally linearly polarized light ($d \approx 100\%$) with horizontal, vertical and oblique E-vector direction, respectively (Fig. S4 of “Electronic supplementary material”). The horizontal test surfaces H-S₋, H-S| and H-S/ were also homogeneous dark grey and reflected nearly totally linearly polarized light ($d \approx 100\%$); however, the E-vector direction of reflected light depended on the direction of view relative to the transmission direction of their horizontal linear polarizers. Thus, a flying tabanid approximating to the horizontal test surfaces H-S₋, H-S| and H-S/ from different directions could perceive horizontal, vertical or oblique E-vector direction of reflected light.

The horizontal black boards used in experiment 4 reflected always horizontally polarized light, whose degree of polarization d was maximal at the Brewster angle (Fig. S5 of “Electronic supplementary material”). The values of d depended on the angle of elevation from the horizontal, but they were always high ($d > 50\%$) at the Brewster angle. Thus, these horizontal test surfaces were strongly attractive to polarotactic, water-seeking tabanids (Kriska et al. 2009).

Attractiveness of test surfaces to tabanids

In 2010, in experiment 1, the canopy trap with a shiny black sphere captured 11.7 times (= 164/14) more tabanids than that with a matte black sphere (Fig. S1c d of “Electronic supplementary material”), which is a statistically significant difference (Table 1; Table S1 of “Electronic supplementary material”). Both traps caught exclusively female tabanids of the following species (Table 1): *Tabanus bovinus*, *Tabanus tergustinus*, *Tabanus quatuornotatus*, *Tabanus bromius*, *Tabanus miki*, *Haematopota pluvialis*, *Silvius vituli*.

These results were corroborated by those obtained in the repetition of experiment 1 in 2011 where the canopy trap with a shiny black sphere captured 9.3 times (= 232/25) more tabanids than that with a matte black sphere, which is a statistically significant difference

Table 1 Species and number (*N*) of female tabanids captured in experiment 1 by canopy traps with a matte or a shiny black sphere as visual target in 2010 and 2011 (no male tabanids were trapped)

Species	Visual target in the canopy trap	
	Matte black sphere	Shiny black sphere
1 August–14 September 2010		
<i>Haematopota pluvialis</i>	2	4
<i>Silvius vituli</i>	1	0
<i>Tabanus bovinus</i>	3	92
<i>Tabanus bromius</i>	0	11
<i>Tabanus miki</i>	0	11
<i>Tabanus quatuornotatus</i>	3	12
<i>Tabanus tergustinus</i>	5	34
Sum	14 (7.9 %)	164 (92.1 %)
19 June–18 September 2011		
<i>Atylotus fulvus</i>	4	2
<i>Atylotus loewianus</i>	0	2
<i>Haematopota italica</i>	0	9
<i>Haematopota pluvialis</i>	1	8
<i>Tabanus autumnalis</i>	12	0
<i>Tabanus bovinus</i>	0	99
<i>Tabanus bromius</i>	6	49
<i>Tabanus tergustinus</i>	1	40
<i>Tabanus</i> sp.	1	23
Sum	25 (9.7 %)	232 (90.3 %)

For data in 2010, the statistical analyses had the following results: χ^2 test: $\chi^2=126.4$, $df=1$, $p<0.0001$ (significant); ANOVA test: $SS_{\text{effect}}=1,875$, $df_{\text{effect}}=1$, $MS_{\text{effect}}=1,875$, $SS_{\text{error}}=806.7$, $df_{\text{error}}=10$, $MS_{\text{error}}=80.7$, $F=23.2$, $p=0.0007$ (significant). For data in 2011, the statistical analyses had the following results: χ^2 test: $\chi^2=166.7$, $df=1$, $p<0.0001$ (significant); ANOVA test: $SS_{\text{effect}}=2,142.5$, $df_{\text{effect}}=1$, $MS_{\text{effect}}=2,142.5$, $SS_{\text{error}}=1,314.1$, $df_{\text{error}}=18$, $MS_{\text{error}}=73.0$, $F=29.3$, $p<0.0001$ (significant)

(Table 1). Both traps caught again only females of the following species (Table 1): *Tabanus autumnalis*, *T. bovinus*, *T. tergustinus*, *T. bromius*, *H. pluvialis*, *Haematopota italica*, *Atylotus fulvus*, *Atylotus loewianus*.

Similar results were obtained in experiment 2 using black cylinders as visual targets; however, the difference between the catches was not as large as that in experiment 1 using black spheres. In experiment 2, the canopy trap with a shiny black cylinder captured 2.2 times (= 69/31) more tabanids than that with a matte black cylinder, which is a statistically significant difference (Table 2; Table S2 of “Electronic supplementary material”). Both traps caught only females of the following species (Table 2): *T. autumnalis*, *T. tergustinus*, *T. bromius*, *H. pluvialis*, *A. fulvus*.

In experiment 3, the vertical sticky test surfaces V-S_⊥, V-S_∥ and V-S_/ with horizontal, vertical and oblique transmission direction of their linear polarizers captured

155 (34.0 %), 161 (35.3 %) and 140 (30.7 %) tabanid flies, respectively (Table 3; Table S3 of “Electronic supplementary material”). These tabanids were exclusively females. On the other hand, the corresponding horizontal sticky test surfaces H-S_⊥, H-S_∥ and H-S_/ trapped 3.5, 3.9 and 4.2 times more tabanids of both sexes (Table 3), respectively: H-S_⊥=550 (31.20 %), H-S_∥=623 (35.34 %), H-S_/= 590 (33.46 %). Neither Tukey multiple-comparison test (Table S4 of “Electronic supplementary material”) nor ANOVA (Table S5 of “Electronic supplementary material”) test resulted in statistically not significant differences in the catches of test surfaces of the same orientation (vertical or horizontal). During experiment 3, the oil-filled trap captured the following tabanid species (both females and males): *T. tergustinus*, *T. bromius*, *T. bovinus*, *T. autumnalis*, *A. fulvus*, *A. loewianus*, *A. rusticus*, *H. italica*. The same species could also be trapped by the sticky polarizing test surfaces used in experiment 3.

According to Fig. 3 (Table S6 of “Electronic supplementary material”), in experiment 4, the black spheres captured practically female tabanids only (the one to two males trapped are negligible in the case of test surface pairs III and IV): the number of females caught was 163, 202, 115 and 308 in the case of test surface pairs I, II, III and IV, respectively. If the horizontal black boards were in the air, they trapped only zero and four tabanids in the case of test surface pairs II and III, respectively, numbers which are negligible relative to the catch of other test surfaces. However, if the boards were on the ground, they captured 53 males + 97 females and 59 males + 99 females in the case of test surface pairs I and IV, respectively. Hence, the

Table 2 Species and number of female tabanids captured in experiment 2 by canopy traps with a matte or a shiny black cylinder as visual target (no male tabanids were trapped)

Species	Visual target in the canopy trap	
	Matte black cylinder	Shiny black cylinder
<i>Tabanus autumnalis</i>	2	12
<i>T. tergustinus</i>	2	8
<i>T. bromius</i>	15	31
<i>Atylotus fulvus</i>	7	16
<i>Haematopota pluvialis</i>	5	2
Sum	31 (31 %)	69 (69 %)

The statistical analyses had the following results: χ^2 test: $\chi^2=14.4$, $df=1$, $p<0.0002$ (significant); ANOVA test: $SS_{\text{effect}}=72.2$, $df_{\text{effect}}=1$, $MS_{\text{effect}}=72.2$, $SS_{\text{error}}=207.8$, $df_{\text{error}}=18$, $MS_{\text{error}}=11.5$, $F=6.25$, $p=0.02$ (significant)

Table 3 Number of tabanids (*T. tergestinus*, *T. bromius*, *T. bovinus*, *T. autumnalis*, *A. fulvus*, *A. loewianus*, *A. rusticus*, *H. italica*) captured in experiment 3 by the horizontal and vertical test surfaces performed on a horse farm at Szokolya in Hungary

	Horizontal test surfaces			Vertical test surfaces		
	H-S _⊥	H-S	H-S _/	V-S _⊥	V-S	V-S _/
Females	356	390	371	155	161	140
Males	194	233	219	0	0	0
Sum	550 (31.20 %)	623 (35.34 %)	590 (33.46 %)	155 (34.00 %)	161 (35.31 %)	140 (30.69 %)

The results of statistical tests (χ^2 test and ANOVA) can be seen in Tables S4 and S5 of “Electronic supplementary material”. The differences in the catches of test surfaces of the same orientation (vertical or horizontal) are statistically not significant

H horizontal test surface, *V* vertical test surface, *S*_⊥ test surface with horizontal transmission direction of its linear polarizer, *S*_| test surface with vertical transmission direction of its linear polarizer, *S*_/ test surface with oblique transmission direction of its linear polarizer

horizontal black boards were attractive to both male and female tabanids if they were on the ground, while they were unattractive when they were in the air. On the other hand, the black spheres were attractive exclusively to female tabanids, and their attractiveness was larger if they were in the air (163 and 115 on the ground versus 202 and 308 in the air). All these differences are statistically significant (Table S7 of “Electronic supplementary material”).

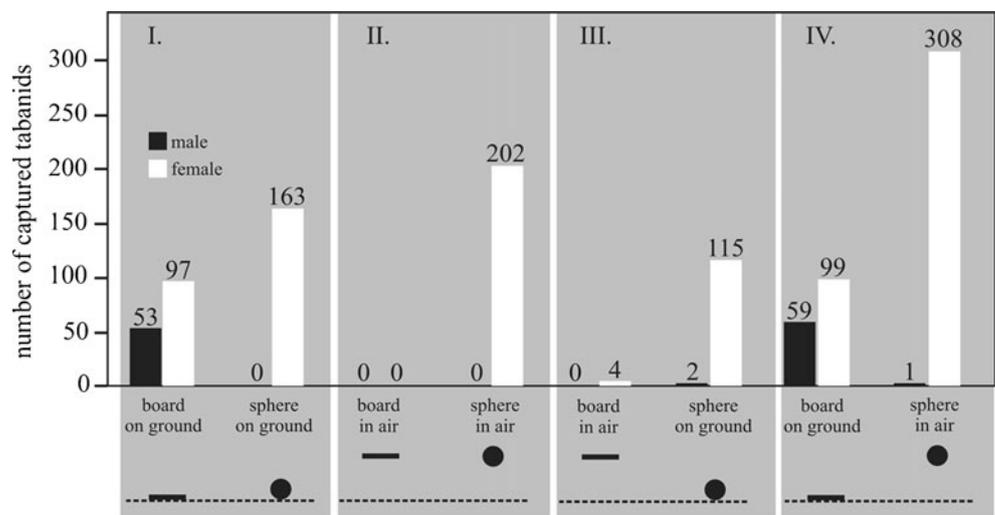
Discussion and conclusions

In experiments 1 and 2, the canopy traps with shiny black spherical or cylindrical visual targets captured significantly more tabanid flies than the same traps with the corresponding matte black visual targets. This difference in catches can only be explained by the different attractiveness of the matte and shiny visual targets of the same shape (sphere or cylinder) to tabanids. Although the shiny black test surfaces used in our experiments reflect a much larger amount of light than

the matte black surfaces, in earlier experiments it has been shown that in such situations the attractiveness of tabanids cannot be explained by means of positive or negative phototaxis (Horváth et al. 2008, 2010a,b; Kriska et al. 2009; Egri et al. 2012). In these experiments, the attraction of tabanids to differently polarizing colourless (black, grey, white) test surfaces placed on the ground or elevated in the air is governed mainly by polarization rather than the intensity of target-reflected light. Independently of the shape (sphere or cylinder) and surface roughness (smooth/shiny or rough/matte) of the targets, the direction of polarization of target-reflected light changed from vertical through tilted to horizontal as functions of the angle of reflection and viewing direction. The only relevant difference in the state of polarization of target-reflected light occurred in the degree of linear polarization *d*: matte black targets reflecting light with low *d*-values, while shiny black targets reflecting light with high *d*-values at and near the Brewster angle.

From this, we conclude that the canopy traps with shiny black visual targets captured much more tabanids than those

Fig. 3 Number of male and female tabanids captured by the test surfaces in the four groups (I, II, III and IV) used in experiment 4



with matte black targets because the attractiveness of their shiny targets to tabanids was much larger than that of matte targets due to the much higher degree of polarization d of target-reflected light, independently of the E-vector direction. This behaviour of polarotactic tabanids is a new kind of polarotaxis based on the degree of polarization of reflected light.

This conclusion is corroborated by the results of experiment 3 in which we found that the attractiveness of the practically totally polarizing vertical test surfaces to tabanids was the same, independently of the E-vector direction of reflected light. The same was true for the horizontal test surfaces used in experiment 3. The vertical test surfaces trapped only female tabanids, while the horizontal ones captured both female and male tabanid flies. Thus, we hypothesize that the vertical test surfaces imitated host animals attacked only by female tabanids that looked for a blood meal. On the other hand, the horizontal test surfaces mimicked water surfaces if they were seen from appropriate directions of view. This was the situation when the transmission direction of the horizontal polarizer was exactly or nearly perpendicular to the vertical symmetry axis of a flying, water-seeking tabanid, the eyes of which were thus stimulated by exactly or nearly horizontally polarized light. Both female and male tabanids find water (to drink or to cool their heated-up body or to seek for a rendezvous place in the immediate vicinity of water) by the horizontal polarization of light reflected from the water surface.

The results of experiment 4 support our conclusion that highly polarizing dark visual targets (e.g. black spheres and cylinders), independently of their height above the ground and of the E-vector direction of reflected light, are attractive only to host-seeking female tabanids. We hypothesize that, if the visual target is on the ground, its visibility may be smaller than when it is in the air; thus, its attractiveness to females could be reduced. Furthermore, experiment 4 corroborates our other conclusion that highly and horizontally polarizing dark visual targets (e.g. horizontal black boards) on the ground are attractive to both male and female tabanids looking for water. If the horizontally polarizing visual target is in the air (higher than about 0.5 m), it is not sensed as water by tabanids because a horizontally polarizing water surface is always on ground level. Although there are also elevated water surfaces in nature, filled rain barrels, for example, according to our many-year field experience, these do not attract tabanid flies. This was also corroborated in our other field experiments, the results of which are still unpublished: (1) In experiments lasting for 1–3 months at four different Hungarian test sites, oil-filled black trays at heights of 0.5, 1.0, 1.5 and 2.0 m above the ground did not capture any tabanid fly, while the same traps on the ground caught several tens or hundreds tabanids; (2) An oil-filled black tray on the ground captured 20 times more tabanids than the

same trap at a height of 20 cm from the ground (G. Horváth: unpublished data).

In our experiments, the traps always captured more females than males, even on the horizontal polarizing surfaces on the ground (Tables 1, 2 and 3; Tables S6 and S7 of “Electronic supplementary material”; Fig. 3). These differences were significant. As the difference between males and females lies at the core of our work (i.e. the new polarotactic behaviour is only found in females), this is an important factor. The reason for the phenomenon that the horizontally polarizing surfaces on the ground captured more females than males could be the following: Male and female tabanids are attracted to horizontally polarized light if they look for mates or for water to drink or to bath (to cool their body). Furthermore, females are also attracted to such light if they want to lay their eggs into/near water/mud. Thus, females may have more motivation to seek horizontally polarizing surfaces on the ground.

The well-known polarotaxis in tabanids and many other aquatic insects and insects associated with water is governed by the horizontal E-vector direction of light reflected from the water surface. This kind of polarotaxis serves the remote visual detection of water by means of the horizontal polarization of water-reflected light. Water insects are attracted only to nearly horizontally polarized light because water surfaces reflect such light. In this case, the degree of polarization d must be higher than a species-dependent threshold d^* (Kriska et al. 2009). If $d > d^*$, then polarotaxis is governed by the direction of polarization: if the deviation of the angle of polarization α from the horizontal is smaller than a threshold $\Delta\alpha^*$, a polarotactic aquatic insect is attracted to the partially linearly polarized light.

The new kind of polarotaxis governed by the degree of polarization d serves the finding of host animals by female tabanids in search of blood. Since the different body parts of a host animal reflect light with different E-vector directions, this positive polarotaxis cannot be elicited exclusively by horizontally polarized light. In this case, the E-vector direction of host-reflected light is irrelevant, and only d is important. Earlier studies (Horváth et al. 2010b; Egri et al. 2012) showed that, the darker the coat of the host, i.e. the higher the d of host-reflected light, the larger its attractiveness to tabanids.

Hence, tabanid flies possess two kinds of polarotaxis: (1) Both female and male tabanids find water by means of polarotaxis governed by E-vector direction (they look for horizontally polarized light indicating water); (2) Female tabanids additionally possess the ability for polarotaxis governed by the degree of polarization d , which is used for host choice (the higher the d of host-reflected light, the more attractive the host). To understand this result, there are at least two possibilities: Female tabanids (1) could switch between these two types of polarotaxis when they look for

host or water or (2) they could be attracted to whatever they see first (host or water). However, it is more probable that motivational state influences which of these two mechanisms guides the behaviour of female tabanid flies.

The sense of polarotaxis governed by the degree of polarization serving host choice is similar to that of polarotaxis governed by the direction of polarization serving water detection. The latter has evolved in aquatic insects because water surfaces cannot be unambiguously detected by means of the intensity and colour of reflected light: Non-aquatic natural surfaces can also reflect light with intensities and colours similar to those of water-reflected light. However, non-aquatic natural surfaces usually do not reflect horizontally polarized light in an extended field of view like water surfaces do. Thus, water bodies can unambiguously be found by means of this horizontally polarized optical cue. Similarly, host animals cannot be unambiguously detected by means of their brightness and colour: Although dark/bright hosts in front of a bright/dark background can be easily detected, dark/bright hosts in front of a dark/bright background can be recognized only with difficulty. However, since the (usually vegetation) background reflects only weakly polarized light, a dark host animal can be easily detected by means of the highly polarized light reflected from its coat. This may be the reason why female tabanids prefer host animals with highly polarizing darker coats (Horváth et al. 2010b) in spite of the fact that the blood of darker or brighter hosts would be equivalently appropriate for female tabanids to ripe their eggs.

It is still unknown in which spectral range the polarization vision of tabanid flies functions. Since tabanids also possess UV-sensitive photoreceptors, their polarization sensitivity can also function in the UV. Although we could not measure the reflection–polarization patterns of our test surfaces and visual targets in the UV, their polarization characteristics in the UV should be similar to those in the visual spectral range measured by imaging polarimetry because these surfaces were colourless (white, grey or black), and the sunlight and skylight illuminating them had natural UV component.

In experiments 1–3, practically the same tabanid species occurred (Tables 1, 2 and 3; Table S3 of “Electronic supplementary material”). Thus, the visual phenomena studied here may be general and valid throughout tabanids. Experiment 4 was performed at a different site, and taxonomical data were not available.

Finally, we would like to emphasize that our results presented in this work explain the enigmatic attractiveness of black spheres to tabanid flies used as visual targets in many tabanid traps. A common feature of many tabanid traps is that they are made of a visual target suspended underneath a tent-like canopy. The function of the visual target is to attract tabanids from a remote distance by means

of optical cues. When the attracted tabanids land on the target and find that it is not a potential meal, a proportion of them fly upward into the funnel-like end of the canopy, where they are trapped by a glass/plastic container. One of the most frequently used visual targets is a black sphere (e.g. a black rubber ball or an inflatable vinyl beach ball spray-painted with glossy black paint) swinging freely from a rope and moving when the wind blows (Fig. 1a, b; Fig. S1a of “Electronic supplementary material”). It is generally believed that the black sphere (1) may imitate the dark silhouette of a host animal and (2) if it is flapping in the wind, its motion might mimic that of the host (e.g. Thorsteinson et al. 1965; Lehane 2005). Although the attractiveness of a black sphere to tabanids is unquestionable (Malaise 1937; Gressitt and Gressitt 1962; Catts 1970; von Kniepert 1979; Wall and Doane 1980; Hribar et al. 1992; Mihok 2002), its explanation has been failed until now.

On the basis of our results, it is very important that such a black sphere must be highly polarizing in order to attract tabanids maximally. This can be ensured only by smooth, that is, shiny, black spheres. Consequently, weakly polarizing matte black spheres are disadvantageous for this purpose. As far as we know, this is a new practical information in the design of tabanid canopy traps. Nevertheless, the spherical visual targets used in such traps are always shiny due to the techniques of their production: these black spheres are made either of rubber or plastic materials, possessing necessarily a smooth, that is, highly polarizing, surface.

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