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Stripes disrupt odour attractiveness to biting horseflies: Battle between ammonia, CO₂, and colour pattern for dominance in the sensory systems of host-seeking tabanids

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HIGHLIGHTS

- Blood-sucking female tabanids search for host animals by visual and olfactory cues.
- Striped coat patterns are visually less attractive to tabanids than homogeneous targets.
- Tabanids are strongly attracted by CO₂ and ammonia emitted by their hosts.
- In experiments we found that the poor visual attractiveness of stripes to tabanids is not overcome by olfactory attractiveness.
- This demonstrates the visual protection of striped patterns against tabanids transmitting lethal diseases to ungulates.

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ABSTRACT

As with mosquitoes, female tabanid flies search for mammalian hosts by visual and olfactory cues, because they require a blood meal before being able to produce and lay eggs. Polarotactic tabanid flies find striped or spotted patterns with intensity and/or polarisation modulation visually less attractive than homogeneous white, brown or black targets. Thus, this reduced optical attractiveness to tabanids can be one of the functions of striped or spotty coat patterns in ungulates. Ungulates emit CO₂ via their breath, while ammonia originates from their decaying urine. As host-seeking female tabanids are strongly attracted to CO₂ and ammonia, the question arises whether the poor visual attractiveness of stripes and spots to tabanids is or is not overcome by olfactory attractiveness. To answer this question we performed two field experiments in which the attractiveness to tabanid flies of homogeneous white, black and black-and-white striped three-dimensional targets (spheres and cylinders) and horse models provided with CO₂ and ammonia was studied. Since tabanids are positively polarotactic, i.e. attracted to strongly and linearly polarised light, we measured the reflection–polarisation patterns of the test surfaces and demonstrated that these patterns were practically the same as those of real horses and zebras. We show here that striped targets are significantly less attractive to host-seeking female tabanids than homogeneous white or black targets, even when they emit tabanid-luring CO₂ and ammonia. Although CO₂ and ammonia increased the number of attracted tabanids, these chemicals did not overcome the weak visual attractiveness of stripes to host-seeking female tabanids. This result demonstrates the visual protection of striped coat patterns against attacks from blood-sucking dipterans, such as horseflies, known to transmit lethal diseases to ungulates.

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1. Introduction

Female tabanid flies searching for blood nourishments are attracted to their host animals by the odours, shape, movement, brightness and colour of the host [1,2,19,20,30,33,34,39], and also by the linear polarisation of host-reflected light [3,9,10,17,18,23]. Tabanids wait for

hosts to appear in shady areas under bushes and trees [37]. Sight is the main host finding mechanism, but body temperature (warmth) and odour (mainly ammonia, carbon dioxide and sweat) also play an important role [31,35]. Moving objects, especially if dark coloured, are most prone to be attacked by tabanids [4,36].

Tabanids are intermittent feeders: their painful bites generally elicit a protective response from the victim so they are frequently forced to move to another host without having the chance to procure a full blood meal. Consequently, they may serve as mechanical vectors

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of some diseases and/or parasites, e.g. anthrax, tularemia, anaplasmosis, hog cholera, equine infectious anaemia, filariasis and Lyme disease transmitted by their bites [11,25,26]. A serious problem can occur in mammals when the blood loss is high due to abundant tabanid bites, i.e. hosts exposed to frequent bites can lose up to 0.5 ml of blood per fly [12], which can severely weaken or even kill them. Thus, numerous painful bites from high populations of tabanids can reduce the fitness of the host animals. Furthermore, tabanids sometimes irritate ungulates so seriously that these host animals cannot graze [24]. Consequently, host animals exposed to tabanids, show strong behavioural responses, such as escape behaviours when approaching flights of tabanid flies are heard. Thus, evolution of a coat pattern with a weak attractiveness to tabanids could be an important selective advantage to mammalian hosts.

Water-seeking male and female tabanid flies are attracted to horizontally polarised light reflected from a water surface [17,23]. Host-seeking female tabanids, using blood as nourishment to increase clutch size and to develop and ripen their eggs, are also attracted to linearly polarised light reflected from the coat of host animals, independent of the direction of polarisation [9,18]. Recently, it was shown that polarotactic tabanids find striped or spotted patterns with intensity alteration (alternating dark and bright stripes or patches) and/or polarisation modulation (stripes or patches with alternating orthogonal directions of polarisation) much less attractive than homogeneous white, grey, brown or black targets. This may be one of the functions of zebra stripes [7,10,32] and spotty animal coats [3]. The attractiveness to tabanids diminishes with decreasing stripe width and spot size. Stripes narrower than a critical width (≈ 5 cm, [10]) and spots smaller than a threshold size (diameter ≈ 10 cm, [3]) are effective enough not to attract tabanids. Ref. [10] demonstrated that stripe widths on the coats of all three extant zebra species (*Equus burchelli*, *Equus grevyi*, *Equus zebra*) fall in a range where the striped pattern is most disruptive, i.e. least attractive to host-seeking tabanids.

Similarly to other ungulates, zebras emit carbon dioxide (CO₂) via their breath and ammonia associated with their urine (ammonia originates from the decay of urine). Tabanid flies are attracted by CO₂ and ammonia, and these chemical attractants are therefore frequently used in tabanid traps (e.g., [13,19,24,28,29,39]). Thus, the question arises whether the weak optical attractiveness of the striped coat pattern of zebras to tabanids can be overcome by the olfactory attractiveness of zebras to tabanids. Could an attractive zebra smell (CO₂, ammonia, sweat) compensate for the poor visual attractiveness of a striped pattern to tabanids, resulting in the loss of the selective advantage of striped coat patterns?

To answer this question, we performed two field experiments, in which we studied the attractiveness to tabanid flies of sticky homogeneous white, black and black-and-white striped three-dimensional targets and horse models provided with CO₂ and ammonia. Since tabanids have positive polarotaxis, we measured the reflection–polarisation characteristics of the test targets used in our experiments.

2. Materials and methods

Experiment 1 was performed between 21 June and 12 September 2012 on a Hungarian horse farm in Szokolya (47° 52' N, 19° 00' E) to investigate the influence of ammonia (the most typical component of bacterially decaying urine) on the attractiveness of sticky three-dimensional visual targets with different surface patterns to tabanid flies. Each target was composed of a sphere (diameter = 50 cm, Fig. 1A–C) and two cylinders (height = 50 cm, the major and minor axis of the elliptical cross-section was 50 cm and 30 cm, respectively, Fig. 1D–F) imitating rounded (spherical) or elongated (cylindrical) body parts of a host animal for host-seeking female tabanids. There were two identical target groups. Each target group was composed of a white, a black-and-white striped and a black target arranged 5 m apart along a straight line (Fig. 1G, H). The homogeneous black and white spheres were common inflatable beach balls sprayed by black and white paint, respectively. The striped sphere was a black-sprayed beach ball onto which white plastic stripes (width = 2 cm) were fixed with adhesive. The cylinders were composed of white plastic buckets. The black cylinders were produced by spraying the white buckets with black paint. The striped cylinders were made with painting black stripes (width = 4 cm) onto the white buckets. Each sphere was fixed at a height of 100 cm to a vertical metal rod stuck into the ground. Two cylinders with the same pattern (white, striped, or black) were impaled onto a vertical metal rod stuck into the ground 50 cm apart from the sphere with the same pattern (white, striped, or black). The height of the lower and higher cylinder was 50 and 100 cm from the ground, respectively. Since the sphere and the two cylinders with the same surface pattern (white, striped, or black) were quite close (50 cm) to each other, they might have been considered by host-seeking flying tabanids to belong to the same (host-imitating) target, thus the numbers of tabanids caught by the spheres and cylinders with the same pattern were pooled (Tables 1, 2). In both target groups all three different targets (white, striped, black) were presented simultaneously (Fig. 1G, H).

One target group was baited with ammonia, while the other group was unbaited. The two target groups were positioned 500 m apart (separation of the odour treatments) at two opposite sides of a grove in such

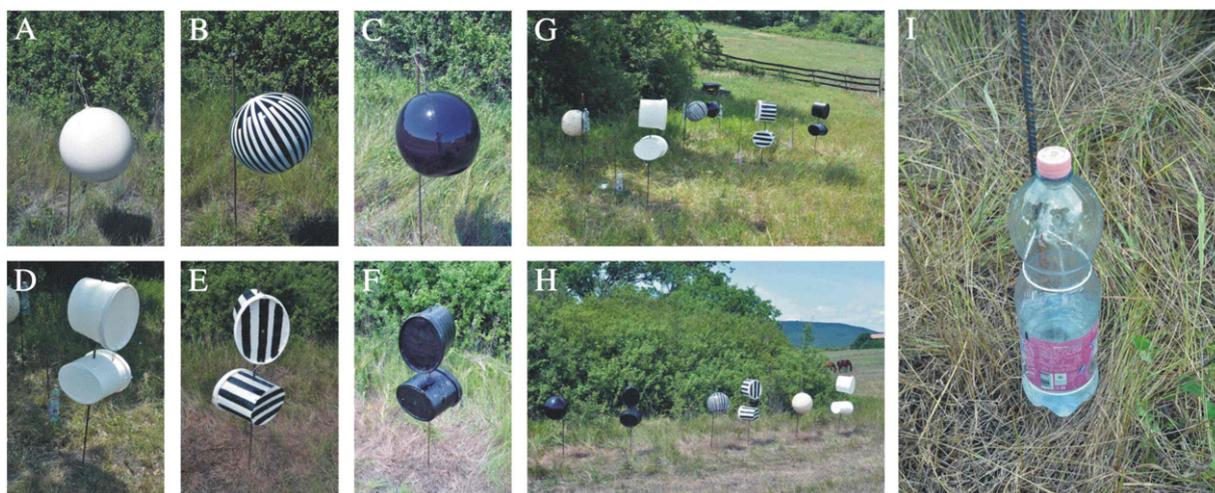


Fig. 1. Sticky white (A, D), black-and-white striped (B, E), and black (C, F) spheres (A–C) and cylinders (D–F) used in experiment 1. Arrangement of the sticky test targets with (G) and without (H) ammonia. (I) A plastic bottle with five small holes on its stopper containing aqueous ammonia as an ammonia source.

Table 1

Number of female tabanids captured by the white, black-and-white striped, and black sticky targets with and without ammonia in experiment 1. Male tabanids were not trapped.

Date (2012)	With ammonia			Without ammonia		
	White	Striped	Black	White	Striped	Black
28 June	17	13	37	8	0	34
1 July	15	3	102	10	1	103
10 July	5	2	160	10	4	141
16 July	1	3	59	2	3	64
25 July	2	1	17	8	1	54
28 July	3	0	9	5	0	19
8 August	4	0	85	4	1	71
15 August	2	0	52	1	0	74
23 August	1	0	25	7	3	129
29 August	1	0	45	3	10	30
4 September	1	0	15	2	0	23
12 September	0	3	16	2	1	31
Sum	52 (7.4%)	25 (3.6%)	622 (89.0%)	62 (7.2%)	24 (2.8%)	773 (90.0%)

a way that from the constant location of a given target group the other group was not visible (Fig. 1G, H). The surface of all targets was covered by transparent, odourless, colourless and weather-proof insect monitoring glue (BabolnaBio mouse trap). The members of a given target group were simultaneously either sunny or shady. Every day both target groups were alternatively (early forenoon and late afternoon) or simultaneously (around noon) sunlit or shady for several hours. Between two data collections one of the target groups was provided continuously with ammonia (this is called the baited hereafter), while the other group was without ammonia (called the unbaited group hereafter). The two (ammonia-baited and unbaited) target groups were at a sufficiently long distance (500 m) from each other that the ammonia originating from the baited group could not influence the area of the unbaited group. To eliminate any site-specific effect, the ammonia sources were periodically switched between the two target groups after each data collection (see Table 1).

Each ammonia source was a plastic bottle (1.5 l) with five small holes on its stopper partly filled with 1 l aqueous household ammonia replenished weekly (Fig. 1I). In the baited target group there was one ammonia source at each target: beneath the cylinders the ammonia sources were placed on the ground fixed with a string to the vertical metal rod, while they were fixed with strings at a height of 1 m to the metal rods holding the spheres, so that ammonia bottles and spheres were at the same height. After a week the solution in the bottles still emitted some ammonia smell. In our ammonia sources (Figs. 1I, 2C) we used a saturated (4% ammonia dissolved in water) household aqueous ammonia, that initially resulted in a strong ammonia odour

Table 2

Statistical comparisons (χ^2 test) between the numbers of female tabanids captured by the white, black-and-white striped and black sticky targets with and without ammonia in experiment 1 (Table 1).

Compared targets	χ^2 test
Black with ammonia versus white with ammonia	df = 1, $\chi^2 = 482.05$, $p < 0.0001$, significant
White with ammonia versus striped with ammonia	df = 1, $\chi^2 = 9.47$, $p = 0.002$, significant
Black without ammonia versus white without ammonia	df = 1, $\chi^2 = 605.41$, $p < 0.0001$, significant
White without ammonia versus striped without ammonia	df = 1, $\chi^2 = 16.79$, $p < 0.0001$, significant
Black with ammonia versus black without ammonia	df = 1, $\chi^2 = 16.34$, $p < 0.0001$, significant
White with ammonia versus white without ammonia	df = 1, $\chi^2 = 0.88$, $p = 0.35$, not significant
Striped with ammonia versus striped without ammonia	df = 1, $\chi^2 = 0.02$, $p = 0.89$, not significant

from a distance of several metres. However, to minimise the risk of reduced attractiveness, the aqueous ammonia was refreshed weekly. Thus, at the beginning of our field experiments the aerial concentration of ammonia around the test targets might have been stronger than that typical for zebras in the field.

The tabanids trapped by the sticky targets were counted and removed periodically (see Table 1) by cleaning the sticky surfaces with petrol. After the complete evaporation of the petrol (the smell of which thus had no effect on the tabanids), the glue was refreshed, the positions of the members of both target groups were rerandomised and the ammonia sources were displaced from one group to the other in order to eliminate site-specific effects.

Experiment 2 was performed between 10 July and 12 September 2012 on the same horse farm as experiment 1 and was designed to investigate the influence of carbon dioxide (imitating the CO₂ exhaled by host animals) and ammonia on the attractiveness of three-dimensional sticky horse models (mock horses) with different optical surface patterns to tabanid flies. We used two pairs of horse models. In each pair a black-and-white striped horse model, called mock zebra (Fig. 2A) and a black mock horse (Fig. 2B) composed of plastic were placed in a normal standing posture on the grassy ground, 5 m apart. The shape and dimensions (length = 160 cm, height = 110 cm, width = 60 cm) of the mock horses were the same. The pattern of the mock zebra was copied from a zebra hide (*E. burchelli*), with a ratio of the black and white surface regions of approximately 50:50.

In the immediate vicinity (50 cm apart) of each mock horse of one of the pairs the same ammonia source (Fig. 2C) as used in experiment 1 was placed. Furthermore, both of these ammonia-baited mock horses were also continuously provided by CO₂ released from a gas tank via a manometer and 2 m of rubber tubing (Fig. 2A, B, D). These mock horses are called the odour-baited models, henceforth. The gas tank (initially 60 bar) was set inside the bushes and the end of the rubber tubing was fixed by string to a vertical metal rod stuck in the ground in such a way that the CO₂ was released at 0.5 l/min (corresponding to the rate of CO₂ exhalation by horses; [5,27]) near the head of each mock horse (Fig. 2A, B). Hence, the CO₂ concentration was similar to the natural situation around breathing zebras. The continuous emission of CO₂ was checked by gas bubbles visible when the end of the rubber tubing was put into a bottle of water. The CO₂ tanks were regularly replaced on depletion.

At 500 m from the odour-baited mock horses another model pair (composed of the same zebra-striped and black mock horses placed 5 m apart) without ammonia and CO₂ was set at the edge of the grove in such a way that from the constant location of a given model pair the other pair was not visible due to trees and bushes (Fig. 2F,G). These mock horses are called the unbaited models henceforth. The odour-baited and the unbaited model pairs were at large enough distance (500 m) from each other (with numerous trees and bushes in-between), so that ammonia and CO₂ originating from the odour-baited mock horses could not influence the area of the unbaited mock horses.

Covering of mock horses with glue, their illumination conditions (sunlit or shady), replenishment with ammonia, periodic counting of captured tabanids (Table 3), randomised positioning within each target pair and periodic switching of the ammonia and CO₂ sources between target pairs after every data collection to eliminate site-specific effects were the same as in experiment 1.

2.1. Number of repetitions

In both experiments several sticky test surfaces (spheres, cylinders, mock horses and zebras) with different reflection-polarisation characteristics trapped tabanids, which were counted and removed periodically (see Tables 1 and 3). After tabanid counting the order of the test surfaces was randomly changed. Since the captured tabanids and other non-tabanid insects were removed periodically, the influence by the view of insect carcasses on new arrivals was reduced, furthermore, the experimental situation was altered by the randomisation of the

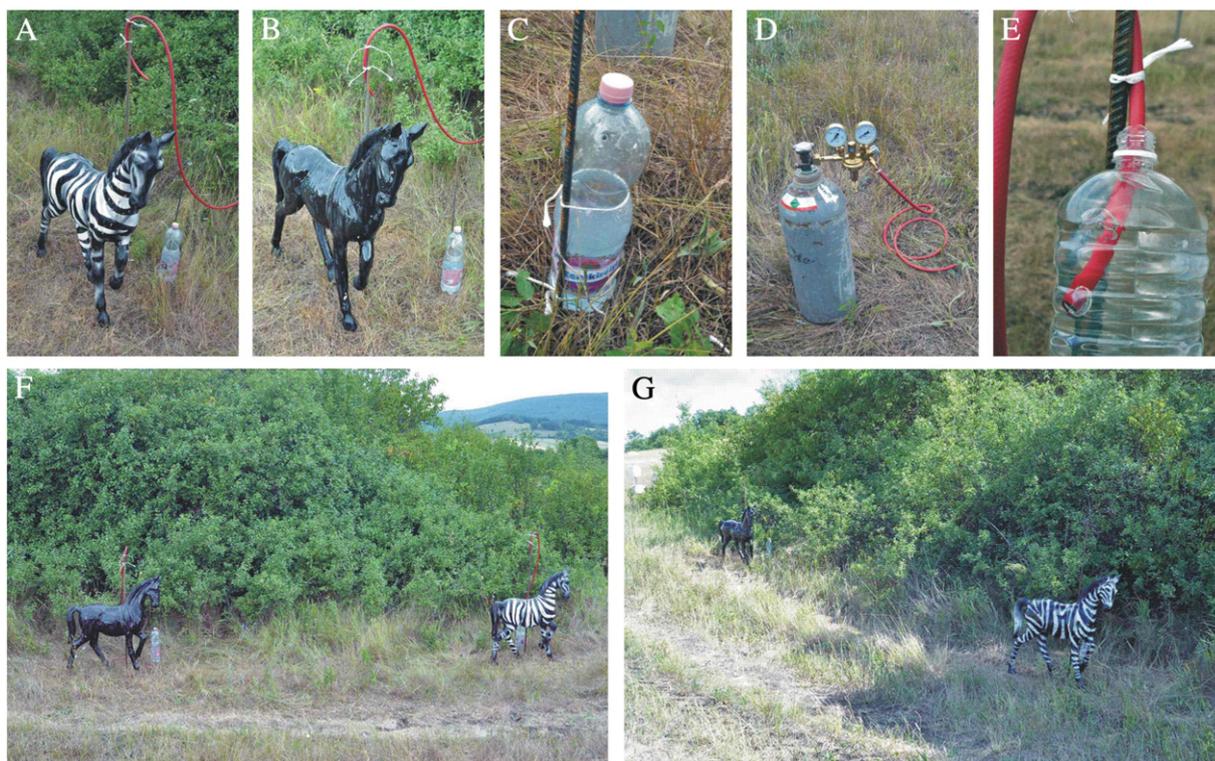


Fig. 2. Black-and-white striped (A) and black (B) sticky horse models provided with carbon dioxide (CO₂) and ammonia in experiment 2. (C) The ammonia source was a plastic bottle with five small holes on its stopper filled partly with aqueous ammonia. (D) The CO₂ originated from a gas tank with a manometer. (E) The continuous emission of CO₂ was checked by gas bubbles visible when the end of the rubber tubing was put into a bottle of water. (F) Arrangement of sticky horse models baited with CO₂ and ammonia. (G) Arrangement of unbaited sticky horse models.

target positions. Thus, following tabanid counting actually, new replicates of experiments began. The number of replicates *R* and number of days *D* of a given experiment were: *R* = 12, *D* = 84 (experiment 1); *R* = 9, *D* = 65 (experiment 2).

2.2. Identification of tabanids

When the trapped tabanids were removed from the insect glue covering the test targets in experiments 1 and 2, their body suffered such serious damages that their taxonomical identification to the species-level was impossible. They were, however, unambiguously identified as tabanid flies (Diptera: Tabanidae). The sex of trapped tabanids was determined on the basis of the anatomical characteristics of their head observed under a magnifying lens (10×): in males the left and right compound eye contact dorsally, whereas they do not contact in females. In

various field experiments performed in the earlier years at the same study site [3,9,10,18] the following tabanid species were captured with a liquid trap: *Tabanus tergustinus*, *Tabanus bromius*, *Tabanus bovinus*, *Tabanus autumnalis*, *Atylotus fulvus*, *Atylotus loewianus*, *Atylotus rusticus*, *Haematopota italica*. Thus, it is normal to suppose that also these tabanid species occurred at the study site during experiments 1 and 2 (Tables 1 and 3).

The reflection–polarisation characteristics of the test targets used in experiments 1 and 2 were measured by imaging polarimetry in the red (650 ± 40 nm = wavelength of maximal sensitivity ± half bandwidth of the CCD detectors of the polarimeter), green (550 ± 40 nm) and blue (450 ± 40 nm) spectral ranges. The method of imaging polarimetry has been described in detail by Refs. [15,16]. Here we present only the polarisation patterns measured in the blue part of the spectrum. Practically the same patterns were obtained in the red and green spectral ranges as in the blue range, because the sticky test targets were white and/or black.

2.3. Statistical analyses

The binomial χ^2 tests [40] were performed with the use of the programme Statistica 7.0. Since the distributions of our tabanid catches were too far from normal (which therefore cannot be compensated by any preliminary data transformation), parametric ANOVA tests could not be used. For our main conclusions drawn the most important data are the total numbers of tabanids captured by the different treatments (odour-baited versus unbaited, white versus black versus striped). To establish the significance of differences between these tabanid catches (to compare two different total catch numbers) it was enough and appropriate to use the binomial χ^2 test. We performed also Kruskal–Wallis tests [40], but we obtained the same results as with the binomial χ^2 tests. Thus, in this work we do not present these Kruskal–Wallis tests.

Table 3

Number of female tabanids captured by the black-and-white striped and black sticky horse models with and without ammonia and carbon dioxide (CO₂) in experiment 2. Male tabanids were not trapped.

Date (2012)	With ammonia + CO ₂		Without ammonia + CO ₂	
	Striped	Black	Striped	Black
16 July	12	38	1	12
25 July	0	16	1	23
28 July	0	8	1	5
8 August	6	61	2	12
15 August	3	30	0	11
23 August	3	23	0	7
29 August	2	8	0	11
4 September	0	10	0	9
12 September	0	6	0	10
Sum	26 (11.5%)	200 (88.5%)	5 (4.8%)	100 (95.2%)

Table 4

Statistical comparisons (χ^2 test) between the numbers of female tabanids captured by the black-and-white striped and the black sticky horse models with and without odour (ammonia and carbon dioxide) in experiment 2 (Table 3).

Compared horse models	χ^2 test
Black with odour versus striped with odour	df = 1, $\chi^2 = 133.96$, $p < 0.0001$, significant
Black without odour versus striped without odour	df = 1, $\chi^2 = 85.95$, $p < 0.0001$, significant
Black with odour versus black without odour	df = 1, $\chi^2 = 33.33$, $p < 0.0001$, significant
Striped with odour versus striped without odour	df = 1, $\chi^2 = 14.23$, $p = 0.0002$, significant

3. Results

In experiments 1 and 2, the sticky visual targets (spheres plus cylinders, mock horses and mock zebras) trapped only female tabanids (Tables 1, 3). From this and our optical measurements we concluded that these test targets imitated visually well the host animals of blood-sucking female tabanid flies. In experiment 1 most tabanids were trapped by the sticky black targets: 89% ($N = 622$) with ammonia, and 90% ($N = 773$) without ammonia (Table 1). According to Table 2, these differences between the baited and unbaited black targets are statistically significant. The white targets caught only 7.4% ($N = 52$) and 7.2% ($N = 62$) of tabanids with and without ammonia, respectively, not statistically different from one another (Table 2). The least tabanids were captured by the black-and-white striped targets: 3.6% ($N = 25$) with ammonia, and 2.8% ($N = 24$) without ammonia, not statistically different from one another (Table 2). The differences between the numbers of tabanids trapped by the black compared to the white targets were significant, independent of provision with ammonia (Table 2). The same was true for the numbers of tabanids trapped by the white and striped targets without ammonia (Table 2).

From experiment 1 we conclude that host-imitating three-dimensional targets with black-and-white stripes did not lose their weak visual attractiveness to tabanids when provided with tabanid-attracting ammonia. These striped targets attracted practically the same small numbers (24 and 25) of tabanids, independent of provision with ammonia.

In experiment 2, the sticky black mock horses captured significantly more tabanids than the mock zebras, independent of the ammonia and CO₂ provision (Tables 3, 4): the baited black horse model trapped 200 (88.5%) and the odourless black mock horse caught 100 (95.2%) tabanids, while the baited and odourless mock zebras captured only 26 (11.5%) and 5 (4.8%) tabanids, respectively. On the other hand, the baited mock zebra trapped significantly more tabanids (26) than the odourless mock zebra (5). The difference between the numbers of tabanids trapped by the baited (200) and odourless (100) black mock horses was significant (Table 4). From experiment 2, we conclude that although the combined emittance of ammonia and CO₂ enhanced the attractiveness of black horse models and mock zebras to tabanids, the mock zebra kept its poor visual attractiveness to tabanids in spite of its emittance of tabanid-attractants.

The sticky white targets (spheres and cylinders) used in experiment 1 reflected practically unpolarised ($d < 5\%$) light, independent of the illumination condition (shady, or sunlit). The same was true for the white stripes of the sticky striped target. On the other hand, the sticky black targets used in experiment 1 reflected strongly ($70\% < d < 90\%$) linearly polarised light at the Brewster angle [$\theta_{\text{Brewster}} = \arctan(n)$ from the local normal vector of the surface, where n is the refractive index of the black paint], depending on the illumination (shady, or sunlit). Independent of the illumination, the direction of polarisation of light reflected from the sticky black targets was perpendicular to the plane of reflection. The same was true for the black stripes of the sticky striped targets.

The sticky black and zebra-striped shady and sunlit horse models used in experiment 2 had quite similar reflection–polarisation characteristics (Fig. 3) as the black and striped targets in experiment 1

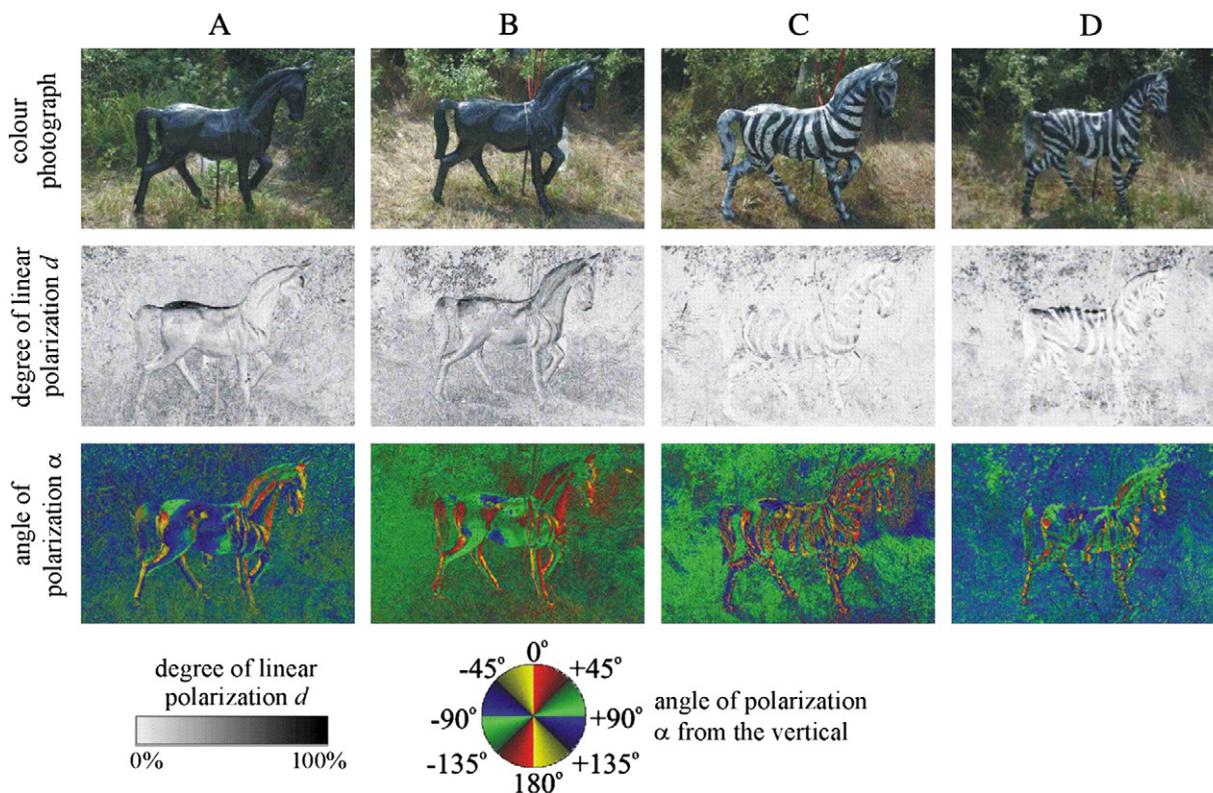


Fig. 3. Colour photographs and patterns of the degree of linear polarisation d and the angle of polarisation α (clockwise from the vertical) of the sunlit sticky black and black-and-white striped horse models used in experiment 2 measured by imaging polarimetry in the blue (450 nm) spectral range. The angle of elevation of the optical axis of the polarimeter was -20° from the horizontal. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and the coats of real horses and zebras whose reflection–polarisation patterns were presented by Refs. [10,18]. At the Brewster angle the black mock horses and the black stripes of the mock zebras reflected linearly polarised light strongly ($70\% < d < 90\%$) with directions of polarisation normal to the local plane of reflection, while the white stripes of the mock zebras reflected practically unpolarised ($d < 5\%$) light.

4. Discussion

Our study examined how ammonia and CO₂ scents influenced the responses of tabanid flies to objects simulating various patterns, including those with characteristic zebra stripes. Cattle and many other ungulates are ruminant animals, while zebras and other horses are not. Although the chemical components of the gas mixture exhaled by these two different ungulate groups more or less differ, all ungulates emit CO₂ and have an ammonia scent. This was the reason why we used these two typical ungulate-related odour types in our field experiments. Our results suggest that although both of these scents that are associated with zebras were attractive to tabanids, they did not reduce the efficacy of the poorly attractive nature of the black-and-white zebra stripes to tabanids. We do not suggest that zebras evolved stripes exclusively as a visual defence against tabanid fly bites, but we propose that one of the advantages of striped (or spotted) coat patterns is that it significantly reduces the visual attraction to tabanids. Apart from the old tsetse fly hypothesis [38], this is the only experimentally tested and proven suggestion on the evolutionary importance of striped and spotted coats. All these are thoroughly discussed by Refs. [3,10].

Since our field experiments were performed in Hungary, we had to work with Hungarian tabanids. This is, however, not a serious problem, because all tabanids that lay their eggs in the immediate vicinity of water or mud are in all probabilities positively polarotactic, like aquatic insects in general [16,23]. Thus, we expect that the tabanids co-occurring with zebras and other ungulates in Africa exhibit similar polarotactic behaviour to the Hungarian species.

In their field experiments, Ref. [10] used such two-dimensional vertical test surfaces and three-dimensional mock zebras, that possessed vertical, tilted as well as horizontal stripes. These mock zebras and those used in our present experiment had such stripe patterns that were copied from real zebras. In these experiments we could not establish any preference of tabanids for particular directions of stripes. Thus, the unattractiveness of striped coat patterns to tabanids seems independent of the stripe direction.

In this work, we show that three-dimensional black-and-white striped targets are significantly less attractive to host-seeking female tabanids than homogeneous white or black targets, even if they emit CO₂ and/or ammonia. Ammonia and CO₂ increased the attractiveness to tabanids 2 times in the case of black mock horses and 5 times in the case of mock zebras. Although the baited black mock horses attracted over 8 times more tabanids than striped ones, a 5-fold increase in tabanid capture with the striped targets baited with ammonia and CO₂ shows that the poor optical attractiveness of striped targets can be overcome to a certain degree by adding a combination of tabanid-attractant chemicals.

After comparing the reflection–polarisation patterns of the sticky black, white and striped targets (spheres and cylinders) and mock horses used in experiments 1 and 2 (Fig. 3) with those of the coats of real white and black horses and zebras [10,18], we concluded that our three-dimensional targets in experiments 1 and 2 imitated well the reflection–polarisation characteristics of the body surface of real horses and zebras.

We compared mock zebras with black mock horses, since in our earlier field experiments [10,18], we found that among equids black horses are the most attractive to host-seeking tabanids. Hence, we wanted to test how many times can tabanid-attractant chemicals increase the tabanid-attractiveness of mock zebras relative to that of black mock

horses. We tested the effect of two odour baits: (i) ammonia for the spherical–cylindrical targets, and (ii) CO₂ with ammonia for the mock horses. Although we did not test the effect of CO₂ alone, this is not a serious problem, since the synergistic interaction of ammonia and CO₂ baits could not overcome the weak visual attractiveness of mock zebras to tabanids. Thus it can be expected that the effect of a single CO₂ bait would be even much weaker. Only if the simultaneous use of tabanid-attracting ammonia and CO₂ baits had compensated the unattractiveness of the striped pattern of the mock zebras, would it have been worth testing the effect of CO₂ separately from ammonia, in order to establish the contributions of these attracting chemicals.

According to Tables 1 and 3, there was a week-to-week variability in the numbers of tabanids caught. The reason for this may be the fluctuation of weather, as under cool, cloudy conditions the flight activity of tabanids considerably declines [6,8,17,22,24]. In the literature, tabanid flies are generally described as lovers of sunlight, warmth and moisture. Ref. [6] reported that temporary cloudiness, rain, or a drop in temperature caused a cessation of tabanid activity. According to Ref. [8], a relatively short diurnal activity of tabanids was apparently given by the deep changes in temperature and relative humidity of the air between night- and day-time in the studied area. Multiple regression analysis by Ref. [22] showed that the peak of the biting activity for five tabanid species was significantly influenced by meteorological factors: when the weather was cold and cloudy, very few specimens were collected.

Since the sticky test targets used in our field experiments trapped only female tabanids, it is pertinent to suppose that these tabanids considered our targets as potential host animals, and they landed on these targets intending to suck blood. Hence the trapped female tabanids sought hosts, and were attracted by the intensity and polarisation of target-reflected light as well as by the odour of the applied white, black and black-and-white striped targets.

In both of our experiments, we used ammonia being a typical attractant of tabanid flies [19,24,28,29]. Mammalian urine contains only a limited amount of ammonium salt, thus the urine itself does not have an ammonia smell. However, ammonia originates from the bacterial decay of urine [14]. The ammonia odour is associated with ungulates for the following reasons: (i) When drinking from more or less constant water bodies, ungulates frequently urinate. Due to the later bacterial decay of urine, the urine-impregnated soil emits more or less ammonia (depending on the soil moisture), signalling that ungulates frequently visit the sites to seek water. (ii) During urination, certain body parts (mainly the legs) of ungulates are unavoidably contaminated with a small amount of urine, that later emits ammonia, especially when the animal is sweating. Consequently, ammonia odour is a typical olfactory marker of host animals or their vicinity for host-seeking female tabanid flies. These are the reasons why tabanids are attracted to ammonia [19,24,28,29]. In the future, it would be worth testing whether tabanids should also see the hosts from the outset to forage more intensely in the vicinity of host excretions.

At the beginning of our experiments, the attractiveness of ammonia odour to tabanids was probably over-represented relative to the natural situation. However, this was not problematic, because it turned out that attractive CO₂ and ammonia do not overcome the weak visual attractiveness of zebra stripes to tabanids. Therefore, the strong initial ammonia concentration around our test targets still supports our main conclusion. We were convinced that the perhaps initially strong ammonia concentration could not repel tabanid flies in our field experiments, since the stronger the ammonia odour, the greater the attractiveness to tabanids. This fact is frequently exploited in chemically-baited tabanid traps [13,19,24,28,29,34]. For example, traps baited with ammonium hydroxide captured four times more tabanids than unbaited traps [21].

While stripes may deter tabanids, ungulates can avoid them by moving into shade, or by tail swishing, grazing during cool times of the day, or using a mixed strategy of grazing in the sun and periodically retreating into shade [18,24].

In experiment 1, the difference in the catches of striped and white targets is not very large (Table 1), but it is significant (Table 2): the ammonia-baited and unbaited white targets captured 52/25 \approx 2.1 and 62/24 \approx 2.6 times more tabanids than the corresponding striped targets. This also demonstrates that tabanid fly bites may be a selective force, and in this respect white coats are more advantageous than black ones, but less advantageous than striped furs. Why have not more mammals evolved white fur? In the opinion of Ref. [18], the advantage of wearing a white coat (visual unattractiveness to tabanids) may be overwhelmed by the disadvantage that a white animal is very striking for predators as well as preys. A white coat is furthermore, less protective against damaging UV light, causing skin tumours in white horses. About the problem of whiteness see further discussion in Ref. [18].

Experiment 1 started on 21 June 2012, while experiment 2 on 10 July 2012, thus they ran parallel since 10 July 2012. According to Table 1, the tabanid catches in experiment 1 became much lower after the start of experiment 2. One of the reasons for this could be that the tabanids trapped by the test targets of one of the experiments could not obviously have been captured by those of the other experiment.

Our findings presented here raise the following questions: Why do so many African sympatric artiodactyls have non-striped coats? Note, however, that in fact there are a number of striped and partially striped mammals in Africa, such as brindled gnu (*Connochaetes taurinus*), bongo (*Tragelaphus eurycerus*), nyala (*Tragelaphus angasi*), Lesser kudu (*Tragelaphus imberbis*), striped hyena (*Hyaena hyaena*), okapi (*Okapia johnstoni*) and giraffe (*Giraffa camelopardalis*), for example. Why are Eurasian equids not striped even though they also suffer tabanid attacks? Another interesting question that could be studied in the future is whether the body heat of zebras (that may attract tabanids, [35]) could or could not overcome the advantage (visual unattractiveness to tabanids) of stripes. Thus, the enigma of why zebras have striped coats is not completely solved and demands additional research.

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