Zebra stripes disrupt the odour attractiveness to biting horseflies: Attractive CO_2 and ammonia do not neutralize the reduced visual attractiveness of zebra stripes to host-seeking tabanids

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Abstract

Female tabanid flies are dependent on blood meals for egg production and search for mammalian hosts by visual and olfactory cues. Polarotactic tabanid flies find striped or spotted patterns with intensity and/or polarization 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 zebra stripes and spotty coat patterns in ungulates. Zebras and other ungulates emit CO_2 via their breath, while ammonia originates from their decaying urine. As host-seeking female tabanids are strongly attracted by CO_2 and ammonia, the question arises as to whether the poor visual attractiveness of zebra stripes to tabanids is or is not overcome by the olfactory attractiveness of zebras to tabanids. 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 CO_2 and ammonia was studied. Since tabanids are positively polarotactic, i.e. are attracted to strongly and linearly polarized light, we measured the reflection-polarization patterns of the test surfaces used and demonstrated that these patterns were practically the same as those of real horses and zebras. We show here that zebra-striped targets are significantly less attractive to host-seeking female tabanids than homogeneous white or black targets, even when they emit tabanid-luring CO_2 and ammonia. Although CO_2 and ammonia increased the number of attracted tabanids, they did not neutralize the weak optical attractiveness of zebra stripes to host-seeking female tabanids. This result demonstrates the visual protection of zebra-striped coat patterns against attacks from blood-sucking dipterans, such as horseflies, known to transmit lethal diseases to ungulates.

Keywords: zebra, tabanid fly, horsefly, striped pattern, parasite protection, ammonia,
carbon dioxide, olfactory cues, polarization vision, polarotaxis, visual ecology

Introduction

Water-seeking male and female tabanid flies are attracted to horizontally polarized light reflected from a water surface (Horváth *et al*., 2008; Kriska *et al*., 2009). Host-seeking female tabanids, using blood meals to increase clutch size and to develop and ripen their eggs, are also attracted to linearly polarized light reflected from the coat of host animals, independent of the direction of polarization (Horváth *et al*., 2010; Egri *et al*., 2012a). Recently, it was shown that polarotactic tabanids find striped or spotted patterns with intensity alteration (alternating dark and bright stripes or patches) and/or polarization modulation (stripes or patches with alternating orthogonal directions of polarization) much less attractive than homogeneous white, grey, brown or black targets. This may be one of the functions of zebra stripes (Egri *et al*., 2012b) and spotty animal coats (Blahó *et al*., 2012). The attractiveness to tabanids decreases with decreasing stripe width and spot size, with stripes narrower than a critical width and spots smaller than a threshold size effective enough in not attracting tabanids. Egri *et al*. (2012a) demonstrated that stripe widths on zebra coats fall in a range where the striped pattern is most disruptive, i.e. least attractive to host-seeking tabanids.

Tabanids are vectors for numerous dangerous pathogens transmitted via their bites (Foil, 1989; Hall *et al*., 1998). Thus, there is a strong selective advantage to avoid being bitten by tabanids for the host animals. Furthermore, tabanids sometimes irritate ungulates so seriously that these host animals cannot graze (Lehane, 2005). All these mean potentially negative fitness consequences for all animals that attract tabanid flies. Consequently, it is an evolutionary advantage not to attract these blood-sucking parasitic insects either visually or by odour. Some hosts of tabanids seem to have evolved an optical weak attractiveness or even unattractiveness to tabanids by developing striped or spotted coat patterns (Blahó *et al*., 2012; Egri *et al*., 2012b), while others may change grazing behaviours and keep themselves protected in the shadow at day-time. However, the latter will lead to reduced chances to forage and may lead to competitive disadvantages and reduced fitness.

Similar to other ungulates, zebras emit carbon dioxide (CO_2) in breath and ammonia associated with their urine (ammonia originates from the decay of urine). Tabanid flies are attracted by CO_2 and ammonia, and these chemical attractants are therefore frequently used in tabanid traps (e.g. Wilson *et al.*, 1966; Hribar *et al.*, 1992; Hall *et al.*, 1998; Lehane, 2005; Mihok and Mulye, 2010; Mihok and Lange, 2011). 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 (CO_2, ammonia, sweat) over-compensate for the poor visual attractiveness of zebra stripes 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 CO_2 and ammonia. Since tabanids have positive polarotaxis, we measured the reflection-polarization characteristics of the test targets used in our experiments.

Materials and Methods

Experiment 1 was performed between 21 June and 12 September 2012 on a Hungarian horse farm in Szokolya (47^o 52' N, 19^o 00' E) to investigate the influence of ammonia (the most typical component of bacterially decaying urine) on the attractiveness of sticky spheres and cylinders with different surface patterns to tabanid flies. These spheres and cylinders imitated host animals for host-seeking female tabanids. There were two identical groups of visual targets. Each target group was composed of a white, a black-and-white striped and a black sphere (diameter = 50 cm, Fig. 1A-C) and two white, two black-and-white striped and two black plastic cylinders (height = 50 cm, the major and minor axis of the elliptical cross-section was 50 cm and 30 cm, respectively, Fig. 1D-F). 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. The height of the lower and higher cylinder was 50 and 100 cm from the ground, respectively. In a given target group the distance between the targets placed along a straight line was 2 m.

The two target groups were positioned 500 m apart at two opposite sides of a grove in such a way that from the site 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. 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 target groups were at enough distance (500 m) from each other that the ammonia originating from the baited group could not influence the area of the unbaited group.

Each ammonia source was a plastic bottle (1.5 liter) with five small holes in its stopper partly filled with 1 liter 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 by 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 a strong ammonia smell. In our ammonia sources (Figs. 1I and 2C) we used a saturated (4% ammonia dissolved in water) household aqueous ammonia, that resulted in a strong ammonia odour from a distance of several tens of meters, even after one week. However, to minimize the risk of reduced attractiveness, the aqueous ammonia was refreshed weekly. Thus, in 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 (by cleaning the sticky surfaces with petrol) periodically (see Table 1), the glue was refreshed, and the positions of the members of both target groups were rerandomized.

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 CO_2 exhalated 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 zebra-striped horse model (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 zebra-striped horse model was copied from a zebra hide, 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. These mock horses are called the baited models, henceforth. Furthermore, each baited mock horse was also continuously provided by CO_2 released from a gas tank via a manometer and 2 m of rubber tubing (Fig. 2A,B,D). 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 CO_2 was released at 0.5 liter/minute (corresponding to the rate of CO_2 exhalation by horses; Marlin and Nankervis, 2002; Brega, 2005) near the head of each mock horse (Fig. 2A,B). Hence, the CO_2 concentration was similar to the natural situation around breathing zebras. The continuous emission of CO_2 was checked by gas bubbles visible when the end of the rubber tubing was put into a bottle of water. The CO_2 tanks were regularly replaced on depletion.

500 m from the baited mock horses another model pair (composed of the same zebra-striped and black models placed 5 apart) without ammonia and CO_2 was set at the edge of the grove in such a way that from the site 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 two model pairs were at enough distance (500 m) from each other (with numerous trees and bushes inbetween), so that ammonia and CO_2 originating from the baited mock horses could not influence the area of the odourless mock horses.

Covering of treatments with glue, their positioning, replenishment with ammonia and periodic counting of flies (Table 3) was as in experiment 1 above.

Number of repetitions: In both experiments several sticky test surfaces (spheres, cylinders, mock horses and zebras) with different reflection-polarization 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, the new arrivals were not influenced by the view of insect carcasses, furthermore, the experimental situation was altered by the randomization of the 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).

Identification of tabanids: When the trapped tabanids were removed from the insect glue covering the treatments 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 (Horváth *et al.*, 2010; Blahó *et al.*, 2012; Egri *et al.*, 2012a,b) the following tabanid species were captured with a liquid trap: *Tabanus tergestinus*, *T. bromius*, *T. bovinus*, *T. autumnalis*, *Atylotus fulvus*, *A. loewianus*, *A. 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-polarization 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 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 as in the blue range, because the sticky test targets were white and/or black.

Statistical analyses using binomial χ^2 test were calculated by the program Statistica 7.0.

Results

In experiments 1 and 2 the sticky visual targets (spheres, cylinders, mock horses and zebras) trapped only female tabanids (Tables 1 and 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. According to Table 1, in experiment 1 most tabanids were trapped by the sticky black spheres and cylinders: 89% (*N* = 622) with ammonia, and 90% (*N* = 773) without ammonia. According to Table 2, these differences between the baited and unbaited black targets are statistically significant. The white spheres and cylinders 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 spheres and cylinders: 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 concluded 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 zebra-striped horse models, independent of the ammonia and CO_2 provision (Tables 3 and 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 zebra-striped horse models 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 zebra model (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 concluded that although the combined emittance of ammonia and CO_2 enhanced the attractiveness of black horse models and mock zebras to tabanids, the mock zebra kept its poor visual attractiveness to tabanids in spite its emittance of tabanid-attractants.

The sticky white spheres and cylinders used in experiment 1 reflected practically unpolarized ($d < 5\%$) light, independent of the illumination condition (shady, or sunlit). The same was true for the white stripes of the sticky striped spheres and cylinders. On the other hand, the sticky black spheres and cylinders used in experiment 1 reflected strongly ($70\% < d$

< 90%) linearly polarized light at the Brewster angle [$\theta_{Brewster}$ = arc tan (*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 polarization of light reflected from the sticky black spheres and cylinders was perpendicular to the plane of reflection. The same was true for the black stripes of the sticky striped spheres and cylinders.

The sticky black and zebra-striped shady and sunlit horse models used in experiment 2 had quite similar reflection-polarization characteristics (Fig. 3) as the black and striped spheres and cylinders in experiment 1: At the Brewster angle the black mock horses and the black stripes of the mock zebras reflected linearly polarized light strongly ($70\% < d < 90\%$) with directions of polarization normal to the local plane of reflection, while the white stripes of the mock zebras reflected polarized ($d < 5\%$) light only very weakly.

After comparing the reflection-polarization patterns of the sticky black, white and striped spheres, 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 (Horváth *et al.*, 2010; Egri *et al.*, 2012b), we concluded that our three-dimensional targets in experiments 1 and 2 imitated well the reflection-polarization characteristics of the body surface of real horses and zebras.

Discussion

Our study examined how ammonia and CO_2 scents influenced the responses of tabanid flies to objects simulating various patterns, including those with characteristic zebra stripes. 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.

Female tabanid flies searching for blood meals are attracted to their host animals by the odours, shape, movement, brightness and colour of the host (Wilson *et al.*, 1966;

Thompson, 1969; von Kniepert, 1979; Allan and Stoffolano, 1986; Allan *et al.*, 1991; Hribar *et al.*, 1992; Moore *et al.*, 1996; Sasaki, 2001), but also by the linear polarization of host-reflected light (Horváth *et al.*, 2008, 2010; Kriska *et al.*, 2009; Blahó *et al.*, 2012; Egri *et al.*, 2012a,b). Tabanids wait for hosts to appear in shady areas under bushes and trees (Vale and Phelps, 1974). Sight is the main host finding mechanism, but body temperature (warmth) and odour (mainly ammonia, carbon dioxide and sweat) also play an important role (Thorsteinson, 1958; Roberts, 1977). Moving objects, especially if dark coloured, are most prone to be attacked by tabanids (Bracken *et al.*, 1962; Thorsteinson *et al.*, 1966).

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 of some diseases and/or parasites, e.g. anthrax, tularemia, anaplasmosis, hog cholera, equine infectious anemia, filariasis and Lyme disease transmitted by their bites (Luger, 1990; Maat-Bleeker and Bronswijk, 1995). A serious problem can occur in smaller animals when the blood loss is high due to abundant tabanid bites, i.e. hosts exposed to frequent bites can lose up to 300 milliliter of blood in a single day, 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. 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 would be an important selective advantage to mammalian hosts. Egri *et al.* (2012b) showed that odourless targets with narrow black-and-white stripes are only slightly attractive to tabanid flies. They proposed that this may be one of the many suggested functions (Ruxton, 2002; Caro, 2009) of zebra stripes. However, as tabanids are able to use information other than visual for host detection, the question arises, whether this weak optical attractiveness of the striped coat pattern to tabanids is neutralized, or even overcome by the odours (ammonia and CO_2) attractive to host-finding female tabanids.

In this work we show that three-dimensional zebra-striped targets are significantly less attractive to host-seeking female tabanids than homogeneous white or black targets, even if they emit CO_2 and/or ammonia. Ammonia and CO_2 increased the attractiveness to tabanids 2 times in the case of black mock horses and 5 times in the case of zebra-striped models. Although the baited black mock horses attracted over 8 times more tabanids than striped ones, a 5-fold increase in tabanid capture with the zebra-striped targets baited with ammonia and CO_2 shows that the poor optical attractiveness of zebra-striped targets can be overcome to a certain degree by adding a combination of tabanid-attractant chemicals.

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 to suck blood. Hence the trapped female tabanids sought hosts, and were attracted by the intensity and polarization 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 claimed to be an attractant of tabanid flies (e.g. Hribar *et al*., 1992; Lehane, 2005; Mihok and Mulye, 2010; Mihok and Lange, 2011). 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 (Hill *et al.*, 2012). The ammonia odour is strongly associated with ungulates for the following reasons: (i) During grazing or resting, 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), signaling that ungulates may be in the vicinity. (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 sweeting. Consequently, ammonia odour is a typical olfactory marker of host animals or their vicinity for host-seeking female tabanid flies. These may be the reasons why tabanids are attracted to ammonia (Hribar *et al*., 1992; Lehane, 2005; Mihok and Mulye, 2010; Mihok and Lange, 2011). In the future, it would be worth testing whether tabanids should also see the hosts from the outset to forage more intensively in the vicinity of host excretions.

In our experiments the attractiveness of ammonia odour to tabanids was probably over-represented relative to the natural situation. In our opinion, however, this was not problematic, because it turned out that attractive CO_2 and ammonia do not neutralize the weak optical attractiveness of zebra stripes to tabanids. Therefore, the perhaps too strong ammonia concentration around our test targets had the benefit to further support our main conclusion.

In this work we show that having a striped coat has the benefit that such coat patterns disrupt (Waage, 1981; Egri *et al*., 2012b) the attraction of blood-sucking female tabanids by odours, such as ammonia and CO_2, for example, that are indicative of possible host animals. By this visual trick zebras and other more or less striped animals can reduce their optical attractiveness to tabanids. Other ungulates (e.g. horses), bearing non-striped coat patterns possess other behavioural responses to tabanid attacks: (i) Hiding in shade: When possible, this is a successful tactic for hosts as tabanids prefer direct sunlight and avoid shady areas (Lehane, 2005; our own observations), because their flight muscles need a higher air temperature to facilitate fast escape responses in order to be quick enough to escape from the body surface of a host animal when it tries to remove, e.g. by tail swings when biting. (ii) Grazing between sunset and sunrise: Tabanids do not fly, and thus do not attack their hosts between sunset and sunrise, when the air temperature is too low for them to fly. (iii) Grazing for a short time in sunshine and then running into the shade periodically: Such a typical behaviour of horses attacked by numerous tabanids on a sunlit meadow near a forest has recently been described by Horváth *et al*. (2010).

Our findings presented here raise the following questions: Why African sympatric artiodactyls have non-striped coats? Why Eurasian horses are not striped even though they also suffer tabanid attacks? Thus, the enigma, why do zebras wear striped patterns, is not completelely solved. In this topic further research is welcome.

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Tables

Table 1: Number of female tabanids captured by the white, black-and-white striped, and black sticky spheres (S) and cylinders (C) with and without ammonia in experiment 1. Male tabanids were not trapped.

date (2012)	**with ammonia**						**without ammonia**					
	white		**striped**		**black**		**white**		**striped**		**black**	
	S	**C**	**S**	**C**	**S**	**C**	**S**	**C**	**S**	**C**	**S**	**C**
28 June	7	10	7	6	12	25	4	4	0	0	13	21
1 July	8	7	0	3	14	88	3	7	1	0	57	46
10 July	4	1	0	2	20	140	3	7	2	2	63	78
16 July	0	1	3	0	9	50	0	2	1	2	48	16
25 July	1	1	0	1	3	14	5	3	0	1	23	31
28 July	3	0	0	0	5	4	2	3	0	0	14	5
8 August	2	2	0	0	32	53	2	2	1	0	52	19
15 August	0	2	0	0	12	40	1	0	0	0	45	29
23 August	0	1	0	0	8	17	6	1	2	1	82	47
29 August	0	1	0	0	15	30	2	1	10	0	5	25
4 September	1	0	0	0	6	9	2	0	0	0	5	18
12 September	0	0	3	0	11	5	2	0	0	1	14	17
sum	26	26	13	12	147	475	32	30	17	7	421	352
total	**52 (7.4%)**		**25 (3.6%)**		**622 (89.0%)**		**62 (7.2%)**		**24 (2.8%)**		**773 (90.0%)**	

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

compared test surfaces	**χ^2 test**
black (S+C) with ammonia *versus* white (S+C) with ammonia	df=1, χ^2=482.05, p<0.0001, **significant**
white (S+C) with ammonia *versus* striped (S+C) with ammonia	df=1, χ^2=9.47, p=0.002, **significant**
black (S+C) without ammonia *versus* white (S+C) without ammonia	df=1, χ^2=605.41, p<0.0001, **significant**
white (S+C) without ammonia *versus* striped (S+C) without ammonia	df=1, χ^2=16.79, p<0.0001, **significant**
black (S+C) with ammonia *versus* black (S+C) without ammonia	df=1, χ^2=16.34, p<0.0001, **significant**
white (S+C) with ammonia *versus* white (S+C) without ammonia	df=1, χ^2=0.88, p=0.35, **not significant**
striped (S+C) with ammonia *versus* striped (S+C) without ammonia	df=1, χ^2=0.02, p=0.89, **not significant**

Table 3: Number of female tabanids captured by the zebra-striped and black sticky horse models with and without ammonia and carbon dioxide (CO_2) in experiment 2. Male tabanids were not trapped.

date (2012)	**with ammonia + CO_2**		**without ammonia + CO_2**	
	zebra	**black**	**zebra**	**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 zebra-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* zebra with odour	df=1, χ^2=133.96, p<0.0001, **significant**
black without odour *versus* zebra without odour	df=1, χ^2=85.95, p<0.0001, **significant**
black with odour *versus* black without odour	df=1, χ^2=33.33, p<0.0001, **significant**
zebra with odour *versus* zebra without odour	df=1, χ^2=14.23, p=0.0002, **significant**

Figures with Legends

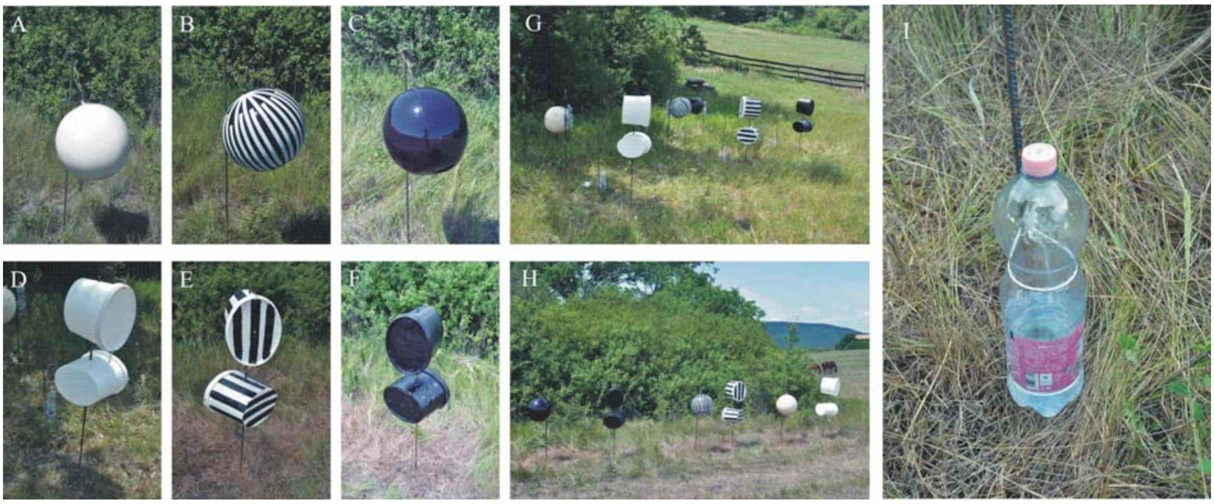

Figure 1: Sticky white (A, D), black-and-white striped (B, E), and black (C, F) spheres (A-C) and cylinders (D-F) used in experiment 1. Arrangement of the sticky test targets with (G) and without (H) ammonia. (I) A plastic bottle with five small holes in its stopper containing aqueous ammonia as an ammonia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

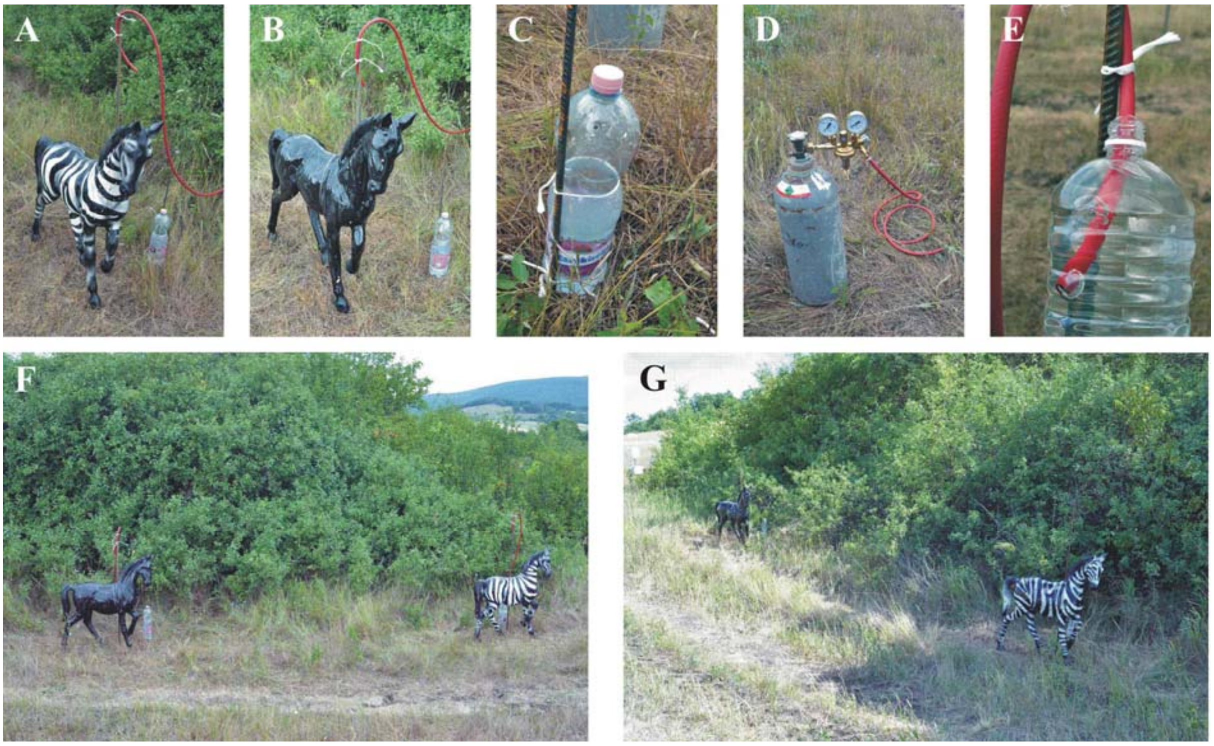

Figure 2: Zebra-striped (A) and black (B) sticky horse models provided with carbon dioxide (CO_2) and ammonia in experiment 2. (C) The ammonia source was a plastic bottle with five small holes in its stopper filled partly with aqueous ammonia. (D) The CO_2 originated from a gas tank with a manometer. (E) The continuous emission of CO_2 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 CO_2 and ammonia. (G) Arrangement of unbaited sticky horse models.

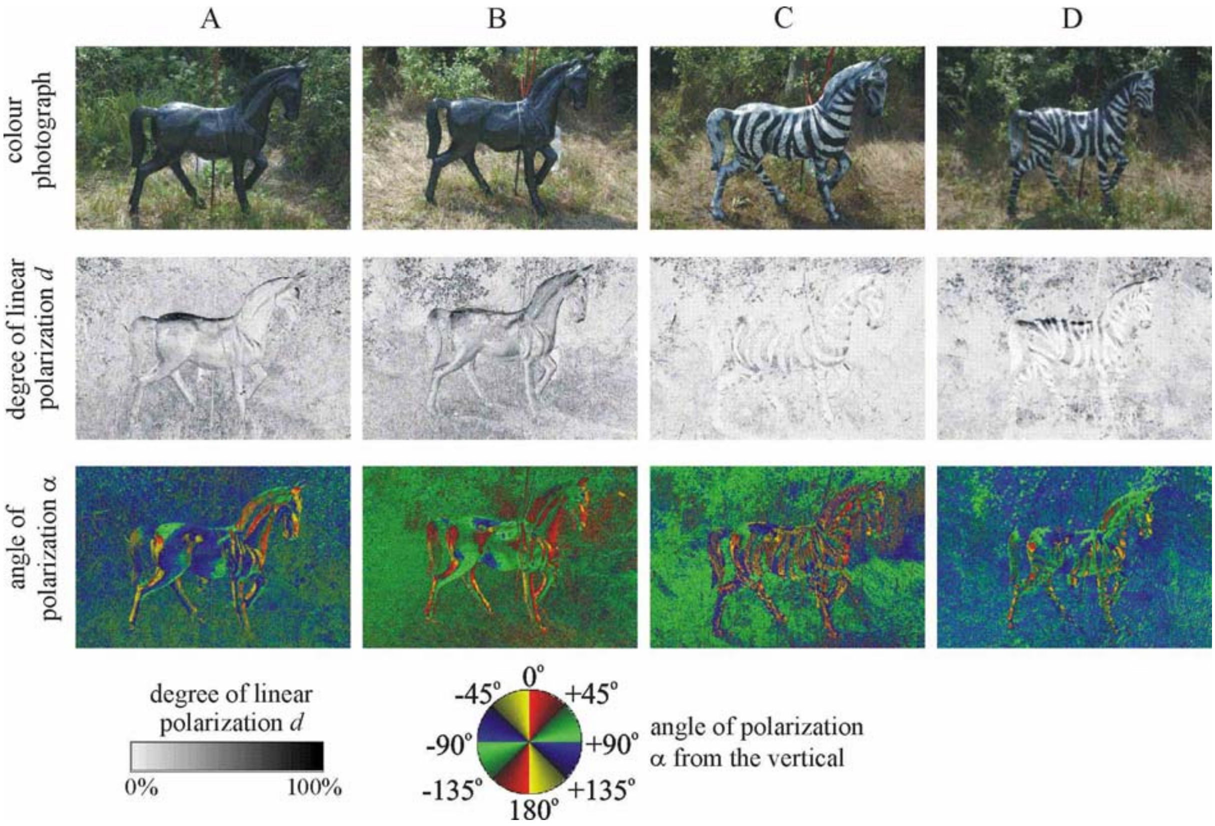

Figure 3: Colour photographs and patterns of the degree of linear polarization *d* and the angle of polarization α (clockwise from the vertical) of the sunlit sticky black and zebra-striped horse models used in experiment 2 measured by imaging polarimetry in the blue (450 nm) spectral range. The angle of elevation of the optical axis of the polarimeter was -20^{o} from the horizontal.