NKFIH K-123930
entitled

Experimental Study of the Functions of Zebra Stripes:
A New Thermophysiological Explanation

FINAL REPORT: SUMMARY OF RESULTS

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by

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1) A kutatási eredmények rövid, tényszerű összefoglalása magyarul

Terepi képalkotó polarimetriás mérésekkel kiderítettük, hogy miért szükséges polarizációérzékelés a nőstény bögölyöknek a vérszívásra alkalmas gazdaállatok kereséshez. Egy termodinamikai terepkísérlettel megcáfoltuk a zebracsíkok feltételezett hűtő hatásának széles körben elterjedt hipotézisét. Laboratóriumi Schlieren-optikás kísérletekkel kimutattuk, hogy a napsütötte zebracsíkok fölött nem alakul ki olyan légörvénysor ami a forróságban hűtené a zebrákat. Ezzel megcáfoltuk az örvénysorral kapcsolatos, ugyancsak elterjedt hipotézist. Terepkísérletekben kimutattuk, hogy a nőstény bögölyök vérszívásra a napsütötte sötét (meleg), fényesen csillogó (erősen fénypolarizáló) gazdaállatokat részesítik előnyben. Terepkísérletekben kiderítettük, hogy a nőstény bögölyök azért szeretik megtámadni a melegebb kültakarójú gazdaállatokat, mert a gazdák vérszívás okozta fájdalma által kiváltott ellenreakciói elől e parazita legyek könnyebben tudnak elmenekülni, mint a hűvösebb testfelszínű állatokról. Terepkísérletekben igazoltuk a zebracsíkok szerepéről felállított új magyarázatunkat, miszerint a csíkos kültakarójú állatokra azért nem szállnak a vért szívni szándékozó nőstény bögölyök, mert napsütésben a rengeteg fekete és fehér csík határvonalainál keletkező hőmérséklet-grádiensek megzavarják azt, hogy a bögölyök hőérzékeléssel találják meg a testfelszín alatt húzódó, környezetüknél kissé melegebb vérereket.

2) A kutatási eredmények rövid, tényszerű összefoglalása angolul

Using imaging polarimetry in the field, we revealed why blood-seeking female horseflies need polarization vision for detection of their host animals. With a thermodynamic field experiment we refuted the wide-spread hypothesis of the cooling effect of zebra stripes. Using Schlieren optics in laboratory, we showed that convective air eddies do not form above sunlit zebra stripes that could cool the zebra's body. In field experiments we demonstrated that female horseflies prefer to suck blood on sunlit dark (warm) and shiny (strongly polarizing) host animals. In field experiments we showed that the escape success of horseflies decreases with decreasing target temperature, that is escape success is driven by temperature. This explains the behaviour of biting horseflies that they prefer warmer hosts against colder ones. Our results also explain why horseflies prefer sunlit dark hosts against bright ones, and why these parasites attack their hosts usually in sunshine, rather than under shaded conditions. In field experiments we corroborated our new hypothesis explaining why biting horseflies avoid host animals with striped pelages: Since the borderlines of sunlit white and black stripes can hamper the thermal vessel detection by blood-seeking female horseflies, striped host animals are unattractive to these parasites which prefer hosts with homogeneous coat, on which the temperature gradients above blood vessels can be detected more easily.

3) Scientific Results Published in Peer-reviewed International Journals

During the 5-year research project NKFIH K-123930 (Experimental Study of the Functions of Zebra Stripes: A New Thermophysiological Explanation) we obtained the following results which have been published or are under consideration/review in scientific journals:

[1] Gábor Horváth, Tamás Szörényi, Ádám Pereszlényi, Balázs Gerics, Ramón Hegedüs, András Barta, Susanne Åkesson (2017) Why do horseflies need polarization vision for host detection? *Royal Society Open Science* 4: 170735 (doi: 10.1098/rsos.170735)

https://arago.elte.hu/sites/default/files/TabanidDarkPolarizedHost_RSOS.pdf

Horseflies (Tabanidae) are polarotactic, being attracted to linearly polarized light when searching for water or host animals. Although it is well known that horseflies prefer sunlit dark and strongly polarizing hosts, the reason for this preference is unknown. According to our hypothesis, horseflies use their polarization sensitivity to look for targets with higher degrees of polarization in their optical environment, which as a result facilitates detection of sunlit dark host animals. In this work, we tested this hypothesis. Using imaging polarimetry, we measured the reflection–polarization patterns of a dark host model and a living black cow under various illumination conditions and with different vegetation backgrounds. We focused on the intensity and degree of polarization of light originating from dark patches of vegetation and the dark model/cow. We compared the chances of successful host selection based on either intensity or degree of polarization of the target and the combination of these two parameters. We show that the use of polarization information considerably increases the effectiveness of visual detection of dark host animals even in front of sunny–shady–patchy vegetation. Differentiation between a weakly polarizing, shady (dark) vegetation region and a sunlit, highly polarizing dark host animal increases the efficiency of host search by horseflies.

[2] Gábor Horváth, Ádám Pereszlényi, Dénes Száz, András Barta, Imre M. Jánosi, Balázs Gerics, Susanne Åkesson (2018) Experimental evidence that stripes do not cool zebras. *Scientific Reports* 8: 9351 (doi: 10.1038/s41598-018-27637-1)

https://rdcu.be/0sQ9

https://arago.elte.hu/sites/default/files/ZebraBarrels_SciRep.pdf

There are as many as 18 theories for the possible functions of the stripes of zebras, one of which is to cool the animal. We performed field experiments and thermographic measurements to investigate whether thermoregulation might work for zebra-striped bodies. A zebra body was modelled by water-filled metal barrels covered with horse, cattle and zebra hides and with various black, white, grey and striped patterns. The barrels were installed in the open air for four months while their core temperature was measured continuously. Using thermography, the temperature distributions of the barrel surfaces were compared to those of living zebras. The sunlit zebra-striped barrels reproduced well the surface temperature characteristics of sunlit zebras. We found that there were no significant core temperature differences between the striped and grey barrels, even on many hot days, independent of the air temperature and wind speed. The average core temperature of the barrels increased as follows: white cattle, grey cattle, real zebra, artificial zebra, grey horse, black cattle. Consequently, we demonstrate that zebra-striped coats do not keep the body cooler than grey coats challenging the hypothesis of a thermoregulatory role of zebra stripes.

[3] Gábor Horváth, Ádám Pereszlényi, Tímea Tóth, Szabolcs Polgár, Imre M. Jánosi (2019) Attractiveness of thermally different uniformly black targets to horseflies: *Tabanus tergestinus* prefers sunlit warm shiny dark targets. *Royal Society Open Science* 6: 191119 (doi: 10.1098/rsos.191119) + electronic supplement

https://arago.elte.hu/sites/default/files/TabanidColdWarmBarrels_RSOS.pdf

https://arago.elte.hu/sites/default/files/TabanidColdWarmBarrels_RSOS-supplement.doc

From a large distance tabanid flies may find their host animal by means of its shape, size, motion, odour, radiance and degree of polarization of host-reflected light. After alighting on the host, tabanids may use their mechano-, thermo-, hygro- and chemoreceptors to sense the substrate characteristics. Female tabanids prefer to attack sunlit against shady dark host animals, or dark against bright hosts for a blood meal, the exact reasons for which are unknown. Since sunlit darker surfaces are warmer than shady ones or sunlit/shady brighter surfaces, the differences in surface temperatures of dark and bright as well as sunlit and shady hosts may partly explain their different attractiveness to tabanids. We tested this observed warmth preference in field experiments, where we compared the attractiveness to tabanids (*Tabanus tergestinus*) of a warm and a cold shiny black barrel imitating dark hosts with the same optical characteristics. Using imaging polarimetry, thermography and Schlieren imaging, we measured the optical and thermal characteristics of both barrels and their small-scale models. We recorded the number of landings on these targets and measured the time periods spent on them. Our study revealed that *T. tergestinus* tabanid flies prefer sunlit warm shiny black targets against sunlit or shady cold ones with the same optical characteristics. These results support our new hypothesis that a blood-seeking female tabanid prefers elevated temperatures, partly because her wing muscles are more rapid and her nervous system functions better (due to faster conduction velocities and synaptic transmission of signals) in a warmer microclimate, and thus, she can avoid the parasite-repelling reactions of host animals by a prompt take-off.

[4] Benjamin Fritz, Gábor Horváth, Ruben Hünig, Ádám Pereszlényi, Ádám Egri, Markus Guttmann, Marc Schneider, Uli Lemmer, György Kriska, Guillaume Gomard (2020) Bioreplicated coatings for photovoltaic solar panels nearly eliminate light pollution that harms polarotactic insects. *Public Library of Science One* 15: e0243296 (doi: 10.1371/journal.pone.0243296) + electronic supplement

https://arago.elte.hu/sites/default/files/RosePetalPol_PLoS-One.pdf

https://arago.elte.hu/sites/default/files/RosePetalPol_PLoS-One_supplement.docx

Many insect species rely on the polarization properties of object-reflected light for vital tasks like water or host detection. Unfortunately, typical glass-encapsulated photovoltaic modules, which are expected to cover increasingly large surfaces in the coming years, inadvertently attract various species of water-seeking aquatic insects by the horizontally polarized light they reflect. Such polarized light pollution can be extremely harmful to the entomofauna if polarotactic aquatic insects are trapped by this attractive light signal and perish before reproduction, or if they lay their eggs in unsuitable locations. Textured photovoltaic cover layers are usually engineered to maximize sunlight-harvesting, without taking into consideration their impact on polarized light pollution. The goal of the present study is therefore to experimentally and computationally assess the influence of the cover layer topography on polarized light pollution. By conducting field experiments with polarotactic horseflies (Diptera: Tabanidae) and a mayfly species (Ephemeroptera: *Ephemera danica*), we demonstrate that bioreplicated cover layers (here obtained by directly copying the surface microtexture of rose petals) were almost unattractive to these species, which is indicative of reduced polarized light pollution. Relative to a planar cover layer, we find that, for the examined aquatic species, the bioreplicated texture can greatly reduce the numbers of landings. This observation is further analyzed and explained by means of imaging polarimetry and ray-tracing simulations. The results pave the way to novel photovoltaic cover layers, the interface of which can be designed to improve sunlight conversion efficiency while minimizing their detrimental influence on the ecology and conservation of polarotactic aquatic insects.

[5] Gábor Horváth, Ádám Pereszlényi, Ádám Egri, Benjamin Fritz, Markus Guttmann, Uli Lemmer, Guillaume Gomard, György Kriska (2020) Horsefly reactions to black surfaces: attractiveness to male and female tabanids versus surface tilt angle and temperature. *Parasitology Research* 119: 2399-2409 (doi: 10.1007/s00436-020-06702-7) + electronic supplement

https://arago.elte.hu/sites/default/files/TabanidTiltedTraps_ParRes.pdf

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Tabanid flies (Diptera: Tabanidae) are attracted to shiny black targets, prefer warmer hosts against colder ones and generally attack them in sunshine. Horizontally polarised light reflected from surfaces means water for water-seeking male and female tabanids. A shiny black target above the ground, reflecting light with high degrees and various directions of linear polarisation is recognised as a host animal by female tabanids seeking for blood. Since the body of host animals has differently oriented surface parts, the following question arises: How does the attractiveness of a tilted shiny black surface to male and female tabanids depend on the tilt angle δ? Another question relates to the reaction of horseflies to horizontal black test surfaces with respect to their surface temperature. Solar panels, for example, can induce horizontally polarised light and can reach temperatures above 55 °C. How long times would horseflies stay on such hot solar panels? The answer of these questions is important not only in tabanid control, but also in the reduction of polarised light pollution caused by solar panels. To study these questions, we performed field experiments in Hungary in the summer of 2019 with horseflies and black sticky and dry test surfaces. We found that the total number of trapped (male and female) tabanids is highest if the surface is horizontal (δ = 0°), and it is minimal at δ = 75°. The number of trapped males decreases monotonously to zero with increasing δ, while the female catch has a primary maximum and minimum at δ = 0° and δ = 75°, respectively, and a further secondary peak at δ = 90°. Both sexes are strongly attracted to nearly horizontal (0° ≤ δ ≤ 15°) surfaces, and the vertical surface is also very attractive but only for females. The numbers of touchdowns and landings of tabanids are practically independent of the surface temperature T. The time period of tabanids spent on the shiny black horizontal surface decreases with increasing T so that above 58 °C tabanids spent no longer than 1 s on the surface. The horizontally polarised light reflected from solar panels attracts aquatic insects. This attraction is adverse, if the lured insects lay their eggs onto the black surface and/or cannot escape from the polarised signal and perish due to dehydration. Using polarotactic horseflies as indicator insects in our field experiment, we determined the magnitude of polarised light pollution (being proportional to the visual attractiveness to tabanids) of smooth black oblique surfaces as functions of δ and T.

[6] Gábor Horváth, Ádám Pereszlényi, Ádám Egri, Tímea Tóth, Imre Miklós Jánosi (2020) Why do biting horseflies prefer warmer hosts? Tabanids can escape easier from warmer targets. *Public Library of Science One* 15: e0233038 (doi: 10.1371/journal.pone.0233038) + electronic supplement

https://arago.elte.hu/sites/default/files/TabanidThermalTrapping_PLoS-One.pdf

https://arago.elte.hu/sites/default/files/TabanidThermalTrapping_PLoS-One-supplement.doc

Blood-sucking horseflies (tabanids) prefer warmer (sunlit, darker) host animals and generally attack them in sunshine, the reason for which was unknown until now. Recently, it was hypothesized that blood-seeking female tabanids prefer elevated temperatures, because their wing muscles are quicker and their nervous system functions better at a warmer body temperature brought about by warmer microclimate, and thus they can more successfully avoid the host's parasite-repelling reactions by prompt takeoffs. To test this hypothesis, we studied in field experiments the success rate of escape reactions of tabanids that landed on black targets as a function of the target temperature, and measured the surface temperature of differently coloured horses with thermography. We found that the escape success of tabanids decreased with decreasing target temperature, that is escape success is driven by temperature. Our results explain the behaviour of biting horseflies that they prefer warmer hosts against colder ones. Since in sunshine the darker the host the warmer its body surface, our results also explain why horseflies prefer sunlit dark (brown, black) hosts against bright (beige, white) ones, and why these parasites attack their hosts usually in sunshine, rather than under shaded conditions.

[7] Ádám Pereszlényi, Dénes Száz, Imre Miklós Jánosi, Gábor Horváth (2021) A new argument against cooling by convective air eddies formed above sunlit zebra stripes. *Scientific Reports* 11: 15797 (doi: 10.1038/s41598-021-95105-4) + electronic supplement

https://www.nature.com/articles/s41598-021-95105-4.epdf

https://arago.elte.hu/sites/default/files/ZebraSchlieren_ScientificReports.pdf

There is a long-lasting debate about the possible functions of zebra stripes. According to one hypothesis, periodical convective air eddies form over sunlit zebra stripes which cool the body. However, the formation of such eddies has not been experimentally studied. Using schlieren imaging in the laboratory, we found: downwelling air streams do not form above the white stripes of light-heated smooth or hairy striped surfaces. The influence of stripes on the air stream formation (facilitating upwelling streams and hindering horizontal stream drift) is negligible higher than 1-2 cm above the surface. In calm weather, upwelling air streams might form above sunlit zebra stripes, however they are blown off by the weakest wind, or even by the slowest movement of the zebra. These results forcefully contradict the thermoregulation hypothesis involving air eddies.

[8] Péter Takács, Dénes Száz, Miklós Vincze, Judit Slíz-Balogh, Gábor Horváth (2022) Sunlit zebra stripes may confuse the thermal perception of blood vessels causing the visual unattractiveness of zebras to horseflies. *Scientific Reports* 12: 10871 (doi: 10.1038/s41598-022-14619-7) + electronic supplement

https://www.nature.com/articles/s41598-022-14619-7

https://arago.elte.hu/sites/default/files/TabanidBlackGreyBarrel_SciRep.pdf

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Multiple hypotheses have been proposed for possible functions of zebra stripes. The most thoroughly experimentally supported advantage of zebra stripes is their visual unattractiveness to horseflies (tabanids) and tsetse flies. We propose here a plausible hypothesis why biting horseflies avoid host animals with striped pelages: In sunshine the temperature gradients of the skin above the slightly warmer blood vessels are difficult to distinguish from the temperature gradients induced by the hairs at the borderlines of warmer black and cooler white stripes. To test this hypothesis, we performed a field experiment with tabanids walking on a host-imitating grey test target with vessel-mimicking thin black stripes which were slightly warmer than their grey surroundings in sunshine, while under shady conditions both areas had practically the same temperature as demonstrated by thermography. We found that horseflies spend more time walking on thin black stripes than surrounding grey areas as expected by chance, but only when the substrate is sunlit. This is because the black stripes are warmer than the surrounding grey areas in the sun, but not in the shade. This is consistent with the flies' well-documented attraction to warmer temperatures and provides indirect support for the proposed hypothesis. The frequent false vessel locations at the numerous black-white borderlines, the subsequent painful bitings with unsuccessful blood-sucking attempts and the host's fly-repellent reactions enhance considerably the chance that horseflies cannot evade host responses and are swatted by them. To eliminate this risk, a good evolutionary strategy was the avoidance of striped (and spotted) host animals.

[9] Dénes Száz, Péter Takács, Ádám Egri, Gábor Horváth (2022) Blood-seeking horseflies prefer vessel-imitating temperature gradients on host-mimicking targets: experimental corroboration of a new explanation of the visual unattractiveness of zebras to tabanids. ***International Journal for Parasitology*** **(submitted: 15 September 2022)**

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

4) Dissemination in Popular Scientific Papers

We wrote the following popular scientific papers:

[P1] Szörényi Tamás, Pereszlényi Ádám, Horváth Gábor, Barta András, Gerics Balázs, Hegedüs Ramón, Susanne Åkesson (2018) Miért kell polarizációérzékelés a gazdaállat-kereséshez? *Fizikai Szemle* 68: 164-171

https://arago.elte.hu/sites/default/files/PolarosSotetGazdaAllat_FSz.pdf

[P2] Horváth Gábor, Pereszlényi Ádám, Száz Dénes, Barta András, Jánosi Imre Miklós, Gerics Balázs, Susanne Åkesson (2019) Zebracsíkok feltételezett hűtő hatásának kísérleti cáfolata. *Fizikai Szemle* 1. rész 69: 117-121, 2. rész 69: 147-154 + címlap

https://arago.elte.hu/sites/default/files/ZebraHordok_FSz.pdf

[P3] Pereszlényi Ádám, Száz Dénes, Jánosi Imre, Horváth Gábor (2021) A zebrák léghűtőjének kísérleti cáfolata. Van-e légörvénysor a zebracsíkok fölött? *Természet Világa* 152: 348-354

https://arago.elte.hu/sites/default/files/ZebraSchlieren_TV.pdf

[P4] Horváth Gábor, Pereszlényi Ádám, Száz Dénes, Egri Ádám, Jánosi Imre (2022) Zebracsíkok termofiziológiai vizsgálata: új magyarázat a zebracsíkok szerepére. *Természet Világa* (benyújtva: 2022. szeptember 3.)

5) PhD Thesis

The following PhD thesis has been written from some of the results:

[D1] Pereszlényi Ádám (2020) *Összetett vizuális ökológiai csapdák és a zebracsíkos bőrfelületek ökológiai és fiziológiás tulajdonságainak vizsgálata*. PhD értekezés, ELTE Biológiai Fizika Tanszék, Budapest, 112 oldal, témavezetők: Horváth Gábor, Kriska György, védés: 2020.11.19, *summa cum laude*