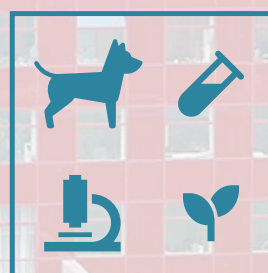




Eötvös Loránd University
Doctoral School of Biology
Conference 2022

PROGRAM & ABSTRACTS



1st December 2022, Budapest



ANNUAL MEETING OF EÖTVÖS LORÁND UNIVERSITY DOCTORAL SCHOOL OF BIOLOGY



DSB

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WELCOME



Greetings to all participants!

It is our great pleasure to welcome you to DSB Conference 2022. The aim of this event is to bring together PhD students in Biology Doctoral School to discuss their work, but also to find out about lectures of broader interest and to get to know each other. This event is open for all students enrolled in a PhD programme at Eötvös Loránd University. Thanks to your enthusiastic participation, DSB Conference 2022 will be a memorable event full of exciting moments and surprising experiences. Get ready for stunning science coming your way.

We wish you all a successful and enjoyable meeting!

Renáta Hamar, Head of the Organizing Committee and Prof. László Nyitray, Head of the Biology Doctoral School

Renáta Hamar

Nyitray L.



INTRODUCTION

SCIENTIFIC COMMITTEE

Head	Dr. László Nyitrai	Professor of department of Biochemistry, ELTE
Members	Dr. Balázs Rosivall	Professor of department of Systematic Zoology and Ecology, ELTE
	Dr. Árpád Dobolyi	Professor of department of Physiology and Neurobiology, ELTE
	Dr. Péter Lőw	Professor of department of Anatomy, Cell and Developmental Biology, ELTE
	Dr. Péter Pongrácz	Professor of department of Ethology, ELTE

ORGANIZING COMMITTEE

Head	Renáta Hamar	PhD student of department of Genetics, ELTE
Members	Kinga Balassa	PhD student of department of Plant Physiology and Molecular Plant Biology, ELTE
	Luca Eszter Balog	PhD student of department of Systematic Zoology and Ecology, ELTE
	Loretta László	PhD student of Institute of Enzymology, TTK Research Center

THE VENUE



Research Centre for Natural Sciences (Main lecture room)
Budapest, Magyar Tudósok Körútja 2, 1117



CONTACT US

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USE OF SOCIAL MEDIA

The official hash tag of the event is #DSBConf2022. The organizers encourage attendees to tweet about the amazing science they experience during the meeting. However, please be polite and respectful of others in all of your online activities.



SCIENTIFIC PROGRAM

8:30-9:00 Registration
9:00-9:15 Conference Opening

Session 1 Chair: Dr. Árpád Dobolyi

9:15-9:45 Keynote Lecture
Dr. Péter Horváth: Life Beyond the Pixels: Image Analysis and Machine Learning Methods for Single-Cell Analysis

9:45-10:00 Anita Marx: Liquid biopsy-based monitoring of residual disease in multiple myeloma

10:00-10:15 Álmos Becz: On the occurrence and microhabitat of four poorly-known Metazoan species from the Ráckeve-Soroksár-Danube

10:15-10:30 Janka Szinyákovics: EDTP suppresses autophagy and decreases lifespan in *Drosophila melanogaster*

10:30- 10:45 Kata Vékony: Assessment of the mini C-BARQ for multi-dog households in Hungary

10:45-11:15 Coffee break

Session 2 Chair: Dr. Péter Lőw

11:15-11:30 Loretta László: Multiverse of scaffold proteins - What role has the absence of Tks4 scaffold protein in tumor biology?

11:30-11:45 Erika Mária Juhász: Foraging strategy and impact of a reintroduced ecosystem engineer species (*Eurasian beaver*) in the presence of invasive woody plants

11:45-12:00 Szilvia Oláh: Neuronal networks involved in regulation of maternal behavior in rodents

12:00-12:15 Máté Sági-Kazár: The molecular background and regulation of chloroplast iron homeostasis

12:15-12:30 Dániel Mittli: The prefrontal cortex and neuroimmunology: from single-cell sequencing to *in vivo* electrophysiology

12:30-13:30 Lunch break

Session 3 Chair: Dr. Balázs Rosivall

13:30 -14:00 Keynote Lecture
Dr. László Zsolt Garamszegi: The beauty of behavioural ecology: snapshots from past studies and future directions

14:00-14:15 Anna Biró: From surface to caves and back - Morphological changes related to adaptation to a novel environment

14:15-14:30 János Pálkás: The topoisomerase III α -RMI1-RMI2 complex orients human Bloom's syndrome helicase for efficient disruption of D-loops

14:30-14:45 Paula Pérez Fraga: Comparing human-oriented communicative abilities of the family dog and the family pigs

14:45-15:00 Péter Berek-Nagy: Secondary metabolites from two grass root endophytic fungal genera: *Flavomyces* and *Darksidea*

15:00-15:30 Coffee break

Session 4 Chair: Dr. Péter Pongrácz

15:30-16:30 Flash posters

16:30-16:45 Award ceremony and farewell thoughts

KEYNOTE SPEAKERS



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POSTER FLASH TALKS PROGRAM

Flash talks are a great way to give an introduction to your work, and whet people's appetite for your research. Each talk lasts for 3 minutes, and presenters are allowed only one simple PowerPoint slide.

1	Ágnes Turóci	Introduced slug species in Hungary
2	Hana Kaci	Food components modify the function of drug transporter Organic anion transporting polypeptides (OATPs)
3	Zoltán Kovács	A novel mechanism for spatial and temporal regulation of SSB protein pools during genome stress via liquid-liquid phase separation
4	Noémi Borbély	Genetic study of modern and medieval Székely Land
5	Kata Tóth	"Why did you choose this dog?": factors affecting the decision-making of prospective dog owners
6	Andrea Kásler	Heated refuges for tadpoles lower the severity of fungal infection
7	Yusuf Can Özdemir	Understanding the genetic history of medieval period Altai region through aDNA
8	Dorottya Sárkány	Effect of <i>Lactobacillus</i> inoculation on the diversity of yeast communities in the experimentally <i>Aspergillus flavus</i> contaminated corn silages
9	Katalin Kuffa	Side-specific cell surface biotinylation to reveal the apico-basal distribution and accessible regions of transmembrane proteins in polarized cells
10	Orsolya Oravecz	Placental galectins: key regulators of innate and adaptive immune responses in pregnancy
11	Varsha Rani	Climate change and diet shift in invasive mysid species
12	Thu Huong, Huynh Ngoc	Trait-based responses of zooplankton communities to different warming scenarios
13	Bálint Halpern	Testing the effects of captive environment on individual variation in feeding behaviour of juvenile Hungarian meadow vipers (<i>Vipera ursinii rakosiensis</i>)
14	Orsolya Ungvári	A fluorescence-based competitive counterflow assay to investigate multispecific OATPs involved in ADME-Tox
15	Sára Sztruhala	Population divergence in aggregation and sheltering behaviour in surface and cave-adapted <i>Asellus aquaticus</i> (Crustacea: Isopoda)



ABSTRACTS OF ORAL PRESENTATIONS

Liquid biopsy-based monitoring of residual disease in multiple myeloma

Anita Marx

The need for sensitive monitoring of minimal/measurable residual disease in multiple myeloma emerged as novel therapies led to deeper responses. As multiple myeloma is characterized by clonal malignant plasma cell infiltration of the bone marrow, the potential benefits of blood-based analyses, the so-called liquid biopsy is prompting more and more studies to investigate its feasibility. To this end, we aimed at assessing and comparing different methods that are currently used (multiparametric flow cytometry, quantitative PCR) or may be suitable for residual disease monitoring (next-generation sequencing, droplet digital PCR). Our objective is to establish a liquid biopsy-based molecular monitoring system based on the rearranged immunoglobulin genes.

We analyzed a small group of myeloma patients with the high-risk t(4;14) translocation, using next-generation sequencing of the immunoglobulin genes, and droplet digital PCR of patient-specific IgH sequences. Moreover, well established monitoring methods such as multiparametric flow cytometry and RT-qPCR of the fusion transcript IGH-MMSET were utilized to evaluate the feasibility of these novel molecular tools. Serum measurements of M-protein and free light chains together with the clinical assessment by the treating physician served as routine clinical data. Our findings were in line with similar studies as they indicated a good correlation of clinical parameters and molecular data from our system, using Spearman correlations. During disease monitoring, the applied methods yielded complementary information thus increasing MRD evaluation probability. We detected indications of early relapse before clinical signs, although this implication needs verification in a larger patient cohort.

On the occurrence and microhabitat of four poorly-known Metazoan species from the Ráckeve-Soroksár-Danube

Becz Álmos

Four poorly-known macroscopic invertebrate species are recorded from the Ráckeve-Soroksár-Danube near the town of Szigetszentmiklós: *Craspedacusta sowerbii* Lankester, 1880 (Cnidaria, Limnomedusae), *Cordylophora caspia* Pallas, 1771 (Cnidaria, Clavidae), *Urnatella gracilis* Liedy, 1851 (Entoprocta) and *Prostoma graecense* Böhmig, 1892 (Nemertea). Sampling was carried out between February 2019 and October 2022.

Prostoma graecense has been known in Hungary from the Lake Balaton and River Tisza since the first discovery by Gelei, in 1928. However, there were no additional records until now. The first record of *Urnatella gracilis* was made more than 60 years ago from River Tisza. Later the species has been reported from the Ráckeve-Soroksár-Danube in 1970. *Cordylophora caspia* is a Ponto-Caspian non-indigenous species, known from the Lake Balaton and River Tisza, but it has not been recorded from the Hungarian section of the Danube until our study. *Craspedacusta sowerbii* is also a non-indigenous species that originates from the Upper Yangtze River Valley. The medusa form of *Craspedacusta sowerbii* has been reported from different Lakes in Hungary (mostly the medusa form), but it was still unknown from the Ráckeve-Soroksár-Danube. We recorded both the medusa and polyp forms of it.

Due to the very few records, good quality video and photo documentation of these species were missing in Hungary. Beyond establishing their presence, the present study aims to characterize their microhabitat and produce photo and video documentation for science communication and education.

EDTP suppresses autophagy and decreases lifespan in *Drosophila melanogaster*

Janka Szinyákovichs

Understanding why neurons become increasingly sensitive to demise at advanced ages remains a fundamental problem in neurobiology, with significant medical implications. Aging is driven by the progressive accumulation of molecular damage interfering with cellular homeostasis. Such damages mainly include misfolded, oxidized and aggregated proteins, and can predominantly be degraded by autophagy, a major catabolic process of eukaryotic cells. Many neurodegenerative diseases are characterized by the formation of protein aggregates in neurons, which



can lead to different neurological dysfunction. Therefore, studying autophagy is of great importance from a biomedical point of view as well. In *Drosophila*, the myotubularin-related lipid phosphatase EDTP, the sole fly ortholog of human MTMR14/Jumpy, is known to downregulate the autophagic process by antagonizing phosphatidylinositol-3 kinase, which catalyzes the formation of autophagic membranes. This inhibitory step helps the cell to maintain autophagic activity at an optimal level. However, it is well known that the capacity of autophagy declines during aging, resulting in the progressive accumulation of toxic protein aggregates. Using a Western blot analysis, semi-qPCR and a trojan gene trap system, we show that EDTP gradually accumulates in brain neurons during the adult life span, thereby establishing an age-associated gradual decline in the capacity of autophagy in these cells. Consistently, genetic inhibition of EDTP delays the development of age-related neuropathological symptoms. Inhibition of EDTP did not just improve climbing ability but also resulted in lifespan extension in treated animals. The orthologous human protein MTMR14 displays a similar tendency to accumulate during aging. These results reveal that after a certain life period EDTP and MTMR14 turn into endogenously toxic proteins promoting brain aging. Thus, age-related brain deterioration is a genetically determined process.

Assessment of the mini C-BARQ for multi-dog households in Hungary

Vékony Kata, Faragó Tamás, Pongrácz Péter

Behavioural problems are among the most common reasons behind dog relinquishment to shelters. Our knowledge about the risk factors, such as lack of socialization or previous trauma is steadily growing. Many of the commonly occurring problem behaviours are social in nature, such as aggression and social fear. It is likely that they have associations with other social attributes of dogs. In groups of social animals, including dogs, one of the most important dynamic feature of group members is the rank they possess in the group hierarchy. The most commonly used method to assess problem behaviours is the C-BARQ questionnaire with more than 100 questions on the behaviour of the dog. In 2014, a short version of C-BARQ was validated in English. The aim of our study was two-fold: a) to validate the short version of the C-BARQ in Hungarian on dogs from multi-dog households, and b) to find possible associations between social problem behaviours and the rank relationship between co-habiting family dogs. We used a questionnaire to assess the rank of each dog in multi-dog households: we asked owners about behaviours of their dogs related to i) obtaining resources; ii) leading and defending the group and iii) signals used to communicate rank. We also translated the short version of the C-BARQ to Hungarian, and asked owners to complete it for the aforementioned dogs. We found similar factors in the short (Hungarian) C-BARQ to the original one. We found that higher ranking dogs are more aggressive towards other dogs and more prone to resource guarding, but they are also less fearful of unfamiliar dogs and are more trainable. This study provided a useful tool in Hungarian to assess problem behaviours of dogs, and probably even more importantly, first time revealed it that the social rank of dogs in multi-dog households might affect other social behaviours in non-competitive contexts (like fear) and even behaviours towards the owner (trainability).

Multiverse of scaffold proteins- What role has the absence of Tks4 scaffold protein in tumor biology?

Loretta László^{1,2}, Tamás Takács^{1,2}, Álmos Tilajka^{1,2}, Anita Kurilla¹, Julianna Novák¹, László Buday¹, Virág Vas¹

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Lung cancer is one of the most common types of cancer worldwide. The 5-year survival rate is low compared to other tumor types (e.g., colon cancer), so early detection, prevention, and appropriate therapy of lung cancer is the primary target. Tks4 is a scaffold protein with four SH3 domains, which has a role in the EGFR signaling pathway and cell motility. Several other functions of the Tks4 protein were first described by our group, such as the role of Tks4 in adipose tissue homeostasis, in the regulation of osteoporosis, and in the regulation of mesenchymal and embryonic stem cells differentiation. However, an intriguing cancer formation-related feature of Tks4 is that in case of the absence of Tks4 protein epithelial-mesenchymal transition (EMT)-like events are induced in colon cancer cells. To shed light on the role of Tks4 also in lung cancer biology, my work aims to investigate the effect of Tks4 protein in human lung cancer formation using in vitro and in vivo measurements.



First, I measured the Tks4 gene expression levels in human lung cancer patient's samples. Then, the CRISPR-Cas9 gene editing system was used to create a Tks4-KO human lung cancer cell line. EMT markers were measured by RT-qPCR to investigate if the absence of Tks4 induce EMT-like phenotype. Furthermore, our goal was to describe new tumor formation-related partner molecules of Tks4 and validate the observed molecular interactions. Therefore, I used mass spectrometry analysis and via proximity ligation assay (PLA) and immunoprecipitation (IP) assay I confirmed the presence of the binding of Tks4 with novel partner molecules.

We found that the Tks4 gene expression levels were significantly decreased in all of the human lung tumor stages, especially in the metastatic phase of the tumor. EMT marker gene expression levels were upregulated in the Tks4-KO clones compared to the wild type cells, indicating that the absence of Tks4 induces an EMT-like phenotype. With mass spectrometry we managed to find new partners of Tks4, for example CAPZA1 (F-actin-capping protein subunit alpha-1) molecule. The interaction of Tks4 and CAPZA1 was confirmed using PLA and IP in human lung cancer cell line. The cancer promoting effect of the newly identified Tks4/CAPZA1 interaction is not elucidated, yet therefore during the last year of my PhD work I will study its potential significance.

In summary, our results showed that Tks4 plays a prominent role also in lung tumorigenesis. Moreover, based on the result of our human patients sample analyses we concluded that Tks4 level changes might be promising prognostic biomarkers in early tumor detection.

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Foraging strategy and impact of a reintroduced ecosystem engineer species (Eurasian beaver) in the presence of invasive woody plants

Erika Mária Juhász

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The Eurasian beaver (*Castor fiber*) became extinct in Hungary in the middle of the 19th century. As a result of reintroduction programmes (1996-2008) and spontaneous migration (1991-), the species started to recolonize the country and, according to our estimates, its population size reached 10-11,000 specimens in 2020. Meanwhile, the habitats of the species were significantly altered due to the river regulations and the spread of invasive woody species. Our aim was to understand the foraging strategy and impact of the native ecosystem engineer species in this novel environment.

Altogether, 20 study sites were selected along 6 rivers in Hungary. At each site, we registered the woody plant supply and its utilization in 100 sampling circles with 2 m in radius. The utilization ratio of native softwoods (willows – *Salix* spp. and poplars – *Populus* spp.) and that of invasive woody species (ash-leaved maple – *Acer negundo*, green ash – *Fraxinus pennsylvanica*, and false-indigo bush – *Amorpha fruticosa*) were compared using Mann-Whitney U tests. We examined the effect of taxon, trunk diameter, and distance from water on the foraging decisions of the beaver by building generalized linear mixed models.

Softwood species were generally preferred over the invasive species. Larger diameter classes were utilized with lower ratio and only in the case of softwood species. The diameter influenced also the type of utilization, the thicker units were carved or debarked rather than felled. The foraging intensity was significantly higher along the first line of woody plants than in a 10 m distance from the riverbank.

The beaver exerts a greater direct impact on softwood species than on the invasive species. Thus, the ecosystem engineer rodent may exacerbate the ongoing progress of biological invasion in degraded floodplain habitats. A conservation conflict is arising between the protection of the Eurasian beaver (EU Habitat Directive/Annex II, IV) and that of softwood gallery forests (Annex I). In the light of this, special attention should be paid to creating more favourable hydrological conditions for the sprouting and renewal of native softwoods through floodplain habitat reconstruction.



Neuronal Networks Involved in Regulation of Maternal Behavior in Rodents

Szilvia Oláh

Pregnancy and lactation have important consequences on the hormonal and neuronal systems of mothers. Lactation has double action on nervous system; pup stimuli exert their actions on the mother through direct neuronal activation of maternal regulatory circuits and elevation of prolactin level in response to suckling. It has been a debate for decades if pup stimulus exerts its action on the mother by sensory inputs or rather the pup-induced elevated prolactin levels. To address this question, we directly compared the distribution of prolactin-sensitive neurons to those neuronally activated by pup suckling measured by the c-Fos technique. A substantial divergence of the two different activation patterns was found as only a portion of the neurons was influenced by both pathways, suggesting independently activated neuronal populations in mother mice. The distribution of the differentially labelled cells revealed that most brain areas containing prolactin-sensitive neurons also included neuronally activated cells, suggesting a local network-level interaction between the different inputs in the adaptation of the brain to motherhood. However, some brain areas including the posterior intralaminar thalamic nucleus (PIL) showed only direct neuronal activation in its neurons expressing the neuropeptide parathormon 2 (PTH2). PTH2 neurons of the PIL have been suggested to relay somatosensory input from the nipple to the forebrain during suckling. We showed that the PIL of mice also contains PTH2-negative calbindin neurons. To investigate their functions, excitatory, inhibitory and control designer receptors exclusively activated by designer drugs (DREADDs) were expressed selectively in calbindin neurons of the PIL with viral gene transfer. Maternal, anxiety, and depression-like behaviours of mice were analysed in response to injection of Clozapine-N-oxide (CNO), the designer ligand of DREADDs. PIL calbindin-positive neurons inhibited maternal behavior and promoted anxiety but did not affect depression-like behavior suggesting that PIL calbindin neurons are involved in the behavioural adaptation of mother mice complementing the actions of prolactin.

The molecular background and regulation of chloroplast iron homeostasis

Máté Sági-Kazár¹, Csaba Hegedűs¹, Brigitta Müller¹, Helga Zelenyánszki¹, Barnabás Cseh¹, Nóra Radics¹, Éva Sárvári¹, Katalin Solymosi², Ádám Solti¹

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Iron (Fe) is an essential micronutrient for all living organisms. In plants Fe is most important for the formation of the photosynthetic apparatus and chlorophyll biosynthesis. For the operation of one single linear photosynthetic electron transport chain a total of 22 Fe atoms, in the form of Fe-S clusters, haem groups and non-haem Fe ions are required, thus the majority of the mesophyll cells' Fe content is located in the chloroplasts.

For the development of photosynthetic structures, chloroplasts operate a reduction-based Fe uptake system, facilitated by Ferric Reductase Oxidase (FRO) 7. A similar well-characterised system operates on the surface of the roots of *Arabidopsis thaliana*, however the regulation of chloroplast Fe uptake is much less understood. The Fe need of chloroplasts is saturated with the full development of photosynthetic structures. This also leads to the downregulation of the Fe uptake system as well as the decrease of chloroplast Fe content during leaf aging suggesting the existence of chloroplast Fe export systems. Maintaining proper chloroplast Fe levels thus requires the strict control of chloroplast Fe transport mechanisms.

Since chloroplasts represent the largest pool of Fe in plants, the regulation of chloroplast Fe homeostasis greatly impacts the Fe status of the whole plant. During Fe deficiency mainly the Fe content of chloroplasts is affected which leads to the upregulation of the root Fe uptake system and the long-distance Fe transport system. During leaf senescence a shift in the plant Fe status can be observed where the chloroplasts of senescent leaves are degraded, and their Fe content is remobilised to younger tissues. There is however only limited information available on the Fe sensing of mesophyll cells and their chloroplasts as well as the long-distance signalling of leaf Fe status.



Here we studied effects of Fe nutrition on chloroplast Fe homeostasis and chloroplast Fe transport mechanisms. Using *Arabidopsis thaliana*, *Brassica napus*, and *Brassica oleracea* models we followed the change in plastidial Fe content, studied the expression pattern of proteins involved in plastidial Fe transport and Fe metabolism using QPCR, and mapped the Fe distribution and Fe content of leaves with X-ray fluorescence imaging and ICP-MS under various Fe nutrition conditions. We also studied how leaf developmental status impacts chloroplast Fe status and Fe mobilisation. We hope to identify how plant Fe nutrition affects chloroplast Fe homeostasis and the regulative effect of chloroplast Fe status on cellular Fe distribution and (re)mobilisation.

Acknowledgements. This work was supported by the ÚNKP-20-3-I-ELTE-862, ÚNKP-21-3-I-ELTE-680 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund. This work was supported by the grant financed by the National Research, Development and Innovation Office, Hungary (NKFIH K-124159; K-135607). Investigations were principally carried out in the frame of ELTE Thematic Excellence Program 2020 supported by National Research, Development and Innovation Office - TKP2020-IKA-05.

The prefrontal cortex and neuroimmunology: from single-cell sequencing to in vivo electrophysiology

Dániel Mittli

ELTE Eötvös Loránd University, Institute of Biology, Laboratory of Proteomics
 Doctoral School of Biology, Program of Molecular Cell Biology and Neurobiology

The investigation of the molecular background of neuro-immune interactions in the brain is an important issue for understanding physiological and pathological processes in the central nervous system. Thus, we first analyzed the single-cell sequencing data of our earlier transcriptomic work, obtained from electrophysiologically identified pyramidal cells and interneurons of the murine prefrontal cortex (PFC). We filtered out the genes that may be associated with the direct communication between immune cells and neurons and examined their expression pattern in PFC neurons. The expression of some of these genes by cortical neurons has not yet been reported. The vast majority of antimicrobial (~53%) and immune cell protein (~94%) mRNAs was identified in the neuronal transcriptomes, owing to the high sensitivity of single-cell sequencing.

Next, to reveal functional alterations in the PFC associated with neuroimmunological processes, we performed electrophysiological and proteomic experiments in brain slices and lipopolysaccharide (LPS)-treated mice, since it is well known that peripheral infections often lead to neuropsychiatric symptoms related to the impairment of PFC-regulated cognitive functions. Based on the gene expression differences of the coreceptor (*Il1rap*) of interleukin-1 beta (IL-1 β) between neuron types in our single-cell sequencing data, we compared the electrophysiological effects of IL-1 β on PFC pyramidal cells and interneurons. We found that pyramidal cells are more responsive to IL-1 β , as could be presumed from the transcriptomic differences. The possible circuit-level correlates of these neuronal changes were investigated by frontal electroencephalographic (EEG) recordings and fronto-occipital functional connectivity analyses in LPS-treated mice and significant changes were found in the fronto-occipital EEG correlation and coherence. The upregulation of the prefrontal IL-1 system after LPS treatment was shown by immunoassays simultaneously with the observed EEG changes. Finally, we investigated the LPS-induced alterations of the synaptic proteome in the PFC using 2-D differential gel electrophoresis and mass spectrometry and found 48 altered proteins mainly related to cellular signaling, cytoskeletal organization, and carbohydrate/energy metabolism. Thus, our results indicate remarkable electrophysiological and molecular changes in the PFC related to acute systemic inflammation that may explain some of the concomitant behavioral and physiological symptoms. Furthermore, our transcriptomic data may initiate further experimental and *in silico* studies on the neuronal expression of immune genes and the potential role of the encoded proteins in neuroimmunological processes.



From surface to caves and back - Morphological changes related to adaptation to a novel environment

Anna Bíró

The colonisation of markedly different environments is generally followed by detectable adaptations. Taxa that inhabit both cave and surface environments create excellent model systems to understand the general rules of these adaptations, as the cave-surface habitat pair represents strikingly different environments in short geographical distances. The properties of caves are unique among habitats as they have close to stable temperature all year round, limited food sources, constant darkness and harbour simple communities. Typically, surface species colonise caves, although there are examples for the opposite direction colonisation as well. As a result, cave colonising species go through parallel or convergent evolution while species colonising surface habitats are expected to go through divergent evolution connected to dissimilar microhabitat properties.

To understand how morphology is affected by the cave-surface environmental sift, we examined the effects of colonisation in both directions using two model organisms. The *Asellus aquaticus* species complex a primarily surface-dwelling species that colonised caves on several independent occasions and the *Niphargus* genus which is predominantly subterranean but have stable populations in surface springs. We compared nine surface and six cave populations of *A. aquaticus* (N cave: 279; N surface: 377), and four cave and four surface species in case of *Niphargus* (N cave: 220, N spring stream: 246). To understand whether the habitat shift has an effect on sexual dimorphism, we examined both sexes. We measured 17 functional morphological traits of *A. aquaticus* and 15 of *Niphargus*. We found marked morphological differences between the habitats in 10 traits of *A. aquaticus* and differences in sexual dimorphism between the habitats in both taxa (*A. aquaticus*: 8, *Niphargus*: 4) but no sole habitat effect in case of the *Niphargus* species. Therefore, we can only speculate that although habitat related morphological changes could be expected in both directions colonisation of ecotones might not represent such an environmental shift as expected or that the time since colonisation is too short to detect habitat related changes. At the same time the habitat dependent sexual dimorphism found in case of both direction colonisation shows that changes in forces in sexual selection between the habitats play an important role in shaping the morphology of the colonisers. As basic knowledge on lifestyle, abundance and sex-ratio of cave dwelling populations is hard to gather, functional morphology could open new ways to understand environmental shift induced changes in cave-surface model systems. In a broader view it can also help to understand the drivers behind phenotypic variation in the wild.

The topoisomerase III α -RMI1-RMI2 complex orients human Bloom's syndrome helicase for efficient disruption of D-loops

János Pálkás

With the process of homologous recombination (HR) cells are able to repair double-strand DNA breaks (DSB) and promote the formation of cross-overs during meiotic division. The main step during HR is the formation of the D-loop (Displacement-loop) DNA structure, which contains both the broken and the homologous template strands. D-loops can be processed in different ways that could lead to the extension or disruption of the strand invasion. Extension is favorable for meiotic crossing over whereas disruption is beneficial during DNA repair. RecQ family helicases play crucial role in the pathway selection of HR by processing D-loops. Based on our lab's previous results, the *E. coli* RecQ helicase disrupts D-loops very efficiently, thus promoting DSB repair. However, the D-loop processing profile of the human BLM (Bloom-syndrome) helicase and its precise role in human HR still remains unknown,

In our work, we investigated D-loop processing by the BLM helicase with an improved version of the transient kinetic assay used previously for examining the *E. coli* RecQ helicase. Furthermore, we tested the effect of the TRR (TOPOIII α -RMI1-RMI2) complex on the BLM's D-loop processing activity, since TRR is a well-known interaction partner of BLM during HR. Based on our results, BLM maintains a balance between D-loop disruption and extension, showing great difference compared to *E. coli* RecQ helicase, which is highly efficient in D-loop disruption. The different behaviour of the two enzymes likely comes from subtle differences between the conserved domain structures. While the *E. coli* RecQ owes its disruptive feature to the HRDC (additional single-stranded DNA binding) domain, the same domain in BLM allows the enzyme to maintain its balance between the two distinct pathways. Furthermore, the TRR complex was shown to modulate D-loop extension by BLM via specific protein-protein interactions between the two enzymes.



Compared to *E. coli* RecQ, this complex activity of the BLM helicase allows the enzyme to fulfill its role in human HR events during DSB-repair and meiosis as well. By modulating D-loop processing of BLM, TRR may be able to indirectly control the outcome of HR.

Comparing human-oriented communicative abilities of the family dog and the family pigs

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Selective pressures during dogs' (*Canis familiaris*) domestication seem to have enhanced dogs' human-oriented communicative skills, although experience also plays a role. Other domestic species perform similar human-oriented behaviours but there is a lack of direct comparisons. Domestic pigs (*Sus scrofa domesticus*) are also highly social, as companion animals occupy a similar role as the dog, but little is known about their interspecific communicative abilities.

Here we compare human-raised dogs' and miniature pigs' human-directed communication. This approach could be informative about the contribution of species-specific traits to the emergence of human-oriented communicative skills. In a test battery (i.e. face-orientation test, unsolvable task paradigm, and out-of-reach reward test) consisting of neutral and food-related contexts, we observed human-oriented behaviours of young dogs (n=12) and pigs (n=11). Both species oriented similarly much to a human face in a begging-for-food context, but dogs did so more than pigs in a neutral context (p=0.011). In the unsolvable task, only dogs increased their orientation-alternation between the target and the human compared to a baseline (p<0.0001), although during baseline there was no species difference. Contrarily, pigs persisted more in trying to solve the task (p=0.011). When they faced an out-of-reach reward, dogs alternated their orientation between the target and the owner more than pigs (p < 0.001).

Together, family dogs and family pigs exhibited similar human-oriented behaviours across different contexts, indicating that these are not unique to dogs. However, the observed results suggest that species' predispositions in communicative behaviours may be determinant for dogs' success in initiating interspecific interactions.

Secondary metabolites from two grass root endophytic fungal genera: *Flavomyces* and *Darksidea*

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Endophytic fungi, colonizing plants internally and asymptotically, are considered valuable but unexplored sources of diverse natural products, because of their interactions with plants and other microorganisms via secondary metabolites (SMs). During investigations on root endophytic fungal communities of semiarid sandy grasslands in Hungary, we isolated representatives of the dark septate endophytic fungal genera *Flavomyces* and *Darksidea* (Pleosporales), characterized by melanized and septate hyphae. We also obtained numerous congeneric, predominantly *Darksidea* isolates from similar ecosystems in different regions of the world, resulting in a remarkable collection of fungal isolates, also representing novel species. As the metabolite composition of these fungal genera



has not been determined yet, we aimed to identify SMs from *Flavomyces* and *Darksidea* by metabolite profiling all available isolates.

Metabolite composition of the fungal isolates was analysed by high-performance liquid chromatography hyphenated with diode array and high-resolution tandem mass spectrometry detection (HPLC-DAD-HRMS/MS). In addition to the HRMS/MS identifications, chemical structures of the isolated compounds were confirmed by nuclear magnetic resonance (NMR) spectroscopy.

As a result, we identified SMs from *Flavomyces* and *Darksidea* for the first time, from fungal genera that proved to be valuable sources of numerous novel or rarely occurring compounds. These compounds represent diverse structural groups, such as polyketides, terpenes, quinones and tetramic acid derivatives. In case of *Darksidea*, comprising several species, correlations were found between the phylogeny and metabolite diversity of the genus, confirming the practical utility of closely related isolates for the high-yield production of certain compounds.

SMs from the genera investigated and correlations between the phylogeny and metabolite diversity will be presented at the conference. Further experiments are in progress to test the biological activities of the identified compounds.

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ABSTRACTS OF POSTERS

Introduced slug species in Hungary

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The Hungarian molluscan fauna is thoroughly explored (Varga et al., 2005). Slugs, however, usually ignored in ecological and faunistic studies, due to two main reasons. Firstly, their storage in ethanol is more complicated compared to dry snail shells. Secondly, their identification is difficult because of the few reliable identification guides (Rowson et al. 2014, Cameron, 2016) and the fact, that dissection is essential for a number of species groups. Our aim is to write an identification book of Hungarian slugs, including photographs of living slugs and anatomical characters as well. During this work, we discovered 6 new species to the Hungarian fauna, 2 of them are already published (Turóci et al., 2020). The increasing number of introduced molluscs is traditionally explained by the climate change (Mainka & Howard, 2010) and the increased international transportation of goods (Hulme, 2009), mostly agricultural and horticultural products. Since 3 out of the 6 introduced species are still restricted to horticultural areas, it seems that the latter is the key factor. Slugs can easily hide in the pots of horticultural plants and tolerate long distance journeys lingering in the soil. In the future we will monitor the distribution of the non-native slug species, and collect data on the horticultural damage they make.

Food components modify the function of drug transporter Organic anion transporting polypeptides (OATPs)

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Introduction: Human organic anion transporting polypeptides (OATPs) are membrane proteins expressed in several tissues of the human body. OATPs mediate the ATP-, and sodium independent cellular uptake of large organic molecules including many clinically important drugs. Multispecific members of the OATP family are key determinants of pharmacokinetics and can be victims of drug-drug or food-drug interactions. The xenoestrogenic mycotoxin zearalenone (ZEN) is a Fusarium-derived food and feed contaminant. Consumption of contaminated food can result in food-drug interactions (FDI) via competing multispecific OATPs. The natural polyphenol resveratrol (RES) and the flavonoids luteolin (LUT) and naringenin (NAR) found in various foods/beverages have various beneficial health effects. The intake of a large doses of RES, LUT, and NAR can result in micromolar concentration of their sulfate and glucuronate conjugates in circulation. RES, LUT and NAR are known inhibitors of drug transporter OATPs, however our knowledge about its metabolites in regard of OATP interaction is limited. Therefore, I have investigated the interaction of ZEN and RES metabolites with multispecific OATPs, 1A2, 1B1, 1B3 and 2B1 and LUT, NAR conjugates with OATP1B1 and OATP2B1.

Methods and results: The inhibitory effects of ZEN, RES, LUT, NAR and their derivatives on the uptake of fluorescent probes was tested on A431 (human epidermoid carcinoma) cells overexpressing human OATPs (OATP1A2, OATP1B1, OATP1B3, or OATP2B1). In addition,

we also followed the uptake of flavonoids in A431 cells overexpressing OATP2B1, using 2- aminoethoxydiphenyl borate (2-APB) as a fluorescence enhancer. We found that zearalenone 14-sulfate is the most potent inhibitor of OATPs, and OATP2B1 was also inhibited by all ZEN derivatives with micromolar IC₅₀ values. RES and its metabolites, with the exception of dihydro-resveratrol, also inhibited the function of OATPs, 1B1, 1B3 and 2B1.



Furthermore, OATP2B1 transport activity was strongly inhibited by LUT and NAR conjugates. The assay developed for measuring flavonoid uptake by OATPs provides a novel method for investigating OATP food-drug interactions. Conclusion: Our data revealed that not only the parent compounds (ZEN, RES, LUT, and NAR), but some of their metabolites can also interact with OATPs. Therefore, high intake of these food components or contaminants may interfere with the pharmacokinetics of OATP substrate drugs.

Keywords: Zearalenone, Resveratrol, luteolin, naringenin, food-drug interactions, OATP transporters.

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A novel mechanism for spatial and temporal regulation of SSB protein pools during genome stress via liquid-liquid phase separation

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Single-stranded DNA binding proteins (SSBs) are ubiquitous and abundant proteins playing crucial roles in genome maintenance in all domains of life. The best characterized SSB is the homotetrameric SSB found in *E. coli*. EcSSB rapidly covers nascent single-stranded (ss) DNA to protect it against degradation, reannealing, and undesired secondary structure formation. In addition, SSB interacts with more than a dozen proteins playing roles in genome maintenance, coordinating their activities and spatial organization. An SSB monomer consists of an N-terminal ssDNA-binding OB fold and a C-terminal region. The latter can be further divided to an intrinsically disordered linker (IDL) and a conserved C-terminal peptide (CTP) mainly responsible for protein-protein interactions. Here we reveal a novel mode of higher-order spatial organization for SSB in which it forms protein condensates *via* liquid-liquid phase separation (LLPS). This process requires all structural regions of EcSSB and is regulated by specific interaction of the CTP with the OB fold. ssDNA can enter SSB condensates, but the saturation of the SSB binding sites by ssDNA eliminates LLPS as ssDNA and the CTP compete for the same binding site on the OB folds. Binding partners of SSB are specifically enriched inside the droplets, whereas control proteins are excluded. Based on these findings we propose a model in which during normal (stress free) conditions the bigger fraction of SSBs is located near the inner membrane forming protein condensates, only a little fraction takes part at the replication forks. During genome stress the amount of ssDNA is exposed triggering the relocation of SSBs from the inner membrane bound condensates to solution phase. SSBs immediately cover these nascent ssDNA regions and deliver repair proteins to their site of action. The dynamic nature of SSB condensates allows the rapid respond to genomic stress and enables timely repair of damaged DNA.

Population divergence in aggregation and sheltering behaviour in surface- versus cave-adapted *Asellus aquaticus* (Crustacea: Isopoda)

Sára Sarolta Sztruhala, Gergely Horváth, , Gergely Balázs and Gábor Herczeg

Aggregation (gathering together) and sheltering (hiding in cover) are basic behaviours that can reduce the risk of predation. However, both behaviours have costs, such as increased competition over resources and high prevalence of contact-spread parasites (aggregation) or lost opportunities for foraging and mating (sheltering). Therefore, variation in these behaviours is expected between populations with varying levels of predation risk. We compared aggregation and sheltering in surface- (various predators) and cave-adapted (no predator) populations of the isopod



Acellus aquaticus in a common garden experiment. Given that the cave environment is constantly dark, we also tested for population variation in light-induced behavioural plasticity. Variation in sheltering was explained by habitat type: cave individuals sheltered less than surface individuals. We found high between-population variation in aggregation with or without shelters and their light-induced plasticity, which was not explained by habitat type. Cave individuals decreased (habituation) whereas surface individuals increased sheltering with time (sensitization). We suggest that population variation in sheltering is driven by predation, whereas variation in aggregation must be driven by other, unaccounted environmental factors, in a similar manner to light-induced behavioural plasticity. Based on habituation/sensitization patterns, we suggest that predation-adapted populations are more sensitive to disturbance related to routine laboratory procedures.

"Why did you choose this dog?": Factors Affecting the Decision-Making of Dog Owners

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There are many hypotheses about why people choose a specific dog, but empirical data is missing. Preference for dog's specific (inner or outer) qualities can differ depending on the prospective owner's previous experiences, circumstances and expectations. We asked Austrian owners to fill in an online questionnaire about why they chose their dog and use their own words. Based on their free answers, we identified 21 dog traits and quantified their frequency in the sample. In our preliminary analysis, we first investigated how parenthood is associated with the choice for a particular dog. We assumed that parents (N=236) would choose "smarter" and more docile dogs than non-parents (N=917). Binomial regression indicated that indeed, "smartness" and friendliness (nonaggression) were more important for parents. In addition, it was less frequent that they adopted a dog from a shelter and more common that another person chose the dog for the family. Future directions can address the effect of the owner's education level, place of residence or the owner's age, all factors that could possibly also have an influence on dog choice.

Heated refuges for tadpoles lower the severity of fungal infection

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Chytridiomycosis is an infectious disease of amphibians, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Bd). In the last fifty years it has led to drastic population declines and extinctions globally. According to previous studies, the critical thermal maximum of Bd is lower than that of most amphibian hosts, which provides the possibility to use thermal-treatment against chytridiomycosis. Moreover, it has been shown that some amphibian species show behavioral fever, a phenomenon that can further enhance the effectivity of such thermal-treatments. In this experiment we aimed to test the effects of the availability of heated refuges on Bd prevalence and infection intensity in tadpoles kept under semi-natural conditions. We entered Bd-exposed and control common toad (*Bufo bufo*) tadpoles at two different densities (15 vs 60 tadpoles) into 120-L mesocosms that contained an immersed refuge.



These refuges were either heated to 30 °C for six days or were left unheated, and tadpoles could enter and leave refuges at their will. We repeatedly monitored the activity of tadpoles to assess refuge-use and to detect behavioral changes during the experiment. We found that the availability of heated refuges did not lower the prevalence of Bd, but it lowered the intensity of infection at both densities. Mean body mass of tadpoles was lower in the high density than in the low density treatment, and this was independent of the thermal treatment. The effect of density was stronger in the uninfected control groups, than in the infected ones. We observed more tadpoles using the heated refuges at higher than at lower densities, but infection status of the tadpoles did not have an effect. Our results show that provisioning of heated refuges may help amphibian populations to lower the Bd infection burden, even under natural conditions and at high densities.

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Understanding the Genetic History of Medieval Period Altai Region Through DNA

Yusuf Can Özdemir, Tamás Szeniczey, Gergely Csiky, Alexey Tishkin, Balázs Mende, Anna Szécsényi-Nagy

The Altaic region of East Eurasia is a mountainous area which separates Western and Eastern steppes but also acts as a passage between these two regions.

Despite the great impacts of how the Eastern Eurasian populations have shaped human history, much less ancient genetics studies on these populations have been carried out than the ones published for the understanding of West Eurasian history.

The 4th-14th centuries East Eurasia is understudied from population genetics aspects, therefore novel studies are due. New samples from the Altaic region could shed light on the movements and population events of the highly mobile Medieval communities in that area. This poster presents the aims and goals of a new project launched in the Institute of Archaeogenomics Research Centre for the Humanities in Budapest, in cooperation with the Barnaul University, Russia.

The inhabitants of the Altai in the Medieval period showed a multiethnic profile, being variously related to North-East Siberian Asian steppe people. A series of nomadic polities ruled the region and these peoples. Having similar political structures; the Rourans, Turks, Uighurs, Kyrgyz and Khitans established their realms successively. This study researches the potential migration and admixture events, happened along the political changes in the Altai.

Ancient DNA was extracted and sequenced in a dedicated clean laboratory facility of the Institute of Archaeogenomics, RCH, following well-established ancient DNA workflow protocols. NGS sequencing was performed on Illumina platforms. Y haplogroup and mtDNA haplogroup predictions and genome wide data analyses were done using state-of-the-art bioinformatics tools. Preliminary biostatistical results suggest that East Eurasian ancestry increased significantly in the Altai region in the period after the establishment of Turkic Khaganate in 552 CE, accompanied by the occurrence of new paternal (Y chromosomal) haplogroups, previously not present in the area.

Effect of *Lactobacillus* inoculation on the diversity of yeast communities in the experimentally *Aspergillus flavus* contaminated corn silages

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The presence of *Lactobacillus* species has an emerging role in silages. *Lactobacillus* population affects not only the consistence and quality of the silage, but also has an effect on the composition and the levels of microbiom.

In our research, we investigated the effect of the high dose of *Lactobacillus* treatment on the community of yeasts. In the beginning of the experiment and after an 8-week-long maturing we quantitatively cultivated the samples from the silage which was inoculated with the mixture of 3 strains of *Lactobacillus* on agar plates containing Rose Bengal and chloramphenicol. In the starting mixture, the majority of the yeast community originated from the phylloplane, the number of the yeasts was $4.8 \cdot 10^7$ CFU/g. Despite the high yeast level in the raw material the 8-week-old silage had



one scale smaller number of yeast propagule (2.9×10^6 CFU/gram), which value has decreased after the *Lactobacillus* treatment (7.1×10^5 CFU/gram). Based on the phenotypical attribution and nucleotide sequences of ITS1-ITS4 region and D1/D2 domain of the 100 isolates from the treated silages, we were able to detect 10 different yeast species. In the control silage we detected only 4 species in contrast with the isolated 8 species of the 8-week-old silage and we were able to separate only 3 species in the *Lactobacillus*-treated silage. In the starting mixture, the most common species were *Cystobasidium slooffiae* and *Kregervanrija fluxuum*. The amount of both of these two species significantly decreased and even disappeared in the control silage. The taxonomical diversity of the yeast community of the starting mixture was relatively high (Index of Shannon = 2.27), which amount during the maturing phase significantly decreased (control silage = 1.93; silage treated with *Lactobacillus* = 1.54). Besides the diversity decrease, the *Lactobacillus* treatment significantly changed the community of yeasts. *Saccharomyces cerevisiae* and *Pichia kudriavzevii* were the most common species in the control silage, while in the lactobacillus-treated silage *Pichia fermentans* also became abundant beside *Saccharomyces cerevisiae*.

Based on our results we can conclude that the lactobacillus treatment, which is widespread in silage practice, has a moderate effect on yeasts considered as autochthon microorganisms in silages.

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Side-specific cell surface biotinylation to reveal the apico-basal distribution and accessible regions of transmembrane proteins in polarized cells

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Cell polarity is an asymmetric distribution of cellular compartments and their components (such as proteins, phospholipids and different complexes). One of the most extensively studied form is the apical-basal polarity of epithelial cells, which plays a crucial role in adhesion, migration or transcellular transport functions of these cells.

Transmembrane proteins (TMPs) are an important class of proteins, that are in different distribution in the apical and basolateral side of the polarized cells. Experimental polarity information about different transmembrane proteins is scattered in the literature and limited to a few hundred proteins based on PolarProtDb (<http://polarprotdb.ttk.hu>). Most of the currently existing techniques provide polarity data about single proteins and are often time-consuming and the interpretation of the results are often not straightforward, especially in those cases, when the primary sequence of the protein was modified and the localization or function of these proteins were changed.

To overcome these technical and interpretation difficulties, here we presented a rapid, high-throughput method that is able to identify polarity data for hundreds of transmembrane proteins at the same time in their native environment. In our experiments we would like to characterize the distribution and accessible segments of different transmembrane or other (such as secreted, anchored) proteins in the polarized MDCK (Madin-Darby Canine Kidney) cells using side-specific cell surface labelling by membrane-impermeable, primary amine-reactive biotinylation agent. **The main steps of the process:** The cells were grown on transwells until formed a fully confluent monolayer, then biotinylated selectively the apical or basolateral surface, after that lysed and plasmamembrane fractions were prepared. The preparations were solubilized, digested and resulted peptides purified on affinity column, finally the eluted labelled peptides were sequenced and quantified by tandem mass spectrometry.

The intensity of resulted peptides/proteins from the different sides of the cells can be used as polarity data in PolarProtDb and labelled sites of the peptides can be used as extracellular constraints in our group developed CCTOP algorithm to refine the topology prediction of these TMPs. Moreover, analysis of the resulting data could pave the way to a better understanding of cell polarization and the molecular mechanisms that maintain it.



Placental galectins: key regulators of innate and adaptive immune responses in pregnancy

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Galectins are β -galactoside binding soluble proteins belonging to the lectin family, with the common property of being able to specifically bind glycoconjugates containing beta-galactoside carbohydrates. They are very diverse in terms of their function and expression and are involved in numerous physiological processes, including apoptosis, cell proliferation, cell adhesion, and cytokine production. Placental galectins such as galectin-13 (gal-13) and galectin-14 (gal-14) are solely expressed by the placenta and contribute to maternal-fetal immune tolerance by promoting apoptosis of activated T cells and polarization of neutrophils towards an immune-regulatory phenotype. Beside placental galectins play a role in proper trophoblast invasion and vascular development of the placenta and their decreased placental expression is linked to pregnancy complications including preeclampsia and miscarriages. However, our knowledge on the immunoregulatory role of placental galectins is still not complete. The purpose of this study was to examine the effects of recombinant gal-13 and gal-14 on cell viability, apoptosis, and cytokine production of peripheral blood mononuclear cells (PBMCs), as well as the signaling pathways involved. Here, we show that gal-13 and gal-14 bind to the surface of non-activated PBMCs (monocytes, NK cells, B cells, and T cells) and increase their viability while decreasing the rate of apoptosis without promoting cell proliferation. We also demonstrate that gal-13 and gal-14 induce the production of IL-8, IL-10, and IFN- γ cytokines in a concentration-dependent manner in PBMCs. The parallel activation of Erk1/2, p38, and NF- κ B signaling in PBMCs, as indicated by phosphorylation, shows that these pathways are involved in the regulation of galectin-affected immune cell functions. These findings provide further evidence on how placenta-specific galectins assist in the establishment and maintenance of a proper immune environment during a healthy pregnancy.

Climate change and diet shift in invasive mysid species

Varsha Rani

The unprecedented increase in mean global temperature has a huge effect on the physiology of organisms, especially in ectotherms. Recent studies showed that omnivorous ectotherms increase their uptake of plant-based carbohydrate-rich diet to accommodate their shifting metabolic demands. This comes with a shift in diet preference from a more carnivorous to a more herbivorous diet. *Limnomysis benedeni* is an omnivorous invasive aquatic predator that has successfully spread into the main river systems and adjacent lakes in Europe from its Ponto-Caspian native range. Its high population growth rates and diet largely based on pelagic resources suggest potentially high impacts on plankton community composition and functioning. The current study aimed to test the change in diet preference of *Limnomysis benedeni* using mixtures consisting of an autotrophic prey (*Cryptomonas* sp.) and three species of zooplankters with increasing size (a rotifer; *Brachionus calyciflorus* sp., and a cladoceran; *Daphnia magna* sp. 24-36 hours old juveniles). Ten-hour grazing experiments at three temperatures (16°C, 23°C, 30°C) were conducted with adult mysids. The results show significantly higher consumption of autotrophic food than heterotrophic with



increasing temperature thereby supporting that *Limnomysis benedeni* has a temperature-dependent diet preference. This has important implications for the structure and functioning of communities and ecosystems, serving as a basis for future studies with more natural settings.

Trait-based responses of zooplankton communities to different warming scenarios

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The prominent aspects of global climate change are the gradual increase in annual mean temperatures and an increased frequency and intensity of extreme heatwaves, which threaten freshwater ecosystems, including shallow lakes. These change stressors can differently affect the taxonomic and trait composition, as well as the functioning of plankton communities, but comparative studies are largely lacking. Therefore, we conducted a four-week-long mesocosm experiment to test the effects of constantly increased temperatures – CW (+3°C) and recurring heatwaves – HW (+6°C) in a setup where both treatments received an identical energy input. As a model community, we used the natural plankton of the large shallow Lake Balaton. We found significantly higher Chl *a* concentrations in the HW treatment than in the CW and control treatments, during and after the first simulated heatwave. Besides, we found higher zooplankton biomass and a lower ratio between Rotifera and Copepoda than in the HW and control treatments. We also found that HW had a significant effect on zooplankton traits, specifically it was associated with small-bodied taxa. Our findings show that HW can have a more immediate effect on biodiversity and trophic interactions, while the effects of CW become only visible in the longer term, in line with the concept of press and pulse disturbances.

Testing the effects of captive environment on individual variation in feeding behaviour of juvenile Hungarian meadow vipers (*Vipera ursinii rakosiensis*)

Bálint Halpern

The Hungarian meadow viper (*Vipera ursinii rakosiensis*) conservation program started captive breeding of the species in 2004. Over the past years altogether over 4300 vipers were born in the Hungarian Meadow Viper Conservation Centre and over 750 vipers were released to ten habitats in Kiskunság and Fertő-Hanság National Parks in Hungary. As captive environment potentially affects development of individual behavior and consequently, future survival of reintroduced vipers, we aimed to test effect of various environmental variables with the intention to fine-tune future captive breeding techniques. Using 32 juvenile vipers, representing 10 families, we assessed whether different prey items, origin and vipers' sex and structural diversity of captive environment affect individual feeding behavior. Vipers were kept in individual terraria and were offered crickets (*Acheta domestica* and *Gryllus assimilis*) every 4 days over a 6-week period. Feeding behavior was observed in a standardized way: we recorded individual timing of the first reaction and frequency of strike(s), holding and swallowing the prey item. According to first results, behavioral variables showed no or low repeatability indicating the lack of individual behavior strategies, which might root in the simple environment. Sexual difference was observed as males were generally faster in the first strike. We also detected feeding preference for house crickets over banana crickets, which is probably in line with substantial differences observed between prey species.



A fluorescence-based competitive counterflow assay to investigate multispecific OATPs involved in ADME-Tox

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Organic anion transporting polypeptides (OATP) classified within the Solute Carrier for Organic Anions (SLCO) family, are Na⁺- and ATP-independent exchangers of large (>300 Da) amphipathic molecules. Endogenous substrates of OATPs are bilirubin, bile acids, thyroid and steroid hormones and prostaglandins. Moreover, multispecific OATPs also recognize xenobiotics, including drugs (antivirals, chemotherapeutics, anti-inflammatory agents, antibiotics and statins). Multispecific OATPs are OATP1A2, OATP1B1, OATP1B3 and OATP2B1, recognizing and transporting a wide variety of chemically diverse molecules. These OATPs are key players of intestinal absorption (OATP2B1), hepatic clearance (OATP1B1 and OATP1B3) and blood to brain penetration (OATP1A2, OATP2B1) of their drug substrates. Co-administration of OATP substrates and inhibitors may lead to altered pharmacokinetics or even toxicity. Therefore, investigation of at least the hepatic OATPs, 1B1 and 1B3 during drug development is recommended by international regulatory agencies (EMA, FDA and PMDA). Radioactive and fluorescent probes of OATPs are usually applied in indirect assays, measuring the inhibition of probe uptake, but not distinguishing between substrates and inhibitors. However, recently the Competitive Counterflow (CCF) assay was developed to differentiate between the competitive inhibitor substrates and non-transported inhibitors of OATPs. However, a fluorescence-based CCF was not available for OATPs, 1A2, 1B3 and 2B1. In my work, using previously identified fluorescent substrates of these OATPs, I developed the CCF for OATP1A2 with sulforhodamine 101 as a probe, and for OATP1B1, OATP1B3 and OATP2B1 using pyranine as an indicator. The CCF was validated by testing the effect of known substrates and inhibitors. Using the newly developed CCF, we identified several novel OATP substrate and inhibitor molecules, of which pentamidine, an FDA-approved anti-protozoal agent administered in African trypanosomiasis, Leishmaniasis, and in Pneumocystis pneumonia seemed to be a specific OATP1A2 substrate. Hence, OATP1A2 may contribute to pentamidine accumulation in the brain. This novel fluorescent method may allow the high throughput screening of OATP's interaction with the analysis of large molecular libraries.

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Genetic study of modern and medieval Székely Land

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We examine DNA of ancient and modern-day Székely individuals from the Udvarhelyszék- region, in order to gather genetic information from Hungarian-speaking long-time residents of Transylvania. The aim of the research is to unveil genetic relationships between ancient and recent populations not only from the Carpathian Basin but from the historically relevant areas. We generated high quality uniparental and whole-genome data, aiming to monitor the continuity and relationships of the region's populations.

The ancient sample set we analysed contains nearly a hundred medieval (12- 16. century) individuals from the region of Székelyudvarhely, mostly from church-related burials, whereas the modern sample set consists of 115 Hungarian-speaking Székely individuals originating from isolated villages of the same region.

We analyzed maternal lineages with whole mtDNA next-generation sequencing, compared the results to published modern and ancient mitogenomic datasets. The majority of the modern and medieval Székely population belongs to European mtDNA haplogroups, but the presence of a small number of Asian-derived haplotypes is evident. Phylogenetic analyses confirmed the presumed eastern origin of certain lineages and in some cases, they can be linked to ancient DNA data of early Hungarians. Among the Y-chromosome haplogroups mostly the European-related ones are dominant and a few samples are assigned to haplogroups with Asian-origin, which may reflect some level of Central Asian connections.

The whole genome results demonstrate the homogeneity and continuity of the medieval Székely population up to the modern times. This study is funded by the NKFIH FK-127938 research grant.



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