4th CONGRESS OF CROATIAN GENETICISTS

with international participation

BOOK OF ABSTRACTS

Krk, Island of Krk, Croatia
September 26-29, 2018

CroatianGeneticSociety

www.genetika.hr

2018

4th CONGRESS OF CROATIAN GENETICISTS

with international participation

BOOK OF ABSTRACTS

Krk, Island of Krk, Croatia
September 26-29, 2018

CroatianGeneticSociety

www.genetika.hr

2018

4th Congress of Croatian Geneticists with International Participation

Book of Abstracts

Published by

Croatian Genetic Society
Zagreb, Croatia

Venue

Hotel Valamar Koralj
Krk, Island of Krk, Croatia
26-29 September 2018

Editors

Hrvoje Šarčević
Đurđica Ugarković
Dušica Vujaklija
Ivan Krešimir Svetec
Marina Svetec Miklenić

On-line publication

www.HGDcongress.eu
www.genetika.hr

ISBN 978-953-57128-1-7

1st internet edition, published September 24th, 2018

Organizing committee

Hrvoje Šarčević, president
Anamarija Štafa, secretary
Jasna Franekić
Grozdana Kuleš
Ivan Krešimir Svetec
Marina Svetec Miklenić
Đurđica Ugarković
Dušica Vujaklija

Scientific committee

Đurđica Ugarković, president
Višnja Bačun-Družina
Hrvoje Fulgosi
Verica Garaj-Vrhovac
Davorin Kajba
Ivica Rubelj
Ivan Krešimir Svetec
Hrvoje Šarčević
Zlatko Šatović
Dušica Vujaklija
Ksenija Zahradka

General sponsors

Eppendorf AG.
INEL – medicinska tehika d.o.o.
Labena d.o.o.

Sponsors

CruX d.o.o.
Diagnostica Skalpeli d.o.o.
Kemomed d.o.o.

Supported by

Ministry of Science, Education and Sports

CONTENTS

PROGRAMME

Wednesday, 26.09.2018.

14:00 – 19:00 Registration

19:00 – 20:30 CONGRESS OPENING CEREMONY
Opening lecture: **Roberto Kolter** (USA): **A journey through the microbial world**

20:30 – 22:00 Welcome party

Thursday, 27.09.2018.

08:30 – 11:30 MICROBIAL GENETICS AND ECOLOGY SESSION
Chairs: Višnja Bačun-Družina, Dušica Vujaklija

08:30 – 09:00 IL1 - **Franz Klein** (Austria): Coordinated cuts remove small chromosome pieces in meiosis
09:00 – 09:30 IL2 - **Ivan Matić** (France): Transcription-translation coupling: the Achilles' heel of the bacterial chromosome
09:30 – 10:00 IL3 - **María Mercedes Zambrano** (Columbia): Exploring microbial ecology to expand product discovery
10:00 – 10:15 OP1 - **Jelena Repar** (Croatia):Elevated rate of genome rearrangements in radiation resistant bacteria
10:15 – 10:30 OP2 - **Davor Zahradka** (Croatia): RecBCD- RecFOR-independent pathway of homologous recombination in *Escherichia coli*

10:30 – 11:00 Coffee break and poster viewing

11:00 – 11:15 OP3 - **Marina Svetec Miklenić** (Croatia): Recombinogenicity of perfect palindromes and quasipalindromes with short central spacers *in vivo*
11:15 – 11:30 OP4 - **Ivana Ivančić Baće** (Croatia): Interplay of CRISPR adaptation, recombination and host nucleases in *Escherichia coli*

11:30 – 12:45 MICROBIAL AND VIRAL PATHOGENS SESSION
Chairs: Ivan Matić, Ksenija Zahradka

11:30 – 12:00 IL4 - **Yossef Av-Gay** (Canada): Modulators of innate immunity as novel host derived therapies against *Mycobacterium tuberculosis*
12:00 – 12:30 IL5 - **Igor Jurak** (Croatia): MiRNAs - tiny but mighty regulators of virus replication
12:30 – 12:45 OP5 - **Momir Futo** (Croatia): Specificity of microbiota-mediated immune priming in insects

12:45 – 14:30 Lunch break

14:30 – 16:30 BIODIVERSITY AND PLANT BREEDING SESSION
Chairs: Hrvoje Šarčević, Zlatko Šatović

14:30 – 15:00 IL6 - **Fred van Eeuwyjk** (Netherlands): New phenotyping techniques offer an opportunity for improved modelling of genotype by environment interactions

15:00 – 15:30 IL7 - **Ian Mackay** (UK): Approaches to the low cost introduction of genomic selection in plant breeding programmes

15:30 – 16:00 IL8 - **Marcos Malosetti** (Netherlands): QTL detection using actual breeding populations

16:00 – 16:15 OP6 - **Želimir Kurtanjek** (Croatia): "DecisionGS" algorithm for optimization of genetic potential; Case of improvement of common wheat *Triticum aestivum*

16:15 – 16:30 OP7 - **Domagoj Šimić** (Croatia): Natural genetic variation of photosynthetic performance in the context of molecular plant breeding

16:30 – 17:00 Coffee break and poster viewing

17:00 – 18:45 PLANT GENETICS SESSION
Chairs: Hrvoje Fulgosi, Jasna Puizina

17:00 – 17:30 IL9 - **Hrvoje Fulgosi** (Croatia): 15 years of photosynthesis research in Croatia – cracking the TROL

17:30 – 18:00 IL10 - **Nenad Malenica** (Croatia): Ku70-tagged CENH3 fusions induce haploids in *Arabidopsis thaliana* and reveal a CENH3 functionality gradient

18:00 – 18:15 OP8 - **Dunja Leljak Levanić** (Croatia): AtBPM1 protein is involved in RNA-directed DNA methylation in plants

*18:15 – 18:30 Promotional lecture - **Pavlina Mueller Kroupa** (Eppendorf Austria, GmbH): Leachables; substances that escape the laboratory plasticware and influence the reactions*

*18:30 – 18:45 Promotional lecture **– Josip Brajković** (Labena d.o.o.): Droplet Digital PCR (ddPCR): Introduction to technology and applications*

18:45 – 19:45 Poster discussion

Friday, 28.09.2018.

09:15 – 10:30 ANIMAL GENETICS SESSION
Chairs: Jasna Franekić, Ivica Rubelj

09:15 – 09:45 IL11 - **Siniša Volarević** (Croatia): The role of ribosomal proteins L5 and L11 in tumor suppression

09:45 – 10:15 IL12 - **Vlatka Zoldoš** (Croatia): Epigenetic regulation of IgG glycosylation using CRISPR/dCas9-based tools

10:15 – 10:30 OP9 - **Ivica Rubelj** (Croatia): Skin homeostasis and regeneration

10:30 – 11:00 Coffee break

11:00 – 12:30 OMICS AND COMUTATIONAL BIOLOGY SESSION
Chairs: Antonio Starčević, Fran Supek

11:00 – 11:30 IL13 - **Fran Supek** (Spain): Differential DNA repair across human chromosomes shapes somatic mutation landscapes

11:30 – 12:00 IL14 - **Antonio Starčević** (Croatia): Semantic ion vectors - deep learning applied to mass spectrometry

12:00 – 12:15 OP10 - **Vesna Boraska Perica** (Croatia): Apoptosis-antagonizing transcription factor AATF and chromatin-remodelling SMARCA2 are associated with thyroid volume in Hashimoto's thyroiditis patients

12:15 – 12:30 OP11 - **Luka Brčić** (Croatia): Genome-wide association analysis suggests novel loci underlying thyroid antibodies in Hashimoto's thyroiditis

13:00 – 18:00 Excursion (optional) - lunch included

21:00 – 24:00 Congress dinner

Saturday, 29.09.2018.

09:00 – 10:30 POPULATION GENETICS AND EVOLUTION SESSION
Chairs: Nevenka Meštrović, Tomislav Domazet Lošo

09:00 – 09:30 IL15 - **Tomislav Domazet Lošo** (Croatia): Evolution of multicellularity and genome complexity

09:30 – 10:00 IL16 - **Đurđica Ugarković** (Croatia): Modulation of gene expression by satellite DNA: evolutionary implications

10:00 – 10:15 OP12 - **Nevenka Meštrović** (Croatia): Root-knot nematodes as a model for centromere (epi)genomics and satellitome research

10:15 – 10:30 OP13 - **Helena Bilandžija** (Croatia): The mechanisms and causes of pigmentation loss in cave adapted animals

10:30 – 11:00 Coffee break

***11:00 – 12:30* CONGRESS CLOSING CEREMONY**

Closing lecture: **Miroslav Radman** (Croatia): **From the genetics of birth to the proteomics of death**

ABSTRACTS

OPENING LECTURE

A journey through the microbial world

Kolter Roberto

Department of Microbiology, Harvard Medical School, Boston, MA, USA

E-mail: rkolter@hms.harvard.edu

Although they are largely invisible, microbes are present in every corner of the biosphere. As a result they have a huge influence on our daily lives, often in unexpected ways. Starting from the familiar surroundings of our homes, Professor Kolter will guide the audience through a virtual tour in which he will analyze in detail the remarkable and wonderful universe of microbes. From the invisible chefs who prepare many of our favorite foods, to the microbes that inhabit our bodies and keep us healthy, to the innumerable ways in which microbes have molded the natural history of our planet, the journey will make it clear that planet Earth is really The Planet of the Microbes.

CLOSING LECTURE

From the genetics of birth to the proteomics of death

Radman Miroslav

MedILS – Mediterranean Institute for Life Sciences, Split, Croatia

Ageing is not a disease, but it causes (or shares the cause of) age-related diseases predisposed by particular inborn oxidation-sensitive "silent" mutations (protein polymorphisms). Such inborn "weak links" become phenotypic with increasing age/oxidation, whereas inborn loss-of-function (knock-out) mutations in the same genes cause disease at birth, called syndromes.

Ageing is considered here as the snowballing phenotype of increasingly damaged, progressively malfunctioning proteomes, and particular silent mutations (polymorphisms that sensitize proteins to oxidative damage) as inborn predispositions to particular age-related diseases. Malfunction of damaged proteins *initiates* latent diseases at the genome level. Long latency of early stage age-related diseases and conditions is maintained by *cellular parabiosis,* which is a phenotypic suppression of cellular dysfunction by inter-cellular molecular traffic (ranging from ions and metabolites to entire organelles). Chronic inflammation *promotes* the onset of pathologies by the interruption of cellular parabiosis via activated extracellular proteases.

IL1

Coordinated cuts remove small chromosome pieces in meiosis

Klein Franz*, Prieler Silvia, Chen Doris, Mayrhofer Elisa, Huang Lingzhi

Department of Chromosome Biology, Max F. Perutz Laboratories, University of Vienna, Vienna, Austria

**E-mail: franz.klein@univie.ac.at*

According to current knowledge sexual reproduction is accompanied by meiotic recombination, induced by DNA breaks (DSBs) catalyzed by Spo11, a eukaryotic relative of TopoVIA from archebacteria. During the breakage reaction, Spo11 becomes covalently attached to the 5'-termini of the break hotspots, a reaction that is reversible in other topoisomerases, but for Spo11 proceeds by releasing it with a single stranded, covalently attached oligo to free the 3'-end for further homologous repair. As a result, Spo11-attached oligos can be isolated from meiotic cells and can be mapped back to their origins.

Here we present our dogma breaking discovery, following our observation, that not only Spo11-oligos can be purified from meiotic extracts, but also dsDNA attached to Spo11. We characterized these dsDNA fragments and showed that initiation of recombination is not exclusively mediated by DSBs, but also by removing chromosomal pieces through the formation of coordinated DSB pairs (double-DSBs or dDSBs). dDSBs occur both in wild type meiosis and in certain mutants. Their recovery is facilitated in repair mutants, which don't remove Spo11 from DNA termini – and which consequently do not degrade dDSB fragments by that mechanism. We have measured the halflife of dDSB fragments in wt and resection deficient mutants. Much of the differences between isolated fragments from wt and mutants can be explained by the differences in their stability.

Several novel conclusions derive from our observations. Most importantly, our results show that two breaks can be carried out in coordination – as their preferred distances are spaced by full DNA turns. Evidence points to a role of DNA secondary structure in the formation of dDSBs and perhaps in the formation of single DSBs as well. We also show that not all DSBs are part of hotspots, a substantial part of them being quite dispersed. In addition, the gaps resulting from dDSBs contribute to meiotic gene conversion, again, challenging the conventional view that gene conversion may exclusively arise from heteroduplex correction. If gaps are subjected to non-homologous repair, deletions with serious consequences could occur, especially in repair compromised individuals. In addition, excised pieces could transpose to other places, if NHEJ pathway would be allowed to contribute to repair.

IL2

Transcription-translation coupling: The Achilles' heel of the bacterial chromosome

Matić Ivan

INSERM U1001- Faculté de Médecine Paris Descartes Université Paris Descartes, Sorbonne Paris Cité, France

E-mail: ivan.matic@inserm.fr

Most bacterial species possess multiple rRNA operons, which are among the most highly transcribed genomic loci during growth. It is generally assumed that multiple rRNA operons copies are needed for rapid growth. Bacterium Escherichia coli has seven rRNA operons, but they are not fully saturated with RNAP even at highest growth rates. In addition, deleting up to three rRNA operons does not show significant impact on growth rate. Consequently, the question is what for rRNA operons redundancy has evolved? We examined how deletions of rRNA operons impact DNA replication. For this, we used strains deleted for 1 to 6 rRNA operons. We found that DNA replication is severely impacted in cells with ≥4 rRNA operon deletions. In these mutants, we observed strong SOS induction, as well as increased: quantity of DNA strand breaks, reactive oxygen species production, mutagenesis and mortality rates. We showed that the primary cause of these perturbations is blockage of the DNA replication forks by R-loops. R-loops are generated by the disruption of transcription-translation coupling due to the reduced number of ribosomes. We also observed that the modulation of the translation capacity by the environmental factors impact genome stability of the wild-type cells by the same mechanism. Therefore, rRNA operon redundancy, besides assuring rapid growth, can be considered to be the genome stability insurance during perturbations induced by nutrient availability and stress.

IL3

Exploring microbial ecology to expand product discovery

Zambrano Maria M.

Molecular Genetics, Corpogen Research Institute, Bogotá, Colombia

E-mail: mzambrano@corpogen.org

Despite their efficacy against infectious diseases, antibiotics are rapidly becoming obsolete due to the rise of drug resistant microorganisms that pose a global threat to human health. The high mountain Páramo ecosystem of the South American Andes, with its high biological diversity and strategic environmental role, offers a unique opportunity for studying microbial communities and uncovering novel microbial products. We therefore studied soil, plant and lichen- associated communities with the aim of exploring these microbiomes and their metabolic potential. All niches analyzed contained highly diverse communities and microorganisms that could be further exploited in the lab. While plant-associated isolates were capable of withstanding extreme environmental conditions, such as temperature and UV exposure characteristic of these mountain ecosystems, several isolates from soil and lichen communities produced antimicrobials. These strains are being further analyzed using genomics tools to determine their novelty and molecular biology approaches to optimize production of particular metabolites.

IL4

Modulators of innate immunity as novel host derived therapies against *Mycobacterium tuberculosis*

Peña-Díaz Sandra, Poirier Valerie, Chao Joseph, Av-Gay Yossef *

Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada

**E-mail: yossi@mail.ubc.ca*

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB) infects, survives, and replicates in the phagosomes of host alveolar macrophages. Mtb interacts with macrophage proteins to subvert the host antimicrobial mechanisms and thus evade the immune response. For instance, our lab has shown that PtpA, a tyrosine phosphatase secreted by Mtb, facilitates infection by interfering with the macrophage proteins V-ATPase, VPS33B, and GSK3α. Furthermore, while residing in the phagosome, Mtb is isolated from many antibiotics used for treatment of infection, thus creating a challenge for anti-TB drug discovery. Therefore, novel approaches that consider the intracellular nature of Mtb and its interactions with the host are a promising new avenue for drug discovery.

In our studies, we used an array of genetics, biochemical and immunological methods to examine the interaction of PtpA with selected macrophage targets. We discovered that PtpA interacted *in vivo* with GSK3β. Additionally, we took a host directed therapeutic approach and targeted GSK3β in Mtb-infected macrophages to determine whether the disruption of the host protein restricts the growth of Mtb. We performed an intracellular high-throughput screening (HTS) of a GSK3 inhibitor library and identified 32 compounds that can kill intracellular Mtb in a percentage higher than 70% at a compound concentration of 20 μM. Selected drug candidates showed dose dependent activity against Mtb at low micro Molar concentrations. Our data highlights the importance of the macrophage for the growth of Mtb and holds a promising strategy for the identification of potential anti-TB drugs with a new mechanism of action.

.

IL5

MiRNAs - tiny but mighty regulators of virus replication

Jurak Igor

Department for Biotechnology, University of Rijeka, Rijeka, Croatia

E-mail: igor.jurak@biotech.uniri.hr

Herpes simplex virus 1 (HSV-1) is an important and one of most studied human pathogens. HSV-1 has two distinct replication phases, productive and latent phase. During the productive phase, the virus expresses its genes and generates viral progeny. On the other hand, during the latent phase virus is dormant and largely inactive. The molecular mechanisms that control the fate of virus replication, although intensively studied, are not yet fully understood. The recent discoveries of virus-encoded miRNAs have introduced a novel paradigm for the control of virus gene expression and replication. HSV-1, similar to other herpesviruses, encodes many miRNAs, some of which are differentially expressed during productive and latent phases of infection, indicating their function in these phases. Interestingly, the most abundant HSV-1 miRNAs target important virus genes, indicating a self-suppression to establish and maintain the latent phase of infection. In addition, HSV-1 infection triggers a massive perturbation of cell metabolism, including the levels of cellular miRNAs, to establish an efficient infection. Nonetheless, although the field is very progressive, there are many remaining fundamental questions and technical challenges to address the exact biological roles of miRNAs in virus infection. An overview of the current understanding of functions of miRNAs in HSV-1 infection will be presented.

IL6

New phenotyping techniques offer an opportunity for improved modelling of genotype by environment interactions

van Eeuwijk Fred[1]*, Bustos-Korts Daniela[1], Millet Emilie J.[1], Boer Martin[1], Kruijer Willem[1], Thompson Addie[2], Malosetti Marcos[1], Iwata Hiroyoshi[3], Quiroz Roberto[4], Kuppe Christian[5], Muller Onno[5], Blazakis Konstantinos N.[6], Yu Kang[7,8], Tardieu Francois[9], Chapman Scott[10]

[1]Biometris, Wageningen University & Research Centre, P.O. Box 16, 6700 AC Wageningen, The Netherlands; [2]Department of Plant, Soil and Microbial Sciences, Michigan State University, 1066 Bogue St, East Lansing, MI 48824 USA; [3]Department of Agricultural and Environmental Biology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan; [4]International Potato Center (CIP), P.O. Box 1558, Lima 12, Peru; [5]Institute for Bio-and Geosciences, IBG-2: Plant Sciences, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany; [6]Department of Horticultural Genetics and Biotechnology, Mediterranean Agronomic Institute of Chania (MAICh), Alsylio Agrokipiou, PO BOX 85, 73100 Chania-Crete, Greece; [7]Crop Science, Institute of Agricultural Sciences, ETH Zurich, Switzerland; [8]Remote Sensing & Terrestrial Ecology, Department of Earth and Environmental Sciences, KU Leuven, Belgium; [9]Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux, UMR759, INRA, 34060 Montpellier, France; [10]CSIRO Agriculture and Food, Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, QLD 4067, Australia and School of Agriculture and Food Sciences, The University of Queensland, Gatton, QLD 4343, Australia

**E-mail: fred.vaneeuwijk@wur.nl*

New types of phenotyping tools generate large amounts of data on many aspects of plant physiology and morphology with high spatial and temporal resolution. These new phenotyping data are potentially useful for a better understanding and prediction of complex traits like yield that are characterized by strong environmental context dependencies, i.e., genotype by environment interactions. For an evaluation of the utility of new phenotyping information, we will look at how this information can be incorporated in different classes of genotype-to-phenotype (G2P) models. G2P models predict phenotypic traits as functions of genotypic and environmental inputs. In the last decade, easy access to high-density SNP and sequence information has boosted the development of a class of G2P models called genomic prediction models that predict phenotypes from genome wide marker profiles. The question now is to build G2P models that incorporate simultaneously extensive genomic information alongside with new phenotypic information. Beyond the modification of existing G2P models, new G2P paradigms are required. We present candidate G2P models for the integration of genomic and new phenotyping information and illustrate their use in examples. Special attention will be given to the modelling of genotype by environment interactions. The G2P models provide a framework for model based phenotyping and the evaluation of the utility of phenotyping information in the context of breeding programs.

IL7

Approaches to the low cost introduction of genomic selection in plant breeding programmes.

Mackay Ian[1,2], Powell Wayne[1]

[1] SRUC, Peter Wilson Building, Kings Buildings, West Mains Road, Edinburgh, UK
[2] IMplant Consultancy Ltd., 14 Queens Road, Chelmsford, UK.

Genomic selection is a maturing technology in which large numbers of genetic markers are used to predict traits for individuals that are not themselves phenotyped. Selection is on these predicted values. This process has revolutionised large animal breeding programmes and is being adopted by the major seed companies. Implementation of genomic selection typically requires high marker densities and extensive phenotyping. For many smaller breeding plant breeding programmes, a complete switch to genomic selection can be too expensive to implement and may be judged as too risky since success or failure cannot be judged for a number of years. In this talk we give examples of cases where genomic selection can be used with low marker densities and reduced phenotyping. These limit disruption to current breeding methods which could then be phased out following demonstrable success of genomic selection.

IL8

QTL detection using actual breeding populations

Malosetti Marcos[1]*, Zwep Laura[1], Forrest Kerrie[2], Hayden Matthew[2], van Eeuwijk Fred[1], Dieters Mark[3].

[1] Biometris - Department of Applied Statistics, Plant Sciences Group, Wageningen University & Research, Wageningen, The Netherlands

[2] Agriculture Victoria Research, Agribio, Bundoora, Vic 3083, Australia

[3] School of Agriculture & Food Sciences, Faculty of Science, The University of Queensland, Australia

**E-mail: marcos.malosetti@wur.nl*

The literature is very rich in published QTLs for all sort of complex traits in plants. QTL detection is typically done using experimental populations such as bi-parental and multi-parental crosses (linkage analysis) or diversity panels (GWAS studies). However, detected QTLs have not necessarily found an application in breeding programs. Differences in genetic backgrounds is a common explanation. Here we turn the problem around, and detect QTL directly using a breeding population while conventional selection for the target trait was applied. The underlying aim is to use QTL to shed light about the genome regions under selection. What are the relevant genomic regions driving trait variation? What are the sources of favourable alleles at those regions? We illustrate the approach with a wheat pre-breeding program aimed at pyramiding resistance alleles to Fusarium crown rot into an Australian elite material. The population structure is akin to a MAGIC population but with stages of selection. A significant change in the resistance level was observed in the population, and by a GWAS approach we were able to identify 17 chromosome regions associated with that response. In addition, we were able to trace back the sources of the favourable alleles, and by using graphical displays identified superior lines by their allele constitution. We conclude that QTL detection in a breeding population is a useful exercise providing information that can be used in future selection decisions.

IL9

15 years of photosynthesis research in Croatia - cracking the TROL

Fulgosi Hrvoje *, Vojta Lea, Rac Anja

Laboratory for Molecular Plant Biology and Biotechnology, Division of Molecular Biology, Institute Ruđer Bošković, Bijenička cesta 54, HR-10 000 Zagreb, Croatia

**E-mail: Hrvoje.Fulgosi@irb.hr*

Oxygenic photosynthesis, performed by plants, algae, and cyanobacteria, is arguably the most important autotrophic process on Earth. Photosynthetic light-to-chemical energy conversion involves numerous electron transfer steps that require rapid and streamlined reactions in order to maintain efficacy and prevent energy leaks to undesignated acceptors, mainly oxygen. Flavoenzyme FNR (ferredoxin:NADP+ oxidoreductase) catalyzes the last step of energy transfer in the linear photosynthetic chain, namely electron transfer from ferredoxin to NADP+. FNR utilizes two reduced ferredoxins to produce one molecule of NADPH. This reaction must be performed with high fidelity in order to prevent ferredoxin from passing electrons to other molecular targets or pathways. FNR is tethered to photosynthetic membranes of vascular plants via an integral membrane protein TROL (thylakoid rhodanase-like protein). Another soluble chloroplast protein Tic62 also participates in FNR sequestration. TROL binds FNR at the vicinity of photosystem I and prioritizes linear electron transfer. When FNR is released from the TROL, highly efficient electron sink is activated. This pathway is faster than methyl viologen-mediated ROS propagation and represents so far undescribed mechanism of photosynthesis regulation.

IL10

Ku70-tagged CENH3 fusions induce haploids in *Arabidopsis thaliana* and reveal a CENH3 functionality gradient

Malenica Nenad

University of Zagreb, Faculty of Science, Department of Molecular Biology, Horvatovac 102a, 10000 Zagreb, Croatia

E-mail: nenad.malenica@biol.pmf.hr

CENH3 is a histone found at kinetochores of higher plants promoting proper chromosome segregation during cell division. Being an essential gene, its loss-of-function is embryo-lethal. CENH3 fusion proteins like GFP-CENH3 or GFP-tailswap, CENH3 orthologs or CENH3 point mutations can complement the cenh3 knockout mutant in Arabidopsis thaliana. Furthermore, such complemented lines when cross-pollinated with wild type result in seed abortion and aneuploid or haploid progeny. Recent studies predominantly tested outcomes of crosses where lines with CENH3-modified kinetochores were crossed to lines with wild type kinetochores. In this study, we performed crosses in which each parent had a different non-wild type CENH3 variant. We hypothesized that such crosses would produce haploids with the more functional CENH3 variant. The less functional i.e. "weaker" CENH3 variant would thus be eliminated.

For that purpose, we constructed a series of *A.thaliana* CENH3 fusion proteins with Ku70, a member of the non-homologous end joining DNA repair pathway. Each construct had a full-length CENH3 protein tagged with a differently-sized Ku70 domain. Only two of seven Ku70-CENH3 constructs complemented the cenh3 loss-of-function phenotype and could induce haploids in crosses with wild type. To test our hypothesis, these two novel haploid-inducing lines were cross-hybridized with each other and with the previously characterised GFP-CENH3 and GFP-tailswap variants. In performed crosses, we could indeed identify haploid plants among the progeny in which just one type of modified CENH3 prevailed, whereas the other „weaker" CENH3 variant was lost. This indicated that a functionality gradient of a given set of engineered CENH3 variants could be defined for *A.thaliana*. Additionaly, with the aim to develop a Ku70/Ku80 interaction-based haploid induction system, we tested the capacity of our Ku70-CENH3 fusion proteins to bind their Ku80 partner in a Y2H screen and then tested whether such interaction could induce CENH3 loss of function in planta.

IL11

The role of ribosomal proteins L5 and L11 in tumor suppression

Volerević Siniša

Department of Molecular Medicine and Biotechnology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

E-mail: sinisa.volarevic@medri.uniri.hr

The exposure of cells to various DNA-damaging stressors activates p53 to preserve cellular and genetic stability, preventing tumor development in mice and humans. The critical role of p53 in tumor suppression is supported by the observation that approximately 50% of all human cancers have mutations within this gene. Although it was largely accepted that common to all p53-activating stresses is DNA damage, research over the last decade has shown that disruption of ribosome biogenesis promotes binding of several distinct ribosomal proteins (RP) to Mdm2 resulting in inhibition of its E3 ubiquitin ligase activity towards p53. As a result, p53 accumulates within the cell and transcriptionally activates genes that regulate apoptosis, cell cycle checkpoints, metabolism and senescence. We have recently shown that RPL5 and RPL11 play a major role in p53 activation upon ribosome biogenesis impairment. Given the importance of RPL5 and RPL11 in p53 activation, we initiated a project to understand their role in the development of malignant tumors. Our efforts led to the identification of a large number of cancer-associated mutations in the RPL5 and RPL11 genes. Our current research focuses on the characterization of functional significance of these newly identified mutations in p53 regulation and tumorigenesis.

IL12

Epigenetic regulation of IgG glycosylation using CRISPR/Cas9-based tools

Zoldoš Vlatka

University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Horvatovac 102A, 10 000 Zagreb, Croatia

E-mail: vzoldos@biol.pmf.hr

Aberrant IgG glycosylation is a feature of chronic inflammatory diseases. Alternative glycosylation can convert IgG from anti-inflammatory to pro-inflammatory antibody, and as such is involved in inflammation. Our efforts are focused on revealing mechanisms underlying the process of IgG glycosylation which occur during B cell development into plasma cells, secretors of IgG. Several genes were previously associated by genome wide association studies with both IgG glycosylation and inflammatory diseases. In order to investigate their functional roles, we applied CRISPR/Cas9-based tools to manipulate their regulation in model cell lines and analyse subsequent phenotypes. For this purpose, we designed and validated dCas9-DNMT3A and dCas9-TET1 fusions for targeted DNA methylation and demethylation, as well as dCas9-KRAB and dCas9-VPR fusions for direct gene silencing and activation. We fused catalytic domains of DNMT3A and TET1 to catalytically inactive Cas9 (dCas9) orthologs from *S. aureus* and *S. pyogenes*, so that we can manipulate methylation in both directions within the same cell. We were able to demonstrate increase and decrease of methylation level in two different pairs of candidate genes within the same cell up to the level of 60% at the targeted CpG sites. Analogous fusions were made using KRAB and VPR domains to the dCas9 orthologs and both pair of genes were successfully manipulated within the same cell. Our newly developed "EpiToolbox" represents an excellent molecular tool for a range of applications in gene regulation and epigenome editing because it is highly modular and easily reconfigured. It can be applied immediately to biological questions related to regulation of IgG glycosylation, inflammation, and in general to molecular mechanisms involved in human complex diseases.

IL13

Differential DNA repair across human chromosomes shapes somatic mutation landscapes

Supek Fran [1]*, Lehner Ben [2,3,4]

[1] *Institut de Recerca Biomedica (IRB Barcelona), Barcelona Institute of Science and Technology, Barcelona, Spain.*
[2] *Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology, Barcelona, Spain.*
[3] *Universitat Pompeu Fabra (UPF), Barcelona, Spain.*
[4] *Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.*

**E-mail: fran.supek@irbbarcelona.org*

Cancer genome sequencing has revealed considerable variation in somatic mutation rates across the human genome, with mutations accumulating faster in heterochromatic late replicating regions and slower in early replicating euchromatin. We identify variable DNA mismatch repair (MMR) as the basis of this domain-scale variation. While regional mutation rates are broadly stable between tumors, there are systematic differences that accurately reflect the cancer cell-of-origin, related to changes in replication timing and gene expression. However, mutations arising after the inactivation of MMR are no longer enriched in late replicating heterochromatin relative to early replicating euchromatin. This implicates differential DNA repair as the primary cause of the large-scale regional mutation rate variation across the human genome (Supek and Lehner, 2015 Nature).

At the smaller, sub-megabase scale, we find that differential MMR also shapes gradients in mutation rates across gene bodies. In particular, we examined clustered mutation patterns across >1,000 tumor genomes and identified a novel and prevalent mutational signature matching the error-prone DNA polymerase eta (POLH). Such clustered mutations occur in tumors associated with carcinogen exposure and they target H3K36me3-marked chromatin, commonly found at 3' ends of transcribed genes. In the absence of carcinogens however, the H3K36me3 regions have a considerably lower mutation rate. This is because the canonical, error-free MMR is normally targeted towards the H3K36me3 histone mark, protecting active genes from alterations. Carcinogens therefore not only increase mutation burden, but can also redistribute mutations to the more important regions of the genome by activating error-prone DNA repair. We predict this can contribute a substantial mutation load in many tumors such as melanoma, gastrointestinal tract and lung cancers, with high potential to yield driver mutations (Supek and Lehner, 2017 Cell).

IL14

Semantic Ion Vectors - deep learning applied to mass spectrometry

Starčević Antonio[1]*, Zućko Jurica[1], Damir Oros[1], Diminić Janko[1], Cindrić Mario[2]

[1]Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia
[2]Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

**E-mail: astar@pbf.hr*

Protein mass spectrometry is the dominant method used for protein characterization. Peptide mass fingerprinting (PMF) is a phrase given to application of mass spectrometry (MS2) to protein identification. In case of PMF, peptides do not fragment sequentially and the process is not entirely random, with some fragmentations being more preferred over others. The resulting fragmentation spectrum captures fragment ions.

A novel approach which relies on Deep learning techniques to capture distributed representations of ions into Paragraph Vectors using unsupervised algorithm was employed to predict subset of informative b and y ions and distinguish them from the “noise” ions. Unlike Word embedding this approach has taken a different turn. Numerical data were turned into words (tokens), which were grouped into sentences and afterwards embedded using Paragraph Vectors. Several tokenization schemes have been implemented and performance has been tested under different parameters. Sole fitness criteria used was the performance of simple binary classification of into informative “ b-y ions” and “noise” (the rest). The best performing combination produced Semantic Ion Vectors, which were fed into classifier of choice and served as a model to make predictions.

Resulting Semantic Ion Vectors can be used in variety of classification tools and provide accurate predictions of informative ions. Tokenization method developed, efficiently reduced complexity of this task. Intensity patterns and other information, commonly used as a set of rules in a synthetic manner (more suited to organic brain) were replaced by simple tokens and embedded into Paragraph Vectors.

IL15

The evolution of multicellular complexity

Domazet-Lošo Tomislav

Ruđer Bošković Institute, Bijenička cesta 54, HR-10000 Zagreb, Croatia

E-mail: tdomazet@irb.hr

The evolutionary life cycle of genes is powered by the process of gene birth and death. This mechanism directly impacts the genome content by generating the gain and loss of entire gene families; a process which dynamic within the phylogeny of eukaryotes is largely unclear. To trace gene family gain and loss one can in principle use phylostratigraphic approach. In this talk, I will show how we get a comprehensive and synchronized estimate of gene family gain and loss across phylogeny of eukaryotes using hundreds of eukaryotic and prokaryotic genomes. These results allowed us to estimate rates of gene family gain and loss over evolutionary time and to reconstruct the ancestral genomes at critical points of multicellular evolution. To achieve these goals, we deployed novel computational strategy within the phylostratigraphic framework. Our next step is to link the gene-and-loss patterns to the evolution of phenotypes.

IL16

Modulation of gene expression by satellite DNA: evolutionary implications

Ugarković Đurđica[1]*, Feliciello Isidoro[1,2], Akrap Ivana[1]

[1]Department of Molecular Biology, Ruđer Bošković Institute, Bijenička 54, HR-10000 Zagreb, Croatia
[2]Dipartimento di Medicina Clinica e Chirurgia, Universita' degli Studi di Napoli Federico II, via Pansini 5, I-80131 Napoli, Italy

**E-mail: Djurdjica.Ugarkovic@irb.hr*

Non-coding repetitive DNAs constitute a considerable portion of most eukaryotic genomes and their function is intensively investigated. Here we analyse a gene-modulatory role for tandemly repeated satellite DNA which is the major building element of pericentromeric and centromeric heterochromatin in many eukaryotes. We use as a model system the beetle *Tribolium castaneum* which has a major satellite DNA preferentially located in pericentromeric heterochromatin but satellite repeats are also dispersed in the vicinity of protein-coding genes within euchromatin. Our results demonstrate for the first time the role of satellite DNA in the modulation of protein-gene expression after long-term heat stress, and reveal the molecular mechanism of their gene-modulatory activity. The influence of satellite DNA on neighbouring genes is epigenetic in nature, based on the transient heterochromatin formation at satellite repeats and their proximal regions, which is induced by specific changes in the environment. Differences in the pattern of distribution of satellite repeats contribute to gene expression diversity among *T. castaneum* strains after long-term heat stress. Based on this, the impact of satellite DNAs on adaptation to different environmental conditions as well as their role in the evolution of gene regulatory networks is proposed.

OP1

Genome rearrangements in radiation-resistant bacteria

Repar Jelena[1]*, Supek Fran[2], Klanjscek Tin[3], Warnecke Tobias[4,5], Zahradka Ksenija[1], Zahradka Davor[1]

[1]Laboratory for Molecular Microbiology, Division of Molecular Biology, Ruder Boskovic Institute, Zagreb, Croatia

[2]Genome Data Science Group, Structural & Computational Biology Unit, Institute for Research in Biomedicine, Barcelona, Spain

[3]Laboratory for Informatics and Environmental Modelling, Division for Marine and Environmental Research, Ruder Boskovic Institute, Zagreb, Croatia

[4]Molecular Systems Group, MRC London Institute of Medical Sciences, London, UK

[5]Institute of Clinical Sciences, Faculty of Medicine, Imperial College London, London, UK

**E-mail:jrepar@irb.hr*

A number of bacterial, archaeal, and eukaryotic species are known for their resistance to ionizing radiation. One of the challenges these species face is a potent environmental source of DNA double-strand breaks, potential drivers of genome structure evolution. Efficient and accurate DNA double-strand break repair systems have been demonstrated in several unrelated radiation-resistant species and are putative adaptations to the DNA damaging environment. Such adaptations are expected to compensate for the genome-destabilizing effect of environmental DNA damage and may be expected to result in a more conserved gene order in radiation-resistant species. However, we show that rates of genome rearrangements, measured as loss of gene order conservation with time, are higher in radiation-resistant species in multiple, phylogenetically independent groups of bacteria. Furthermore, we explore whether detected high rearrangement rates in radiation-resistant species constitute a survival strategy or are a passive consequence of environmental DNA damage.

OP2

RecBCD- RecFOR-independent pathway of homologous recombination in *Escherichia coli*

Buljubašić Maja[1,2], Zahradka Ksenija[1], Hlevnjak Ana[1,3], Repar Jelena[1], Đermić Damir[1], Zahradka Davor[1]*

[1]*Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia*
[2]*University of Split, Split, Croatia*
[3]*Pliva, Zagreb, Croatia*

**E-mail: zahradka@irb.hr*

The RecA protein is a key recombinase in bacteria that catalyzes pairing and strand exchange between homologous DNA duplexes. In Escherichia coli, the RecBCD and RecFOR protein complexes mediate RecA assembly on DNA. Thus, two recombination pathways, RecBCD and RecFOR, are distinguished in E. coli. Inactivation of both recombination pathways by recB(CD) recF(OR) mutations results in severe recombination deficiency. Here we describe a novel, RecBCD- RecFOR-independent (RecBFI) recombination pathway that is active in ΔrecBCD sbcB15 sbcC(D) ΔrecF mutants. In transductional crosses, these mutants show only three-fold decrease of recombination frequency relative to the wild-type strain. At the same time, they recombine 50- to 100-fold better than their sbcB+ sbcC+ and ΔsbcB sbcC counterparts. The RecBFI pathway strongly depends on recA, recJ and recQ gene functions, moderately depends on recG and ruvABC functions, whereas inactivation of dinI, recX, recN, radA, and uvrD genes has a negligible effect. After exposure to UV and γ-irradiation, the ΔrecBCD sbcB15 sbcC ΔrecF mutants show moderately increased DNA repair proficiency relative to their sbcB+sbcC+ and ΔsbcB sbcC counterparts. Inactivation of 3′-5′ exonucleases ExoVII, ExoIX and ExoX does not mimic the effect of the sbcB15 mutation suggesting that protection of 3’ overhangs itself is not sufficient to enable RecBFI pathway. Rather, our results suggest that SbcB15 protein plays an active role in formation of the RecA filament.

OP3

Recombinogenicity of perfect palindromes and quasipalindromes with short central spacers *in vivo*

Svetec Miklenić Marina, Štafa Anamarija, Žunar Bojan, Svetec Ivan Krešimir*

[1]*Laboratory for Biology and Microbial Genetics, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia*

**E-mail: iksvetec@pbf.hr*

A palindromic sequence in DNA with two identical inverted repeats and no central spacer region is called a perfect palindrome. Otherwise the palindrome is referred to as a quasipalindrome. Both perfect palindromes and quasipalindromes can constitute fragile sites in the genome prone to chromosome breakage which can result in potentially dangerous mutations and rearrangements. Several conditions in humans (such as the Emanuel syndrome) are caused by recombinogenic palindromic sequences in the genome. Palindrome recombinogenicity is caused by their ability to form secondary structures – hairpins in ssDNA and cruciforms in dsDNA. Using yeast *Saccharomyces cerevisiae* as a model, we compared the recombinogenicity of a perfect palindrome and quasipalindromes with short central spacers (1-10 bp) *in vivo.* We determined that a spacer as short as 7 bp significantly reduces the recombinogenicity, while a 10 bp spacer can completely stabilize palindromes up to 150 bp long, most likely by disabling cruciform formation. Furthermore, we found that in the absence of Sgs1 helicase the recombinogenicity of a perfect palindrome and of otherwise stable quasipalindrome with a 10 bp spacer is increased by the same factor, indicating that these two sequences have the same probability for hairpin formation in the lagging strand during replication. On the other hand, the absence of Rad27 nuclease (involved in Okazaki fragment maturation) dramatically increases the recombinogenicity of a perfect palindrome, but not a quasipalindrome with a 10 bp spacer. This result indicates that in the case of Rad27 absence the hairpin might form in the relatively short 5' flap displaced by Pol δ during Okazaki fragment synthesis.

OP4

Interplay of CRISPR adaptation, recombination and host nucleases in *Escherichia coli*

Radovčić Marin[1], Wettstein Lukas[2], Killelea Tom[2], Bolt Edward L.[2], Ivančić Baće Ivana[1]*

[1]University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Horvatovac 102a, 10000 Zagreb, Croatia

[2] School of Life Sciences, Queen's Medical Centre, Medical School, University of Nottingham, Nottingham NG7 2UH, United Kingdom

**E-mail: iibace@biol.pmf.hr*

CRISPR-Cas system is a prokaryotic adaptive immune system against foreign genetic elements. Immunity is acquired through insertion of small fragments (spacers) of invader DNA into a CRISPR array. This process is called adaptation, and can be mediated solely by the protein complex Cas1-Cas2 ("naïve adaptation"). In *E. coli*, RecBCD is thought to aid naïve adaptation by generating single-stranded DNA intermediates that are reannealed and further processed by Cas1-Cas2, and then integrated into the CRISPR array. In this work, we wanted to better understand the role of RecBCD and other host exonucleases in the process of prespacer preparation. Our genetic analysis shows that nuclease activity of RecBCD enzyme is not required for spacer preparation and that RecA inhibits adaptation probably because it prevents DNA processing and stimulates homologous recombination. However, helicase activity of RecBC(D) is required and is helped by 3'-5' host ssDNA exonucleases to occasionally generate appropriate DNA substrates for Cas1-Cas2 binding. Our in vitro analysis implies that Cas1-Cas2 forms a stable complex on DNA substrates with 5' overhangs and catalyses their cutting. Overall, our data suggest that 5' overhangs are important as substrates for adaptation and that these may be bound and processed by Cas1-Cas2.

OP5

Specificity of microbiota-mediated immune priming in insects

Futo Momir[1]*, Kurtz Joachim[2]

[1]Laboratory of Evolutionary Genetics, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia
Institute for Evolution and Biodiversity, University of Münster, Germany

**E-mail: mfuto@irb.hr*

Recent evidence indicates that invertebrates are capable of an unexpected degree of immune memory, i.e. the immune priming. However, the underpinnings of this phenomenon are still largely unknown and the mechanisms may differ from case to case. As microbiota has been suggested to shape immunological responses of various hosts, the main aim of this study was to elucidate the mechanisms behind the already observed phenomenon of oral priming. Our hypothesis was that the eradication of commensal bacteria from insect larvae might lead to loss of the priming effect. As a model we used a well-established host-parasite system, the red flour beetle *Tribolium castaneum* and its natural entomopathogen, *Bacillus thuringiensis*. Our results confirmed our hypothesis and undoubtedly proved the essential role gut microbes play in the oral immune priming in *T. castaneum*, although the molecular and mechanistic underpinnings behind this phenomenon need further research. Furthermore, we showed that the oral priming in our host-pathogen model comes with a certain level of specificity. Our results represent a significant contribution to the growing body of knowledge on the topic of insect immunity and immune memory through the aspect of insect microbiota.

OP6

"DecisionGS" algorithm for optimization of genetic potential; Case of improvement of common wheat *Triticum aestivum*

Kurtanjek Želimir

Faculty of Food Technology and Biotechnology (retired), University of Zagreb, Croatia

E-mail: zelimir.kurtanjek@gmail.com

Advances of artificial intelligence AI machine learning ML algorithms enable modeling and systems analysis of biopotential based on whole genome wide associations GWAS and selected phenotype features. Knowledge extraction and statistical validation of model inferences critically depends on availability of big data sets. Here is proposed application of boosted iterative algorithm of decision tree forest for genome selection "DecisionGS" and possible application of CRISPR technique for genome modification of food stuffs avoiding GMO legal issues. The complexity of plant genome sequencing is significantly reduced by genotypization. In this work applied are GWAS wheat data generated by DArT (Diversity Array Technology) available from CIMMYT International Maize and Wheat Improvement Center. The data set has 1279 DArT markers corresponding to presence of single nucleotide perturbations (SNP), for 600 wheat (*Triticum aestivum*) varieties, and 8 corresponding trait properties. Extraction of the key DArT markers related to focused phenotype is assessed on the variable drop out algorithm. Importance of genome interaction (nonlinearity) is based on between DecisionGS and linear elastic nets models. Accuracy of DecisionGS is comparable to deep learning algorithm. Numerical simulated predictions indicate possible 15 % increase in protein production per hectare of land compared to the best observed trait from the CIMMYT data set.

OP7

Natural genetic variation of photosynthetic performance in the context of molecular plant breeding

Šimić Domagoj[1,2]*, Franić Mario[2,3], Galić Vlatko[1], Mazur Maja[1], Jambrović Antun[1,2], Zdunić Zvonimir[1,2].

1 Department of Maize Breeding and Genetics, Agricultural Institute Osijek, Osijek, Croatia
2 Centre of Excellence for Biodiversity and Molecular Plant Breeding, Zagreb, Croatia
3 Institute of Agriculture and Tourism, Poreč, Croatia

**E-mail: domagoj.simic@poljinos.hr*

Photosynthesis research has been implemented scarcely into molecular plant breeding practice. Opposed to variation generated in the laboratory via mutagenesis or cis/transgenesis, our research group at the Agricultural Institute Osijek was focused on intraspecific spontaneously generated (natural) genetic variation of photosynthetic performance (PP) found in maize (Zea mays). Over the past decade, the maize inbred lines B73 and Mo17, and their biparental IBM population acted as an excellent resource for genetic studies of natural variation of photosynthesis in the context of molecular plant breeding. Firstly, the IBM population was evaluated *per se* for a set of PP parameters. A quantitative trait loci (QTL) analysis revealed 10 significant QTLs of which five were co-localized when combined over the environments indicating polygenic inheritance. One pleiotropic locus on the chromosome 7 coincided with the gene *gst23* that may be associated with efficient photosynthesis. Recently, we completed quantitative genetic analysis of the IBM population testcrosses evaluated in a series of stress and non-stress environments. QTL analysis of the IBM testcrosses detected several common QTLs for PP and yield under the moderate heat scenario. It suggests that PP parameters could be efficient secondary breeding traits for selection under heat stress. The next step of our genetic research on photosynthesis will be comprehensive genome-wide association study in a panel of maize inbred lines.

OP8

AtBPM1 protein is involved in RNA-directed DNA methylation in plants

Bauer Nataša, Jagić Mateja, Škiljaica Andreja, Markulin Lucija, Leljak-Levanić Dunja*

Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

**E-mail: dunja@zg.biol.pmf.hr*

DNA methylation is one of several epigenetic mechanisms used by cells to control gene expression. Plant DNA methylation occurs at CG, CHG and CHH (H is A, C or T) sequences. Nonsymmetrical CHH methylation cannot be sustained by the maintenance and require de novo methylation in each cell cycle through a process called RNA-directed DNA methylation (RdDM). In this prosess the DDR complex target the methylation machinery to specific regions in the genome but the mechanism of DDR complex possitioning is still not clear. AtBPM1 belongs to the small family of *Arabidopsis* MATH and BTB domain proteins that are known as substrate-specific adaptors in the cullin3 dependent ubiquitin proteasome pathway. Tandem affinity purification and yeast two hybrid assay revealed interaction of BPM1 with DMS3 and RDM1, crucial components of RdDM. Fluorescently tagged BPM1 and its truncated version with deletion of MATH or BTB domain showed that both domains are essential for accumulation of the protein in nucleolus, whilst co-localization show significant overlap of BPM1 with aforementioned RdDM components. Yeast two hybrid assay using truncated BPM1 protein missing MATH or BTB domain revealed that the cullin3-binding BTB domain had higher affinity for RDM1 interaction while both, MATH and BTB domains proved to be equally important for DMS3 interaction. New insights into BPM1 protein and its interaction partners indicate an important, cullin independent function of MATH-BTB protein family in RdDM.

OP9

Skin homeostasis and regeneration

Rubelj Ivica[1]*, Nanić Lucia[1]

[1]Ruder Boskovic Institute, Zagreb, Croatia

**E-mail: rubelj@irb.hr*

Aging is slow and progressive biological process characterized by continuous decrease of functions at all levels in the organism; from cells, tissues and organs to the whole body. It results in reduced capacity of tissue regeneration accompanied by the disruption of normal homeostasis and accumulation of tissue damage. Experimental evidence suggests that cellular senescence has a critical influence on human aging primarily controlled by telomere shortening. Accumulation of senescent cells causes tissues and organs to lose their ability to regenerate, including reduction of the stem cell renewal potential. Since senescent cells impair normal function of neighbouring healthy cells by altering their microenvironment, using a novel technology for micro-transplantation of young cells into old rat skin, we aim to increase ratio of young cells in the skin and alter skin homeostasis towards younger phenotype. This research will give us an answer on how we can influence skin homeostasis by inducing a profile of young physiology in an old skin and to which extent we can restore the regenerative potential of the skin.

OP10

Apoptosis-antagonizing transcription factor *AATF* and chromatin-remodeling *SMARCA2* are associated with thyroid volume in Hashimoto's thyroiditis patients

Brčić Luka[1], Barić Ana[2], Brekalo Marko[2], Gračan Sanda[2], Škrabić Veselin[3], Kaličanin Dean[1], Novak Ivana[4], Zemunik Tatijana[1], Barbalić Maja[1], Punda Ante[2], Boraska Perica Vesna[1]*

[1]Department of Medical Biology, University of Split, School of Medicine, Split, Croatia
[2]Department of Nuclear Medicine, University Hospital Split, Split, Croatia
[3]Department of Pediatrics, University Hospital Split, Split, Croatia
[4]Department of Immunology and Medical Genetics, University of Split, School of Medicine, Split, Croatia

**E-mail: vboraska@mefst.hr*

Thyroid volume of patients with Hashimoto's thyroiditis (HT) varies in size over the course of disease and is defined by delicate balance between thyrocyte proliferation and apoptosis. We performed the first genome-wide association study (GWAS) of thyroid volume with the hypothesis that changes in thyroid volume of HT patients reflect changes in number of thyrocytes and biological activity of thyroid gland. We performed thyroid ultrasound and calculated thyroid volume in 359 HT patients recruited at University Hospital Split. GWAS of thyroid volume was performed on 6 007 322 genetic variants using linear mixed model implemented in GEMMA. We found two loci associated with thyroid volume on genome-wide significant level: rs7212416 inside apoptosis-antagonizing transcription factor *AATF* ($P= 8{,}95\times10^{-9}$) and rs10738556 near chromatin-remodeling *SMARCA2* ($P=2{,}83\times10^{-8}$). As increased apoptosis of thyrocytes is one of the landmarks of thyroid tissue of HT patients, our results suggest that the magnitude of apoptosis may be genetically regulated by *AATF* in pathological microenvironment of thyroid gland. SMARCA2 functions as transcription regulator of certain genes by chromatin remodeling. Further, it interacts with HDAC1 that may be generally inhibited by AATF, thus linking these two genes. We identified two highly plausible genetic loci, *AATF* and *SMARCA2,* which seem to be involved in the processes of transcriptional regulation that drives thyrocytes to apoptosis in HT patients.

OP11

Genome-wide association analysis suggests novel loci underlying thyroid antibodies in Hashimoto's thyroiditis

Brčić Luka[1], Barić Ana[2], Gračan Sanda[2], Torlak Lovrić Vesela[2], Brekalo Marko[2], Škrabić Veselin[3], Zemunik Tatijana[1], Barbalić Maja[1], Punda Ante[2], Boraska Perica Vesna[1]*

[1]Department of Medical Biology, University of Split, School of Medicine, Split, Croatia
[2]Department of Nuclear Medicine, University Hospital Split, Split, Croatia
[3]Department of Pediatrics, University Hospital Split, Split, Croatia

**E-mail: vboraska@mefst.hr*

Thyroid antibodies against thyroglobulin (TgAb) and thyroid peroxidase (TPOAb) are key markers of Hashimoto's thyroiditis (HT). To explore genetic background of thyroid antibodies, we performed the first genome-wide association study (GWAS) of thyroid antibodies levels in 405 HT patients.

GWAS analyses on TgAb and TPOAb levels were performed separately on a set of 6 007 542 genetic variants using GEMMA. To identify genetic variants that predispose to both thyroid antibodies, we additionally performed a bivariate analysis using MultiABEL.

We detected two suggestively associated genetic variants with TgAb, rs6972286 close to *ANKRD7* and *LSM8* ($P=2.34\times10^{-7}$) and rs756763 inside *CA10* ($P=6.05\times10^{-7}$); one with TPOAb, rs12507813 between *TRIM61* and *TRIM60* ($P=4.95\times10^{-7}$); and three with both antibodies (bivariate analysis), rs13190616 inside *RP11-138J23.1* ($P=2.01\times10^{-6}$), rs561030786 close to *DUBR* ($P=7.33\times10^{-6}$) and rs12713034 inside *FSHR* ($P=7.66\times10^{-6}$). All identified genomic regions have a substantial literature record of involvement with female-related traits, immune-mediated diseases and personality traits that are all characterized by increased thyroid antibody levels.

Our findings demonstrate the existence of genetic overlap between thyroid autoimmunity in HT and several non-thyroid diseases characterized by the presence of thyroid antibodies. We also suggest that genetic variants that regulate antibody levels may differ between HT patients and individuals with normal thyroid function.

OP12

Root-knot nematodes as a model for centromere (epi)genomics and satellitome research

Despot Slade Evelin[1], Pavlek Martina[1], Mravinac Brankica[1], Castagnone-Sereno Phillipe[2], Plohl Miroslav[1], Meštrović Nevenka[1]*

[1] Division of Molecular Biology, Ruđer Bošković Institute, Bijenička street 54, 10000 Zagreb, Croatia
[2] INRA/UNSA/CNRS, Institut Sophia Agrobiotech, 400 Route des Chappes, BP167, 06903 Sophia Antipolis, France

**E-mail: nevenka.mestrovic@irb.hr*

Root-knot nematodes (genus *Meloidogyne*) are established as important model organisms of plant parasites. Their characteristics, such as holocentric chromosomes, polyploidy and parthenogenesis make them an ideal system to study genome evolution, speciation processes, and centromere structure and function. In this regard, our analysis of centromere specific histone H3 (CENH3) in the genome of *M. incognita* reveals three divergent CENH3 variants instead of the commonly present only one. All CENH3 variants are completely conserved in related species, and transcriptome data show their developmental stage-specific expression. Immunostaining of chromosomes with antibodies specific for CENH3 variants reveals holocentric distribution pattern for each of them. Given that the centromere tends to be established upon satellite DNA arrays we further focus on their characterization in related *Meloidogyne* genomes. Using next-generation sequencing combined with novel bioinformatics tools we detected a remarkable collection of satellite DNAs (satellitome) in each genome. Comparative analyses of satellitomes indicate the species evolutionary history in accordance with a recent hypothesis which suggests a multiple hybridization events in species origin. All results will be discussed in terms of coexistence and possible roles of different CENH3 variants in holocentric centromeres, and correlated with trends in satellitome evolution.

OP13

The mechanisms and causes of pigmentation loss in cave adapted animals

Bilandžija Helena[1,2], Ćetković Helena[1], Jeffery William R[2].

[1] *Laboratory of Molecular Genetics, Division of Molecular Biology, Ruđer Bošković Institute, Croatia*
[2] *Department of Biology, University of Maryland, College Park, MD 20742 USA*

**E-mail: hbilandz@irb.hr*

The loss of pigmentation is one of specialized traits which evolved as an adaptation to subterranean habitats. It appeared in almost all groups with cave-adapted species, but not much is known about the molecular and evolutionary mechanisms of its appearance. The exception is the teleost model system *Astyanax mexicanus*, in which a deletion in the *oca2* gene underlies albinism in several independently evolved cavefish populations. We have investigated albinism in diverse cave species and discovered that 1. pigment cells are invariably present and 2. there is a lesion in the first step of melanin synthesis pathway. The preservation of pigment cells is suggestive of their function in processes other than pigmentation. Further examination confirmed the role of melanocytes in vertebrate immunity and maintenance of melanin synthesis capacity as a part of the innate immune response in albino cave arthropods. The non-random inactivation of melanin synthesis suggests that there must be an adaptive benefit. The cessation of pigment production could be selected for as a result of minimizing energy expenditure for production of unnecessary substances, or as an indirect effect since many pleiotropic phenotypes are intertwined with pigmentation. We found evidence for both of these hypotheses implicating that natural selection may be involved in the evolution of albinism, contrary to traditional views of pigmentation being a trait that is simply discarded in the absence of light.

PP1

Molecular diversity of narrow-leaved ash (*Fraxinus angustifolia* Vahl) plus trees in Croatia

Andrić Ivan[1], Šatović Zlatko[2], Kajba Davorin[1]*

[1]*Department of Forest Genetics, Dendrology and Botany, Faculty of Forestry, University of Zagreb, Croatia*
[2]*Department of Seed Science and Technology, Faculty of Agriculture, University of Zagreb, Croatia*

**E-mail: davorin.kajba@zg.htnet.hr*

Research into the molecular diversity was conducted in two clonal seed orchards (Čazma, Nova Gradiška) on 92 plus trees belonging to the two seed regions (seed region upper Sava river, middle Croatia and Kupa river and seed region middle part of the Sava river). Genetic analysis was revealed with eight microsatellite markers (Femsatl 4, Femsatl 8, Femsatl 10, Femsatl 11, Femsatl 16, M2-30, FR639485, FR646655). The aim of the study was to determine the level of molecular diversity of narrow-leaved ash from this two seed regions. Since the plus trees go through a very narrow phenotypic selection the comparison of the obtained genetic parameters from this study and with other research in natural stands concluded that there were no significant interpopulation variability. The highest genetic variability belongs to the intrapopulation level of genetic structure. Bayesian analysis of the population structure of selected genotypes with consideration to the genomic share can not be clearly separated into two seed regions. They are artificially demarcated based on the ecological conditions in which the narrow-leaved ash populations exists, but the obtained genetic structure and variability of selected plus trees indicate their individual variability.

PP2

Data mining reveals silent genomic mutations to be important for pathotype determination in pepper mild mottle virus

Banović Đeri Bojana[1]*, Pajić Vesna[2], Dudić Dragana[2]

[1]*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, PO Box 23, 11010 Belgrade, Serbia*
[2]*Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia*

**E-mail: bojanabanovic@imgge.bg.ac.rs*

Pepper mild mottle virus (PMMoV) is a single-stranded RNA virus that significantly reduces yields in pepper. According to its ability to infect pepper 5 pathotypes are discerned, ranging from P_0 strains of the weakest to $P_{1,2,3,4}$ strains of the strongest pathogenicity. To test data mining and bioinformatics tools potential in discovering new knowledge on plant viruses from the existing genomic data, we extracted 231 PMMoV sequences from public databases on March 2015. The dataset included complete and partial genomic sequences obtained by sequencing of real PMMoV strains that occurred over 30 years worldwide and were never compared to each other. Our approach revealed that nucleotide content at 5 positions in the replicase encoding genes and 3 positions in the coat protein encoding gene can be used to discern specific PMMoV genotype variant associated to $P_{1,2}$ or $P_{1,2,3}$ pathotype. Interestingly, due to containing silent mutations these sites have never been detected as informative before. The model was tested for predicting pathotypes of 10 PMMoV sequences deposited after March 2015, confirming it is a reliable asset for discrimination among $P_{1,2}$ and $P_{1,2,3}$ pathotypes based solely on the nucleotide content at 8 newly detected informative genomic positions. Such knowledge enables developing of fast and cost-effective pathotype screening tests and holds potential for finding more eco-friendly solutions to detect, control and suppress pepper mild mottle virus in the near future.

PP3

Insights into the origins of animal complexity from a basal metazoan: Genome of the endemic cave sponge *Eunapius subterraneus*

Ćetković Helena[1], Bilandžija Helena[1]*, Halasz Mirna[1], Fabijanić Maja[2], Pleše Bruna[1], Vlahoviček Krisitan[2]

[1]Laboratory of Molecular Genetics, Division of Molecular Biology, Ruđer Bošković Institute, Croatia
[2]Bioinformatics Group, Department of Molecular Biology, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia

**E-mail: hbilandz@irb.hr*

The amazing complexity of metazoan genomes originated early in the evolution of the animal kingdom. Rapid advances of next-generation sequencing (NGS) technologies and bioinformatic data analysis are enabling elucidation of early metazoan gene regulation and development. Sponges have a unique evolutionary position of being the simplest and arguably the earliest branching metazoans, and are therefore critical for understanding the genomic traits of the ancestor of metazoa - the first multicellular animal. However, available genomic data from the phylum Porifera is still very scarce.

We sequenced the genome of the freshwater sponge *Eunapius subterraneus,* Sket & Velikonja, 1984 using a combination of NGS methods (Illumina and Oxford Nanopore) and assembled a draft version of its genome. *Eunapius subterraneus* is the only cave-adapted sponge species, known from only a small region in Croatia. The sponge possess unique morphological and ecological features. Using the -omics methodologies in combination with experimental approaches we aim to advance our understanding of 1. basic processes involved in multicellular development and differentiation and 2. the process of adaptation to different environmental conditions.

Financial support by the Croatian National Science Foundation (Project No. 6400) is gratefully acknowledged.

PP4

Intra-varietal variability of the olive cultivar ‘Piculja’

Bolarić Snježana[1], Batelja Lodeta Kristina[2]*, Vokurka Aleš[1], Benčić Đani[2]

[1]*Department of Plant Breeding, Genetics and Biometrics, Laboratory of Biotechnology, University of Zagreb Faculty of Agriculture, Croatia*
[2]*Department of Pomology, University of Zagreb Faculty of Agriculture, Croatia*

**E-mail: kbatelja@agr.hr*

Croatian Adriatic coast is rich in germplasm of local olive (*Olea europaea* L. subsp. *europaea* var. *europaea*) varieties that has not been fully explored yet. One of these varieties, cv. ‘Piculja’, is grown only in the south Dalmatian region (the Dubrovnik-Neretva County in the south of Croatia). It is not among the leading cultivars, but besides its significant role in the production of high-quality olive oil, it has also important economic and technological role as a pollinator for other olive varieties. The aim of ‘pilot’ research was to get insight into genetic variability within limited, but hypothetically diverse set of four old trees of ‘Piculja’. Genetic variability within the cultivar may have an important role as a basis for clonal selection, production of reproductive material and clonal recognition. The insight into genetic variability may also contribute to the knowledge of cultivar evolution and development. The analyses were conducted by eleven consensual SSR markers which revealed the variations in the three SSR loci and seven allelic combinations. The significant differences were also detected by analysis of variance of morphological data for phenotypically stabile traits: the shape of the leaf blade, the length and the width of the stone, but not in the shape of the stone. Significant differences in these traits, altogether with genetic SSR variability is a strongpoint for confirmation of clonal diversity within the cultivar ‘Piculja’.

PP5

Cryptic speciation in European riffle beetles (Insecta: Coleoptera: Elmidae) as revealed by molecular phylogenetic analyses

Bruvo Mađarić Branka[1]*, Jäch Manfred A.[2], Mičetić Stanković Vlatka[3], Brojer Michaela[2], Ugarković Đurđica[1]

[1] *Ruđer Bošković Institute, Zagreb, Croatia*
[2] *Natural History Museum, Vienna, Austria*
[3] *Croatian Natural History Museum, Zagreb, Croatia*

**E-mail: bruvo@irb.hr*

Riffle beetles (Insecta: Coleoptera: Elmidae) inhabit fast running waters worldwide and are often used as bioindicators of water quality and in monitoring of running water ecosystems. In Europe, Elmidae are represented by ten genera, with about 40 species recognized so far. However, certain species of elmids are very difficult to identify by their morphology and recent investigations suggest that there is a large number of cryptic, so far unrecognized, species.

We present the results of phylogenetic analysis of the typical European genera *Elmis* Latreille, 1978 and *Limnius* Illiger, 1802 by using mitochondrial and nuclear genes. Molecular data suggest the existence of nine new, so far undescribed species within each of the genera *Elmis* and *Limnius*. Beside molecular data, most of the putative new species may be distinguished by certain morphological characters as well. This finding almost doubles the number of the species so far recorded in these two genera in Europe and adjacent regions.

Our results contribute to the knowledge of freshwater biodiversity of Europe and represent the base for future investigations on the processes of the speciation of water beetles, being essential in establishing projects on conservation of aquatic species and aquatic biotopes.

PP6

SNP genotyping of croatian common bean landraces

Carović-Stanko Klaudija[1,2]*, Liber Zlatko[2,3], Vidak Monika[1], Gunjača Jerko[1,2], Grdiša Martina[1,2], Lazarević Boris [2,4], Šatović Zlatko[1,2]

[1]University of Zagreb, Faculty of Agriculture, Department of Seed Science and Technology, Svetošimunska cesta 25, HR-10000 Zagreb, Croatia
[2]Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Svetošimunska cesta 25, HR-10000 Zagreb, Croatia
[3]University of Zagreb, Faculty of Science, Department of Botany, Marulićev trg 9A/ II, HR-10000 Zagreb, Croatia
[4]University of Zagreb, Faculty of Agriculture, Department of Plant Nutrition, Svetošimunska cesta 25, HR-10000 Zagreb, Croatia

**E-mail: kcarovic@agr.hr*

Common bean (*Phaseolus vulgaris* L.) is economically the most important among five domesticated species from genus *Phaseolus*. In Croatia, common bean is a traditionally grown in low input production systems. The production is based on landraces displaying high levels of diversity. In order to assess the genetic diversity of Croatian common bean landraces, 174 accessions were analysed using a DArTseq method of SNP genotyping. Out of 17,514 polymorphic markers, 8,092 (46%) had high scoring reproducibility (>0.95), high call-rate (>0.90) and the minor-allele frequency higher than 5%. Genomic regions flanking SNPs were than aligned against the reference genome of *P. vulgaris*. From the 8,092 SNP sequences, 6,599 (82%) were aligned to 11 chromosomes of common bean. The average number of SNPs per chromosome was 599.91, ranging from 403 on chromosome 4 to 834 on chromosome 2. Mean number of SNPs per Mbp was 12.85 or on average one SNP every 77,828 base pairs. The average expected heterozyogsity was 0.373, ranging from 0.096 to 0.500 while the polymorphism information content ranged from 0.091 to 0.373 with an average of 0.298. The model-based analysis of population structure was carried out using 923 SNPs in linkage equilibrium. The results were compared to those obtained by microsatellite markers. The SNP set will be used in genome-wide association studies (GWAS) concerning bioactive nutrient content.

PP7

Comparative satellitome analysis reveals the polypoid hybrid origin in holocentric nematodes

Despot Slade Evelin[1], Širca Saša[2], Plohl Miroslav[1], Meštrović Nevenka[1]*

[1] *Division of Molecular Biology, Ruđer Bošković Institute, Bijenička street 54, 10000 Zagreb, Croatia*
[2] *Agricultural institute of Slovenia, Ljubljana, Slovenija*

**E-mail: nevenka.mestrovic@irb.hr*

Satellite DNAs (satDNAs) are tandemly repeated non-coding DNA sequences, and the most common constituents of every functional centromere. Holocentric chromosomes and multiple genome copies establish nematodes of the genus *Meloidogyne* as an attractive model for studying centromere-associated DNA sequences. In order to identify a pool of satDNAs that may be involved in genomics of the holocentromere, we have analyzed the satellitome (whole-genome complement of satDNAs) in four different publicly available *Meloidogyne* genomes using RepeatExplorer computational tool. Our results of genome-wide analyses revealed the presence of a remarkable collection of 38-80 different satDNAs in each examined genome. In addition, comparative analyses of satelitomes show three main groups of satDNAs; common for all four studied species, and shared by subsets of three or only two species. This distribution of satDNAs supports a recent hypothesis based on comparative analyses of coding regions, that speciation processes in *Meloidogyne* may be a result of additive interspecific hybridizations. Our preliminary analysis of satDNA distribution on chromosomes of studied species also speaks in favor of such unusual genome evolution in *Meloidogyne*.

PP8

A new role of RecA protein in *Escherichia coli?*

Đermić Edyta[1], Zahradka Davor[2], Vujaklija Dušica[2], Đermić Damir[2]*

[1] Department of Plant Pathology, Faculty of Agriculture University of Zagreb, Svetošimunska 25, 10 000 Zagreb, Croatia
[2] Division of Molecular Biology, Ruđer Bošković Institute, Bijenička 54, 10 000 Zagreb, Croatia

**E-mail: dermic@irb.hr*

The ubiquitous and evolutionary conserved RecA protein binds to single-stranded DNA (ssDNA), producing a nucleoprotein filament. RecA has three known roles in *E. coli*: RecA nucleoprotein filament enables repair of DNA double-strand breaks (DSBs) and exchange of similar DNA molecules by promoting homologous recombination; also, RecA nucleofilament facilitates autocleavage of LexA repressor, hence enabling induction of SOS response; and finally, during SOS induction, RecA activates a mutagenic DNA polymerase V. Another role of RecA exists in *E. coli*, namely RecA inhibits DNA degradation. However, no relation of the latter RecA function to the three established ones has been explored previously. Here, we introduced DSBs (by gamma-irradiation) into *E. coli* chromosome labelled by [^{3}H]thymidine and monitored its degradation. We have shown that RecA inhibition of DNA degradation depends on RecA's concentration and its rate of association with ssDNA. Also, our results indicate that RecA suppression of DNA degradation is separable from the other three roles of RecA, thus suggesting it represents a novel RecA function in *E. coli,* namely regulation of DSB processing.

PP9

A unique sterile CMS-S type of onion cytoplasm identified in the Croatian clone of *A. × cornutum* Clementi ex Visiani, 1842

Fredotović Željana, Šamanić Ivica, Puizina Jasna

Faculty of Science, Department of Biology, University of Split, Croatia

**E-mail: puizina@pmfst.hr*

Cytoplasmic male sterility (CMS) is a natural phenomenon in which plant does not produce functional anthers or pollen, but the female part of the flower is functional and can be fertilized by a foreign pollen. CMS is extremely suitable for breeding and hybrid seed production. Triploid onion, *Allium × cornutum* Clementi ex Visiani, 1842 (2n = 3x = 24) is an established minor garden crop with a complex genome structure with three parental species, *A. cepa* L., *A. pskemense* B.Fedtsch., and *A. roylei* Stearn. Two of its mostly studied clones are Croatian 'Ljutika' and the Indian 'Pran' which are shown to be genetically highly similar. A CMS-S type of cytoplasm has been previously confirmed in 'Pran'. To investigate whether 'Ljutika' might also possesss the S-type of cytoplasm we analysed three chloroplast genes *accD, atpF, petB* and one mitochondrial gene *cob* in 'Ljutika' and its three parental species. PCR-RFLP analysis of the chloroplast genes *accD, atpF, petB* and the mitochondrial gene *cob*, as well as the sequence analysis of two cpDNA markers, *matK* gene and *atpB-rbcL* spacer regions, showed that 'Ljutika' possesses the male-sterile S-type of cytoplasm. The phylogenetic analysis of *atpB-rbcL* and *matK* sequences of 'Ljutika' and its parental species suggested that *A. roylei* could be a donor of S-cytoplasm and a female parent of *A.× cornutum*, 'Ljutika'.

PP10

Preliminary morphological description of cultivated and wild olives in Croatia

Glavinović Višeslav, Tadić Josip, Radunić Mira, Klepo Tatjana*

Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, Split, Croatia
Centre of Excellence for Biodiversity and Molecular Plant, Svetošimunska 25, Zagreb, Croatia

**E-mail: Tatjana.Klepo@krs.hr*

Olive growing is one of the most important crops in Mediterranean part of Croatia. Several Croatian research groups studied olive diversity with molecular markers revealing a great diversity of olive cultivars grown in Croatia. However, morphological evaluation of cultivated, especially wild olives in Croatia is still lacking. Here, we present preliminary results of morphological evaluation of 66 different olive genotypes: 27 autochthonous and 14 introduced cultivars, and 24 wild olives (four feral and 20 preliminary genuine wild from island of Hvar, Pelješac and Lastovo). Qualitative morphological characterization of fruit and endocarp of examined olive genotypes were performed according to the IOC protocol, while quantitative morphological characterization ware performed with image analysis software (WinFOLIA Pro 2014a). Data analysis revealed significant differences between all sample groups (autochthonous, introduced olives and wild olives). As expected, for all morphological traits cultivated olives showed significantly higher values in comparison to the wild olives. For instance, average fruit weight for autochthonous and introduced olives were 3.62 g and 3.16 g, respectively while for wild olives was 0.51 g. Results here presented revealed high morphological variability between olive cultivars (*ex situ*) and wild olives (*in situ*).

PP11

Low doses of cadmium and phthalates – cytotoxic and genotoxic properties

Huđek Ana[1], Rumenova Raychinova Mirela[1], Ćurčić Marijana[2], Mozo Javier Esteban[3], Bačun-Družina Višnja[1], Durgo Ksenija[1]*

[1]*Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia*
[2]*Faculty of Pharmacy, University of Belgrade, Serbia*
[3]*Institute of Bioengineering, Miguel Hernández University of Elche, Spain*

**E-mail: kdurgo@pbf.hr*

Environmental contaminants often occur in the nature at low concentrations that are not considered to be toxic. Anyhow, their presence and, in some cases, persistence, can cause significant side effects on members of affected ecosystem. Cadmium and phthalates are often found in the environment in nanomolar concentrations. Cadmium is classified to be human carcinogen and phthalates are not directly acting genotoxic compounds. If there are indices of their tumorigenicity it is supposed that it is a result of nongenotoxic mechanism of action. Bis-2 ethylhexyl phthalate is widely used phthalate, and there are no experimental data concerning genotoxicity of environmentally significant concentrations. In this work, genotoxic potential of environmentally significant concentrations of both compounds and their mixtures were investigated on different human cell lines; tongue carcinoma cell line, human colon carcinoma cells and human hepatocellular carcinoma cells during 2 and 4 hours of incubation. Expectedly, results of testing for cytotoxicity were negative, but mixtures of cadmium and DHEP caused cell proliferation. Also, it was noticed that mixtures caused formation of free radicals and certain DNA damage after different times of treatment, so it can be concluded that mixtures of nontoxic concentrations of contaminants, cadmium and DHEP, caused genotoxic effect.

PP12

Evolution of single nucleotide polymorphisms in fat mass and obesity associated gene

Huđek Ana[1], Škara Lucija[1], Smolkovič Barbara[1], Ravlić Sanda[2], Nanić Lucia[2], Matovinović Martina[3], Rubelj Ivica[2], Bačun-Družina Višnja[1]*

[1] *Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia;*
[2] *Ruđer Bošković Institute, Zagreb, Croatia*
[3] *University Hospital Centre, Zagreb, Croatia*

**E-mail: bacun-druzina.visnja@pbf.hr*

Obesity is, in most cases, a multifunctional disease resulting from the interaction of changes in the human genome and environmental factors affecting the development of the disease throughout one's life. Single nucleotide polymorphisms (SNPs) are genetic variants that occur with a different frequency in the human genome. Fat mass and obesity associated (*FTO*) gene and genetic association of *FTO* SNPs are related to obesity and genetic aging.

In this work, we investigated three common SNPs in the *FTO* gene in women from Zagreb County. In cases with obesity present, the frequencies of the risk genotypes AA rs9939609, CC rs1421085 and GG rs17817449 of the *FTO* gene were significantly higher. Interestingly, 60.87% of the obese women are not homozygous carriers of even one risk allele opposite the rest 39.13% of the obese women who are triple-homozygous carriers of the risk alleles for all three studied SNPs. Considering the bimodal distribution of the investigated SNPs in obese women, the other genetic, metabolic, physiological, behavioural, sociocultural and / or environmental factors that can contribute to female obesity are a matter of great interest. Furthermore, it is a long way to go before we may answer questions about which factors affect the occurrence and evolution of SNPs in the complex genetics of human diseases.

PP13

Drought tolerance of soybean genotypes at early vegetative stage

Kereša Snježana*, Šarčević Hrvoje, Lovrić Ana, Žulj Mihaljević Maja, Đuretec Krešimir, Čavlovićak Snježana, Pejić Ivan

University of Zagreb Faculty of Agriculture, Department of Plant Breeding, Genetics and Biometrics, Svetošimunska cesta 25, 10000 Zagreb, Croatia

**E-mail:skeresa@agr.hr*

Soybean (*Glycine max* L. Merr.) is one of the major sources of protein for human and animal nutrition as well as a source for vegetable oil. Drought is a major limiting factor that can drastically reduce soybean yield. Due to climatic changes, periods of severe drought can occur at germination and early vegetative stage. The objective of this study was to screen 32 soybean genotypes for drought tolerance in early vegetative stage in controlled conditions. Seeds were germinated in moist silica sand and seedlings transplanted in tubes filled with vermiculite. The tubes were placed in a containers filled with a half strength Hoagland solution (control) or the same solution with 6% of diluted polyethylene glycol (PEG) 8000 (drought). Plants were grown in growth chamber at 22°C, 60% air humidity under cool-white light and 16/8 day/night photoperiod for 26 days. The following traits were measured: root and shoot length, fresh weight and dry weight. PEG-treated plants showed decrease in all measured traits with significant difference among genotypes. Drought tolerant genotype no. 3, 8, 11, 15, 22 and 25 with the highest drought tolerance efficiency (DTE) and the lowest decrease of shoot and root fresh and dry weight in drought were identified. These traits were highly positively correlated while root length negatively correlated with them. In conclusion, soybean genotypes that attained high root biomass without compromising shoot biomass are considered as drought tolerant.

PP14

Transcriptome and proteome measures of evolutionary origin and divergence

Koska Sara, Domazet-Lošo Tomislav*

Laboratory of Evolutionary Genetics, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia

**E-mail: tdomazet@irb.hr*

Transcriptome age index (TAI) is a combination of phylostratigraphy and stage-specific gene expression data which reflects the evolutionary age of the transcriptome at a given ontogenetic stage. It quantifies the mean evolutionary age of a transcriptome, whereas the evolutionary age of each gene is weighted by its expression level. On the other hand, transcriptome divergence index (TDI) is based on the ratio between the number of synonymous and non-synonymous substitutions between two species. This index is an indicator of selective pressure within protein-coding regions, reflecting thus the natural selection. It represents the mean sequence divergence of a transcriptome, where the sequence divergence of each gene is weighted by its expression level. TAI is a measure of long-term evolutionary changes, as it includes time span since the origin of life, whereas TDI reflects short-term evolutionary changes covering the period since the divergence of focal species. These indices have been used in evolutionary genetics, e.g. in detecting molecular hourglass pattern in animal, plant and fungal development, or determining evolutionary origin of genes. Modeled on TAI and TDI, we have developed two novel indices that use proteome expression levels for calculations – the proteome age index (PAI) and the proteome divergence index (PDI). Combining them with the transcriptome indices, we were able to identify the differences and connections between the transcriptome and proteome.

PP15

Concomitant resistance to paclitaxel in an ovarian cancer cell variant selected with carboplatin

Kralj Juran[1]*, Duran George E. [2], Stupin Polančec Darija [3], Bačić Niko[4], Sikic Branimir I.[2], Brozovic Anamaria[1]

[1]Division of Molecular Biology, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, HR-10000, Croatia
[2]Oncology Division, Stanford University School of Medicine, Stanford, CA 94305, USA
[3]Fidelta Ltd., Zagreb, HR-10000, Croatia
[4]Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, HR-10000, Croatia

**E-mail: juran.kralj@gmail.com*

Most epithelial ovarian cancer patients are diagnosed with advanced-stage disease due to the late appearance of symptoms and lack of early diagnostic methods/markers. The major problem for successful therapy is the development of tumour drug resistance during carcinogenesis (20-30%) and upon exposure to chemotherapy. The ovarian cancer cell line OVCAR-3/CBP was established by treatment of the ovarian adenocarcinoma cell line OVCAR-3 with long-term, stepwise selection in carboplatin (CBP) up to 25 μM. The variant is ~1.5-2-fold resistant to CBP, with cross-resistance to paclitaxel (TAX, ~4-fold), and presents with a mesenchymal-like phenotype. The increased expression of *NHE-1*, *ATP7-B* and decreased expression of *ABCC2* and *CTR-1* implied decreased total cell platination as a possible mode of CBP resistance, which was confirmed by flame atomic absorption spectrometry. Despite the increased level of *ABCB1* transcripts, OVCAR-3/CBP did not efflux [^{3}H]-docetaxel differently compared to parental cells, and the P-glycoprotein inhibitor PSC-833 did not alter these drug accumulation profiles. This indicates that the TAX resistance in OVCAR-3/CBP is non-MDR, but is associated with elevated TUBB3 (class III beta-tubulin) content along with total α- and β-tubulin relative to parental OVCAR-3 cells. In summary, drug selection with carboplatin in an ovarian cancer cell line resulted in non-MDR cross-resistance to paclitaxel. Experiments investigating the functional significance of altered tubulin content and microtubule dynamicity in response to drug exposure in OVCAR-3/CBP are on-going.

PP16

Genetic diversity of maize inbred lines from Croatian Gene Bank revealed by SSR markers

Lovrić Ana[1]*, Šarčević Hrvoje[1,2], Pejić Ivan[1,2], Kozumplik Vinko[2], Bolarić Snježana[1]

[1]*Department of Plant Breeding, Genetics and Biometrics, Faculty of Agriculture, University of Zagreb, Svetošimunska 25, 10000 Zagreb, Croatia*
[2]*Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Faculty of Agriculture, University of Zagreb, Svetošimunska 25, 10000 Zagreb, Croatia*

**E-mail: alovric@agr.hr*

The use of exotic materials in maize breeding is essential to solve the narrow genetic base of the commercial germplasm. Assessing the genetic diversity and heterotic pattern of existing exotic material can help in its implementation in modern breeding programs. Croatian gene bank (CGB) maintain a collection of inbred lines (accessions) in whose pedigrees the germplasm comprising several landraces from South Eastern Europe is significantly involved. The aim of the present study was to assess genetic diversity of inbred lines from CGB collection using simple sequence repeat (SSR) markers. Seventy-seven accessions from the CGB collection together with four standards (elite US inbred lines) were genotyped using 40 SSRs evenly distributed over ten maize chromosomes. A total of 220 alleles were found across 40 loci in the 81 inbred lines. The number of alleles per locus varied from 2 to 16 with an average of 5.5. Polymorphism information content (PIC) values varied from 0.24 to 0.80 with an average of 0.51. One or two private alleles per genotype were found in 20 accessions and 3 private alleles were found in one standard. Cluster analysis clearly separated all genotypes from each other assigning them into four major groups with one standard inbred appeared in each group. The results of clustering were only partially in accordance with the known pedigrees. The results of the present study will be useful in effective utilization of studied lines in hybrid maize breeding programs.

PP17

The effect of recurrent selection on genetic structure of a synthetic maize population

Mandić Ana[1]*, Šarčević Hrvoje[2], Kozumplik Vinko[3]

[1]*Department of Plant Breeding, Genetics, Seed Production and Biometrics, Faculty of Agriculture and Food Technology, University of Mostar, Biskupa Čule bb, 88000 Mostar, Bosnia and Herzegovina*
[2]*Department of Plant Breeding, Genetics and Biometrics, Faculty of Agriculture, University of Zagreb, Svetošimunska 25, 10000 Zagreb, Croatia*

**E-mail: ana.mandic@aptf.sum.ba*

The Maksimir 3 Synthetic maize population (Zea mays L.) was created by intercrossing of 12 domestic inbred lines, which origin traces back to several landraces. The population underwent four cycles of recurrent selection for grain yield and lodging resistance. The objective of the present study was to examine the effect of selection on simple sequence repeat (SSR) marker allele frequencies and population structure. The SSR analysis revealed no significant changes in the mean number of alleles per locus and the mean expected heterozygosity after four cycles of selection. The proportion of selectively nonneutral loci in single cycles of selection, based on Waples' test of selective neutrality, varied between 16% and 37%. Some of nonneutral loci co-located with previously published QTLs controlling important agronomic traits. Between 5% and 29% of loci were found to be in significant Hardy-Weinberg (HW) disequilibrium with majority of them showing an excess of homozygosity. Excess of homozygosity at several loci was highly consistent across cycle populations suggesting positive assortative mating as the possible cause of the observed HW disequilibrium. Linkage disequilibrium (LD) test was not significant for the most pairs of SSR loci. The proportion of pairs of loci in significant LD varied across cycles of selection between 0.1% and 1.8% probably due to the effects of genetic drift and epistatic selection.

PP18

Temperature-dependent structural changes in Cas3 protein in *Escherichia coli*

Markulin Dora[1], Peharec Štefanić Petra[1], Čulo Anja[1], Pandžić Marta[1], Matković Marija[2], Ivančić Baće Ivana[1*]

[1]Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Horvatovac 102A, 10 000 Zagreb, Croatia
[2]Division of Organic Chemistry and Biochemistry, Rudjer Boskovic Institute, Bijenicka c. 54, 10 000 Zagreb, Croatia

**E-mail: ivana.ivancic.bace@biol.pmf.hr*

The CRSISPR-Cas system is a significant mechanism of bacteria and archea that provide adaptive immunity against viruses and plasmids. It consists of DNA repeats separated by spacers of foreign origin (CRISPR locus), and *cas* genes responsible for various stages of defense. In *E. coli*, Cas3 protein is involved in a degradation of invader DNA as a last stage of defense. Recent studies showed that Cas3 could be the limiting factor for regulation of the CRISPR-Cas immunity due to its unusual property – loss of activity at 37°C, unless the protein is present in abundance. In this work we wanted to investigate if the loss of Cas3 activity is caused by structural change of protein which is temperature-dependent. We monitored structural changes in the purified protein by measuring a change of ellipticity using circular dichroism and by measuring intrinsic tryptophan fluorescence using fluorescence spectrometry. Both methods gave the same result, a subtle conformational change in helical region at 35°C which is in agreement with the protein activity change *in vivo*. This is probably the first experimental evidence that Cas3 activity from *E. coli* is temperature-dependent due to the change in protein conformation. Also, similar structural change was observed in archaeal Cas3 suggesting that this trait is preserved in other species as well. The results of this research will contribute to better understanding of regulation of Cas3 activity as well as to the progress of the CRISPR-Cas field.

PP19

HOXB13 germline mutation and DNA mismatch repair genes in the pathogenesis of Croatian prostate cancers

Matić Zrinka[1], Frobe Ana[2], Ugarković Đurđica[1], Đermic Damir[1], Feliciello Isidoro[3]*

[1] *Department of Molecular Biology, Ruder Boskovic Institute, Zagreb, Croatia*
[2]*Department of Oncology and Nuclear Medicine, UHC Sestre Milosrdnice, Zagreb, Croatia*
[3]*Dipartimento di Medicina Clinica e Chirurgia, Universita degli Studi di Napoli Federico II, Naples, Italy*

**E-mail: isidoro.feliciello@unina.it*

HOXB13 is a homeobox protein transcription factor that is required for prostate development. Genetic mutations of this gene are expected to lead to loss of prostate homeostasis and promote prostatic cancer, one of the most common malignancies in Croatia. HOXB13 mutations might also alter the physical interactions with the androgen receptor (AR), inducing the AR malignancy shift in human prostate epithelial cells. DNA mismatch repair (MMR) genes preserve genetic stability by correcting errors during DNA synthesis and several studies indicated that they may play important roles in the development of human prostate cancer. There are no data about both single nucleotide polymorphism genotyping of HOXB13 gene and MMR gene expression analysis in Croatian prostate cancer patients. Thus, the present study investigated the gene expression profile of seven major MMR genes (MLH1, MLH3, MSH2, MSH3, PMS1, PMS2 and MSH6) and five HOXB13 germline missense mutations (G84E, Y88D, A128D, G135E and L144P) in Croatian population and their association to prostate cancer susceptibility.

PP20

Effects of melatonin and resveratrol on telomere dynamics in liver and kidneys in 1- and 2-year-old Wistar rats†

Nanić Lucia[1], Gerić Marko[2], Gajski Goran[2], Jurasović Jasna[3], Karaica Dean[4], Ljubojević Marija[4], Micek Vedran[5], Novak Jovanović Ivana[6], Orct Tatjana[3], Peraica Maja[6], Rašić Dubravka[6], Vrhovac Madunić Ivana[4], Sabolić Ivan[4], Breljak Davorka[4], Rubelj Ivica[1]*

Ruđer Bošković Institute, Zagreb, Croatia: [1]Division of Molecular Biology, Laboratory for Molecular and Cellular Biology; Institute for Medical Research and Occupational Health, Zagreb, Croatia: [2]Mutagenesis Unit, [3]Analytical Toxicology and Mineral Metabolism Unit, [4]Molecular Toxicology Unit, [5]Laboratory Animals Unit, [6]Toxicology Unit

†*Supported by the Croatian Science Foundation project IP-11-2013-1481.*

**E-mail: rubelj@irb.hr*

Telomeres are the main guardians of genome stability. During DNA replication, progressive shortening of telomeres takes place with each cell division. Critically short telomeres induce cell cycle arrest or apoptosis. Oxidative stress also plays an important role in telomere dynamics. Recent evidence highlights direct molecular connection between telomere attrition and mitochondrial dysfunction. Although reactive oxygen species (ROS) are normal products in intermediary metabolism, and are crucial for different intracellular signalling pathways, a disbalance resulting in ROS excess induces oxidative stress and accelerates telomere shortening. It is assumed that with ageing, production of ROS may be increased causing faster telomere shortening, and that both may be ameliorated by antioxidants. To test this hypothesis, we treated male and female Wistar rats for 9 or 21 months with melatonin and resveratrol and investigated their effect on telomere attrition rate in liver and kidney (various zones) tissues. Telomere length was assessed by Southern blotting of genomic DNA. The results in 1- and 2-year-old male and female rats showed an absence of significant differences in telomere length in the tissues from both organs after treatment with antioxidants compared to control (vehicle-treated) animals (N=4 per group). Based on obtained results, we did not observe beneficial properties of tested antioxidants towards age-dependent telomere shortening.

PP21

Idetification of *A. flavus* and *A. parasiticus* in different crops using molecular genetic methods

Nikolić Ana, Nikolić Milica, Ignjatović-Micić Dragana, Marković Ksenija, Stanković Slavica

Maize Research Institute, Slobodana Bajića 1, 11185, Belgrade, Serbia

**E-mail: anikolic@mrizp.rs*

The main aflatoxin (AF) producing fungi are *A. flavus* and *A. parasiticus*. All previous studies carried out under the production conditions of Serbia showed no presence of *A. parasiticus.* The significance of direct losses as a consequence of crop grain (maize, wheat) infection, as well as potential contamination with aflatoxins, have pointed out the need to determine the presence of toxigenic potential of isolates originating from Serbia. Morphological, toxicological and molecular traits were considered in order to identify species of the *Aspergillus* genus present in this area using grain samples from different crops. Besides standard methods, PCR-RFLP technique was used for species identification. *A. flavus* and *A. parasiticus* species can be distinguished based on number of restriction sites for *BglII* in the intergenic spacer (IGS) for the AF biosynthesis genes *aflJ* and *aflR*. A total of 56 isolates were submitted to PCR-RFLP and five were proven to be *A. flavus* and 50 *A. parasiticus*. Also, one isolate did not show either of the expected profiles indicating presence of some other species of the *Aspergillus* genus. Further in-depth analysis will include sequencing of specific gene regions in order to confirm obtained data using GCPSR method and maybe enable identification of other species of this genus.

PP22

Assessment of genetic diversity of garlic accessions from Croatia by SSR markers

Poljuha Danijela[1]*, Kralj Ines[1], Šatović Zlatko[2,4], Ban Dean [1,4], Toth Nina[2], Dumičić Gvozden[3], Kereša Snježana[2], Goreta Ban Smiljana[1,4]

[1]Institute of Agriculture and Tourism, Karla Huguesa 8, 52440 Poreč, Croatia
[2]Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia
[3]Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, 21000 Split, Croatia
[4]Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Svetošimunska cesta 25, 10000 Zagreb, Croatia

**E-mail: danijela@iptpo.hr*

Garlic (*Allium sativum* L.) is a vegetatively propagated vegetable extensively studied due to its beneficial health effects. The cultivation and use of garlic is a part of Croatian tradition and each region or even village has their own landraces preserved for generations. In a relatively small area, a high phenotypic diversity could be observed and therefore the aim of this study was to assess a genetic diversity of collected Croatian garlic accessions. We genotyped 43 accessions from three Croatian regions (Istria, Dalmatia and continental Croatia) along with 14 foreign landraces using six polymorphic SSR markers. The number of distinct genotypes has been determined and the genetic structure and relationships among the accessions have been accessed. This is the first attempt to evaluate a large set of garlic accessions maintained by Croatian institutions as a part of the National program for conservation and sustainable use of plant genetic resources for food and agriculture. Accompanied by morphological and biochemical description, the results of this study will contribute to better knowledge on garlic genetic resources and foster effective and sustainable management of Croatian collections.

PP23

Genetic biodiversity of Croatian olives (*Olea europaea* L.) analyzed by SSR markers: A step toward the sustainable management of national genetic resources

Poljuha Danijela[1]*, Kralj Ines[1], Krapac Marin[1], Klepo Tatjana[2,8], Strikić Frane[7], Radunić Mira[2], Benčić Đani[3], Marinović Peričević Marija[4], Žeravica Domagoj Ivan[4], Car Marijo[4], Marić Mara[4], Vujević Predrag[5], Culek Mirta[6], Šatović Zlatko[3,8].

[1] *Institute of Agriculture and Tourism, Karla Huguesa 8, 52440 Poreč, Croatia*
[2]*Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, 21000 Split, Croatia*
[3]*Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia*
[4]*Department of Mediterranean Cultures, University of Dubrovnik, Marka Marojice 4, 20000 Dubrovnik, Croatia*
[5]*Institute of Pomology, Croatian Center for Agriculture, Food and Rural Affairs, Gorice 68b, 10000 Zagreb, Croatia*
[6]*Plant Genetic Resources Divison, Croatian Center for Agriculture, Food and Rural Affairs, Usorska 19, 31000 Osijek, Croatia*
[7]*University of Split, Ruđera Boškovića 37, 21000 Split, Croatia*
[8]*Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Svetošimunska cesta 25, 10000 Zagreb, Croatia*

**E-mail: danijela@iptpo.hr*

Croatia excels in a valuable and rich, but insufficiently investigated olive biodiversity. Significant efforts have been made in the last decade to collect national genetic resources to ensure their long-term conservation in *ex situ* collections, and thus, their sustainable management. In Croatia there are four *ex situ* olive germplasm collections, with 88 accessions officially registered in the Croatian Plant Genetic Resources Database. In all countries with centuries-long tradition of olive growing, there are numerous traditional cultivars, as well as a pronounced problem of homonymy, synonymy and intravarietal variability. The aim of this study, carried out within the National programme for conservation and sustainable use of plant genetic resources for food and agriculture, was to characterize the collections and to assess the genetic diversity of collected olive germplasm. We genotyped 69 accessions of 39 traditional cultivars, along with five introduced cultivars, using 14 polymorphic SSR markers. The number of distinct genotypes, duplicated accessions, and the cases of homonymy and synonymy have been determined and the genetic structure and relationships among Croatian traditional olive cultivars have been accessed. Results obtained in this study can be used for germplasm conservation, breeding programs, nursery production improvement and for optimizing a national strategy for the management of olive genetic resources.

PP24

Assessment of genetic diversity in red clover breeding populations selected in abiotic stress conditions using AFLP markers and fodder quality traits

Primorac Jurica[1], Šarčević Hrvoje[2], Kozumplik Vinko[2], Knezović Zrinka[1], Bolarić Snježana[2].

[1]University of Mostar, Faculty of Agriculture and Food Technology, Mostar, Bosnia and Herzegovina
[2]University of Zagreb, Faculty of Agriculture, Zagreb, Croatia

**E-mail: Jurica.primorac@aptf.sum.ba*

Changes in the global climate pose demands for the breeders in creating red clover cultivars tolerant to abiotic stress. The aim of this study was to evaluate the most important fodder quality traits of red clover breeding populations developed under the abiotic stress conditions during two experimental years and to estimate genetic diversity with AFLP markers. In the field trials, 12 red clover populations were included, of which nine were Croatian breeding populations, two Japanese cultivars and one standard Polish cultivar 'Viola'. Analysis of variance (ANOVA) found significant differences between populations in all investigated quality properties in both research years. In the second, drier year, the average content of structural carbohydrates was lower and the average water-soluble carbohydrate content (WSC) increased from 27.82 g kg^{-1} in the first year to 54.66 g kg^{-1} in the second year. AFLP marker analysis revealed a high level of polymorphism and the proportion of polymorphic loci across populations was 72.4 %. An analysis of molecular variance (AMOVA) revealed that the largest proportion of variation (92.27 %) resides at the within population level. An UPGMA dendrogram based on genetic distances divided populations into two groups. One group contained, with one exception, all populations selected in low temperature conditions, while the other contained populations selected in dry conditions and under conditions without abiotic stress.

PP25

Interplay of CRISPR adaptation, recombination and host nucleases in *Escherichia coli*

Radovčić Marin[1], Wettstein Lukas[2], Killelea Tom[2], Bolt Edward L.[2], Ivančić Baće Ivana[1]*

[1]*University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Horvatovac 102a, 10000 Zagreb, Croatia*
[2]*School of Life Sciences, Queen's Medical Centre, Medical School, University of Nottingham, Nottingham NG7 2UH, United Kingdom*

**E-mail: iibace@biol.pmf.hr*

CRISPR-Cas system is a prokaryotic adaptive immune system against foreign genetic elements. Immunity is acquired through insertion of small fragments (spacers) of invader DNA into a CRISPR array. This process is called adaptation, and can be mediated solely by the protein complex Cas1-Cas2 ("naïve adaptation"). In *E. coli*, RecBCD is thought to aid naïve adaptation by generating single-stranded DNA intermediates that are reannealed and further processed by Cas1-Cas2, and then integrated into the CRISPR array. In this work, we wanted to better understand the role of RecBCD and other host exonucleases in the process of prespacer preparation. Our genetic analysis shows that nuclease activity of RecBCD enzyme is not required for spacer preparation and that RecA inhibits adaptation probably because it prevents DNA processing and stimulates homologous recombination. However, helicase activity of RecBC(D) is required and is helped by 3'-5' host ssDNA exonucleases to occasionally generate appropriate DNA substrates for Cas1-Cas2 binding. Our *in vitro* analysis implies that Cas1-Cas2 forms a stable complex on DNA substrates with 5' overhangs and catalyses their cutting. Overall, our data suggest that 5' overhangs are important as substrates for adaptation and that these may be bound and processed by Cas1-Cas2.

PP26

QTL analysis for pre-harvest sprouting resistance in bread wheat

Rajković Bruno[1,2]*, Lovrić Ana[1], Šarčević Hrvoje[1,2], Kereša Snježana[1], Maričević Marko[3], Gunjača Jerko[1,2], Šatović Zlatko[2,4], Novoselović Dario[2,5]

[1]Department of Plant Breeding, Genetics and Biometrics, Faculty of Agriculture, University of Zagreb, Svetošimunska 25, 10000 Zagreb, Croatia
[2]Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Faculty of Agriculture, University of Zagreb, Svetošimunska 25, 10000 Zagreb,Croatia
[3]Bc Institute for Breeding and Production of Field Crops, Dugoselska 7, Rugvica, 10370 Dugo Selo, Croatia
[4]Department of Seed Science and Technology, Faculty of Agriculture, University of Zagreb, Svetošimunska 25, 10000 Zagreb, Croatia
[5]Department for Breeding and Genetics of Small Cereal Crops, Agricultural Institute Osijek, Južno predgrađe 17, 31000 Osijek, Croatia

**E-mail: brajkovic@agr.hr*

Pre-harvest sprouting (PHS) in wheat can cause a reduction in grain yield, test weight and grain quality. The level of seed dormancy at harvest maturity is the main component of PHS resistance in wheat. In the present study, recombinant inbred line (RIL) mapping population developed by single seed descent from the cross 'Bezostaja 1' (weak PHS resistance, non-dormant) × 'Klara' (high PHS resistance, dormant) was used to analyse seed dormancy. The RIL population and parental genotypes were grown in replicated trials in two different environments. The genetic and the genotype × environment component of variance accounted for 71 and 20% of the phenotypic variance for seed dormancy, respectively. The estimated heritability of the trait was 0.85. In the RIL population, transgressive segregation was observed mostly in the direction of low seed dormancy, while only a few lines had more pronounced seed dormancy than the dormant parent 'Klara'. A genetic map has been developed based on the RIL population while QTL analysis using composite interval mapping (CIM) and multiple interval mapping (MIM) allowed the identification of putative quantitative trait loci (QTLs) controlling seed dormancy.

PP27

Transcriptional activity of repetitive DNA families in the beetle *Tribolium castaneum*

Sermek Antonio[1], Feliciello Isidoro[1,2], Ugarković Đurđica[1]*

[1] *Department of Molecular Biology, Ruđer Bošković Institute, Bijenička 54, HR-10000 Zagreb, Croatia*
[2] *Dipartimento di Medicina Clinica e Chirurgia, Universita' degli Studi di Napoli Federico II, via Pansini 5, I-80131 Napoli, Italy*

**E-mail: ugarkov@irb.hr*

Satellite DNAs are tandemly repeated sequences clustered within heterochromatin. However, in some cases such as the major TCAST1 satellite DNA from the beetle *Tribolium castaneum*, they are found partially dispersed within euchromatin. Such organization together with transcriptional activity enables TCAST1 to modulate the activity of neighbouring genes and plays a role in heterochromatin remodelling during development and environmental stress response. In order to explore if other *T. castaneum* repetitive families have features which could provide them with a possible gene-modulatory role we analyse here transcription activity of ten distinct TCAST families. Transcriptome sequencing (RNA-seq) was performed on total RNA extracted from adult *T. castaneum* grown at 25°C as well as from adults subjected to 24 h heat stress at 42°C, with 30 min and 1 h of recovery, respectively. For oocytes and embryos we used publicly available dataset (SRA accession number: SRP050428). After sequencing, adapters and low quality reads were removed and mapped using Bowtie2 to dimers of repetitive families. Normalization method used was fragments per kb of transcript per million mapped reads (FPKM) which we calculated manually. Results from RNA-seq were confirmed by qPCR. Our study reveals that low copy TCAST repetitive families, partially dispersed within euchromatin, are transcribed. Their transcriptional activity is increased after heat stress and differs among stages of embryogenesis.

PP28

Compatibility of ten frequently used fluorescent proteins with the EVOS Floid microscope

Stošić Irin Antun, Žunar Bojan, Svetec Miklenić Marina, Štafa Anamarija, Svetec Ivan-Krešimir*

Laboratory for Biology and Microbial Genetics, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

**E-mail: iksvetec@pbf.hr*

The first isolated and most commonly used fluorescent protein is the green fluorescent protein (GFP). However, fluorescent proteins that cover the whole visible spectrum have been available and used as indispensable tools in a large spectrum of bioscience research. Fluorescent microscopes illuminate samples with the light of one colour, but detect only the light emitted by the fluorophores in the sample. Microscopes have been constantly developing, and one of the major advancements was the use of light emitting diodes (LED) as the source of light. The advantages of LEDs are uniform illumination, less dissipated heat and an extremely long life time. However, LEDs are limited to certain fixed wavelengths unlike the previously used arch lamps that emit white light. Therefore, the aim of this study was to determine the compatibility of ten commonly used fluorescent proteins (mTagBFP2, mTFP1, mEGFP, mCitrine, tdTomato, mTagRFP, mCherry, mKate, mPlum, and E2Crimson) with the EVOS Floid Imagining Station fluorescence microscope which uses blue, red and green light emitting diodes (LEDs) as a light source. We found out that, although the EVOS Floid Imagining Station fluorescence microscope has only three LEDs emitting specific wavelengths of the spectrum, it can detect a wide range of fluorescent proteins. Out of ten tested proteins only the mTagRFP was not detected. Furthermore, it is possible to distinguish five proteins in the same sample, one blue (mTagBFP2), one turquoise (mTFP1), one orange (mKate), one of three green (mEGFP, mCitrine or tdTomato) and one of three red (mCherry, mPlum or E2 Crimson) fluorescent proteins.

PP29

Interplay between epigenetic and genetic variation in Dalmatian sage (*Salvia officinalis* L.)

Šatović Zlatko[1,2]*, Jug Dujaković Marija[3], Radosavljević Ivan[2,4], Bogdanović Sandro[1,2], Grdiša Martina[1,2], Liber Zlatko[2,4].

[1]*University of Zagreb, Faculty of Agriculture, Zagreb, Croatia*

[2]*Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Zagreb, Croatia*

[3]*Institute for Adriatic Crops and Karts Reclamation, Split, Croatia*

[4]*University of Zagreb, Faculty of Science, Zagreb, Croatia*

**E-mail: zsatovic@agr.hr*

To understand the role of epigenetic processes in plant adaptation, existing epigenetic variation of natural plant populations has been assessed and compared to genetic variation. Twenty-five Dalmatian sage (*Salvia officinalis* L.) populations, each consisting of 20 to 25 plants, were analysed using epigenetic Methylation-Sensitive Amplification Polymorphism markers (MSAP) and genetic AFLP markers. Epigenetic and genetic diversity was compared by analysing within-population diversities and pairwise genetic differentiation between populations. The importance of epigenetic processes in divergence and adaptation of Dalmatian sage was analysed by comparing the number of outlier epiloci/loci under divergent selection and by the assessment of the association between epiloci/loci and bioclimatic variables. The identification of F_{ST} outlier epiloci/loci was carried out using a Bayesian method while the spatial analysis was used to test the probability of presence of an epiallelic/allelic variant given the environmental conditions of the sampling locations.

PP30

Overexpression of *YAP1* gene increases the resistance of yeast strains to inhibitors found in lignocellulosic hydrolysates

Štafa Anamarija[1], Djedović Elvis[1], Moguš Leo[1], Žunar Bojan[1], Svetec Miklenić Marina[1], Šantek Božidar[2], Svetec Ivan Krešimir[1]*

[1]*Laboratory for Biology and Microbial Genetics, Faculty of Food Technology and Biotechnology, Pierottijeva 6, University of Zagreb, Croatia*
[2]*Laboratory for Biochemical Engineering, Industrial Microbiology and Malting and Brewing Technology; Faculty of Food Technology and Biotechnology, Pierottijeva 6, University of Zagreb, Croatia*

**E-mail: iksvetec@pbf.hr*

Due to the constant increase in energy consumption, the production of biofuels from renewable sources, such as lignocellulosic waste, has become more frequent in the last several decades. Lignocellulosic waste consists of cellulose, hemicellulose and lignin and the yeast *S. cerevisiae* is not able to ferment it directly. Therefore, lignocellulosic waste is usually hydrolysed to release compounds that can be used for fermentation. However, the pretreatment also generates compounds that act as growth and fermentation inhibitors such as acetic and levulinic acid and 2-furaldehyde. In this study, newly constructed intraspecies hybrid diploids, obtained by crossing natural *S. cerevisiae* isolates, showed higher resistance to acetic and levulinic acid. Selected strains were also transformed with replicative vectors overexpressing yeast genes (e.g. *ATR1, CTA1, FLR1, YAP1, ZWF1*) encoding proteins involved in a stress response and the overexpression of *YAP1* gene further increased the resistance to inhibitors. Therefore, our results suggest that the construction of intraspecies hybrids coupled with genetic engineering is a promising approach for construction of new biotechnologically relevant yeast strains.

PP31

Screening of Croatian grape varieties to downy mildew sensitivity

Štambuk Petra[1,2], Preiner Darko[1,2], Šikuten Iva[1], Marković Zvjezdana[1], Andabaka Željko[1], Stupić Domagoj[1], Maletić Edi[1,2], Karoglan Kontić Jasminka[1,2]

[1]*University of Zagreb Faculty of Agriculture, Department of Viticulture and Enology, Zagreb, Croatia*
[2]*Centre of Excellence for Biodiversity and Molecular Plant Breeding, Zagreb, Croatia*

**E-mail: pstambuk@agr.hr*

In spite of being a small country, Croatia presents an important source of grapevine biodiversity which can be interesting for wine industry and breeding. In last two decades our research activities are focused on preservation, genotyping and evaluation of production characteristics of native varieties. To define full potential of native varieties there is a need to define the level of their susceptibility to fungal diseases. The aim of this research was to define the level of susceptibility/resistance of Croatian grapevine varieties to downey mildew (*Plasmopara viticola)* using modified field screening method. Altogether 57 genotypes/varieties were included in the research: 52 Croatian native varieties, two susceptible international *V. vinifera* L. cultivars (Cabernet Sauvignon, Riesling), 2 resistant cultivars (Solaris, Regent) and 2 *Vitis* species (*Vitis ripara, Vitis amurensis*). Own-rooted cuttings were planted in drip irrigated pots in field conditions close to the production vineyard at experimental station "Jazbina", Department of Viticulture and Enology, Faculty of Agriculture Zagreb. After initial growing of shoots to the certain stage the monitoring of disease symptoms was carried out according to OIV descriptor 452 (degree of resistance on leaves). Results showed small difference among native varieties and majority of them have very low and low level of resistance to powdery mildew, as expected.

PP32

Investigating the roles of a chromatin acylation reader in *Saccharomyces cerevisiae*

Vidan Nikolina[1,2], Qi Wang Bella[2], Verma-Gaur Jiyoti [2], Traven Ana[2] , Sopta Mary[1]*

[1]*Laboratory for Molecular and Cell Biology, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia*
[2]*Infection and Immunity Program and the Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Melbourne, Australia*

**E-mail: msopta@irb.hr*

Chromatin regulation by histone lysine acetylation is important for transcriptional regulation. In addition to enzymes that append or remove histone acetylations, «reader» proteins interact with the modified histones and recruit chromatin regulators and transcriptional complexes to DNA to enable biological outcomes. The YEATS domain proteins constitute an evolutionarily conserved family of histone acylation readers that are able to read not only histone lysine acetylation, but also the novel histone mark, lysine crotonylation. The genome of the model eukaryote, the yeast *Saccharomyces cerevisiae*, encodes three YEATS domain proteins - Taf14, Sas5 and Yaf9. These proteins are subunits of various chromatin remodelling complexes including RSC, INO80 and SWI/SNF and, in the case of Taf14, also of the general transcriptional factors TFIID and TFIIF. While the human homologs, such as AF9, have been implicated in oncogenic transformation, the precise cellular roles of the YEATS proteins are poorly defined. Here we show that in *S. cerevisiae*, mutants in some of the YEATS protein-encoding genes have roles in DNA damage response and functions in growth in the presence of cell wall damaging agents, membrane affecting agents and oxidative stress.

PP33

Construction of an all-strain applicable construct for integration of DNA sequence into herpes simplex virus 1 genome – work in progress

Vignjević Ana[1,2], Pribanić Matešić Marina[1], Serdar Marina[1,3], Djaković Lara[1,2], Jurak Igor[1]

[1]Laboratory for molecular virology, Department of Biotechnology, University of Rijeka, 51000 Rijeka, Croatia
[2]Master Program in Biotechnology in Medicine, University of Rijeka, Department of Biotechnology
[3]BSc Program in Biomedical Science, School of Chemistry and Biosciences, Faculty of Life Sciences, University of Bradford, Bradford BD7, UK

**E-mail: ana.vignjevic@student.uniri.hr*

Herpes simplex virus 1 (HSV-1) is a well-studied human pathogen involved in many ailments - from mild oral infections to serious health conditions such as keratitis and encephalitis. The replication of HSV-1 consists of a productive (lytic) and a latent phase (latency). During the lytic phase, the virus expresses its genes and generates viral progenies, inevitably destroying host cells. At the same time, the virus infects nearby neurons where active replication is halted and expression of genes is limited only to the region of the genome encoding latency associated transcripts (LAT). Importantly, the virus can reactivate from latency and cause recurrent diseases.

The main goal of this study is to generate a simple construct for an efficient insertion of a sequence of interest into the HSV-1 genome using a cloned virus genome as a bacterial artificial chromosome (BAC) and homologous recombination in bacteria to study latency. We have generated a plasmid vector carrying homologous sequences to a region between UL3 and UL4 of the HSV-1 genome, a selection marker and a multiple cloning site (MCS) for cloning a sequence of interest (SOI). To evaluate and optimize the expression of the inserted SOI within the virus genome for different applications, we have generated several constructs carrying enhanced green fluorescent protein (EGFP) expressed under a strong promoter from cytomegalovirus (CMPp) or under an endogenous HSV-1 promoter. Also, in an effort to minimize the construct, we plan to test if a transcriptional termination signal cloned downstream of the SOI is required for the efficient expression of the inserted genes. The project and the current work in progress will be presented.

PP34

A Genetic structure and hybridization risk assessment for the wildcat (*Felis silvestris silvestris*) population in Croatia

Vladušić Tomislav[1]*, Lindić Petra[1], Jaša-Šangulin Lucija[1], Bielen Ana[1], Hrašćan Reno[1], Peričić Salihović Marijana[2], Gužvica Goran[3], Šver Lidija[1]

[1]*Laboratory for Biology and Microbial Genetics, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia*
[2]*Institute for Anthropological Research, Ljudevita Gaja 32, Zagreb, Croatia*
[3]*Oikon Ltd. - Institute for Applied Ecology, Trg senjskih uskoka 1-2, Zagreb, Croatia*

**E-mail: tomislav.vladusic@pbf.hr*

The european wildcat *Felis silvestris silvestris* inhabits wide areas in Europe and is considered a subspecies genetically close to the domestic cat *Felis silvestris catus*. The two subspecies have different habitat preference, but as human activity causes undisturbed areas to disappear the two subspecies come increasingly into contact, allowing for hybridization and the disappearence of the genetically pure wildcat populations. While hybridization has been confirmed throughout Europe, the genetic structure of wildcats in Croatia has not been previosly studied. With relatively conserved forest habitats, Croatia might contain a wildcat population largely unaffected by hybridization. If present, such population is important in maintaining of the wildcat genetic structure for both conservation of the existing wildcat populations and reintroduction of the genetically pure wildcats into areas from which they have disappeared. Muscle, blood and oral mucosa samples have been obtained from both wildcats and domestic cats. After DNA extraction, microsatellite markers have been amplified by high fidelity DNA polymerase using multiplex touchdown PCR and analysed by capilary electrophoresis. Bayesian clustering using Structure 2.3.4 clearly separated the wildcat, domestic and hybrid cat populations and the genetic variability of each population has been analyzed using Arlequin 3.5.2.2. and CLUMPAK software. The results show low incidence of hybrids, and the existance of subpopulations in both wildcats and domestic cats. These results indicate the need for further wildcat habitat preservation, and might be used as a basis in tracking, monitoring and conservation of the wildcat population in Croatia.

PP35

Croatian buckwheat landrace populations and genetic diversity assessment via microsatellite markers

Žulj Mihaljević Maja[1]*, Habuš Jerčić Ivanka[1], Bogović Mara[2], Pejić Ivan[1], Gunjača Jerko[1]

[1]*University of Zagreb, Faculty of Agriculture, Department of Plant Breeding, Genetics and Biometrics*
[2]*Agricultural extension service, Varaždinska county, Varaždin*

**E-mail: mzulj@agr.hr*

Buckwheat (*Fagopyrum esculentum* Moench) is an old, gluten free pseudo-cereal originating from Asia whose importance increased in Croatia as well due to healthy diet trends. It is produced as a main or post-crop after winter cereals, with very low inputs which makes it attractive for the farmers. In Croatia it is traditionally grown in the northwestern part – Croatian Zagorje, Podravina and in Međimurje. With increasing interest for buckwheat production and consumption, lack of the adequate reproduction material becomes an issue since in Croatia there is no buckwheat breeding program or seed trade. There is therefore a need for collecting, genotyping and revitalizing the remaining Croatian buckwheat germplasm. Accordingly, the aim of this research was to determine genetic profiles and genetic structure of local buckwheat landrace populations that were collected earlier. Molecular genetic analysis of 12 microsatellite (SSR) markers was done for progeny obtained from reselection (25 individuals per population) as well as for international reference varieties (10 individuals per variety). Low level of intervarietal genetic variability was determined and thus it was hard to clearly separate populations. Due to high intravarietal variability further reselection of chosen populations is needed. Reference varieties were genetically more homogenous as expected.

AUTHOR INDEX

A

B

C

K

L

LJ

M

N

O

P

Q

R

S

Š

T

U

V

W

Y

Z

Ž

SPONSORS

GENERAL SPONSORS

SPONSORS

SUPPORTED BY

Ministry of Science, Education and Sports

CroatianGeneticSociety
www.genetika.hr
2018

ISBN 978-953-57128-1-7