# Plant Chromosome Biology
## Cytogenetics meeting 2023

## Book of abstracts

September 11–13, Brno

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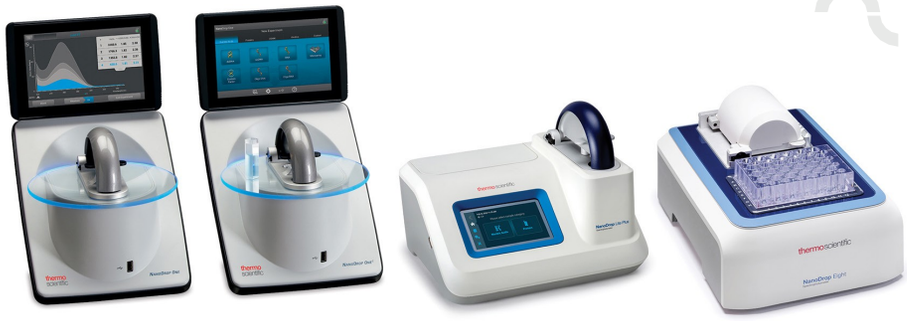

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# Plant Chromosome Biology
## Cytogenetics meeting 2023

### September 11-13, Brno

The conference brings together researchers investigating all aspects of plant genomes. Considering the ongoing progress in the field of chromosome biology, the focus of this meeting will be the structure of plant chromosomes and genome dynamics in the light of epigenomic stability. The program addresses a broad spectrum of fundamental and applied aspects of these topics.

Like the years before, the meeting provides excellent opportunities to stimulate scientific discussions regarding chromosome engineering, advanced chromosome biology and plant breeding.

Organizers:

**Roman Hobza**, Department of Plant Developmental Genetics, Institute of Biophysics of the Czech Academy of Sciences

**Andreas Houben**, Society for Plant Breeding e.V. (GPZ), section "Cytogenetic and Chromosome Analysis".

plantcytobrno.ibp.cz

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# Programme

## 11 September 2023:

8:00 - 8:30:
Registration

8:30 - 9:00:
Opening ceremony

**9:00 - 10:25:**
**Session 1: Genome stability, nucleus and chromosome architecture**

10:25 - 10:50:
Coffee Break

**10:50 - 12:10:**
**Session 1: Genome stability, nucleus and chromosome architecture**

**12:10 - 12:30:**
**Session 1: Poster pitches**

12:30 - 13:20:
Lunch

**13:20 – 13:40: Partners presentations**

**13:40 - 14:45:**
**Session 2: Genome and chromosome evolution, environmental response and plasticity**

14:45 – 15:10:
Coffee break

**15:10 - 16:10:**
**Session 2: Genome and chromosome evolution, environmental response and plasticity**

**16:10 – 16:40:**
**Session 2: Poster pitches**

16:40 – 16:55:
Break

**16:55 – 18:40:**
**Session 3: New tools, perspectives and applications**

**18:40 – 19:00:**
**Session 3: Poster pitches**

19:00:
Posters Dinner (Session 1, 2, 3)

## 12 September 2023:

**08:30 - 09:55:**
**Session 4: Mitosis, meiosis, gamete formation, polyploidy and apomixes**

09:55 - 10:15:
Coffee break

**10:15 - 11:30:**
**Session 4: Mitosis, meiosis, gamete formation, polyploidy and apomixes**

**11:30 - 11:40:**
**Session 4: Poster pitches**

11:40 - 12:40:
Coffee + Posters (Session 4)

12:40 - 13:30:
Lunch

13:40:
Departure to the Mendel Museum

**13:50 - 14:20:**
**Eva Chocholová: Peas of Mendel´s life revealed by dental calculus**

**14:20 - 14:50:**
**Ingo Schubert: The fate of Mendel's discoveries**

15:00 - 18:00:
Tours of the Mendel Museum / free time

18:00:
Conference Dinner

## 13 September 2023:

**8:30 - 10:05:**
**Session 5: Specialized chromosomes (Sex chromosomes, B chromosomes and others)**

**10:05 – 10:20**
**Session 5: Poster pitches**

**10:20-10:40:**
**Andreas Houben: Plant chromosome biology - Quo vadis?**

**10:40 – 11:40:** Coffee + Posters (Session 5)

**11:40 - 12:10:**
**Awards and closing ceremony**

12:10:
Lunch

## List of talks

| | | | |
|---|---|---|---|
| **Session 1:** | **Genome stability, nucleus and chromosome architecture** | | |
| **Time:** | **2 hours and 45 minutes** | | |
| **Chair:** | **Hana Šimková and Jiří Fajkus** | | |
| 1. **Main speaker:** | **Holger Puchta**: Applying CRISPR/Cas to plants: From gene editing to chromosome engineering | **Abstract 1** | **9:00 – 9:45** |
| 2. **Talk** | **Takayoshi Ishii**: Chromosome elimination during hybrid embryogenesis of Poaceae: wheat/oat × Pennisetum | **Abstract 2** | **9:45 – 10:05** |
| 3. **Talk** | **Jiří Fajkus**: Telomerase RNA gene paralogs: the usual pathway to unusual plant telomeres | **Abstract 3** | **10:05 – 10:25** |
| 4. **Talk** | **Yi-Tzu Kuo**: Mitotic and meiotic centromere dynamics differ in a holocentric species with megabase-sized centromere units | **Abstract 4** | **10:50 – 11:10** |
| 5. **Talk** | **Paula Franke:** Key aspects of the spatial nucleus organization in the Brachypodium genus | **Abstract 5** | **11:10 – 11:30** |
| 6. **Talk** | **István Molnár:** U chromosomes of Aegilops biuncialis are more frequently eliminated than M chromosomes during their introgression into wheat | **Abstract 6** | **11:30 – 11:50** |
| 7. **Talk** | **Mehrdad Shahbazi**: Cytonuclear Interactions in Auto- and Allopolyploids of Festuca-Lolium Complex | **Abstract 7** | **11:50 – 12:10** |
| **Poster pitch session for poster:** | **8, 9, 10, 11, 12, 13, 14, 15** | | |

## List of talks

| | | | |
|---|---|---|---|
| **Session 2:** | **Genome and chromosome evolution, environmental response and plasticity** | | |
| **Time:** | **2 hours and 5 minutes** | | |
| **Chair:** | **Martin Lysák and Ingo Schubert** | | |
| **1. Main speaker:** | **Tony Heitkam**: The Crocus Chronicles: Decoding the Triploid Puzzle of Clonal Saffron Crocus | **Abstract 16** | **13:40 – 14:05** |
| **2. Talk** | **Sreetama Bhadra**: The impact of genome size on trait flexibility, evolutionary radiation and the distribution of angiosperms | **Abstract 17** | **14:05 – 14:25** |
| **3. Talk** | **Bozena Kolano**: Evolutionary Dynamics of tandem repetitive sequences in Crepis sensu lato | **Abstract 18** | **14:25 – 14:45** |
| **4. Talk** | **Terezie Mandáková**: Origin and evolution of diploid and allopolyploid Camelina genomes | **Abstract 19** | **15:10 – 15:30** |
| **5. Talk** | **Hanna Weiss-Schneeweiss:** Prospero's magic: when karyotypic and genomic changes drive diversification (and the phenotype does not keep up) | **Abstract 20** | **15:30 – 15:50** |
| **6. Talk** | **Trude Schwarzacher**: Insights into chromosome and repetitive sequence evolution in Musaceae from long-molecule, t2t genome assemblies and molecular cytogenetics | **Abstract 21** | **15:50 – 16:10** |
| **Poster pitch session for poster:** | **22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34** | | |

## List of talks

| | | | |
|---|---|---|---|
| **Session 3:** | **New tools, perspectives and applications** | | |
| **Time:** | **1 hours and 45 minutes** | | |
| **Chair:** | **Jiří Macas and Jan Bartoš** | | |
| **1. Main speaker:** | **Jaroslav Doležel:** Let them move: flow cytometric chromosome analysis and sorting | **Abstract 35** | 16:55 – 17:20 |
| **2. Talk** | **Hana Šimková:** A 3C view of 3D genome dynamics in barley | **Abstract 36** | 17:20 – 17:40 |
| **3. Talk** | **Petr Novák:** RepeatExplorer tools for genome annotations | **Abstract 37** | 17:40 – 18:00 |
| **4. Talk** | **Nobuko Ohmido:** Unveiling the Nano-World of Plant Chromosomes: Chromatin Fiber Compaction and Scaffold Protein Localization using Advanced Microscopy Techniques | **Abstract 38** | 18:00 – 18:20 |
| **5. Talk** | **Takashi Okamoto:** Wheat-rice hybrid mitochondria in Oryzawheat and their transmission to the next generation | **Abstract 39** | 18:20 – 18:40 |
| **Poster pitch session for poster:** | **40, 41, 42, 43, 44, 45** | | |

## List of talks

| | | | |
|---|---|---|---|
| **Session 4:** | **Mitosis, meiosis, gamete formation, polyploidy and apomixes** | | |
| **Time:** | **2 hour and 40 minutes** | | |
| **Chair:** | **Andreas Houben and Miroslava Karafiátová** | | |
| **1. Main speaker:** | **Jiri Macas:** Comparative genomics of plant centromeres | **Abstract 46** | **8:30 – 8:55** |
| **2. Talk** | **Begoña Quirós-de-la-Peña**: Cytogenetic and molecular evidence for polyclonality and sexual events in allopolyploid apomictic species *Hieracium halleri* (Asteraceae) | **Abstract 47** | **8:55 – 9:15** |
| **3. Talk** | **Ales Pecinka:** Spatial in vivo dynamics of mitotic divisions in barley (*Hordeum vulgare*) | **Abstract 48** | **9:15 – 9:35** |
| **4. Talk** | **Jana Lunerová, Veit Herklotz:** Unraveling the Rosa canina Genome: Cytogenetic, Genomic, and Phylogenetic Insights into Pentaploid Genome Harbouring Asymmetrical Meiosis. | **Abstract 49** | **9:35 – 9:55** |
| **5. Talk** | **Inna Lermontova:** The protein-protein interaction network of KNL2 in Arabidopsis thaliana | **Abstract 50** | **10:15 – 10:35** |
| **6. Talk** | **Suriya Tamilselvan Nattar Amutha:** Single pollen nucleus genotyping and virus tools for meiotic gene studies in barley (*Hordeum vulgare* L.) | **Abstract 51** | **10:35 – 10:55** |
| **7. Talk** | **Stefan Steckenborn:** Dynamics and adaptation of the meiotic recombination landscape among natural and artificial chromosomes in holocentric Rhynchospora plant species | **Abstract 52** | **10:55 – 11:15** |
| **8. Talk** | **Veronika Kolačková**: STED nanoscopy of intra-nuclear chromatin structures in barley (Hordeum vulgare L.) | **Abstract 53** | **11:15 – 11:30** |
| **Poster pitch session for poster:** | **54, 55, 56, 57, 58, 59, 60, 61, 62** | | |

## List of talks

| | | | |
|---|---|---|---|
| **Session 5:** | **Specialized chromosomes (Sex chromosomes, B chromosomes and others)** | | |
| **Time:** | **1 hour and 55minutes** | | |
| **Chair:** | **Roman Hobza and Sònia Garcia** | | |
| **1. Main speaker:** | **Roman Hobza:** Key drivers of plant sex chromosomes knowledge explosion | **Abstract 63** | **8:30 – 8:50** |
| **2. Talk** | **Václav Bačovský**: A complex affair – sex chromosome evolution in plants | **Abstract 64** | **8:50 – 9:10** |
| **3. Talk** | **Lucie Horáková:** Considerable genetic diversity within the hop varieties explains non-Mendelian segregation patterns | **Abstract 65** | **9:10 – 9:25** |
| **4. Talk** | **Miroslava Karafiátová**: Does B chromosome alternate the pollen development in wild sorghum? | **Abstract 66** | **9:25 – 9:45** |
| **5. Talk** | **Sònia Garcia:** Unveiling Reproductive Diversity: Tree of Sex 2.0 Progress Report | **Abstract 67** | **9:45 – 10:05** |
| **Poster pitch session for poster:** | **69, 70, 71, 72, 73, 74, 75, 76** | | |
| **Closing talk** | **Andreas Houben:** Plant chromosome biology - Quo vadis? | **Abstract 68** | **10:20 – 10:40** |

## List of posters

| Session 1: | Genome stability, nucleus and chromosome architecture | |
|---|---|---|
| 1. | **Eszter Gaál**: Crested wheatgrass and the gametocidal genes system to widen the gene pool of wheat | **Abstract 8** |
| 2. | **Anna Nowicka:** Towards an understanding of nuclear morphology and chromosome organization in endoreduplicated nuclei of barley seed tissues | **Abstract 9** |
| 3. | **Ondřej Helia:** Effects of extensive chromosomal rearrangements in terms of genetics and epigenetics in Arabidopsis thaliana model plant | **Abstract 10** |
| 4. | **Alžběta Doležalová:** Variability in mutual chromosome positioning in 3D space of rice nuclei | **Abstract 11** |
| 5. | **Solmaz Khosravi:** Chromosomal rearrangments and epigenetic consequences | **Abstract 12** |
| 6. | **Marcel Hubinský:** Extensive chromosomal changes and seed geometric morphometrics in Silene | **Abstract 13** |
| 7. | **Eva Sýkorová:** Chromosomes at their ends – evolutionary perspectives | **Abstract 14** |
| 8. | **Serhii Mykhailyk:** Nucleolar dominance in the allotetraploid model grass Brachypodium hybridum – exploring molecular mechanisms behind the cytogenetic phenomenon. | **Abstract 15** |

## List of posters

| Session 2: | Genome/Chromosome evolution, environmental response and plasticity | |
|---|---|---|
| 1. | **Nomar Waminal**: Dysploidy in spring crocuses (Crocus series Verni) | **Abstract 22** |
| 2. | **Yile Huang**: The pathways of whole-genome duplications, diploidization, and descending dysploidy in the Biscutelleae (Brassicaceae) | **Abstract 23** |
| 3. | **Hina Shimomai**: Urban environments, chromosome formula and polyploidization in two Commelina species | **Abstract 24** |
| 4. | **Sara Ishiguro**: Major genotype-panels for Hydrangea genomics by comparative repeat analysis | **Abstract 25** |
| 5. | **Xinyi Guo**: The amplification of interstitial telomeric repeats accompanied genome evolution in the East Asian genus Yinshania (Brassicaceae) | **Abstract 26** |
| 6. | **Dana Trunova**: Does the time matter? Revealing the pathways of rRNA gene evolutionin Brachypodium hybridum of different evolutionary age. | **Abstract 27** |
| 7. | **Jana Čížková**: Evolution of the family Musaceae through large chromosomal rearrangements | **Abstract 29** |
| 8. | **Anton Stepanenko**: Nucleotide level structure of 5S rDNA loci in the great duckweed, Spirodela polyrhiza | **Abstract 30** |
| 9. | **Sophie Maiwald**: Evolving together: Cassandra retrotransposons gradually mirror promoter mutations of the 5S rRNA genes | **Abstract 31** |
| 10. | **Milan Pouch**: Polyploidy-driven genome evolution of the South African genus Heliophila (Brassicaceae) | **Abstract 32** |
| 11. | **Thiago Nascimento**: Independent and recurrent dysploidy events in Phaseolus beans | **Abstract 33** |
| 12. | **Nicola Schmidt**: Repeat turnover meets stable chromosomes: Repetitive DNAs mark speciation and gene pool boundaries in sugar beet and wild beets | **Abstract 34** |

## List of posters

| Session 3: | New tools, perspectives and applications | |
|---|---|---|
| 1. | **Tereza Šlajsová**: The application laboratory for agricultural research at the Centre of Plant Structural and Functional Genomics of Institute of Experimental Botany | **Abstract 40** |
| 2. | **Petr Fajkus**: Computational identification of evolutionary conserved non-coding RNAs | **Abstract 41** |
| 3. | **Martina Nešpor Dadejová**: Detection of protein recruitment and protein-protein interactions at DNA lesions in plant protoplasts | **Abstract 42** |
| 4. | **Ludwig Mann**: High-fidelity repeat consensus sequences from short reads | **Abstract 43** |
| 5. | **Bhanu Prakash Potlapalli**: Development of novel CRISPR/Cas9-based tools for visualizing genomic sequences | **Abstract 44** |
| 6. | **Petr Janás**: Reversing ancient chromosomal rearrangements in Cardamine hirsuta (Brassicaceae) using CRISPR/Cas | **Abstract 45** |

## List of posters

| Session 4: | Mitosis, Meiosis, gamete formation, polyploidy and apomixes | |
|---|---|---|
| 1. | **Jana Szecówka:** Both male and female meiosis contribute to non-Mendelian inheritance of parental chromosomes in interspecific plant hybrids (Lolium × Festuca) | **Abstract 54** |
| 2. | **Azahara C. Martin:** ZIP4 is required for normal progression of synapsis and for over 95% of crossovers in wheat | **Abstract 55** |
| 3. | **Astrid Johanna Irena Severyns:** Uncovering the mechanism of cold-induced meiotic restitution in Musa spp. | **Abstract 56** |
| 4. | **Ramakrishna Yadala:** Recurrent plant-specific duplications of KNL2 and its conserved function as a kinetochore assembly factor | **Abstract 57** |
| 5. | **Baicui Wang:** Identification of novel meiotic players based on screening of Axis TurboID candidates (ATC) and SUMO candidates | **Abstract 58** |
| 6. | **Maria Cuacos:** Anti- and pro-CO factors in barley | **Abstract 59** |
| 7. | **Zhenling lyu:** Tetraploid B. rapa are highly tolerant of tetravalent formation and aneuploidy | **Abstract 60** |
| 8. | **Ludmila Oliveira:** Antibodies against kinetochore proteins NDC80 and KNL1 provide highly versatile markers of centromeres in flowering plants (angiosperms) | **Abstract 61** |
| 9. | **Chao Feng:** Proteomic profiling of meiotic chromosome axis in Arabidopsis thaliana based on TurboID | **Abstract 62** |

## List of posters

| Session 5: | Specialized chromosomes (Sex chromosomes, B chromosomes and others) | |
|---|---|---|
| 1. | **Jana Kružlicová:** Weird and wonderful – an origin of sex chromosomes in two races of Rumex hastatulus | **Abstract 69** |
| 2. | **Jianyong Chen:** Identification of the trans-acting factors that control the non-Mendelian drive of the rye B chromosome | **Abstract 70** |
| 3. | **Gihwan Kim:** Identification and functional characterization of genes controlling the process of root-specific B chromosome elimination in Aegilops speltoides | **Abstract 71** |
| 4. | **Pavla Novotná:** Dosage compensation mechanism in plants with evolutionary young sex chromosomes | **Abstract 72** |
| 5. | **Lucie Hloušková:** Characterization of a maize landraces collection concerning the B chromosome | **Abstract 73** |
| 6. | **Joan Pere Pascual-Díaz:** Sex-chrome version 2.0: a database of green plant species with sex chromosomes | **Abstract 74** |
| 7. | **Marcel Hubinský**: Non canonical bases as possible regulators of sex chromosomes in the dioecious plant Silene latifolia | **Abstract 75** |
| 8. | **Pavel Jedlička:** Extended reconstruction of transposable elements in plants using TE-greedy-nester | **Abstract 76** |
| 9. | **Kashif Nawaz:** Assembly of saltgrass genomes reveals unique genomic features of a dioecious, perennial, halophytic grass | **Abstract 78** |

# Session 1

# Genome stability, nucleus and chromosome architecture

## Talks

**1.**

# Applying CRISPR/Cas to plants: From gene editing to chromosome engineering

Holger Puchta[1]

1. *Joseph Gottlieb Kölreuter Institute for Plant Sciences, Karlsruhe Institute of Technology*

Till today programmable nucleases as CRISPR/Cas have been applied to plants mainly on genes for the improvement of traits. However, breeding also requires the breaking or establishing genetic linkages on the chromosome level. Using Cas9, we were able to change genetic linkages by inducing heritable translocations in the Mb range between heterologous chromosomes in Arabidopsis thaliana. Recent improvements in sequence analysis of crop plants reveal that multi Mb long inversions occur with high frequency between different genotypes, leading to crossover suppression. We were not only able to demonstrate that inversions up to almost chromosome size can be achieved in Arabidopsis, but also meiotic recombination can be redirected this way. Thus, on one side a recombination dead region could be reactivated after 5000 years and on the other almost a complete chromosome could be excluded from genetic exchange. In the future, CRISPR/Cas-mediated chromosomal engineering will allow us to restructure plant genomes according to our needs for breeding. Finally, we developed a new technology based on DSB-induced genome elimination for tissue engineering named CRISPR-Kill, allowing us to induce targeted cell death in different organs at select developmental stages. Recently we were able to set up an inducible CRISPR-Kill system, allowing also the temporal control of cell death.

## 2.

# Chromosome elimination during hybrid embryogenesis of Poaceae: wheat/oat × *Pennisetum*

Takayoshi Ishii[1]

1. *Arid Land Research Center, Tottori University*

Wide hybridisation is one of the practical methods for increasing genetic diversity in plant breeding. However, there are several levels of incompatibility during wide hybridisation in different species combinations. Wheat (*Triticum aestivum*) can cross with subfamilies of distant species such as maize (*Zea mays*), sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*). Sperm from these species fertilise the wheat egg. However, non-wheat chromosomes are eliminated during early embryogenesis. In the oat (*Avena sativa*) x pearl millet combination, all the pearl millet chromosomes are retained in the background of the haploid oat genome during embryogenesis and young seedling formation. Maternal and paternal factors influence chromosome elimination. To elucidate the paternal factors influencing the process of chromosome elimination, we crossed several different *Pennisetum* species with wheat and oat. Different degrees of chromosome elimination were found. 1. rapid chromosome elimination, 2. gradual chromosome elimination, 3. retention of a few chromosomes and 4. no chromosome elimination.

## 3.

# Telomerase RNA gene paralogs: the usual pathway to unusual plant telomeres

Mgr. Petr Fajkus, Ph.D.[1], Mgr. Michal Závodník[2], Mgr. Vratislav Peška, Ph.D.[3], Prof. RNDr. Jiří Fajkus, Ph.D.[4]

1. *Institute of Biophysics ASCR, and Masaryk University, CEITEC*
2. *Masaryk University, CEITEC, and Faculty of Science, NCBR*
3. *Institute of Biophysics, ASCR*
4. *Masaryk University, CEITEC, Masaryk University, Faculty of Science, NCBR, and Institute of Biophysics, ASCR.*

Telomerase, telomere DNA and associated proteins represent a complex, finely tuned, and functionally conserved mechanism that ensures genome integrity by protecting and maintaining chromosome ends. Changes of its components can threaten an organism's viability. Nevertheless, molecular innovation in telomere maintenance has occurred multiple times during eukaryote evolution, giving rise to species/taxa with unusual telomeric DNA sequences, telomerase components, or telomerase-independent telomere maintenance. The central component of telomere maintenance machinery is telomerase RNA (TR) as it templates telomere DNA synthesis, its mutation can change telomere DNA and disrupt its recognition by telomere proteins, thereby leading to collapse of their end-protective and telomerase recruitment functions. Using a combination of bioinformatic and experimental approaches, we examine a plausible scenario of evolutionary changes in TR underlying telomere transitions. We identified plants harbouring multiple TR paralogs whose template regions could support the synthesis of diverse telomeres. In our hypothesis, formation of unusual telomeres is associated with the emergence and occurrence of TR paralogs that can accumulate mutations, and through their functional redundancy, allow for the adaptive evolution of the other telomere components. Experimental analyses of telomeres and telomerase in the examined plants demonstrate evolutionary telomere transitions corresponding to TR paralogs with diverse template regions.

## 4.

# Mitotic and meiotic centromere dynamics differ in a holocentric species with megabase-sized centromere units

Yi-Tzu Kuo[1], Amanda Souza Câmara[1], Veit Schubert[1], Pavel Neumann[2], Jiří Macas[2], Jianyong Chen[1], Michael Melzer[1], Jörg Fuchs[1], Takayoshi Ishii[3], André Marques[4], Andreas Houben[1]

1. *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany*
2. *Biology Centre, Czech Academy of Sciences, Institute of Plant Molecular Biology, České Budějovice, Cezch Republic*
3. *Tottori University, Arid Land Research Center, Tottori, Japan*
4. *Department of Chromosome Biology, Max Planck Institute for Plant Breeding Research, Cologne, Germany*

The centromere is the chromosomal region where the spindle microtubules attach during cell division. In contrast to monocentric chromosomes with a single centromere location in most known species, holocentric species exist with a centromere distributed throughout chromosome length. The mechanism that triggered a centromere-type transition might differ among independently evolved holocentric lineages, leading to diverse holocentromere compositions. We found a drastic change in centromere organization between the closely related monocentric *Chamaelirium* and holocentric *Chionographis*. Remarkably, in *Chionographis japonica*, the repeat-based holocentromere is formed based on only a few monocentromere-like megabased-sized centromere units. The composition and distribution of the centromere units influenced the large-scale genome organization and formed distinct eu- and heterochromatic domains. The genome organization in *Chionographis* possesses characteristics of both monocentric and holocentric species, distinct from those in other known holocentric species. Additionally, *Chionographis* performs a monocentromere-like meiosis, in which the multiple centromere units of each chromatid form a single cluster and act like a monocentromere. Strikingly, the sister centromeres are unfused at meiosis I, resulting in four centromere clusters on each bivalent. Our findings broaden the knowledge about the diversity of centromere organizations. We demonstrate the unique value of analyzing non-model species for evolutionary comparisons to reveal novelties in even well-studied structures.

# 5.

## Key aspects of the spatial nucleus organization in the *Brachypodium* genus

MSc Paula Franke[1], MSc Chinedum Anajemba[1], dr Manoj Kumar Solanki[1], dr Joanna Łusińska[1], dr Ewa Robaszkiewicz[1], dr Artur Piński[1], dr Elżbieta Wolny[1], dr Dominika Idziak-Helmcke[1], prof. Robert Hasterok[1]

1. *Plant Cytogenetics and Molecular Biology Group, Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, 28 Jagiellonska Street, 40-032 Katowice, Poland*

The 3-D arrangement of interphase chromatin greatly impacts the processes in the nucleus and the organism. Small-genome grass species of the *Brachypodium* genus are used as models for studying different aspects of chromatin spatial arrangement in plants. Our ongoing studies use 3-D fluorescence *in situ* hybridization (FISH) on isolated leaf and root nuclei of various *Brachypodium* species to address diploid and polyploid genome organization differences and the role of different nuclear envelope proteins in preserving 3-D chromatin structure.

Preliminary analysis of parental subgenome positioning in an allotetraploid *B. hybridum* interphase nuclei with the use of subgenome-specific BAC clones showed that parental subgenomes predominantly occupy distinct nuclear territories, with only little intermingling at the territories' borders. The position and the size of the territories have been characterized.

Analysis of centromeric and telomeric domain number and distribution by FISH in different genotypes of *Brachypodium* species indicated intraspecific variation in domain association. A similar analysis performed on *B. distachyon* SUN2 knock-out mutant suggests that lack of SUN2 envelope protein may also affect specific domain arrangement in the nucleus.

The authors acknowledge financial support from the National Science Centre, Poland (grant no. 2018/31/B/NZ3/01761).

## 6.

# U chromosomes of *Aegilops biuncialis* are more frequently eliminated than M chromosomes during their introgression into wheat

István Molnár[1], Eszter Gaál[1], András Farkas[1], László Ivanizs[1], Balázs Kalapos[1], Edina Türkösi[1], Klaudia Kruppa[1], Éva Szakács[1], Mahmoud Said[2], Abraham Korol[3], Jaroslav Doležel[2], Miroslav Valárik[2]

1. *Department of Biological Resources, Centre for Agricultural Research, Eötvös Lóránd Research Network, 2462, Hungary*
2. *Institute of Experimental Botany of the Czech Academy of Sciences, Centre of Plant Structural and Functional Genomics, Šlechtitelů 31, 779 00 Olomouc, Czech Republic*
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Wild goatgrass, *Aegilops biuncialis* ($U^bU^bM^bM^b$) represents an untapped source of genetic diversity that could be exploited in wheat improvement. However, chromosome mediated gene introgression from wild relatives into wheat has been hampered by the lack of genome refference sequence and scarcity of molecular tools.

A DArTseq genetic map of $F_2$ populationderived from cross of *Ae. biuncialis* MvGB642 x MvGB382 lines revealed well preserved collinearity of the $M^b$-genome with bread wheat, while multiple karyotype reconstructions were identified in the $U^b$-genome. The wheat-*Aegilops* synteny allowed mapping of the DArTseq markers in a wheat x *Ae. biuncialis* $BC_3F_{1\text{-}3}$ population. The DArTseq genotyping in combination with fluorescence *in situ* hybridization (FISH) using U- and M-genomic- and DNA and Afa family, pSc119.2 and 45S rDNA repetitive probes confirmed the presence of *Ae. biuncialis* chromosomes $1M^bS$, $3M^b$, $4M^b$, $5M^b$ and $6M^b$, while a majority of $U^b$ chromosomes was lost and only $1U^b$-, $2U^b$-, $4U^b$- and $7U^b$ chromosomal fragments were present in some lines. The new addition-, substitution and introgression lines together with the chromosome specific markers will facilitate development of new wheat cultivars adapted to the changing environmental conditions.

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# 7.

## Cytonuclear Interactions in Auto- andAllopolyploids of *Festuca-Lolium* Complex

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Some Protein complexes consist of both nuclear and organellar-encoded subunits. For example, the large subunit of Rubisco is encoded by the plastid gene RbcL, while the small subunit is encoded by the nuclear gene RbcS. The subunits of such protein complexes are interdependent and precisely regulated at the genomic, transcriptomic, and post-transcriptomic levels. The stoichiometry between the subunits and their optimal assembly can be disrupted by polyploidization, which increases the ploidy level of the nuclear genome without instant doubling of the organellar genome, and hybridization, which merges two distinct genomes. In this study, we investigate both the stoichiometry restoration mechanisms of Festuca prantesis and Lolium multiflurom autopolyploids and conflicts in genomics, transcriptomics, and structural proteomics resulting from merging two genomes in allopolyploids (with maternally inherited organellar genomes). Our data suggest that increasing chloroplast number and especially chloroplastic gene copy number in autopolyploids are the main contributors to stoichiometry restoration without significant changes in gene expression level. In allopolyploids, we detected that although the chloroplast genome is inherited strictly maternally, the majority of the nuclear genes involved in cytonuclear complexes are expressed equally from both homoeologues, with the exception of PSB domain 7 (PPD7), which was mainly expressed from Lolium multiflurom. Differences in the amino acid composition between the homoeologues did not significantly alter the binding site structure or the electrostatic potential of the surface except in the PPD7 protein, in which substitution of isoleucine (I) with L-threonine (T) can contribute to potential differences in protein stability and hydrogen bonding interactions.

# Session 1

## Genome stability, nucleus and chromosome architecture

## Posters

# 8.

## Crested wheatgrass and the gametocidal genes system to widen the gene pool of wheat

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Gene pool of wheat has narrowed during domestication and cultivation over thousands of years. Wild relatives of wheat are promising source of new genes for wheat improvement. Crested wheatgrass (*Agropyron cristatum* L. Gaertn.) represents a rich source of important genes. Gametocidal (*Gc*) system is an efficient approach to transfer useful traits to wheat from wild relatives. *Gc* genes induce chromosomal rearrangements in gametes lacking them in monosomic addition of the *Gc* chromosome. The utilization of wild genetic diversity has been hampered by low throughput of selection methods and the lack of knowledge on genomes of wild relatives. Conserved Ortholog Set (COS) markers specific for genes conserved throughout evolution define orthologous regions, thus enabling comparison of regions on chromosomes of related species.

We crossed wheat Chinese Spring (CS)-*A. cristatum* 5P and 6P chromosome addition lines with CS-*Ae. cylindrica* 2C chromosome addition line with *Gc* effect to induce structural rearrangements between wheat and 5P or 6P chromosomes. GISH and FISH analysis of $BC_1F_2$ detected seven and two wheat-*Agropyron* translocations for 5P and 6P, respectively. Three 5P-wheat and one wheat-6P translocations were transferred to the next generation. Characterization of selfed progenies indicated that 5P introgressions positively affected grain number per plant. We also used fourteen wheat–*A. cristatum* disomic and ditelosomic additions and the 1PS·1BL translocation line to map COS markers on *Agropyron* and to characterize syntenic relationships. Out of 328 selected COS markers, 279 consistently amplified products in tetraploid *A. cristatum*, and, out of these, 139 were polymorphic between tetraploid crested wheatgrass and wheat. Sixty-nine markers were found to be suitable for the detection of tetraploid *A. cristatum* chromosomes 1P–6P in wheat, ranging from 6 to 17 markers per chromosome. Generally, markers of the same homeologous group were detected on the same chromosome arms.

Acknowledgments:

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## 9.

# Towards an understanding of nuclear morphology and chromosome organization in endoreduplicated nuclei of barley seed tissues

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Cereal grains represent a special type of seeds, with the largest part formed by the endosperm, a plant-unique tissue nourishing the developing and germinating embryo. As a model in our study, we use barley, which has a large genome (2n = 2x = 14, 1C = 4.8 Gbp) with interphase chromosomes organized in Rabl configuration with centromeres and telomeres positioned at the opposite nuclear poles. Using flow-sorted nuclei, we investigated nuclear morphology and chromosome organization in cycling and endoreduplicated nuclei isolated from embryo and endosperm tissues of developing grains. We found that higher nuclear ploidy and developmental progression alter the nuclear shape, cause nucleoli dispersion, generate a positional loss of sister chromatid cohesion, and reduction in centromeric histone CENH3 abundance. This coincided with the centromeres and telomeres dislocations within the nuclear space. Interestingly, all nucleus- and chromatin changes were accompanied by reduced genome stability, progressive loss of cell viability, and cell death. Altogether, this project uncovers the complexity of nuclear and cellular processes during barley grain development.

## 10.

# Effects of extensive chromosomal rearrangements in terms of genetics and epigenetics in *Arabidopsis thaliana* model plant

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A demand for new crop varieties with maximum yield and resistance to biotic and abiotic stress conditions is accentuated by the global environmental changes of recent years. Modern approaches of genetic engineering can contribute to obtaining the desired traits. To this end, the CRISPR/Cas approach can be used. Importantly, it is even possible to simulate large-scale genome rearrangements mimicking those that occurred naturally during plant evolution (*Schindele et al., 2020*). However, it is not yet known if and how these genome changes will be reflected in subsequent generations of modified plants. The focus of our research is the study of effects of large-scale genome modifications using homozygous lines of *Arabidopsis thaliana* with reciprocally translocated ends of long arms of chromosomes 1-2 and 1-5 (*Beying et al., 2020*). The phenotypic diversity was observed in T4 generation of homozygous plants, in contrast to T3 generation, where all translocated plants were comparable to the wild type line. In addition to phenotype monitoring in subsequent generations, analyses of telomere lengths, gene expression and chromatin structure are in progress. These analyses are supposed to reveal whether and how chromatin organization is affected in response to changes in genome organization, and whether and how respective changes are interrelated.

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## 11.

# Variability in mutual chromosome positioning in 3D space of rice nuclei

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Our study focuses on the 3D analysis of chromatin organization in rice (Oryza sativa, 2n = 2x = 24; 489 Mbp/1C). To assess the chromosome positioning in interphase nuclei, chromosome oligo-painting was used to analyze their 3D distribution in G1 nuclei of root meristem and leave tissues. Six different configurations of chromosome territories (CTs) were observed in the root G1 nuclei. In comparison, only four configurations were observed in leaf G1 nuclei. The volume of CTs and frequency of their association varied also between the tissue types. CTs of chromosome 9 were usually separated (87 %) in the root G1 meristematic nuclei. In leaf nuclei, CTs separation was less frequent (37 %). Differences were observed also in the size of the nucleolus and the number and size of 45s rDNA loci. Finally, super-resolution microscopy (STED) revealed a more compact and less relaxed structure of chromatin in differentiated G1 nuclei of leaves compared to root meristem G1 nuclei. The average width of chromatin fiber in leaf G1 nuclei was 240 nm, compared to the root G1 where the width of the chromatin fiber was three times narrower (83 nm).

## 12.

# Chromosomal rearrangments and epigenetic consequences

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There is a strong correlation between the epigenetic state of a DNA sequence and its relative location on a chromosome. Compared to euchromatin referred as gene-rich regions, heterochromatin referred as gene-poor regions show different epigenetic modifications including DNA methylation and histone modifications. To address the question whether the chromosomal position of a sequence has a critical influence on the epigenetic state and activity of genes, CRISPR/Cas-based restructured Arabidopsis thaliana chromosomes will be used.

Using CRISPR/Cas technology, chromosome rearrangements including translocation (rDNA repeats on Chromosome 2 and 4) and inversion (5 Mb pericentromeric region of chromosome 3) events have been introduced. Subsequently, the Arabidopsis lines will be used to decipher whether moving a sequence to a different position on a chromosome might change its epigenetic state and gene expression profile.

To confirm homozygous lines having translocation and inversion events, single-copy-specific FISH probes were designed which will be used on naturally extended pachytene chromosomes. These lines will be used for ChIP-seq and whole genome bisulfite sequencing to investigate whether changing the position of euchromatic and heterochromatic regions can influence the epigenetic patterns of the rearranged regions at the sequence level.

In the next step, the Col0 lines (having inversion of a 5 Mb pericentromeric region on chromosome 3) will be crossed with Ler-1 (having the same inversion) to observe if the epigenetic marks are inherited to the next generation. Considering that only the euchromatic chromosome arms undergo genetic exchange as the basis of breeding during meiosis, we will investigate whether the epigenetic state or chromosome location is the main factor which affects cross over. Using the F1 hybrids and SNP analysis, we will evaluate whether the euchromatic region near the centromere or the heterochromatic region within a chromosomal arm are prone to cross overs, assuming that the epigenetic state is conserved in both ecotypes. As a result, we ought to be able to determine whether or not the epigenetic state is what suppresses cross overs.

## 13.

# Extensive chromosomal changes and seed geometric morphometrics in Silene

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Plants may undergo different natural changes, which dramatically modify their genomes. One is polyploidization, which is the presence of more than two chromosome sets within a cell. The second is hybridization, wich is the process of crossbreeding between genetically dissimilar parents to produce a hybrid. Both are regarded as key factors for plant evolution and reflect differences in fenotype in the different plant organs. In Silene we can find both examples in nature, and this genus has a seed shape diversity long recognized as a valuable source of information for infrageneric classification. Morphometric analysis is the statistical study of shape and size and their covariations with other variables. Shape is commonly described as the property of an object invariant under scaling, rotation, or translation. Traditionally, seed shape description was limited to an approximative comparison with geometric figures (rounded, globular, reniform or heart shape). Seed shape quantification has been based on direct measurements such as area, perimeter, length or width, narrowing statistical analysis. A geometrical approach involves mathematical equations. These equations encompass morphological traits under complex genetic control. Looking at seed images, or at their silhouettes, as geometric objects is a direct way to achieve mathematical accuracy in seed shape description. This is required for the combination of morphology with genetics, ecology or taxonomy. We generated synthetic tetraploids of Silene latifolia and performed controlled crosses between diploid S. latifolia and Silene dioica as an approach to analyse seed morphology. After imaging capture and postprocessing, we applied statistical analysis and we have found differences in the general seed shape both, in the tetraploids and in the hybrids. A detailed inspection through fluorescence microscopy allowed the identification of shape differences in the cells of the seed coat. These differences, in the case of the hybrids, permitted us to identify what was the sex of both of the parents. Morphometry can be applied to any image processing including cell microstructures, and provides mathematical accuracy for statistical analysis.

## 14.

# Chromosomes at their ends – evolutionary perspectives

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When organisms settled for linear chromosomes, the maintenance of their ends became essential. Nucleoprotein complexes called telomeres were established to guard chromosomal ends and genome stability. The telomerase-based mechanism looks like a winner for solving the problem of telomere DNA maintenance. Telomerase adds short tandem repeats that were designated as typical telomere motifs in large groups for long time, e.g. TTAGGG in vertebrates, TTTAGGG in plants. Our mapping of telomere sequences across the eukaryotic tree of life led to identification of novel telomere repeats in various groups including algae, plants and animals, e.g. the unusual telomeric repeat CTCGGTTATGGG in onion. Phylogeny suggested that the telomere repeat TTAGGG is an ancestral telomere type, but our results show that there is much more sequence variability, together with well-defined switchpoints in telomere motif evolution. Currently, a promising progress in plant telomerase research is made after the plant RNA subunit in onion and other plants was discovered in 2019. Further research identified plant TR subunits and defined their evolutionary relationship in the deep origin of plants and ciliates. Subsequently, further research led to the identification of TRs in Hymenoptera which share structural features similar to plant TRs and distinct to vertebrate TRs. However, which features are critical for telomere function (repeat, mechanism, structure, proteins) will be discussed.

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## 15.

# Nucleolar dominance in the allotetraploid model grass *Brachypodium hybridum* – exploring molecular mechanisms behind the cytogenetic phenomenon.

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Nucleolar dominance (ND) is the epigenetic silencing of one set of ancestral 35S rRNA genes (35S rDNA) in plant hybrids and allopolyploids. Despite being studied for decades, the molecular mechanisms selecting a gene set predestined for repression remain elusive. *Brachypodium hybridum*, an allotetraploid grass with a DDSS subgenome composition (D subgenome derived from *B. distachyon*; S subgenome from *B. stacei*), offers a convenient model for investigating ND due to its relatively compact genome, low content of repetitive sequences, and a single chromosomal pair carrying 35S rDNA inherited from each progenitor. Moreover, the presence of diverse genotypes exhibiting distinct 35S rDNA expression patterns, along with genetic resources and sequencing data for multiple *B. hybridum* accessions and ancestral diploid species, strengthens its suitability as a model to study ND.

Here, a population-level analysis of ND in *B. hybridum* was performed. The number and chromosomal distribution of 35S rDNA loci were determined by fluorescence *in situ* hybridisation. The contributions of rDNA homoeologues in different *B. hybridum* genotypes were assessed by Southern hybridisation and bioinformatic analysis of genomic Illumina reads. The expression of rDNA homoeologues at different developmental stages was analysed by RT-qPCR using locus-specific primers and RT-CAPS. Finally, the methylation status of rRNA genes was determined in several *B. hybridum* genotypes exhibiting differential expression of 35S rDNA. We conclude that ND in *B. hybridum* is developmentally regulated in some but not all genotypes, indicating population-specific regulation of ND.

# Session 2

# Genome and chromosome evolution, environmental response and plasticity

## Talks

## 16.

# The Crocus Chronicles: Decoding the Triploid Puzzle of Clonal Saffron Crocus

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Saffron crocus (Crocus sativus L.), a sterile triploid (2n = 3x = 24), is a clonally propagated crop grown for the production of saffron, the highest priced spice of the world. The evolutionary origin of the triploid C. sativus was largely unknown and has been subject of debate since the early 20th century. Recent cytogenetic and comparative next-generation sequencing approaches pointed to ancient Greece as a point of origin for saffron and concluded that C. sativus is an autotriploid species solely derived from heterogeneous cytotypes of C. cartwrightianus (Schmidt et al. 2019, Nemati et al. 2019, reviewed in Kazemi-Shahandashti et al. 2022).

In our ongoing work, we target the processes before and after emergence of triploid saffron crocus. With this, we can look into the past, present and future of this clonal line:

(1) The past: How is chromosomal variability in the progenitor C. cartwrightianus? We detected large differences in haplotype variability among chromosomes, suggesting that some C. cartwrightianus chromosomes have obtained a higher genetic diversity across the Mediterranean than others. This haplotype diversity is fixed in saffron's karyotype, a testimony to the genetic variability of C. cartwrightianus 5000 years ago.

(2) The present: Are there signs of somaclonal variability in the saffron clone? By comparing triploid saffron accessions across the globe, we detect chromosomal variability in three independent instances. Based on the combinations of these three sites of chromosomal variability, we conclude that at least four saffron lineages evolved somaclonally after emergence of triploid saffron crocus. These saffron lineages have likely spread and are most probably retained by vegetative propagation.

(3) The future: Can the global cultivation of the saffron crocus clone serve as a model for the epigenetics of adaptation? As the basis for comparative and epigenetic analyses, we are assembling reference genomes of the triploid saffron crocus and the diploid progenitor C. cartwrightianus. We show the progress of sequencing, assembly and chromosomal anchoring.

## 17.

# The impact of genome size on trait flexibility, evolutionary radiation and the distribution of angiosperms

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Angiosperm diversity is a result of repeated evolutionary radiations. However, it is unclear how genomic factors contribute towards such radiations. Here, we test the hypothesis that genome size acts as a potential driver of 'trait flexibility' i.e., the evolvability of traits over macroevolutionary times, affecting diversification rates. We integrate genome size, functional trait, distribution, and phylogenetic data for palms (Arecaceae)- a pantropical family comprising ca. 2,600 species – showing extraordinary trait and genome size diversity. We used macroevolutionary and structural equation models to show that palm lineages increased diversification rates ca. 20 million years ago, concordant with increased variation in genome sizes and traits. However, rates of genome size evolution were not associated with diversification rates, but with fruit size and stem diameter evolutionary rates. Instead, fast diversification rates were found for species with relatively large genomes, small fruits, and fast rates of stem height, fruit length, or leaf size evolution. Our results provide evidence that genome size can act as an inherent factor predisposing plants to trait flexibility, and fast evolution of traits provides the adaptability for lineages to persist and diversity. This may explain some of the most enigmatic evolutionary radiations across angiosperms and their distribution across the globe.

## 18.

# Evolutionary Dynamics Of Tandem Repetitive Sequences in *Crepis sensu lato*

dr hab Bozena Kolano[1], dr Gülru Yücel[2], dr Natalia Borowska-Zuchowska[1], dr Maciej Bisaga[1], dr Magdalena Senderowicz[1], dr Teresa Nowak[1]

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Repetitive sequences are ubiquitous and fast-evolving elements responsible for genome size variation and the large-scale organisation of plant genomes. *Crepis s.l.* consists of primarily diploid species with relatively small numbers of large and well-differentiated chromosomes and high variation in genome size. This makes it a good model system for investigating the organisation and diversification of repetitive elements. Bioinformatic, molecular and cytogenetic approaches were applied to study the organisation and evolution of repetitive elements in some *Crepis* species. Sequences were classified in terms of type and their abundance in the genome. Comparative analyses of their genomic organisation and chromosomal distribution were performed for groups of closely related species representing different evolutionary lineages of *Crepis*. Most identified repeats were specific to a small group of closely related species. The comparative analyses of satellite repeats showed that most satellite families were characterised by species-specific chromosomal distribution patterns. Usually, the overall patterns of the chromosomal distribution of an individual satDNA were comparable in very closely related species with similar genome sizes. In contrast, the more distantly related species, especially those whose speciation was accompanied by extensive genome size changes, often showed distinct patterns of satDNAs chromosomal distribution.

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## 19.

# Origin and evolution of diploid and allopolyploid *Camelina* genomes

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False flax or gold of pleasure (*Camelina sativa*) is an increasingly popular oilseed crop closely related to arabidopsis and canola. Despite the available sequenced genome and other genomic resources, the origin and putative parental genomes of the allohexaploid *C. sativa* (2*n* = 6*x* = 40) remained unknown. Nearly nothing was known about the origin, genome evolution and phylogenetic relationships of diploid (2*n* = 12 and 14) and polyploid (2*n* = 26, 38, and 40) *Camelina* species. First, we established fine-scale comparative cytogenomic maps for all diploid, tetraploid and hexaploid *Camelina* species and elucidated the origin of polyploid genome and phylogenetic relationships between *Camelina* species. We identified parental genomes of the known allopolyploid species (*C. intermedi*a, *C. microcarpa*, *C. rumelica* and *C. sativa*) and reconstructed the sequence of hybridization events. Besides common structural rearrangements, such as translocations and inversions, complex dysploidal chromosomal rearrangements generated shattered chromosomes in *Camelina* diploids. The nature of these rearrangements was further analyzed using chromosome-level genome assemblies of four diploid *Camelina* genomes. These complex chromosomal alterations, similar to chromoplexies associated with several human disorders, have not been reported in a non-model diploid plant species as yet.

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## 20.

# *Prospero*'s magic: when karyotypic and genomic changes drive diversification (and the phenotype does not keep up)

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The *Prospero autumnale* complex (Hyacinthaceae) is chromosomally remarkably variable. Phylogenetic and genomic analyses of hundreds of individually karyotyped plants from the entire distribution range of the complex revealed that the observed variation is very structured, both geographically and karyotypically. Three major processes creating these intricate and complex patterns of variation were identified: (1) dysploidy on diploid level (chromosome base numbers of *x* = 5, 6, 7 or 8) likely with low levels of karyotype restructuring, (2) recurrent auto- and allopolyploidization accompanied by backcrossing to the parental diploid(s) and/or inter-polyploid crossing, and (3) genome size dynamics due to proportional changes in dispersed repeat lineages and non-proportional changes in tandem repeat families, exclusively found and/or uniquely amplified in individual cytotypes. Yet another level of complexity stems from young and recurrently formed B-chromosomes and evolutionarily older euchromatic supernumerary chromosomal segments (SCSs). This enormous karyotypic variation is, however, not paralleled by phenotypic diversification as morphometric analyses failed to identify any character combinations for unambiguous cytotype identification. The karyotypic variation is not only tolerated, but emerges as a main driver of the evolution of this species complex, which, rather uniquely for bulbous species, propagates only sexually.

## 21.

# Insights into chromosome and repetitive sequence evolution in Musaceae from long-molecule, t2t genome assemblies and molecular cytogenetics

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The family Musaceae, with three genera Musa, Musella and Ensete, includes important crops and ornamental species. To understand their genome evolution, we combined data from chromosome scale assemblies with fluorescent in situ hybridization to describe the chromosomal rearrangements and repetitive DNA composition. Some 50-60% of the genomes are composed of repetitive sequences with LTR-retroelements and DNA transposons predominant and their families distributed among different groups of species. A tandemly repeated 134bp satellite sequence is present at the centromeres of Ensete glaucum, E. ventricosum and Musella lasiocarpa, but no tandem repeat is in Musa centromeres. 45S rDNA and 5S rDNA have variable numbers and chromosomal locations and occupy often otherwise non-syntenic arms. Thus, rDNA sites seem to move independently and associate with different chromosomal environments; the arm carrying 45S rDNA in E. glaucum is depleted in genes, but the arms with 45S rDNA in M. lasiocarpa have a similar gene density to other chromosome arms. The telomere-to-telomere assemblies, including centromeric positions, enabled us to characterize the chromosomal rearrangements. Musella and Ensete, both x=9, show only few whole arm translocations and large regions of synteny supporting the close relationship but separation of genera, while the x=11 Musa species are phylogenetically separate with many more postulated chromosomal rearrangements and genetic differences. This insights into the chromosomal and repetitive sequence evolution of Musaceae along with gene sequences in the assemblies, add to a comprehensive and robust pangenome of Musa and Ensete, valuable to understanding evolutionary mechanisms and for crop improvement.

# Session 2

# Genome and chromosome evolution, environmental response and plasticity

## Posters

## 22.

# Dysploidy in spring crocuses (*Crocus* series *Verni*)

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Spring crocuses, which comprise ten diploid and two polyploid species, as well as a polyploid complex, show diverse chromosome numbers (2*n* = 8, 10, 12, 14, 16, 18, 20, 22, 28), indicating dysploid karyotype changes. Thus, this taxon provides the opportunity to analyze mechanisms driving fast karyotype evolution. Here, we gather insights into dysploidy in *Crocus* ser. *Verni* by analyzing the genomic dynamics of repetitive elements. We characterized the types and abundances of repetitive genomic elements from each taxon and performed a comparative karyotype analysis in a phylogenomic context. *Crocus longiflorus*, which has 2*n* = 28 chromosomes, has the smallest genome size (2C = 3.2 pg), whereas taxa with fewer chromosomes have increased genome sizes. Repeat quantification and FISH analysis of six repeat probes suggest a concerted action of descending dysploidy and a burst of transposable elements (*Ty1/Copia* and *Ty3/Gypsy*) as drivers to the reduction of chromosome number while increasing genome size in more recently evolved taxa. This work provides insights into dysploidy in ser. *Verni* and an opportunity to develop approaches to study other dysploid-rich clades in plants.

## 23.

# The pathways of whole-genome duplications, diploidization, and descending dysploidy in the Biscutelleae (Brassicaceae)

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In plants, whole-genome duplications (WGDs) are often characterized by genome-wide diploidization including descending dysploidy, i.e., chromosomal rearrangements that reduce chromosome number and genome size. However, the genomic basis and evolutionary significance of chromosomal restructuring remain poorly understood. Phylogenetic and comparative genomic analyses revealed that the Biscutelleae consist of one diploid (Megadenia), one neotetraploid (Heldreichia), and three mesotetraploid (Biscutella, Lunaria, and Ricotia) genera, with the ancestral tetraploid genomes arising from independent WGD events involving closely related diploid genomes. Here, we sequenced, analyzed and compared genomes of four Biscutella species (Buckler Mustards) differing by genome sizes (0.7 to 1.1 Gbp) and chromosome numbers (n = 6 and 8). Genome sequence data with long-range scaffolding combined with molecular cytogenetics provided accurate assemblies and annotations at the chromosome level. Highly reshuffled Biscutella genomes have descended from two ancPCK-like ancestral genomes via allopolyploidy followed by extensive descending dysploidy (n = 16 → n = 6 and 8). Such genome restructuring mediating descending dysploidies and speciation appeared to be predominantly non-random, with large chromosomal segments conserved as duplicates, while others exhibited considerable turnover. Our results provide new insights into the role of dysploid changes in plant genome evolution, speciation, and diversification. Czech Science Foundation, project 21-07748L.

## 24.

# Urban environments, chromosome formula and polyploidization in two *Commelina* species

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Urbanization largely changes biodiversity and natural environments. Few studies have investigated plant adaptation to urban environments based on both ecological and molecular cytological approaches. We focus on two annual plants, *Commelina communis* (Cc) and its related species, *C.cfciliata* (Ccf) which grow widely in Asia and are highly diverse both in morphological traits and chromosome numbers (Ccf; 2n=46 and Cc; 2n=88). We demonstrate how plants adapt to urban environments by investigating the distribution in urban-rural gradients, the comparison of stomata and genome size, and chromosome karyotyping. As a result, Cc was distributed widely from rural to urban areas, while Ccf were found only in rural areas. The stomata length of Cc was significantly longer, and Cc had double genome size, compared to that of Ccf. Ccf chromosomes were detected on a part of Cc chromosomes by GISH. From these results, the large stomata size of Cc seems due to polyploidy, and polyploidy might give Cc to have a high potential to adapt to urban environments compared to Ccf. This is the first investigation to clarify the distribution difference relating to urbanization, the difference in stomata and genome size, and to conduct chromosome karyotyping in two *Commelina* species. However, there was no significant relationship between stomata size and urbanization in the regional comparisons. In this presentation, we discuss the mechanism of plant adaptation in urban environments focusing on the relationship between the effects of human activity and genetic traits which leads to the ecological difference in nature plants.

## 25.

# Major genotype-panels for *Hydrangea* genomics by comparative repeat analysis

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*Hydrangea* is a popular garden plant due to its ornamental value. However, hybridization has been performed primarily based on its morphological traits, resulting in unknown genetic diversity, species origin, and phylogeny. Analysis of repetitive elements in *Hydrangea* species is a key for understanding its genetic diversities and achievements of genetic resources for elaborate breeding. In this study, whole genome sequencing (WGS) and comparative graph-based-clustering analyses were performed using Repeat Explorer pipeline to characterize and compare repetitive elements within representative six *Hydrangea* species with different origins and chromosome numbers. The LTR retrotransposons are the most abundant repetitive elements within the *Hydrangea* genomes. Comparative clustering analysis divided into four groups based on the composition and abundance of identified repeats in their genomes; Japanese diploid *Hydrangea* species (GAK, TAM, and EZO), Japanese polyploid *Hydrangea* (NOR), Chinese *Hydrangea* (STR), and American *Hydrangea* (ANN). Tekay elements in *H. paniculata* (NOR) genome are independently developed from Japanese diploid species whereas Ale elements are conserved between NOR and Japanese diploid species. Thus, we demonstrate that NOR is polyploidized and differentiated independently from ancient Japanese diploid species, followed by sharing the abundance and composition of Ale elements by the horizontal transmission. In addition, satellite DNAs (satDNAs) landscape of NOR genome suggests that NOR genome has two genetic genealogies from Japanese diploid *Hydrangea* and Chinese *Hydrangea* species. In this presentation, we will also discuss the results of localize those repetitive satDNAs on *Hydrangea* chromosomes by FISH.

## 26.

# The amplification of interstitial telomeric repeats accompanied genome evolution in the East Asian genus *Yinshania* (Brassicaceae)

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Hybridization plays a key role in genomic divergence and speciation in plants. However, little is known about the variation in karyotypes and repetitive sequences during genome evolution enabled by hybridization. Here, we present an integrative cytogenomic study of the small crucifer genus *Yinshania* (c. six species), endemic to northern and southwestern China. Two infrageneric clades were identified and hybridization was detected both within and between clades. While three species (*Y. acutangula*, *Y. exiensis*, and *Y. wilsonii*) are diploid (2*n* = 12) and two (*Y. microcarpa* and *Y. zayuensis*) are tetraploid (2*n* = 24), *Y. henryi* includes both cytotypes. The diploid genomes have the same structure, which probably originated from an ancestral genome with eight chromosomes by descending dysploidy via two end-to-end translocations. The genomes of *Y. microcarpa* and *Y. zayuensis* are allotetraploid, whereas the tetraploid genome of *Y. henryi* is most likely an autotetraploid. The chromosome-scale genome assembly of *Y. wilsonii* corroborated the structure of the diploid karyotype and revealed slow genic evolution with distinct dynamics of major retrotransposon clades. In addition, we observed high variation in the number of 35 rDNA and tandem repeat loci among species. Interestingly, TTTAGGG-type telomeric repeats and their variants were identified as the most abundant satellite family in all *Yinshania* genomes. The presence/absence of the TTTAGCG variant appears to be associated with infrageneric cladogenesis. These simple tandem repeats were frequently associated with CRM retrotransposons and together form large loci at pericentromeres of all chromosomes. This work was supported by a research grant from the Czech Science Foundation (20-03419Y).

## 27.

# Does the time matter? Revealing the pathways of rRNA gene evolutionin *Brachypodium hybridum* of different evolutionary age.

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Ribosomal RNA genes (rDNA) encode the essential components of ribosomes, which are the cellular machinery responsible for protein synthesis. These genes consist of conserved genic and variable non-coding regions, providing a valuable tool for studying phylogenetic relationships and genome evolution. Previous studies of the rRNA genes in *Brachypodium hybridum* (a model allotetraploid grass originating from the diploid evolutionary ancestors similar to contemporary *B. distachyon* and *B. stacei*) mainly focused on the 35S rDNA. It has been shown that interaction between the ancestral genomes results in nuclear dominance and reduction of the copy number against one of the parental variants of 35S rDNA in numerous accessions of *B. hybridum*. In contrast, little is known about the organization of 5S rDNA in *B. hybridum*. In this study, we focused on two lineages of *B. hybridum* with different ages (0.14 Ma and 1.4 Ma) of origin. The 5S and 35S rRNA gene organization analyses have been performed using molecular (Southern Blot, gCAPS), cytogenetic (FISH) and bioinformatic approaches. The chromosomal distribution of 5S and 35S rDNA showed the uniparental loss of loci in *B. hybridum* Bhyb26, representing an ancient lineage. Furthermore, evidence of homogenization of rDNA was found. Our results shed more light on the evolutionary pathways of rDNA structure and evolution in this important model grass.

The research was funded by the National Science Centre, Poland (project number2020/39/O/NZ8/00184) to BK and DT.

## 29.

# Evolution of the family *Musaceae* through large chromosomal rearrangements

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The evolution of the family *Musaceae* was accompanied by whole genome duplication followed by diploidization and fractionation of the genome. Large-scale chromosomal rearrangements played a key role in diversification and speciation. The family *Musaceae* comprises genera *Musa*, *Ensete* and *Musella*. While *Ensete* (2n=18) and *Musella* (2n=18) are represented by few species, genus *Musa* contains about 70 different species, which are classified into four sections. The largest of them, Eumusa (x=11), comprises most of edible banana cultivars. Closely related section Rhodochlamys (x=11) contains ornamental species. Section Australimusa (x=10) contains a peculiar group of edible banana clones known as Fe'i. The last section, Callimusa (x=9, 10), is the most diverse one and includes species closely related to *Ensete* and *Musella*.

With the aim to identify and compare structural chromosome changes and to shed light on karyotype evolution in *Musaceae*, we used oligo painting FISH. We observed numerous chromosomal rearrangements, some of which were shared in closely related species. Translocation breakpoints were identified in assembled Oxford Nanopore data by a newly developed bioinformatic pipeline. Together with phylogenetic analysis and divergence time estimation of the main clades and selected species of *Musaceae*, we suggested the most probable mode of karyotype evolution.

## 30.

# Nucleotide level structure of 5S rDNA loci in the great duckweed, Spirodela polyrhiza

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The ribosomal DNA (rDNA) plays an important part in eukaryotes by encoding four ribosomal RNAs necessary for ribosome biogenesis, thus regulating organism's growth and development. Although intensively characterized cytogenetically in many plant taxa, the 5S and 35S rDNA loci are usually excluded from description of whole genome sequences because of their redundancy in hundreds to thousands tandemly arranged copies. Taking advantage of the uniquely low rDNA copy number in the monocot aquatic plant great duckweed, *Spirodela polyrhiza* (Lemnaceae), we revealed the detailed molecular organization of the 5S rDNA loci combining molecular cytology, conventional and extra-long Oxford Nanopore (ON) sequencing. While in situ hybridization detected a single 5S rDNA locus on *S. polyrhiza* chromosomes, the ON reads revealed two clusters of 5S rDNA units, one containing 60 copies of tandemly repeated units of about 1100 bp and a second cluster with 40 copies of ~500 bp repeated units. Both clusters are surrounded by highly AT-enriched sequences mapped correspondingly to *S. polyrhiza* chromosomes 13 and 6. The ON sequencing gave a reliable estimate of the 5S rDNA unit copy number, which was confirmed by quantitative PCR, however gene sequences were untypically heterogeneous compared to usually highly homogenized plant rDNA repeats. Validation of the rough ON data by sequencing multiple plasmids containing PCR fragments covering individual 5S rDNA units demonstrated that both clusters contained the conservative 119bp long sequence encoding 5S rDNA followed by the locus-specific intergenic spacer (IGS). In the locus of ChrS13, the 5S rRNA coding sequences were spaced by IGSs of 1056-1069 bp, and by the IGS of 400bp in the locus of ChrS6. To the best of our knowledge, the obtained data on 5S rRNA in *Spirodela polyrhiza*, makes the rDNA loci of this species most completely characterized among the flowering plants.

## 31.

# Evolving together: Cassandra retrotransposons gradually mirror promoter mutations of the 5S rRNA genes

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The 5S rRNA genes are among the most conserved nucleotide sequences across all species. Similar to the 5S preservation we observe the occurrence of 5S-related non-autonomous retrotransposons, so-called Cassandra. Cassandras harbor highly conserved 5S rDNA-related sequences within their long terminal repeats (LTRs), advantageously providing them with the 5S internal promoter. However, the dynamics of Cassandra retrotransposon evolution in the context of 5S rRNA gene sequence information and structural arrangement are still unclear, especially: 1) do we observe repeated or gradual domestication of the highly conserved 5S promoter by Cassandras and 2) do changes in 5S organization such as in the linked 35S-5S rDNA arrangements impact Cassandra evolution? Here, we show evidence for gradual co-evolution of Cassandra sequences with their corresponding 5S rDNAs. To follow the impact of 5S rDNA variability on Cassandra TEs, we investigate the Asteraceae family where highly variable 5S rDNAs, including 5S promoter shifts and both linked and separated 35S-5S rDNA arrangements have been reported. Cassandras within the Asteraceae mirror 5S rDNA promoter mutations of their host genome, likely as an adaptation to the host's specific 5S transcription factors and hence compensating for evolutionary changes in the 5S rDNA sequence. Changes in the 5S rDNA sequence and in Cassandras seem uncorrelated with linked/separated rDNA arrangements. We place all these observations into the context of angiosperm 5S rDNA-Cassandra evolution, discuss Cassandra's origin hypotheses (single or multiple) and Cassandra's possible impact on rDNA and plant genome organization, giving new insights into the interplay of ribosomal genes and transposable elements.

## 32.

# Polyploidy-driven genome evolution of the South African genus *Heliophila* (Brassicaceae)

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The tribe Heliophileae in the family Brassicaceae displays remarkable morphological diversity, that distinguishes it from other crucifer tribes. Endemic to southern Africa, this tribe thrives in biodiversity hotspots where polyploidy is traditionally not associated with diversification and speciation events. Our comprehensive study integrates molecular phylogenetics, genomics, cytogenetics, ecology, and taxonomy to investigate Heliophileae, encompassing about 70 *Heliophila* species with varying chromosome numbers (2*n* = 16 - 80). A large-scale phylogenetic analysis of more than 400 accessions revealed four major infrageneric clades that diversified between 12 to five million years ago. Using chromosome-scale genome assembly of *Heliophila variabilis* (*n* = 11, 334 Mb), we discovered two pairs of differentially fractionated subgenomes, indicating an allo-octoploid origin of the genome and Heliophileae. The putative octoploid ancestral genome (2*n* = 8x = 60) likely emerged from hybridization between two distinct allotetraploids (2*n* = 4x = 30), followed by post-polyploid diploidization and clade-/species-specific descending dysploidies (30 → 16 - 26). Here, we examine chromosome number evolution during diploidization among and within major *Heliophila* clades in South Africa and test the extent to which infrageneric cladogenesis and genome diploidization follow geographic, edaphic and (paleo)climatic patterns as well as ecological life histories.

## 33.

# Independent and recurrent dysploidy events in *Phaseolus* beans

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Beans from *Phaseolus* genus are well known for their importance as a protein source for humans, specially provided by cultivated species as the common bean and lima bean. Most *Phaseolus* species have a conserved karyotype number with *2n* = 22, except for the Leptostachyus group, a ~1.3 My clade comprising three species with *2n* = 20. Its ancestral underwent a descending dysploidy event promoted by a nested chromosome fusion. In this work, we investigated the karyotype evolution of *Phaseolus* species from the Filiformis group, using oligo-FISH painting probes for chromosomes 1, 2, 3 and 5, plus 5S and 35S rDNA. Our data revealed the existence of a new dysploid species outside of the Leptostachyus group, *P. angustissimus* (Filiformis group; ~2,9 My), also with 20 chromosomes; however, other species of this group showed *2n* = 22. *Phaseolus angustissimus* also underwent genome reshuffling, although with less structural rearrangements then *P. leptostachyus*. Our new findings revealed that dysploidy events in *Phaseolus* are recurrent, despite the absence of a previous whole genome duplication; and points to the necessity of further investigations for elucidating chromosome evolution in beans.

## 34.

# Repeat turnover meets stable chromosomes: Repetitive DNAs mark speciation and gene pool boundaries in sugar beet and wild beets

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Sugar beet (*Beta vulgaris*) and all wild beet relatives share a base chromosome number of nine and similar chromosome morphologies. Yet, interspecific breeding is impeded by chromosome and sequence divergence that is still not fully understood. As repetitive DNAs are the fastest evolving parts of the genome, they likely impact genomic variability and contribute to the separation of the beet gene pools. Hence, we ask if innovations and losses in the repeatome can be linked to chromosomal differentiation and speciation.

We traced genome and chromosome-wide evolution across sugar beet and twelve wild beets comprising all sections of the beet genera *Beta* and *Patellifolia*. For this, we combined short and long read sequencing, flow cytometry, bioinformatics and cytogenetics to build a comprehensive framework for our beet panel that spans the complete scale from DNA to chromosome to genome. Both, genome sizes (694 Mbp in the diploid *B. vulgaris* subsp. *maritima* to 2,471 Mbp in the pentaploid *B. intermedia*) and repeat profiles (53-68% repeats), reflect the separation of the beets into gene pools in such a way that specific repeats show contrasting evolutionary patterns: We identified the section- and species-specific repeat emergences and losses, e.g. of the retrotransposons causal for genome expansions in the section *Corollinae/Nanae*. As most genomic variability lies in the satellite DNAs, we then focus on tracing the 19 beetSat families across the three beet sections/genera. These taxa harbor evidence for contrasting strategies in repeat evolution, leading to contrasting satellite DNA profiles and fundamentally different centromere architectures, ranging from chromosomal uniformity in *Beta* and *Patellifolia* species and the formation of patchwork chromosomes in *Corollinae/Nanae* species.

We show that repetitive DNAs are causal for the genome expansions and contractions across the beet genera, providing insights into the genomic underpinnings of beet speciation. Satellite DNAs in particular vary considerably between beet genomes, leading to the evolution of distinct chromosomal setups in the three gene pools, likely contributing to the barriers in beet breeding. Thus, with their isokaryotypic chromosome sets, beet genomes present an ideal system for studying the link between repeats, genomic variability, and chromosomal differentiation and provide a theoretical fundament for understanding barriers in any crop breeding effort.

# Session 3

# New tools, perspectives and applications

## Talks

## 35.

# Let them move: flow cytometric chromosome analysis and sorting

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Condensed mitotic chromosomes have traditionally been studied using microscopy after they are immobilized on a flat surface. However, it is also possible to analyze them during movement in a narrow stream of liquid using flow cytometry. The so-called flow karyotyping, classifies chromosomes according to relative DNA content and/or the amount of particular DNA repeats. As the results are less informative compared to those obtained using molecular cytogenetics, flow karyotyping has been useful only in particular cases. The real power of flow cytometric chromosome analysis lies in the ability to purify mitotic chromosomes by sorting either in bulk, or just a particular chromosome for downstream analysis. Importantly, the morphology of flow-sorted chromosomes is well preserved, their DNA is intact and suitable for a range of applications. Dissecting genomes to individual chromosomes greatly simplified gene mapping and cloning as well as assembling sequences of complex genomes. The ability to purify mitotic metaphase chromosomes made it possible to describe three-dimensional organization of DNA and characterize their protein composition. The examination of purified chromosomes using environmental scanning electron microscopy revealed a unique organization of perichromosomal layer. The analysis and sorting using flow cytometry thus ideally complements microscopic observations and facilitates the study of nuclear genomes and their building blocks – chromosomes.

Keywords: 3D chromatin organization, Chromosome proteome, Chromosome ultrastructure, Genome sequencing, Gene cloning, Perichromosomal layer

## 36.

# A 3C view of 3D genome dynamics in barley

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Chromosomes undergo marked structural metamorphoses during the cell cycle. During interphase, relaxed chromatin is stored in the cell nucleus, being folded at several levels. Its flexible arrangement must ensure that the correct gene expression programs are executed at the right time and in the right cell type and that the chromatin can be rapidly reorganized into highly condensed structures – metaphase chromosomes, which are essential for a safe transfer of genetic information into the next generation. Mechanics of these processes is underexplored in plants. Emergence of novel molecular techniques based on chromatin conformation capture, Hi-C in particular, enable studying the 3D chromatin dynamics on the DNA level.

Taking barley as a model, we coupled Hi-C with flow sorting, which enabled purification of metaphase chromosomes and nuclei at G1, S and G2 phase, respectively. Polymer modelling based on Hi-C data from the metaphase chromosomes suggested helical folding of the DNA with ~30 Mb per turn. Analysis of Hi-C data from G1, S and G2 revealed relatively small changes in chromatin condensation in nuclei from root tips but a striking difference between root-tip G1 and G0/G1 of a leaf tissue, suggesting distinct interphase chromatin arrangement between rapidly cycling and non-cycling cells. Our data, supported by microscopy observations, point to several differences in higher-order chromatin organisation between plants and animals.

# 37.

## RepeatExplorer tools for genome annotations

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The RepeatExplorer Galaxy web server, originally designed to annotate repeats in genomes where assembly was not available, has grown in tandem with advancements in sequencing technologies. As high-quality genome assemblies have become routinely available, the server has incorporated novel tools for annotating repeats in these assemblies. The new tools, including DANTE, DANTE-LTR, and TideCluster, combine assembly-free approaches, similarity-based annotation, and feature-based annotation.

DANTE (Domain-based ANnotation of Transposable Elements) utilizes the REXdb database of conserved domains to detect and classify coding regions of transposable elements within genomes. Going beyond the level of Ty1/copia and Ty3/gypsy superfamilies, it enables more detailed and universally applicable classification of LTR-retrotransposons in higher plant genomes. Compared to previous computational tools, DANTE refines the annotation process to reveal lineage-specific distribution patterns of LTR-retrotransposons along assembled chromosomes.

The DANTE_LTR tool is designed to analyze output from the DANTE pipeline to identify complete LTR retrotransposons, including structural features of retrotransposons such as LTR sequences, primer binding sites, and target site duplications.

The TideCluster tool provides a solution for tandem repeat identification in genome assemblies. It employs TideHunter (Gao et al. 2019) to detect tandem repeats, and performs further processing of the identified repeats by their similarity-based clustering and reconstructing consensus sequences using Tandem Repeat Analyzer (TAREAN).

These tools provide robust and detailed analyses of nucleotide sequences of varying lengths, from complete chromosome assemblies to sequences of only a few hundred base pairs. They generate GFF3-formatted files containing sequence coordinates of annotated regions, which can be visualized on the RepeatExplorer server using the JBrowse genome browser or downloaded for further analysis. The combination of these tools on the RepeatExplorer server enables comprehensive repeat annotation of genome assemblies.

References:

Gao, Y., B. Liu, Y. Wang, and Y. Xing, 2019 TideHunter: efficient and sensitive tandem repeat detection from noisy long-reads using seed-and-chain. Bioinformatics 35: i200–i207.

## 38.

# Unveiling the Nano-World of Plant Chromosomes: Chromatin Fiber Compaction and Scaffold Protein Localization using Advanced Microscopy Techniques

Professor Dr Nobuko Ohmido[1], Dr Channarong Sartsanga[1], Dr Rinyaporn Phengchat[1], Dr Toshiyuki Wako[2], Dr Astari Dwiranti[3], Professor Dr Kiichi Fukui[4]

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Recent advancements in microscopy have revolutionized the analysis of intricate structures at the nano level. In this study, we employed cutting-edge microscopes such as the helium ion microscope (HIM), focused ion beam scanning electron microscope (FIB/SEM), high voltage transmission electron microscope (HVTEM), and ultra-HVTEM with exceptional resolutions to delve into the plant chromosome structure. These advanced tools offer high magnification and contrast properties, enabling us to visualize condensed metaphase chromosomes surface and investigate the precise localization of scaffold proteins within.

Our study specifically focuses on the surface structure of barley metaphase chromosomes, employing the HIM to estimate the unit size of chromatin fiber compaction. Additionally, we explore the mechanism of chromosome condensation by examining the effects of $Ca^{2+}$ depletion using the SEM. Furthermore, we demonstrate the distribution of an important scaffold protein, Topoisomerase II (TopoII), in specific regions of the barley chromosomes using immunogold staining and HVTEM. Our findings reveal that TopoII proteins are distributed along the chromosome arms, with heightened density observed at the centromere and nucleolar organizing regions (NORs). We have achieved a comprehensive understanding of the precise alignment and quantification of TopoII protein at the nano level in barley chromosomes.

## 39.

# Wheat-rice hybrid mitochondria in Oryza wheat and their transmission to the next generation

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4. *Department of Life Sciences, Meiji University, Kanagawa, Japan*

In vitro fertilization (IVF) system has been established in rice, wheat and maize, and combination of these three IVF systems offers the possibility of artificially generating inter-subfamily zygotes among three crops. We produced wheat–rice hybrid zygotes via IVF with various gamete combinations, and allopolyploid zygotes consisting of a wheat egg cell, a rice egg cell and a wheat sperm cell (ReWsWe zygotes), or wheat egg cell, a rice egg cell, a wheat sperm cell and a rice sperm cell (Double zygote, DZ) developed into mature plants. The hybrid nature of these possible wheat-rice hybrids was addressed by FISH-based cytogenetical analyses and genome sequencing. The results indicated that rice chromosomes are eliminated during the development of allopolyploid wheat-rice zygotes, and that the ReWsWe zygote- and DZ-derived plants possess a "wheat" nuclear genome and a "wheat" + "rice" mitochondrial genome. In addition, the inserted rice mitochondrial DNA regions were transmitted to progenies. Oryzawheat, the first hybrid wheat possessing a heterogeneous cytoplasm across subfamilies, can provide a new horizon for utilizing inter-subfamily genetic resources between wheat and rice.

# Session 3

# New tools, perspectives and applications

# Posters

## 40.

# The application laboratory for agricultural research at the Centre of Plant Structural and Functional Genomics of Institute of Experimental Botany

Mgr. Tereza Šlajsová[1], Ing. Radim Čegan, Ph.D.[1], RNDr. Jan Šafář, Ph.D.[1]

1. *Institute of Experimental Botany AS CR, v. v. i., Centre of Plant Structural and Functional Genomics*

The Centre of Plant Structural and Functional Genomics of the Institute of Experimental Botany in Olomouc established the Application Laboratory (AL) in 2017 as the first laboratory of its kind in the Czech Republic. It was founded within The Czech Academy of Sciences initiative called Strategy AV21 and its research programme Foods for the Future. This programme aims to contribute to food safety through agricultural research. The primary mission of this laboratory is to connect researches with plant breeders and commercial companies. It can enhance competitiveness of the breeder companies through transmission of the scientific knowledge and experience with modern methods in molecular biology and genomics. This collaboration is fulfilled at different levels. The AL provides professional consultation, organisation of specialized workshops and practical courses, as well as custom services. These include next generation sequencing, genotyping, flow cytometry, molecular cytogenetics, bioinformatics and data analysis, specific marker development and other comprehensive DNA analysis.

Acknowledgement: Work of the laboratory is funded by Strategy AV21, Programme Foods for the Future (Period: 2020–2024).

## 41.

# Computational identification of evolutionary conserved non-coding RNAs

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Telomerase RNA (TR) is an important non-coding RNA that plays a crucial role in the synthesis of telomere repeats and the assembly of the telomerase ribonucleoprotein complex. However, characterizing TRs is challenging due to their highly variable nature. Recent studies have successfully characterized TRs in various organisms, including plants, algae, protists, and the insect order Hymenoptera (Fajkus NAR 2019, 2021, 2023). To overcome the difficulties in identifying these mysterious RNAs across different taxonomic groups, we have optimized strategies.

Traditional methods, such as blast-based searches, are ineffective in identifying TR homologs due to their low sequence conservation. However, using the Infernal tool, which employs sequence-structure homology searches, significantly improves the identification process on a broader evolutionary scale. Nevertheless, Infernal requires more advanced procedures and programming skills compared to user-friendly web-based tools like blast. To address this issue, we have developed a user-friendly pipeline for performing Infernal analyses. The pipeline only requires two inputs from the user: query sequences in Stockholm format and a subset of NCBI genome assemblies as a database. The results are automatically processed and presented in a comprehensive table, including significant hits and the taxonomy of the organisms.

Since Infernal is central to this analysis, these searches are particularly suitable for identifying homologs of structural non-coding RNAs in a broad evolutionary context. In line with T. Dobzhansky's statement, "Nothing in Biology Makes Sense Except in the Light of Evolution," we present a computational pipeline that simplifies the search for non-coding RNAs.

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# 42.

## Detection of protein recruitment and protein-protein interactions at DNA lesions in plant protoplasts

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Plants are sessile organisms that require to adapt to constantly shifting environmental conditions and face numerous exogenous and endogenous factors that damage their DNA and lead to genetic alterations. However, the range of available tools for investigating the repair processes in plant genomes is limited.

Recently, we have adapted an experimental system that enables us to target DNA lesions in the root cells and protoplasts of Arabidopsis thaliana (Nešpor Dadejová et al., 2022). Using this approach builds upon previous studies conducted on mammalian models we can transiently transform plant protoplasts with proteins of interest, including DNA repair proteins, and consequently use laser irradiation to observe protein recruitment within the nucleus and nucleolus. By employing this method, we can study the dynamic nature of DNA repair in plants following exposure to UV irradiation and observe alterations in protein interactions following DNA damage. Although we initially tested this technique in Arabidopsis thaliana, its applicability can be extended to crop plants, which holds significant implications for practical field applications.

Currently, we are expanding our methodologies to explore the impact of elevated temperatures, chemical agents such as zeocin, and CRISPR-Cas -induced DNA damage on the localization of DNA repair proteins within plant cells. This work opens up new avenues for studying DNA damage response in plants and provides new insights into the subject.

## 43.

# High-fidelity repeat consensus sequences from short reads

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Despite the many cheap and fast ways to generate genomic data, good and exact genome assembly is still a problem, with especially the repeats being vastly underrepresented and often misassembled. As short reads in low coverage are already sufficient to represent the repeat landscape of any given genome, many read cluster algorithms were brought forward that do repeat identification and classification. But how can trustworthy, reliable and representative full-length repeat consensuses be derived from unassembled genomes? Here we combine methods from repeat identification and genome assembly to derive these robust consensuses. We test several use cases, such as (1) consensus building from clustered short reads of non-model genomes, (2) from genome-wide amplification setups, and (3) specific repeat-centered questions, such as the linked vs. unlinked arrangement of ribosomal genes. In all our use-cases, the derived consensuses are robust and representative. To evaluate overall performance, we compare our high-fidelity repeat consensuses to RepeatExplorer2-derived contigs and check if they represent real transposable elements as found in long reads. Our results demonstrate that it is possible to generate useful, reliable and trustworthy consensuses from short reads by a combination from read cluster and genome assembly methods in an automatizable way. We anticipate that this opens the way towards more efficient and less manual repeat characterization and annotation, benefitting all genome studies, but especially those of non-model organisms.

## 44.

# Development of novel CRISPR/Cas9-based tools for visualizing genomic sequences

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The DNA within the nucleus is intricately folded, and visualizing the three-dimensional structure of the genome directly contributes to our understanding of how chromatin is spatially organized and its role in gene expression during normal development and disease. CRISPR-FISH provides a distinct advantage over traditional FISH methods as it eliminates the need for complete DNA denaturation and maintains the inherent chromatin structure. CRISPR-FISH uses target-specific crRNA, fluorescence-labeled tracrRNA, and recombinant dCas9 protein to detect repetitive sequences in fixed plant and animal nuclei and chromosomes treated with formaldehyde or ethanol-acetic acid. Using specific target-specific crRNAs and varying fluorescence-labeled tracrRNAs, different DNA sequences can be selectively labeled. Additionally, this technique can be combined with immunostaining and DNA replication methods to gain further insights. Here we report on our recently developed method called CRISPR-CID (CRISPR/dCas9 mediated chromogenic in situ detection) that combines chromogenic signal detection using alkaline phosphatase or peroxidase enzymes with CRISPR-based imaging. CRISPR-CID applies the horseradish peroxidase enzyme for non-fluorescently labeling of repetitive sequences in fixed nuclei and chromosomes. The resulting chromogenic signals can be analyzed using a conventional bright field microscope. This technique is particularly valuable in educational institutions and science outreach settings that have limited resources and lack access to expensive fluorescence microscopes. Furthermore, we have improved the CRISPR-FISH method by introducing an ALFA-tag fused to recombinant dCas9 protein, resulting in improved signal intensity. We demonstrate target-specific labeling using an ALFA-tagged dCas9 protein combined with a centromere-specific gRNA from A. thaliana and a fluorescence-labeled NbALFA nanobody. Additionally, we achieved enhanced signal intensity of target-specific signals by utilizing a dCas9 protein that contains multiple copies of the ALFA-tag. This was accomplished by combining it with a minibody and a fluorescence-labeled anti-rabbit secondary antibody. The development of a CRISPR-EM strategy for labelling repeats that can be analyzed at the highest resolution by transmission electron microscopy is in progress.

## 45.

# Reversing ancient chromosomal rearrangements in Cardamine hirsuta (Brassicaceae) using CRISPR/Cas

Mgr. Petr Janás[1], Mgr. Milan Pouch[1], RNDr. Terezie Mandáková, Ph.D.[1], prof. Mgr. Martin Lysák, Ph.D., DSc.[1]

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Large-scale chromosomal rearrangements, including deletions, inversions, and reciprocal translocations, have played important roles in plant genome evolution and speciation. By engineering these rearrangements, we can gain valuable insights into the molecular basis and impact of chromosomal aberrations on genotype and phenotype. Here, we employed CRISPR/Cas technology to induce gross chromosomal rearrangements in the genome of hairy bittercress (Cardamine hirsuta, Brassicaceae; n = 8), a widely used model organism to study various aspects of developmental processes, ecological interactions, and evolutionary adaptations. By Agrobacterium-mediated floral dip transformation using a transfer vector carrying SaCas9 driven by an egg-cell-specific promoter and two highly specific gRNAs, we successfully generated primary transformants in the T1 generation. PCR analysis and subsequent Sanger sequencing confirmed the presence of a 3-kb deletion and inversion within chromosome Ch8, as well as 0.1 to 2-Mb reciprocal translocations between chromosomes Ch6 and Ch8. These initial results highlight the effectiveness of CRISPR/Cas tools in manipulating chromosomal structures in C. hirsuta. They provide the basis for further generation of chromosomal rearrangements, reversing modern karyotypes back to their ancestral states, and understanding the effects of these rearrangements.

# Session 4

## Mitosis, meiosis, gamete formation, polyploidy and apomixes

## Talks:

# 46.

## Comparative genomics of plant centromeres

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Centromeres represent the final frontiers of genomics because of their high content of satellite repeats, which in principle are extremely difficult to assemble. However, the recent introduction of accurate long-read sequencing technologies and advanced assembly strategies has led to gapless assemblies of multiple plant genomes, ushering in a new era in centromere research. Although comparison of centromere sequences between phylogenetically distant species is invaluable in revealing overall centromere diversity in plants, it may not be as efficient in elucidating individual stages or evolutionary steps that led to their current variation. In principle, this type of information is only available through the comparative analysis of closely related genotypes, whose conserved synteny can be used as a reference for revealing shifts in centromere positions, changes in associated epigenetic profiles, and the accumulation or elimination of centromeric repeats. This information can in turn be used to elucidate general patterns and associated molecular mechanisms of centromere evolution. In this talk, I will provide an overview of recent advances in comparative genomics of centromeres between multiple genotypes of extensively studied models or crops such as *Arabidopsis*, rice, and maize, made possible by the availability of pangenome assemblies in these species. I will then report on our efforts to establish comparative centromere (cyto)genomics in the legume tribe Fabeae which is of particular interest because of the high sequence diversity and evolutionary dynamics of its centromeres.

## 47.

# Cytogenetic and molecular evidence for polyclonality and sexual events in allopolyploid apomictic species *Hieracium halleri* (Asteraceae)

Begoña Quirós-de-la-Peña[1], Ladislava Paštová[2], Jindřich Chrtek[1], Eliška Záveská[3], Yann J. K. Bertrand[1], Jiřina Josefiová[2], Barbora Tomešová[2], Pavel Zdvořák[4], Alexander Belyayev[2], Patrik Mráz[4]

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*Hieracium* s.str. (Asteraceae) is a highly diverse genus known for its notorious taxonomic complexity, which is caused by widespread past interspecific hybridization. This process is closely linked to polyploidization and apomixis (asexual reproduction by seeds), which has ensured the persistence of otherwise sterile hybrids. As a result, apomictic polyploid species dominate the taxonomic diversity of the genus. However, very little is known about the evolutionary and biogeographical history of polyploid apomictic species.

This study aims to decipher the evolutionary origin of a high mountain apomictic *H. halleri* which probably arose from hybridization between *H. alpinum* and *H. intybaceum*. To achieve this aim, we have applied a combination of cytogenetic (FISH and GISH), flow cytometric (assessment of mode of reproduction) and molecular approaches (RADSeq) on plants collected across the range of the species (the Alps and the Western Carpathians).

Our results show that prevailing triploid *H. halleri* cytotype contains two genomes of *H. alpinum* and one genome of *H. intybaceum*, the pattern explaining much closer morphological affinity of *H. halleri* to *H. alpinum*. We further found that despite strictly apomictic mode of reproduction, *H. halleri* is cytogenetically variable taxon, both in chromosome number (prevailing are triploids, tetraploids are very rare) and karyotype pattern (variation in number and positions of 5s rDNA, 45S rDNA and HintCL-82 repetitive DNA clusters, and unbalanced translocations). These results along with RADseq data clearly indicate a polyclonal character of this hybridogenous species and signs of probably ancient sexual reproduction (meiotically driven intergenomic translocations). We suppose that both multiple origin of *H. halleri* and posthybridization chromosomal rearrangements have been involved in rather unexpected chromosomal and molecular variation of this apomictic species.

## 48.

# Spatial *in vivo* dynamics of mitotic divisions in barley (*Hordeum vulgare*)

Dr. Ales Pecinka[1], Mgr. Katerina Kaduchova[1], Dr. Cintia Marchetti[2], prof. Miroslav Ovecka[2], Dr. Petr Galuszka[2], Dr. Véronique Bergougnoux[2], prof. Jozef Šamaj[2]

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The structure and the dynamics of chromatin organization in cell nuclei are not strict and may change rapidly during the cell cycle, in different tissues, and in response to abiotic and biotic stimuli. The most rapid changes in cell architecture occur during cell divisions. In mitosis, the chromatin arrangement is changed due to its condensation into compact chromosomes, which are subsequently distributed into the newly emerging daughter cells. To study the dynamics of mitosis in plants with large genomes, we developed a series of unique barley marker lines carrying fluorescently labeled fusion proteins that are indicative of specific chromosomal/nuclear domains such as the chromatin (GFP/YFP/CFP-H2B), microtubules (mCHERRY-TUA3) and nucleolus (YFP-FIB1). With these lines, we were able to measure the length of mitotic division in barley root cells and reveal the dynamics of tagged cellular structures. Moreover, we found that the condensation of mitotic chromosomes does not reach its maximum in metaphase but proceeds until telophase where the newly formed daughter cells emerge.

Funding by the Czech Science Foundation project 21-02929S.

# 49.

# Unraveling the Rosa canina Genome: Cytogenetic, Genomic, and Phylogenetic Insights into Pentaploid Genome Harbouring Asymmetrical Meiosis.

Jana Lunerová[1*] (1st speaker) and Veit Herklotz[2**] (2nd speaker), Radka Vozárová[1], André Marquez[3], Meng Zhang [3], Bruno Hüttel[3], Andreas Houben[4], Joerg Fuchs[3], Dörte Harpke [4], Christiane M. Ritz[2] and Aleš Kovařík[1]

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Polyploidy and hybridisation often have profound consequences on the reproductive strategy in plants. An intriguing example represent the odd ploid (2n = 5x = 35) dogroses with their distinctive hemisexual meiosis, differentiating between pairing bivalents, inherited by pollen and egg cells, and non-pairing univalents transmitted by egg cells only.
Here, we combined cytogenetic, phylogenetic, and genomic approaches to analyse dogrose genomes. Using FISH (fluorescence in situ hybridisation) we investigated chromosomes in male canina meiosis of several dogrose species. In meiotic phase I we found differences in the distribution of rDNA and satellite markers between bivalent and univalent chromosomes. All cytogenetic markers could be successfully mapped to the newly developed whole genome assembly of R. canina (sequenced by the PacBio HiFi technology) and their distribution and abundance was well correlated with FISH results. The synteny analysis (SyRi) revealed numerous sizeable inversions, deletions, and other rearrangements on univalents suggesting a possible connection to their non-pairing nature and unique evolutionary trajectories. In addition, the pollen's single-copy loci originating from the two bivalent-forming chromosome sets (2x) exhibited extremely high levels of homology. The remaining three sets showed significant dissimilarity and likely originated from univalent-forming chromosomes. Phylogenetic analyses using diploid rose species revealed that in R. canina two bivalent (homologous) and one univalent forming subgenomes align with the Synstylae clade (named accordingly the “S-type” subgenome) while the remaining two univalent forming genomes have their origin in the Rosa clade (“R-type” subgenome). The genomic composition of 5x R. canina can thus be tentatively written as “SSsRR”. In conclusion, through combined methodical approaches we hope to provide a deeper understanding of the unique meiotic behaviour of chromosomes in R. canina and its allies.

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## 50.

# The protein-protein interaction network of KNL2 in Arabidopsis thaliana

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Centromeres are chromosomal regions where kinetochore proteins interact and form a protein complex that regulates the cell cycle. The licensing of CENH3 to the centromeres is regulated by the kinetochore protein KNL2 in plants and other organisms. In this work, we aim to elucidate and characterize the regulatory factors that interact with KNL2, which will aid in determining the functional role of KNL2 in Arabidopsis. Immunoprecipitation combined mass spectrometry (IP-MS) and yeast-two hybrid (Y2H) library screening performed with full-length and N- and C-terminal parts of KNL2 resulted in identification of 37 proteins from the ubiquitination and sumoylation pathways. Initially, three proteins, UBC19, UBC20 (ubiquitin-conjugating enzymes), and ULP1D (a deSUMOylating factor), were selected from the Y2H library screening as a putative direct interactor of KNL2. The interaction of these proteins has been validated using BiFC interaction analysis in N. benthamiana. UBC19 and UBC20 interact directly with KNL2 and KNL2-N after applying MG115, which aligns with the previous results showing that KNL2 is regulated by the proteasome degradation pathway at its N-terminal part. The interaction of ULP1D and UBC19 with KNL2-C has been shown, which is evident that the level of KNL2 in the mitotic cell cycle (metaphase to anaphase) could be regulated via the SUMO-ubiquitin crosstalk pathway. Further, we identified that the lysine sites (336K and 339K) of KNL2 are the target sites for binding ubiquitin protein through interaction and localization analysis. Thus, the KNL2 protein is regulated via the degradation pathways for the proper cenH3 loading and cell division processes.

## 51.

# Single pollen nucleus genotyping and virus tools for meiotic gene studies in barley (Hordeum vulgare L.)

Suriya Tamilselvan Nattar Amutha[1]

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Meiotic recombination generates genetic variation through reciprocal (crossover, CO) and non-reciprocal (gene conversion, GC) DNA exchanges between homologous parental chromosomes. Typically, COs are positioned towards distal regions of chromosomes, especially in crops with large genomes like barley leaving large portions of the genome untapped during breeding. Therefore, to accelerate barley breeding, novel approaches are required to achieve the desired genetic recombination outcome.

We present here multiplex Crystal digital PCR (dPCR)-based single pollen nucleus genotyping for rapid and high throughput assessment of recombination rate as well as CO interference in genetically linked chromosomal intervals in barley hybrids. We used our dPCR approach to measure an increase in male meiotic recombination rate in hybrid barley defective for the anti-CO factor HvRECQ4 (ATP-dependent DNA helicase). Moreover, natural variation in male meiotic recombination rates in hybrid barley was assessed. For targeted gene editing and transient transcript downregulation in barley, we employed Barley stripe mosaic virus (BSMV)-mediated virus-induced gene editing (VIGE) and virus-induced gene silencing (VIGS) strategies, respectively. Heritably edited barley plants defective for key meiosis genes were isolated using VIGE. In sum, multiplex Crystal dPCR and VIGE/VIGS approaches enable rapid dissection of meiotic gene function in barley and likely also other crops.

## 52.

# Dynamics and adaptation of the meiotic recombination landscape among natural and artificial chromosomes in holocentric Rhynchospora plant species

Stefan Steckenborn[1], Meng Zhang[1], Magdalena Marek[1], Bruno Hüttel[1], Andre Marques[1]

1. *Max Planck Institute for Plant Breeding Research*

The meiotic recombination landscape is highly influenced by chromosome number, as at least one crossover per chromosome occurs in most plants. Additionally, centromeres typically inhibit meiotic recombination, driving crossover formation towards chromosome ends. Therefore, holocentric plant species, with hundreds of centromere units distributed across the length of each chromosome, offer a unique opportunity to study how atypical centromeres can affect the meiotic recombination landscape. Moreover, due to their high tolerance to chromosome fissions and fusions, the genome structure within holocentric plant species is highly dynamic, offering an appealing alternative to analyze the major effects of sudden changes in chromosome number via fission and fusions (either naturally evolved, or artificially generated by DNA damage and Cas9-mediated chromosome engineering) on crossover frequency and distribution. The present project describes how we adapted a methodology of single-cell RNA/ATAC sequencing to build recombination maps using form thousands of pollen nuclei, with the aim of uncovering the differences in CO landscape and revealing the major effects of karyotype evolution on recombination dynamics in non-monocentric organisms.

## 53.

# STED nanoscopy of intra-nuclear chromatin structures in barley (Hordeum vulgare L.)

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Recent development in the field of super-resolution microscopy enabled detailed analysis of cellular or even sub-cellular structures. One of the super-resolution techniques, stimulated emission depletion (STED) microscopy, is based on confocal method and provides an ability to achieve spatial resolution in the range of 30 – 80 nm. However, STED microscopy is extremely sensitive to refractive index mismatch along the optical path. Any mismatch can result in spherical aberrations leading to a significant reduction in resolution and signal loss. Moreover, the combination of excitation laser and STED laser causes fast fluorophore bleaching, thus specific highly stable fluorophores have to be used.

In order to fully utilize the potential of STED analysis in plant chromatin research, we optimized the whole procedure of sample preparation and image acquisition. We used barley (cv. Morex) as model plant to provide a protocol useful for detailed analysis of chromatin structure and organization in highly condensed mitotic metaphase chromosomes and interphase nuclei. To achieve the best sample purity and subsequent resolution, mitotic metaphase chromosomes as well as interphase nuclei were isolated by flow sorting. Pure chromosomes or nuclei were embedded in polyacrylamide gel to preserve their three dimensional (3D) structure. The effect of different commercially available mounting media was studied after 3D-FISH experiments with probes labelled by Abberior STAR 580 and 635P, and chromatin visualization by SPY650-DNA. Our optimized protocol enabled to achieve lateral resolution of ~ 30 – 40 nm in the 3D samples, and to observe unique structure and arrangement of chromatin fibers in the 3D space of interphase nuclei and mitotic chromosomes.

# Session 4

## Mitosis, meiosis, gamete formation, polyploidy and apomixes

## Posters:

## 54.

# Both male and female meiosis contribute to non-Mendelian inheritance of parental chromosomes in interspecific plant hybrids (*Lolium* × *Festuca*)

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2. *John Innes Centre, Norwich, UK*

Elimination of chromosomes from one parental subset is a phenomenon occasionally observed in newly developed interspecific hybrids. This represents a limitation to the speciation of allopolyploids and an obstacle for breeders who use wide hybridization for crop improvement. The mechanisms underlaying uniparental genome elimination are still poorly understood.

Using a cytogenetic approach, we studied the chromosome composition of F2 and reciprocal backcross (BC1) generations of *Festuca* x *Lolium* hybrids, commercially important fodder and turf grass. We found that during female meiosis, *Festuca* chromosomes are replaced by *Lolium* chromosomes, presumably by the mechanism of meiotic drive. Occasional elimination of *Festuca* chromosomes was also observed during male meiosis. Detailed analysis of the first and second male meiotic divisions revealed that *Lolium* univalents are regularly attached to microtubules and transmitted to daughter nuclei, whereas *Festuca* univalents often remain unattached and are subsequently eliminated.

Additionally, we performed meiotic transcriptome analysis and identified two kinetochore genes, *NNF1* and *NDC80*, expressed only from the *Lolium* variants in all meiotic samples, while they were expressed from both genomes in somatic tissues. It is plausible that the elimination of the *Festuca* chromosomes in the hybrid is a consequence of the silencing of the *Festuca* alleles of the kinetochore genes leading to improper kinetochore complex assembly on these chromosomes.

Our findings help to elucidate the mechanisms responsible for chromosome elimination and genome dominance occurring in interspecific hybrids. They are relevant not only to the research community but also to plant breeders to facilitate decision-making in parental line selection.

## 55.

# *ZIP4* is required for normal progression of synapsis and for over 95% of crossovers in wheat

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Tetraploid (AABB) and hexaploid (AABBDD) wheat possess multiple sets of similar chromosomes (homeologs). Thus, the success of meiosis and fertility rely on synapsis and crossover (CO) formation exclusively occurring between homologous chromosomes (homologs). The major meiotic gene *TaZIP4-B2* (*Ph1*) in hexaploid wheat promotes homologous COs while suppressing homeologous COs. In other species, *ZIP4* mutations eliminate approximately 85% of COs, consistent with loss of the class I CO pathway. Tetraploid wheat has three *ZIP4* copies: *TtZIP4-A1* on 3A, *TtZIP4-B1* on 3B, and *TtZIP4-B2* on 5B. We show that disrupting two *ZIP4* copies in *Ttzip4-A1B1* double mutants reduces COs by 76-78%, while disrupting all three copies in *Ttzip4-A1B1B2* triple mutants reduces COs by over 95%. This indicates that either the numbers of class I COs in tetraploid wheat is higher than previously reported, or that the *TtZIP4-B2* copy also affect class II COs. It is possible that the duplication and divergence of *ZIP4* genes during wheat polyploidization have given *TaZIP4-B2* an additional function to stabilize both CO pathways. Moreover, in plants lacking all three *ZIP4* copies, synapsis is delayed and never completed. These findings confirm *ZIP4-B2* (*Ph1*) essential role in synapsis, and indicate stronger effects of *TtZIP4* genes compared to Arabidopsis and rice.

## 56.

# Uncovering the mechanism of cold-induced meiotic restitution in Musa spp.

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Most banana cultivars currently grown are triploid, as these are desired for their superior agronomical quality and high level of sterility leading to seedless fruits. As a result of this, banana plants need to be propagated vegetatively, leading to large monocultures with identical clones that are highly vulnerable to (a)biotic stress. One of the solutions to guarantee sustainable banana production under changing environments is the development of new cultivars that combine productivity of high-quality fruits with adaptation to local climates. In current Musa breeding, new triploid cultivars are created from existing ones via interploidy crossing. This breeding schedule is however laborious and inefficient. An alternative pathway to generating triploid banana genotypes is the exploitation of sexual polyploidization via the ectopic formation of diploid (2n) gametes. This would avoid inefficient interploidy crosses and complex inheritance patterns. We have been able to induce 2n pollen grain formation after a cold treatment of Musa velutina, and are using cytological techniques to uncover the nature of the temperature-induced defect that is observed.

## 57.

# Recurrent plant-specific duplications of KNL2 and its conserved function as a kinetochore assembly factor

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KINETOCHORE NULL2 (KNL2) plays a key role in centromere recognition and kinetochore assembly. To gain insight into the origin and diversification of the plant KNL2 gene, we reconstructed its evolutionary history and classified into two clades; αKNL2 and βKNL2 in eudicots and γKNL2 and δKNL2 in grasses, respectively. KNL2 proteins across species can be identified by the presence of conserved module called SANTA (SANT Associated) domain. The presence of CENPC-k motif apart from the SANTA domain differentiates αKNL2 and γKNL2 from βKNL2 and δKNL2. However, despite the absence of the CENPC-k domain, Arabidopsis βKNL2 exhibits centromeric localization similar to αKNL2. Homozygous βknl2 mutants of Arabidopsis cannot survive beyond the seedling stage, while heterozygous mutants with normal vegetative growth have a high percentage of abnormally developed or aborted seeds. Defects in seed development are most likely not related to the formation of abnormal gametes but to abnormalities in postzygotic cell divisions. To investigate the βKNL2 function and its centromere targeting mechanism, we generated several truncated βKNL2 versions by deleting the conserved motifs and domains. We hypothesize that centromeric localization of βKNL2 is dependent on its interaction with other proteins which might happen through SANTA domain and/or conserved motifs at the C-terminus. The confirmed centromeric localization of βKNL2 and mutant analysis suggests that it is involved in the loading of centromeric histone H3 CENH3 into centromeric nucleosomes, similar to αKNL2. Overall, our study provides insight into the evolutionary diversification of the KNL2 gene and its role in kinetochore assembly and maintenance of genome stability.

## 58.

# Identification of novel meiotic players based on screening of Axis TurboID candidates (ATC) and SUMO candidates

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During meiosis, random assortment of homologous chromosomes and homologous recombination leading to the formation of crossover (CO; reciprocal genetic exchange between homologs) both contribute to the genetic diversity of the offspring. The formation of CO depends on the interplay between a proteinaceous structure called the meiotic chromosome axis, the synaptonemal complex (SC) and meiotic recombination proteins. In addition, posttranslational protein modifications (PTMs) play vital roles in dynamically regulating components of the axis, SC and the recombination machinery.

Despite the key role of the meiotic chromosome axis and the SC for CO formation, a complete picture of the underlying mechanisms and players is still missing. Recently, TurboID-based proteomic profiling of the meiotic chromosome axis identified a list of 39 axis-proximate candidate proteins (ATCs) in Arabidopsis thaliana. Among them, both known and new meiotic proteins are found. Here, male meiotic chromosome behavior was studied in selected T-DNA insertion mutant lines and the candidate gene ATC3 was found to be required for meiosis, i.e. the formation of the obligate CO. Further initial data on the functional role and the localization of ATC3 based on fusion proteins will be presented.

Despite the essential role of PTMS in regulating axis, SC, and CO formation in non-plant species, our knowledge of SUMOylation's involvement in plant meiosis is limited. Hence, mutants for two SUMO genes, SUMO3 and SUMO5, were isolated based on CRISPR in A. thaliana. Initial data on the localization of SUMO5 and SUMO3 based on fusion proteins and of their mutant phenotypes will be shown.

## 59.

# Anti- and pro-CO factors in barley

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Meiosis is the cell division responsible for the generation of gametes. During meiosis, homologous recombination ensures: 1) formation of a physical link between maternal and paternal chromosomes, essential for proper chromosome segregation and fertility; and 2) exchange of genetic information between parental chromosomes, assuring genetic variability harnessed during breeding. The outcome of homologous recombination can be either a crossover (reciprocal exchange of genetic material between homologous chromosomes) or a non-crossover, and this is regulated by antagonistic actions of so-called “pro-crossover” and “anti-crossover” factors.

One limitation of plant meiotic homologous recombination, in particular in cereal crops such as barley (Hordeum vulgare), is that crossovers do not occur homogeneously along the genome. Instead, they form preferentially in (sub-)terminal chromosome regions, leaving large (peri-)centromeric regions unrecombined.

We are interested in better understanding meiosis in cereals, using barley as our model, ultimately aiming to manipulate the frequency and/or distribution of recombination events. We selected three meiotic candidates which have been reported in Arabidopsis and other non-plant species as pro-crossover (HEI10) and anti-crossover factors (FIGL1 and RECQ4) for mutant generation and meiotic characterization. HEI10, as in other species, is needed for crossover formation and its absence leads to mostly unpaired chromosomes during meiosis; FIGL1, opposite to results in Arabidopsis, is needed for crossover formation, being its role as anti-crossover factor still to be determined; and RECQ4 is an anti-crossover factor increasing recombination up to 4-fold in selected chromosomal intervals.

## 60.

# Tetraploid B. rapa are highly tolerant of tetravalent formation and aneuploidy

Zhenling lyu[1], Annaliese Mason[1]

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Although turnip (Brassica rapa var. rapa) cultivars are usually diploid, autotetraploids produced by artificially induced chromosome doubling are also grown as fodder crops, where they are competitive with their (natural) diploid counterparts. To date, induced autopolyploids in Brassica are all thought to have unstable meiosis, but the use of autotetraploid as crops suggest high fertility and putatively meiotic stability. Hence, we aimed to investigate genome structure, meiosis, and fertility in autotetraploid turnips.

We found loss and gain of individual chromosomes (aneuploidy) was common in autotetraploid turnip lines sourced from germplasm banks. Meiotic configurations (number of univalents, bivalents, trivalent, tetravalent, and higher multivalents per cell) were quantitatively assessed at the diakinesis stage, showing an average of 3.1 - 4.9 tetravalents and 7.2 - 9.6 bivalents per meiosis in this material. High pollen viability was observed in both euploids and aneuploids.

High frequencies of meiotic tetravalent formation and observations of aneuploidy without major phenotypic effects or reductions in pollen fertility indicate that tetraploid B. rapa is highly tolerant of chromosomal variation, which may explain its success as a crop despite a lack of conventionally stable meiosis (fully diploidized/ 100% bivalent formation). Possible mechanisms (structural and epigenetic factors) for this effect are under investigation.

## 61.

# Antibodies against kinetochore proteins NDC80 and KNL1 provide highly versatile markers of centromeres in flowering plants (angiosperms)

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The positions of centromeres on mitotic chromosomes can be roughly determined morphologically as primary constrictions. However, accurate determination of functional centromere domains depends on the detection of centromere-associated proteins using specific antibodies. The most commonly used marker for centromeres is the centromere-specific histone CENH3, which replaces the canonical histone H3 in centromeric nucleosomes. CENH3 is very similar to H3, except for the N-terminal tail, which is usually used as an immunogen to produce antibodies. Unfortunately, the N-terminus of CENH3 is highly divergent among species, resulting in the need to develop CENH3 antibodies for each species or genus of interest, which is laborious and costly.

Recently, we developed antibodies against the kinetochore proteins NDC80 and KNL1 in *Cuscuta* species. Sequence analysis of the domains used as immunogens revealed a high degree of similarity across flowering plants, suggesting that the antibodies developed primarily for *Cuscuta* spp. may also work in other plant species. This was confirmed by *in situ* immunodetection of NDC80 and KNL1 in several eudicot, basal dicot, and monocot species. Our findings open up possibilities for broader applications of the developed antibodies in studying centromeres across diverse plant species.

# 62.

## Proteomic profiling of meiotic chromosome axis in *Arabidopsis thaliana* based on TurboID

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The meiotic chromosome axis is a proteinaceous structure along which sister chromatids are arranged in a loop-base array during meiotic prophase I. The chromosome axis is critical for synapsis and meiotic recombination. In plants, the limited number of meiotic cells embedded in floral organs constrains proteomic approaches aiming to unravel the composition and regulation of meiotic chromosome axes.

Here we present the application of TurboID (TbID)-based proximity labeling in meiotic cells of *Arabidopsis thaliana* for two meiotic chromosome axis-associated proteins ASYNAPTIC1 (ASY1) and ASYNAPTIC3 (ASY3). Among 39 identified candidates most known axis-related and novel proteins were identified. After mutant screening based on meiotic chromosome spread analysis, we identified (at least) four of the novel candidates with a meiotic mutant phenotype (presence of univalents, i.e. failure to form the obligate CO). Among them, one candidate was found to be part of the synaptonemal complex (SC). In its absence, SC formation is disrupted and chiasmata formation is reduced while CO levels are increased and CO interference is virtually abolished.

Together, TbID-based proximity labeling is a robust tool for the identification of protein proximate proteins in rare cell types such as meiotic cells in *A. thaliana*.

# Session 5

# Specialized chromosomes (Sex chromosomes, B chromosomes and others)

## Talks:

## 63.

# Key drivers of plant sex chromosomes knowledge explosion

Roman Hobza, Václav Bačovský, Vojtěch Hudzieczek, Radim Čegan, Viktor Tokan, Tomáš Janíček, Marcel Hubinský, Nicolas Blavet, Dmytro Abramov, Lucie Horáková, Pavla Novotná, Markéta Bodláková, Bohuslav Janoušek, Jose Rodriguez, Boris Vyskot, Matěj Lexa, Pavel Jedlička, Marie Krátká, Zdeněk Kubát and Eduard Kejnovský

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For decades, we have been gradually accumulating data about sex chromosomes and their role in genome structure and evolution. Recent advances in NGS data mining, microscopic analysis, epigenetics and functional genetics have significantly accelerated our knowledge about sex chromosomes. Our effort to test long-standing hypothesis and confirm old theories should transform towards raising new biological questions. I will briefly summarize recent developments and the state of the art of sex chromosome research.

This study was supported by a research grant from the Czech Science Foundation (grants no. 22-00301S, 21-00580S and 22-00364S).

## 64.

# A complex affair – sex chromosome evolution in plants

Ing. Václav Bačovský, Ph.D.[1], Ing. Radim Čegan, Ph.D.[1], Mgr. Zdeněk Kubát, Ph.D.[1], Mgr. Lucie Horáková[1], Ing. Pavla Novotná[1], Mgr. Jana Kružlicová[1], RNDr. Roman Hobza, Ph.D.[1]

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Generally accepted theory predicts that the sex chromosomes evolved from a regular pair of autosomes. Through a series of chromosomal rearrangements and cycles of deleterious mutation accumulation, XY chromosomes gain different function and differ in their (epi)genomic organisation leading to functional and structural diversity. Well-differentiated sex chromosome systems have evolved a core set of characteristics in animals that are yet still to be fully explored in plants. We found that plant sex chromosomes undergo large proto-sex chromosome-autosome rearrangements, followed by the evolution of recombination suppression and partial/incomplete dosage compensation. We suggest that epigenetic conflict within the non-recombining region of the Y chromosome is predated by large-scale gene down-regulation that leads to expression imbalances, either in one or both sexes. This process is accelerated by the accumulation of transposable elements and satellites within the non-recombining region as demonstrated in Silene latifolia and Rumex acetosa, or newly in R. hastatulus. The sequence divergence and later morphological differences (heteromorphism) might lead to co-evolution of similar mechanisms termed in animals as sex chromosome meiotic inactivation. In this work, we show that the sex chromosomes are remarkable examples of genomic convergence, although subsequent evolutionary steps are more dependent on epigenetic state and evolutionary history of both sex chromosomes. We show recent discoveries in four well-established plant species – Silene, Rumex, Humulus and Coccinia, and discuss future directions of plant sex chromosome research.

# 65.

## Considerable genetic diversity within the hop varieties explains non-Mendelian segregation patterns

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3. *Hop Research Institute Co. Ltd, Kadaňská 2525, 438 46 Žatec, Czech Republic.*

Humulus lupulus is a dioecious and economically important plant classified into five varieties - var. lupulus, lupuloides, pubescens, neomexicanus, and cordifolius. These varieties have distinct levels of genetic variability following their geographical origin. Despite the long breeding history of hop, genomic studies of male plants that are essential for hop breeders were for a relatively long time neglected. Interestingly, wild male hop displays non-Mendelian segregation patterns that lead to meiotic abnormalities, and further genomic instability in subsequent generations. However, the origin of such meiotic instability and the mechanism through which the new lines show stable chromosome inheritance is still enigmatic.To explore the non-Mendelian segregation in hop, we analyzed centromeric repeat fraction of hop genome using ChIP-sequencing assay with Humulus lupulus-specific CENH3. This allowed us to identify new chromosome-specific marker(s) (namely TR120 and cl293) that were used to study chromosome segregation defects in mitosis and meiosis. Using fluorescence in situ hybridization we found unbalanced segregation patterns that leads to aneuploidy (2n=18-22) in the new generation. The unbalanced segregation pattern is caused namely by dicentric chromosome that is lagging during transition from metaphase I to anaphase I. Our results show structurally diverse genomes of Humulus varieties and suggest an influence of breeding and geographical isolation on evolution of hop genome. These findings may help to improve new hop breeding programs to characterize new hop cultivars.

## 66.

# Does B chromosome alternate the pollen development in wild sorghum?

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B chromosomes, dispensable genomic components of many species, are perceived as peculiar chromosomes thanks to their features, which are puzzling in many ways. They ignore several commonly accepted biological laws and are escaping the evolutionary mechanisms of DNA maintenance. Their survival in the population is one of the striking examples shared with all B chromosomes. Accumulation of B chromosomes via non-disjunction is the most frequently described mechanism with S. purpureosericeum being not an exception. We observed B-drive during the pollen development at first pollen mitosis. Surprisingly, if this division runs in a regular manner and B-sister chromatids segregate, the alternative pathway of polymitosis is triggered off. In this pathway, VCN (carrying B chromosome) is most likely not arrested in the G1 phase but is allowed to complete the cell cycle and divide again. Right in the frame of this division, B chromosomes finally non-disjoin and are passed to a „new“ generative nucleus. This GCN divides regularly producing two sperms with multiplied B chromosome number. „Former“ GCN arose from the standard first division of unicellular pollen remains attached to the pollen wall and becomes an extra nucleus.

The work was supported by The Czech Science Foundation (grant no. 22-02108S) and by The Czech Academy of Sciences (project no. DAAD-22-02).

# 67.

## Unveiling Reproductive Diversity: Tree of Sex 2.0 Progress Report

Dr. Sònia Garcia[1], Dr. Daniel L. Jeffries[2], Dr. Cibele G. Sotero-Caio[3], Dr. Tatiana Giraud[4], Dr. Paul Jay[5], Dr. Lukas Kratochvil[6], Dr. Kamil S. Jaron[3], Enabling Platforms Team[3], Tree of Sex Consortium[7]

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Reproduction is a fundamental aspect of life that has intrigued evolutionary biologists due to the diverse range of reproductive systems in nature. However, previous research has primarily focused on a limited number of species, neglecting many aspects of reproductive diversity. To address this, understanding the full spectrum of reproductive strategies is crucial for unraveling their connections to life history traits, population genetics, and genomic and chromosome characteristics.

To achieve this, the Tree of Sex initiative (v2.0, treeofsex.sanger.ac.uk) has been relaunched. As an open and highly-collaborative initiative, its aim is to gather comprehensive information on reproductive systems in eukaryotic life, including sexual and asexual reproduction, the evolution of separate sexes, and the degeneration of sex/mating-type chromosomes. Over 70 researchers from various institutions and disciplines have joined this international consortium.

Building upon the original Tree of Sex project, the database incorporates extensive updates and additional data on relevant reproductive traits. It also integrates genomic information to keep pace with the expanding field and aligns with resources like Genomes on a Tree (GoaT). Here we discuss the progress made and highlight the evolutionary topics that we aim to explore once the database is populated.

# 68.

## Plant chromosome biology – Quo vadis?

Andreas Houben[1]

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In the field of plant chromosome biology, significant progress has been made based on the development and application of new technologies. I will discuss how methods like whole genome sequencing, CRISPR-Cas, chromosome painting, chromosome engineering and others were used to address fundamental research questions and might help to advance plant breeding tools.

# Session 5

## Specialized chromosomes
## (Sex chromosomes, B chromosomes and others)

## Posters:

## 69.

# Weird and wonderful – an origin of sex chromosomes in two races of *Rumex hastatulus*

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*Rumex hastatulus* is a dioecious plant that evolved into two distinct races with a polymorphic sex chromosome system, XY in Texas (Tx) race and XYY in North Carolina (NC). The time of sex chromosome divergence in the older race (Tx) is estimated to be 9–16 mya, while the younger system (NC) evolved only 0.6 mya. Male plants of Tx race (2n = 10) possess telocentric X chromosome and large metacentric Y chromosome. In contrast to Tx race, NC males (2n = 9) underwent a series of chromosomal rearrangements that led to substantial changes in their genome organization. However, the exact mechanism through which was NC race genome formed and which parts of the autosomes were lost has not been completely understood yet. Using the combination of NGS data and PacBIO technology, we analysed the repeatome of both races and identified sex-specific satellite candidates for further cytogenetic analysis. The difference in the proportion of the sex-specific satellites and their position revealed that the neo-sex chromosome system of NC underwent at least two fusions between the sex chromosomes (X and Y) and one pair of autosomes. These rearrangements were further accompanied by multiple breakages of the ancestral Y chromosome that led to the formation of two neo-Y chromosomes (Y1, Y2) in NC males. NC race genome was further shaped by additional inversion and fusion supported by the localization of satellites on the autosomes. The exact understanding of the chromosomal rearrangements that formed the genome of *Rumex hastatulus* will allow us to study the epigenetic degeneration of neo-Y chromosomes. Further, this system is an ideal model to study dosage compensation evolution in old and new sex chromosome systems with the same evolutionary background and to discover the early mechanisms of sex chromosome evolution.

## 70.

# Identification of the trans-acting factors that control the non-Mendelian drive of the rye B chromosome

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The B chromosome (B) is a dispensable element in the genome of many plants, animals, and fungi. To counteract the elimination of supernumerary chromosomes, many Bs evolved a drive mechanism to transmit themselves at a higher frequency compared to the standard A chromosome. To decipher the chromosome drive process, we have selected the B of rye (Secale cereale) as a model. During the first pollen grain mitosis (PMI), rye B sister chromatids continue to stay cohesive rather than separate, and then both chromatids preferentially enter the generative nucleus. The drive process of the rye B is controlled by the trans-acting B-located non-disjunction control region. Bs lacking the non-disjunction control region (NCR) undergo normal disjunction at the first pollen mitosis and therefore do not drive. The rye B shows an efficient drive also when it is introduced into wheat.

To narrow down the non-disjunction control region, we compared the repeat composition of the long arm of different B variants by fluorescence in situ hybridization (FISH) and identified a subtelomeric region (~8% of the B) that controls the drive of the rye B. To identify the genetic element(s) that control the non-disjunction of the rye B, we assembled the rye B sequences using PacBio, Hi-C, Nanopore sequencing, and optical mapping. In addition, 33 RNA-seq data sets from anthers undergoing PMI of rye and wheat were generated, including of drive-positive and drive-negative specimens. Differential expression analysis identified that only 16 candidates are up-regulate in all the comparisons between drive-positive and drive-negative data. PCR-based mapping revealed that 10 of the 16 candidates locate within the NCR. Their tissue- and stage-specific expression patterns were tested in RNA-seq data from 7 different tissues of wheat with Bs. The drive-associated candidate gene NCR28 shows a PMI-specific and strong expression. NCR28 is encoded by 13 transcriptionally active copies, and FISH confirmed the NCR-specific localization and its multiple copy number. Transient expression of an EYFP-NCR28 construct in Nicotiana benthamiana revealed a microtubule-like pattern in dividing cells. Our results shed light on the mechanism of chromosome drive in plants. Next, CRISPR-Cas9 will be applied to study further the function of NCR28 in the process of chromosome drive.

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## 71.

# Identification and functional characterization of genes controlling the process of root-specific B chromosome elimination in *Aegilops speltoides*

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Genome stability is a crucial feature of eukaryotic organisms since the loss or gain of even a single chromosome can significantly affect normal development and the organism as a whole. Nonetheless, some organisms tend to eliminate specialized chromosomes (such as sex or B chromosomes) either partially or entirely in a certain stage of development. B chromosomes (Bs) are present in some eukaryotic organisms exclusively in addition to standard A chromosome complement. Whereas some organisms carry Bs in every tissue, others do not have Bs in some organs or tissues. The presence or absence of the B chromosome is variable but also depends on the developmental stage of the organism, e.g., the early stage of embryogenesis. The elimination of Bs in goat grass *Aegilops speltoides*, is root-specific and the elimination undergoes at the onset of embryogenesis (embryo 6 days after pollination). Aided by technical advances in microscopic analysis, genome sequencing, and bioinformatics analysis, the mechanism behind B chromosome elimination in Ae. speltoides is beginning to unravel; however, the genes controlling this mechanism remain highly elusive. In this research, we aim to identify the candidate genes that control the process of root-specific B chromosome elimination by comparative RNA-seq analysis of the transcriptome from the tissues undergoing B chromosome elimination compared to the exact same tissue from plants that do not carry B chromosomes. Furthermore, the function of candidate genes will be characterized using virus-induced gene silencing (VIGS).

## 72.

# Dosage compensation mechanism in plants with evolutionary young sex chromosomes

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Some organisms evolved mechanism of dosage compensation (DC) to equalize gene expression between biological sexes. This process affects either the entire X chromosomes or may exhibit local and incomplete X chromosome locus specific deregulation, proceeding gene-by-gene to large chromosomal regions. Despite large advances in the field of sex chromosome biology, the mechanisms through incomplete DC evolves remains unclear. In dioecious plant, *Silene latifolia*, early cytological findings suggested that the maternal X chromosome is being upregulated similar as in mammals. Such evidence was recently supported by transcriptomic expression analysis, showing incomplete DC mechanism in both sexes. Nevertheless, it is not known, which mechanisms affect the early stages of DC evolution and what epigenetic processes set the precise expression balance. Here, we show different replication patterns between Xs in homogametic sex using EdU that incorporates into newly synthesized DNA. Comparing the XY chromosomes and autosomes, we provide hypothetical model of sex chromosome replication timing and show late replication of paternal X chromosome p-arm. We provide a missing link between early and recent transcriptomic evidence, and discuss future possibilities how to test DC in relation to different ploidy level.

## 73.

# Characterization of a maize landraces collection concerning the B chromosome

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B chromosomes are classified as non-essential genomic components whose performance is rather atypical. They bring no clear benefit to the host, act as parasitic and still they persist in populations over the ages. Due to maize agronomical importance, its B chromosome is pioneering the plant B chromosome biology and is one of the most thoroughly studied.[1] However, the diversity as well as distribution of the maize B chromosome within wild landraces are pending exploration. Here, we report on B chromosome distribution across the various maize accessions from the CIMMYT germplasm bank. We designed an effective and reliable pipeline including presence/absence screening of plant individuals using direct PCR followed by a rapid method for scoring a number of B chromosomes based on droplet digital PCR. B-chromosome diversity was analysed by combining the cytogenetic approach of fluorescent in situ hybridization with B-specific repeats and SNPs identified in sequences of a selected set of 100 lines where the B chromosome was detected. Due to maize agronomical importance, its chromosome B is thoroughly studied and is pioneering in the plant B chromosome biology.

The work was supported by Ministry of Education, Youth and Sports (award no. LTT19007) and Czech Science Foundation (award no. 23-04887S).

## 74.

# Sex-chrome version 2.0: a database of green plant species with sex chromosomes

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The majority of plant species are hermaphrodite, bearing male and female reproductive organs within one flower, while only a small number possess distinct sexes. These separate sexes can be found either in different flowers within the same individual (monoecy) or in different flowers within separate individuals (dioecy), although other systems also exist. Due to their infrequent occurrence and wide taxonomic distribution, it is believed that sex chromosomes have independently evolved in plants multiple times. These can exhibit microscopic heteromorphism, as seen in species like *Coccinia grandis*, or they can be homomorphic, as observed in *Asparagus*, *Ecballium*, and *Populus*.

In this context, we present the new release of the Sex-chrom database (www.sexchrom.csic.es), a resource containing sex chromosome related information for green plants, stemming from papers published from 1919 to March 2022 with data for 229 species of dioecious green plants. Among them, 124 species exhibit male heterogamety (XY), 33 species display female heterogamety (ZW) and 55 species of bryophytes show haploid-phase sex determination (UV). This resource serves as a valuable tool for anyone seeking knowledge on sex chromosomes in green plants.

## 75.

# Non canonical bases as possible regulators of sex chromosomes in the dioecious plant *Silene latifolia*

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Enzymatic oxidation products of 5-methylcytosine, known as oxi-mCs, have a broad range of biological functions in mammals. Accumulating data on plants suggests a role for 5-hydroxymethylcytosine (5hmC) in the regulation of transposable elements and association with heterochromatic regions. Silene latifolia is a model plant for sex chromosome evolution. It possesses heteromorphic young sex chromosomes with a degenerating Y chromosome characterized by a size increase due to transposable element accumulation. A global quantification showed high values for 5hmC and 5-formylcytosine (5fC) comparable to mammals but no differences based on sex. Additionally, the global distribution of oxi-mCs in interphase nuclei showed colocalization with chromocenters except for 5hmC. The signal distribution of 5hmC was more uniform with partial enrichment in chromocenter territories, suggesting a euchromatic/heterochromatic character. On metaphasic chromosomes, the differences are observed on the signal distribution between both X sex chromosomes. Chromosome Xf1 displayed overall enrichment of 5hmC and 5fC regarding the chromosome Xf2. Colocalization showed higher correlation between 5mC and 5fC than with 5hmC, which may indicate differential function for 5hmC and 5fC. Immunoprecipitation analysis of selected sex-linked genes and sex biased TEs showed differential regulation based on a clear sex-dependent clustering rather than a gathering based on the cytosine modifications.

## 76.

# Extended reconstruction of transposable elements in plants using TE-greedy-nester

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Transposable elements, including LTR retrotransposons, are mobile genetic elements constituting remarkable portions of plant genomes and significantly contribute to genome structure, size and regulations. Because of high level of their mutual sequence similarity and numerous insertion into one another, the correct identification of full-length LTR retrotranposons is a challenging bioinformatic task.

Our software, TE-greedy-nester (Lexa et al., 2020) takes an advantage of greedy algorithm wich allows to mine increasingly fragmented copies of full-length LTR retrotranposons. We found this tool to be superior in computation time and full-length element recovary in highly nested regions. Using TE-greedy-nester we showed that e.g. nesting of LTR retroelements is not random (Jedlicka et al., 2019); nested elements often have lower LTR similarity than pre-existing ones (Jedlicka et al., 2020); TEs fragmentation and LTR similarity differs in (i) low-recombining Y chromosome of dioecious Silene latifolia; and (ii) in monocentric and holocentric chromosomes of closely relative species of Juncaceae family.

Recent challenge is integration of tools for detection of other types of repetitive elements (e.g., tandem repeats and Miniature Inverted TEs – MITEs). Thererfore we redesigned the entire codebase to support module implementation. Thereafter, we included two other detection tools - Tandem Repeat Finder and MiteFinderII (Benson, 1999 and Hu & Shang, 2018, respectively); and conducted a few initial testing runs on plant genomic sequences.

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## 78.

# Assembly of saltgrass genomes reveals unique genomic features of a dioecious, perennial, halophytic grass

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The climate crisis in agriculture centers on freshwater scarcity. Understanding halophyte adaptation for productivity without freshwater offers new opportunities for crop improvement and neodomestication. Saltgrass (Distichlis spp.) is a unique genus of diecious halophytic C4 grasses, growing in tidal plains and saltmarshes with seawater salinity levels. We present high-quality telomere-to-telomere phased genome assemblies of four saltgrass genets, revealing an allotetraploid species comprising of two syntenic subgenomes of ~300 Mb each. Comparing genets, we identify a novel chromosome fusion between two subgenomes that leads to genetic differentiations of two saltgrass species (D. spicata; 2n=40 and D. stricta; 2n=38). Furthermore, performing extensive population genetics of several saltgrass populations provides the genetic diversity linked with geographical distribution. Additionally, we report a repeat-rich B chromosome (~7 Mb) in two of the genets. Using sex-specific populations, k-mer analyses, we identified genomic location for sex-determination. Saltgrass exhibits high saline resistance, chromosome fusion, B chromosome, and dioecious traits, making it an appealing candidate for neo-domestication in saline environments, and an ideal model plant to study diverse genomic features.

# Partners presentations

# 77.

## Overview of the Cellular Imaging Core Facility (CELLIM) – CEITEC MU

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CELLIM is the Light Microscopy Core Facility of CEITEC MU. It is part of the CzechBioImaging research infrastructure funded by the Ministry of Education, Youth and Sports of the Czech Republic. Internationally, it is part of the Advanced Light Microscopy and Medical Imaging Node Brno CZ - European Research Infrastructure Consortium EuroBioImaging ERIC. CELLIM provides services in the field of light microscopy and offers extensive expertise in sample preparation, image acquisition and data analysis. We provide access to state of the art instrumentation as well as expertise in sample preparation and data analysis. We have expertise/tools for sample preparation of various types of biological samples for widefield/confocal/SIM microscopy. Advanced techniques we offer include 2D single molecule localisation microscopy, 3D STORM, TIRF microscopy, spectral separation of fluorochromes and lightsheet microscopy. We also have expertise in expansion microscopy and sample clearing using various protocols. An integral part of our services is image processing and analysis - deconvolution, 3D rendering, stitching, object segmentation and measurement, etc. using various tools.

Further details about the facility, including contact information, an overview of the equipment, upcoming events and access to the online reservation system can be found on our website: https://cellim.ceitec.cz/. For the latest news from our site, you can al,so follow us on our Twitter acount (@Ceitec_CellimCF).

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