ELIXIR CZ Annual Conference 2022

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Summary of content

About ELIXIR CZ infrastructure

The Czech National Infrastructure for Biological Data, abbreviated ELIXIR Czech Republic or ELIXIR CZ, is a distributed research infrastructure for bioinformatics that has arisen from an advanced computational environment. We are dedicated to organisation, storage, sharing and facilitation of interoperability of life science data for further processing and analysis. We respond to the needs of national scientific community, but we are also a proud member of the pan-European infrastructure for biological data ELIXIR, which brings together life science resources from throughout Europe.

ELIXIR CZ is comprised of 14 research performing organisations across the Czech Republic, with its headquarters in the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences.

Institute of Organic Chemistry and Biochemistry of the CAS (IOCB)
Coordinating body of ELIXIR CZ and administrator of computational resources for bioinformatics research. IOCB develops proteomics resources and a database of small molecules, which are the flagships of ELIXIR CZ.

CESNET
CESNET is one of providers of a large national e-infrastructure for research and development, more specifically, provides communication, computing and storage facilities. CESNET acts as an ambassador of the Czech Republic in GÉANT Project, EGI Federation, and TERENA Association.

Masaryk University: CEITEC, CERIT-SC
CEITEC dedicates its' services to molecular medicine and structural biology, it is also a member of INSTRUCT infrastructure. CERIT-SC is one of providers of an e-infrastructure that provides advanced IT services.

Palacký University Olomouc (UP)
Provider of structural bioinformatics tools. UPOL acts as a liaison point to infrastructure EATRIS.

Charles University (CU)
Developer of tools for diagnostics and prognosis in medicine. UK also provides tools for high-throughput analysis of genomic, proteomic and structural data. UK is active in education and training on aspects of work with biological data.

Institute of Molecular Genetics of the CAS (IMG)

UMG provides DNA and RNA sequence analysis and tools. UMG represents the liaison point to infrastructures INFRAFRONTIER and EU-OPENSCREEN.

Institute of Microbiology of the CAS (IMIC)

MBÚ provides tools for computational biology and bioinformatics as well as models of biological networks.

Institute of Biotechnology of the CAS (IBT)

Provider of bioinformatics tools for structural biology. IBT provides database of DNA structural families.

Biology Centre of the CAS (BC)

BC is dedicated to sequence composition, molecular organisation and evolution of plant genomes and chromosomes.

University of Chemistry and Technology, Prague (UCT)

UCT provides training in the use of tools in cheminformatics and bioinformatics. UCT also provides structural bioinformatics computing tools.

Czech Technical University in Prague – Faculty of Information Technology (CTU)

CTU is dedicated to conceptual modelling and software implementation of conceptual models and development of modelling tools.

University of West Bohemia (UWB)

IT provider for marrow donor analysis and search applications. UWB operates a synthetic biology laboratory that supports tools for efficient assembly protocols and tools for hybrid biochemical reaction simulation.

University of South Bohemia in České Budějovice (USB)

USB represents a genomic centre for plants and microorganisms and applied informatics.

International Clinical Research Center of St. Anne's University Hospital in Brno (FNUSA ICRC)

Developer and provider of novel bioinformatics tools for protein structure analysis and prediction of the effect of mutations on human health.

Scientific Programme

Monday, 19 September

11:30 — 13:30 Registraton
12:00 — 13:30 Lunch

13:30 — 15:00 SESSION 1: Big data and ELIXIR

13:30 — 14:10 Ilia J. Leitch
Genome size diversity and why it matters – insights from the Plant DNA C-values database
14:10 — 14:35 Jiří Vondrášek
ELIXIR CZ meets challenges of data management in Life Sciences
14:35 — 14:50 Petr Novák
ELIXIR CZ supported tools for genome annotations and data sharing

15:00 — 15:30 Coffee break

15:30 — 16:40 SESSION 2: Repetitive DNA

15:30 — 16:00 Tony Heitkam
Repetitive DNA in plants: a guide to genomic change and regulatory innovation
16:00 — 16:20 Nicola Schmidt
Beet 'em up: Unifying 30 years of repeat research in beets and wild beets
16:20 — 16:40 Zdeněk Kubát
LTR retrotransposons preferentially spread through either the male or female plant germline
16:40 — 17:00 Pavel Jedlička
Reconstruction of LTR retrotransposons insertions in plant genomes

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18:10 — 19:40 Dinner

19:40 — 22:10 Posters I

Tuesday, 20 September

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9:00 — 9:30 Andre Marques
How to evolve without centromeres?

9:30 — 9:50 Jiří Macas
Weird but useful: what we can learn from atypical plant centromeres

9:50 — 10:10 Yi-Tzu Kuo
Plasticity in holocentromere organization: The holocentromere of Chionographis is comprised of a few megabase-sized CENH3 positive satellite arrays

10:10 — 10:30 Pavel Neumann
Identification of changes in structural and regulatory kinetochore protein genes in holocentric compared to monocentric Cuscuta species

10:30 — 11:00 Coffee break

11:00 — 12:30 SESSION 4: Chromosomes, meiosis and beyond

11:00 — 11:30 Andreas Houben
Analysis and application of natural and artificially induced chromosome elimination

11:30 — 11:50 Steven Dreissig
Recombination landscape divergence between populations is marked by larger low-recombining regions in domesticated rye

11:50 — 12:10 Václav Bačovský
Analysis of synaptonemal complex in plants: dissection of SCs in Silene latifolia

12:10 — 12:30 Jana Szecówka
Both male and female meiosis contribute to non-Mendelian inheritance of parental chromosomes in interspecific plant hybrids (Lolium × Festuca)

12:30 — 14:00 Lunch

14:00 — 15:00 Posters II

15:00 — 20:00 SPORTS and GAMES
15:00 — 16:30 Orienteering
15:45 — 20:00 Football tennis/Footnet

20:00 — 23:00 Dinner + PARTY

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9:00 — 10:30 SESSION 5: Structure and organization of plant genome

9:00 — 9:30 Ingo Schubert
DNA double-strand breaks as drivers of genome and karyotype evolution

9:30 — 9:50 Martin Kováčik
Developing an atlas of gene expression during barley grain development

9:50 — 10:10 Yile Huang
Structure and evolution of the meso-octoploid genome
of Heliophila variabilis (Brassicaceae)

10:30 — 11:00 Coffee break

11:00 — 12:00 SESSION 6: Specialized chromosomes

11:00 — 11:20 Tomáš Janíček
Back to the Future: Sex chromosomes and flower development

11:20 — 11:40 Miroslava Karafiátová
Mysterious biology of the B chromosome in wild Sorghum

11:40 — 12:00 Extra Lecture

11:40 — 12:00 Petr Smýkal
Towards the understanding of the seed dormancy as the key
domestication trait in legumes

12:00 — 13:00 Lunch

Lectures

Genome size diversity and why it matters – insights from the Plant DNA C-values database

Ilia J. Leitch - The Royal Botanic Gardens, Kew

Across the diversity of life-on-earth, species belonging to Kingdom Plantae are notable for their huge diversity of genome sizes (1C nuclear DNA amount), with values ranging ~11,800-fold from ~12.5 Mbp – 148 Gbp. In seeking to understand the biological significance and ecological consequences of such diversity biologists have been estimating genome sizes for over 70 years. To overcome the challenge of finding published genome size data that were scattered widely across the literature, the Plant DNA C-values database was launched in 2001, with the aim of providing a user-friendly, one-stop shop for plant genome size data available on the internet. With the increasing rate at which genome size data are being published, a further six updates have been released, with the most recent (release 7.1, 2019) containing genome size data for 12,273 species, including representatives from across all the major lineages of land plants and many algal lineages as well. This talk provides an overview of the Plant DNA C-values database and some of the challenges it faces together with some of the future opportunities it provides. In addition, it will highlight some of the large-scale comparative analyses which have been enabled by this resource. These will include insights into how the immense genome size diversity is distributed across the tree of plant life, how it evolved and some recent studies which have used the data to explore the influence of genome size on the distribution and survival of plants across the globe.

ELIXIR CZ meets challenges of data management in Life Sciences

Jiří Vondrášek - Institute of Organic Chemistry and Biochemistry of the CAS, ELIXIR CZ

There is a systematic movement in Life Science Data space towards implementation of FAIR principles. FAIRness of Data is a key factor in their utilization across disciplines and one of the requirements of FAIRness is professional Data Management. ELIXIR CZ in tight collaboration with ELIXIR NL contributed enormously to the solution of the Data Management problem by the development of a complex tool for Data Management Plan. The Data Stewardship Wizard became one of the officially recommended tool for Data Management by European Commission and it is a sophisticated solution for researchers and data managers. One of the important aspects of the process lies in proper metadata and ontology assignment. This will allow integration of data for purposes of EOSC and national repositories, connecting different data resources seamlessly, and including all -omics disciplines with chemical, health, and human data. ELIXIR CZ offers not only the solution for reasonable data management, but it is also the authority providing training and implementation of proper bioinformatics tools. In collaboration with other ELIXIR members, we build up an expert data network to respond to requirements of the research community and to work on challenges of data management.

ELIXIR-CZ supported tools for genome annotations and data sharing

Petr Novák - Biology Centre, Czech Academy of Sciences, Institute of Plant Molecular Biology

The RepeatExplorer Galaxy web server (https://repeatexplorer-elixir.cerit-sc.cz/) was originally developed to annotate repeats in genomes for which assembly was not available. Since the advent of new sequencing technologies, high quality genome assemblies can be obtained routinely, and so the RepeatExplorer server also began to incorporate new tools suitable for annotation of repeats in genome assemblies. New tools available on the RepeatExplorer server include DANTE, DANTE-LTR, and Library-based Annotation Tool and combine assembly-free approaches, similarity-based annotation, and structure-based annotation. Annotation tracks created on the RepeatExplorer server can then be visualized directly on the RepeatExplorer server using the Jbrowse genome browser.

For users who want to make genome annotations publicly available, we also offer the Apollo web server as a ELIXIR-CZ service (https://elmo5-26.hw.elixir-czech.cz). This server can be used either as a simple public genome browser where annotation tracks can be easily visualized, or it can be used for manual curation of genome annotations. Alternatively, annotation files can be shared conveniently through the National Repository provided by CESNET (https://data.narodni-repozitar.cz/) . The advantage of the National Repository is that it provides a unique and persistent identifier for the shared data set (DOI) and continuous access to the data.

These services are provided by BC CAS and CESNET, members of ELIXIR-CZ.

Repetitive DNA in plants: a guide to genomic change and regulatory innovation

Tony Heitkam - Faculty of Biology, Institute of Botany, Technische Universität Dresden

Plant genomes vary in size. Apart from genome duplications, repetitive DNAs are main drivers of genomic growths or shrinkages. They not only fill the space between genes, they (i) impact phenotypes, (ii) form chromosomal backbones, and (iii) serve as epigenetic regulators.

Despite the numerous functions and involvements in physiological processes, repetitive DNAs are still not well understood. This has two main reasons: First, repetitive DNAs can provide evolutionary shortcuts. Due to their degree of sequence repetitions in the genome, repetitive DNAs have fast, different and impactful mechanisms of evolution that are added on top of Mendelian genetics. Second, repetitive DNAs are diverse. Their structural diversity requires integrated genomics, cytogenetics and bioinformatics efforts, so far without community-decided standardization of analyses.

Here, I am introducing current research in the group. I will explain each of the aspects mentioned above and detail how our past, present and to-be-started research contributes to the understanding and application of repetitive DNAs in plant chromosomes, epigenetics and phenotypes.

Beet ‘em up: Unifying 30 years of repeat research in beets and wild beets

Nicola Schmidt - Faculty of Biology, Institute of Botany, Technische Universität Dresden

Sugar beet (*Beta vulgaris*) is the only sugar producing plant growing in temperate climate zones, contributing approx. 20 % of the worldwide sucrose production. Sugar-producing beet varieties were domesticated over only two centuries, resulting in the loss of several valuable traits, including stress and pathogen resistance. Breeding approaches involving beet wild relatives may help broadening the crop’s gene pool to develop improved cultivars. However, despite all beets sharing a common base chromosome number of 9, interspecific breeding is impeded by chromosome and sequence divergence. As repeats belong to the genomic fraction that evolve the fastest, genome and chromosome evolution can be traced in high resolution using comparative repeatomics. Taking advantage of next and third generation sequencing data (Illumina and Oxford Nanopore), we assessed the genome sizes and repeatomes of sugar beet and twelve wild beets comprising all sections of the beet genera *Beta* and *Patellifolia*. While we found that genome sizes and repeat profiles reflect the Betoideae classification into genera/sections, we also observe contrasting evolutionary patterns for specific repeat types: Whereas autonomous and non-autonomous LTR retrotransposons are causal for genome expansions in the section *Corollinae/Nanae*, satellite DNAs have a rather low impact on the genome size. We have identified section- and species -specific emergence of satellite DNAs with wide replacements of the structurally relevant sequences in the centromeres. Taken together, we unify the works on beet repeats spanning the last 30 years and show that the ups and downs of repeat expansions and contractions across the beet genera reflect the genomic divergence of the species and serve to better understand beet evolution.

LTR retrotransposons preferentially spread through either the male or female plant germline

Zdeněk Kubát - Institute of Biophysics of the Czech Academy of Sciences

Transposons are a dominant component of many eukaryotic genomes. In plants, they make up to 90 % of the genome. They are genetic units capable of self-replication and incorporation of newly made copies into the genome. The increase in copy number is thought to occur in waves called 'transposition bursts' punctuated by extended periods of low or no activity. Relatively little is known about at which stage of the plant life cycle transposons are active, but it is thought that most new insertions occur during reproduction, when the genome undergoes what is known as epigenetic reprogramming. Transposons at this stage can disrupt the protein-coding sequences or gene regulation and are therefore potentially harmful, and plants have therefore evolved a range of defence mechanisms to prevent transposon activity at the transcriptional or post-transcriptional level. The regulatory mechanisms known to date differ to some extent in the male and female germlines, and it is therefore possible that the male and female germlines contribute unequally to the copy number increase of transposons in the offspring genomes. However, in the traditional hermaphroditic plant species used to study transposons, it is difficult to study such effects due to the absence of sex-specific chromatin. Here we present results obtained in plants with heteromorphic sex chromosomes of the genera Silene and Rumex. Most of the LTR retrotransposons in these species have unequal copy numbers on the X and Y sex chromosomes and autosomes. The copy number ratios on the sex chromosomes and autosomes suggest that most LTR retrotransposons spread predominantly through either the male or female lineage. In addition, we studied differences between plant populations of the same species and found that the same LTR retrotransposon families spread predominantly through the male lineage in some populations and through the female lineage in other populations. Taken together, these results demonstrate that the insertion activity of LTR retrotransposons occurs predominantly in one of the parental lineages and that transposons can switch between male- and female-specific activity in a short time.

Funding: This research was supported by the Czech Science Foundation (19-15609S and 22-00364S).
Keywords: Silene latifolia, Rumex acetosa, LTR retrotranspozons, sex chromosomes

Reconstruction of LTR retrotransposons insertions in plant genomes

Pavel Jedlička - Department of Plant Developmental Genetics, Institute of Biophysics of the Czech Academy of Sciences

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 (nesting), the correct identification of full-length LTR retrotranposons is a challenging bioinformatic task.

In order to approach the most realistic view of full-length LTR retrotransposons, we created software using a greedy recursive algorithm to mine the increasingly fragmented copies of full-length LTR retrotransposons in assembled genomes and other sequence data – 'TE-greedy-nester' [1]. We found that this tool is superior in computation time and full-length element recovery in the highly nested regions. In follow up studies we reported that nesting of LTR retrotransposons is not random and element integration is correlated with sequence composition, secondary structure and the chromatin environment [2]. Furthermore, we used TE-greedy-nester in comprehensive LTR retrotransposon age estimation study [3]. We found out that LTR similarity depends on LTR length and further that nested elements often have a lower LTR similarity than pre-existing ones. We suggest that gene conversion of LTR regions could be responsible for both phenomena.

This research was supported by the Czech Science Foundation (grant 21–00580S).

[1] Lexa M, Jedlicka P, Vanat I, Cervenansky M, Kejnovsky E. TE-greedy-nester: structure-based detection of LTR retrotransposons and their nesting. Bioinformatics. 2020 Dec 22;36(20):4991-4999. doi: 10.1093/bioinformatics/btaa632.

[2] Jedlicka P, Lexa M, Vanat I, Hobza R, Kejnovsky E. Nested plant LTR retrotransposons target specific regions of other elements, while all LTR retrotransposons often target palindromes and nucleosome-occupied regions: in silico study. Mob DNA. 2019 Dec 14;10:50. doi: 10.1186/s13100-019-0186-z.

[3] Jedlicka P, Lexa M, Kejnovsky E. What Can Long Terminal Repeats Tell Us About the Age of LTR Retrotransposons, Gene Conversion and Ectopic Recombination? Front

How to evolve without centromeres?

André Marques - Department of Chromosome Biology, Max Planck Institute for Plant Breeding Research

The centromere typically defines a single region in most eukaryotic chromosomes. However, several plant and animal lineages assemble holocentromeres along the entire chromosome length. Here, we compare genome organization and evolution as a function of centromere type by assembling the first chromosome-scale holocentric genomes with repeat-based holocentromeres from three beak-sedges (*Rhynchospora pubera, R. breviuscula*, and *R. tenuis*) and their closest monocentric relative, *Juncus effusus*. We demonstrate that transition to holocentricity affected 3D genome architecture by redefining genomic compartments, while distributing centromere function to thousands of repeat-based centromere units genome-wide. We further uncover the complex genome organization of *R. pubera* hiding its unexpected octoploidy and describe the strong chromosome number reduction experienced by *R. tenuis*, a species with only two chromosomes. We show that chromosome fusions, facilitated by repeat-based holocentromeres, promoted karyotype evolution and diploidization. Furthermore, taking advantage of 10X single-cell RNA sequencing of pollen nuclei we uncover the first recombination map for a holocentric plant, revealing new patterns of recombination landscape in non-monocentric organisms. Our study sheds light on several important aspects of genome architecture and evolution affected by differential centromere organization.

Weird but useful: what we can learn from atypical plant centromeres

Jiří Macas - Biology Centre of the Czech Academy of Sciences, Institute of Plant Molecular Biology

Centromeres are chromosome regions that facilitate faithful chromosome segregation during cell division. This is achieved by providing an anchor point for the assembly of the kinetochore, a protein complex that connects centromeric chromatin to spindle microtubules. The position of the centromere on chromosomes of most species is determined epigenetically by the presence of the centromere-specific histone variant CENH3. There are two distinct types of centromere organization, which in turn influence the overall morphology of chromosomes - monocentric and holocentric. Monocentric chromosomes have centromeres restricted to a single specific region that forms a primary constriction during mitosis, whereas holocentric chromosomes are characterized by centromeres distributed along the entire chromosome length. However, there are plant species whose centromeres deviate from these basic types. This provides us with an opportunity to investigate the evolutionary forces and molecular mechanisms that lead to these deviations from the usual patterns, which in turn may shed light on the basic principles of centromere determination. In this talk, I will provide an overview of the two cases of atypical plant centromeres being studied in our lab - the holocentromeres of *Cucuta* and the meta-polycentromeres of *Fabeae*.

Plasticity in holocentromere organization: The holocentromere of Chionographis is comprised of a few megabase-sized CENH3 positive satellite arrays

Yi-Tzu Kuo - Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)

The centromere is the region on a chromosome where the kinetochore assembles and spindle microtubules attach. In addition to monocentric chromosomes, holocentric species with a chromosome-wide centromere distribution exist. We assembled the chromosome-scale reference genome and analyzed the holocentromere and (epi)genome organization of the lilioid *Chionographis japonica*. Strikingly, the holocentromere consists per chromatid of only 7 to 11 evenly spaced megabase-sized CENH3-positive centromere units arranged of palindromic 23 and 28 bp-long satellite arrays. In interphase nuclei, few centromere units associate and form chromocenters. Using polymer simulations, we modeled the cell cycle-dependent formation of the holocentromere in *Chionographis*. Likely, during the process of chromosome condensation, looping and folding of chromatin bring the centromere units next to each other to act as a single centromere. The metaphase chromosomes are compartmented in a medial to lateral arrangement of eu- and heterochromatic domains. We reveal that the epigenetic regulation of repeat-based centromeres in both monocentric and holocentric species is evolutionarily conserved. Our findings broaden our knowledge in plasticity and diversity of holocentromere organization. We demonstrate the unique value of analyzing non-model species for evolutionary comparisons to reveal novelties in even well-studied structures.

Identification of changes in structural and regulatory kinetochore protein genes in holocentric compared to monocentric Cuscuta species

Pavel Neumann - Biology Centre, Czech Academy of Sciences, Institute of Plant Molecular Biology

Proper chromosome segregation during cell division in eukaryotes depends on the centromere, a chromosomal region where the kinetochore forms and the spindle microtubules attach. In eukaryotes, there are two major types of centromere organization: 1) monocentric, in which the mitotic spindle binds to a single region on each chromosome, recognizable as a primary constriction on condensed metaphase chromosomes, or 2) holocentric, in which chromosomes lack primary constrictions and spindle binding sites are distributed along nearly the entire chromosome length. Although the causes and consequences of the transition to holocentricity are elusive, studies of holocentric insect species suggest that the transition may be related to changes in kinetochore protein composition.

The parasitic genus *Cuscuta* (Convolvulaceae) is an exception among plants with respect to centromere organization and includes species with both monocentric and holocentric chromosomes. Studies of chromosomal morphology, behavior during mitosis and meiosis, and distribution of spindle attachment sites have revealed that holocentric *Cuscuta* species occur exclusively in the subgenus *Cuscuta*, whereas monocentric species are restricted to the other two subgenera Grammica and *Monogynella*.

To determine whether and how the kinetochore has changed in holocentric compared to monocentric *Cuscuta* species, we compared the repertoire of kinetochore protein genes between *C. europaea* and *C. epithymum*, representing the holocentric subgenus *Cuscuta*, and three monocentric *Convolvulaceae* species, including *C. australis* and *C. campestris* from the sister subgenus *Grammica*, and *Ipomoea nil*, representing an outgroup genus. Our results demonstrated that the transition to holocentricity in *Cuscuta* species was accompanied by unprecedented changes in both structural and regulatory kinetochore protein genes. The changes included loss of *KNL2α* and *KNL2β* genes, increased divergence rate of *CENH3* genes, truncation of the structural kinetochore protein genes *CENP-C*, *KNL1*, and *ZWINT1*, and extensive disruption of genes involved in the spindle assembly checkpoint (SAC) that regulates kinetochore activity during mitosis. In situ immunodetection of the kinetochore proteins CENH3, CENP-C, KNL1, MIS12, and NDC80 then revealed that all of these proteins were localized to the kinetochore in monocentric *Cuscuta* species, whereas CENH3 became a heterochromatin-associated protein in *C. europea* and the others were not detectable on chromosomes in any of the holocentric *Cuscuta* species. These results suggest that holocentric *Cuscuta* species have lost the ability to form a functional kinetochore of the standard composition and that they do not employ a SAC-dependent mechanism to control the proper attachment of microtubules to chromosomes.

Analysis and application of natural and artificially induced chromosome elimination

Andreas Houben - IPK Gatersleben, Germany

In different plant species and species hybrids, chromosome-type and parent-specific chromosome elimination exist. Our analysis revealed CENH3-dependent and CENH3-independent chromosome elimination processes. Haploid production through uniparental genome elimination through different modifications of CENH3 has been successfully demonstrated in *Arabidopsis thaliana*. However, the method was only applicable to a few crops with low haploid induction frequency. Recently we showed that the nanobody-driven directed degradation of the EYFP-CENH3 fusion protein could be used for the generation of haploid plants.

Recombination landscape divergence between populations is marked by larger low-recombining regions in domesticated rye

Steven Dreissig - Institute of Agricultural and Nutritional Sciences, Martin-Luther-University Halle-Wittenberg, Halle (Saale)

The genomic landscape of recombination plays an essential role in evolution. Patterns of recombination are highly variable along chromosomes, between sexes, individuals, populations, and species. In many eukaryotes, recombination rates are elevated in sub-telomeric regions and drastically reduced near centromeres, resulting in large low-recombining(LR) regions. The processes of recombination are influenced by genetic factors, such as different alleles of genes involved in meiosis and chromatin structure, as well as external environmental stimuli like temperature and overall stress. In our work, we focused on the genomic landscapes of recombination in a collection of 916 rye (Secale cereale) individuals. By analyzing population structure among individuals of different domestication status and geographic origin, we detected high levels of admixture, reflecting the reproductive biology of a self-incompatible, wind-pollinating grass species. We then analyzed patterns of recombination in overlapping subpopulations, which revealed substantial variation in the physical size of LR regions, with a tendency for larger LR regions in domesticated subpopulations. Genome-wide association scans (GWAS) for LR region size revealed a major quantitative-trait-locus (QTL) at which, among 18 annotated genes, an ortholog of histone H4 acetyltransferase *ESA1* was located. Rye individuals belonging to domesticated subpopulations showed increased synaptonemal complex length, but no difference in crossover frequency, indicating that only the recombination landscape is different. Furthermore, the genomic region harbouring rye *ScESA1* showed moderate patterns of selection in domesticated subpopulations, suggesting that larger LR regions were indirectly selected during domestication to achieve more homogeneous populations for agricultural use.

Analysis of synaptonemal complex in plants: dissection of SCs in Silene latifolia

Václav Bačovský - Department of Plant Developmental Genetics, Institute of Biophysics of the Czech Academy of Sciences

Synaptonemal complex (SC) is a tripartite meiotic protein structure and one of the key elements essential for homologous chromosome pairing during prophase I (occurring in leptotene until pachytene/diplotene). The SC is assembled from two axial elements, anchoring each of the two sister chromatids of a homologue. Central element (CE) is attached to axial elements in a zipper-like structure by transverse filaments that span the central region and allowing chromosome synapsis. Despite recent progress and overall characterization of SCs in Metazoan, a little is known about SCs sub-organization in plants. In this work, we present optimized methodological workflow for transmission electron microscopy (TEM) and explain critical steps during tissue preparation that allow to target (3D) ultrastructure of a particular SC in meiotic cell by electron tomography. We present physical properties between synapsed and disrupted pairing regions that differ in width with two CEs, in the dioecious model plant *Silene latifolia*. We show this workflow is useful to study fully synapsed and unpaired regions, and further discuss possible improvements that may allow to decipher detailed SC subdomains.

Acknowledgement: We acknowledge Imaging Methods Core Facility at BIOCEV, institution supported by the MEYS CR (Large RI Project LM2018129 Czech-BioImaging) and ERDF (project No. CZ.02.1.01/0.0/0.0/18_046/0016045) for their support with obtaining imaging data presented in this poster. V.B. acknowledges the fellowship from the Czechoslovak Microscopy Society (CSMS).

Key words: meiosis, electron microscopy, chromosome synapsis, autosomes, pachytene

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

Jana Szecówka - Institute of Experimental Botany of the Czech Academy of Sciences

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

Using 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 those of Lolium, presumably by the mechanism of meiotic drive. This mechanism reflects the asymmetry of female meiosis and allows chromosomes of a 'dominant' genome to be transmitted to an egg cell more often than those of a 'submissive' genome which tend to be placed to polar bodies and thus do not participate in the next generation. Occasional elimination of Festuca chromosomes has been observed also during male meiosis. Detailed analysis of first and second male meiotic divisions revealed that Lolium univalents are regularly attached to microtubules and transmitted to daughter nuclei, while those of *Festuca* are not. They frequently stay unattached, lag in segregation, form micronuclei and are subsequently eliminated.

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

Our findings help to shed light on 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 during parental lines selection.

Funding: Czech Science Foundation grant: 20-10019S; European Regional Development Fund OPVVV project "Plants as a tool for sustainable development": CZ.02.1.01/0.0/0.0/16_019/0000827

DNA double-strand breaks as drivers of genome and Karyotype evolution

Ingo Schubert - Leibniz Institute of Plant Genetics & Crop Plant Research (IPK) Gatersleben

Double-strand breaks (DSBs) represent the most severe DNA damage and are risky for cell survival and stable inheritance of genetic information. A series of mechanisms emerged early during evolution to overcome this risk by re-establishing the original status. Moreover, various routes of DSB induction and/or (mis-)repair not only contribute to stable inheritance and differentiation (chromatin elimination), but also to adapt to environmental challenges (e.g. adaptive immunity), but most importantly they promote genome size dynamics, chromosome rearrangements and speciation. The C-value paradox finds an explanation in different strategies of DSB repair. Eventually, despite the ambivalent nature of DSBs, evolutionary aspects might turn the balance more to advantages than disadvantages.

Developing an atlas of gene expression during barley grain development

Martin Kováčik - Institute of Experimental Botany, Czech Academy of Sciences, Centre of the Region Haná for Biotechnological and Agricultural Research

Cereal seeds are an important source of food, feed and raw materials for biofuels and other technical products. We use barley (*Hordeum vulgare*) to study molecular and cellular mechanisms governing seed development. To provide comprehensive spatiotemporal information about barley grain developmental processes, we performed an RNA-seq transcriptomic study of three main seed tissues (embryo, endosperm, seed maternal tissues) from 4 until 32 days after pollination. Analysis of differential gene expression and gene clustering based on their expression profiles revealed timing of the major biological processes in different grain tissues. Gene co-expression network and motif enrichment analysis pointed out specific groups of genes and transcription factors with possible impacts on the regulation of endosperm development. Furthermore, expression of previously described marker genes for different endosperm compartments indicate that endosperm differentiation occurs even before cellularization. We defined a set of new tissue-specific marker genes and transcription factors that can help understand the major pathways controlling barley seed development. Altogether, our atlas of gene expression during barley grain development will be a useful resource for both basic research scientists and breeders.

Structure and evolution of the meso-octoploid genome of *Heliophila variabilis* (Brassicaceae)

Yile Huang - CEITEC – Central European Institute of Technology and National Centre for Biomolecular Research, Faculty of Science, Masaryk University

There is broad consensus that whole genome duplications (WGDs) followed by post-polyploid diploidization (PPD) have contributed significantly to the evolution of land plants and, in particular, angiosperms. The Heliophileae, the most morphologically diverse lineage in the mustard family, remains largely unknown with respect to its genome origin, evolution, and phylogenetic relationships. Here, we report the chromosome-scale assembly of a first meso-octoploid crucifer genome, the meso-octoploid *Heliophila variabilis* (~300 Mb, 2n=22). Although the *H. variabilis* genome has shrunk considerably and the octoploid chromosome number was reduced, most of the homoeologous chromosomal regions have been identified in four copies, accompanied by biased divergence in gene density and phylogenetic relationships. These four genomic copies were identified as two less fractionated (sub1 and sub2) and two more fractionated (sub3 and sub4) subgenomes. The putative ancestral genomes diverged during Oligocene-Miocene and their subsequent mergers may have occurred rapidly c. 18 million years ago. The biased subgenome fractionation was associated with extensive chromosomal rearrangements that mediated chromosome fusions and the activity of transposable elements. The progressive genome diploidization has enabled evolution of many important traits, including drought tolerance, disease resistance, leaf development, and flower color evolution. Our results provide a deeper understanding of the mid- and long-term evolutionary consequences of polyploidization and post-polyploid diploidization cycles. This work was supported by the Czech Science Foundation (project no. 19-07487S).

Back to the Future: Sex chromosomes and flower development

Tomáš Janíček - Institute of Biophysics of the Czech Academy of Sciences v.v.i, Plant Developmental Genetics

Flowering is one of the most important stages in a plant life cycle, yet flower development as a whole is far from being completely understood. Developmental reprogramming takes place in a small number of cells, triggering highly regulated spatio-temporal gene expression, which makes flowering an especially difficult mechanism to study. Flower development is controlled by a combination of both genetic (transcription activators and repressors, miRNAs) and epigenetic factors (chromatin remodelers), to achieve the final goal of sexual reproduction. Dioecious plants, such as *Silene latifolia*, are dealing with the additional challenge of establishing a unisexual flower by repressing development of the reproductive organs. This process is encoded by sex-determining loci in the genome, which can span from non-recombining regions on autosomal chromosomes to fully evolved sex chromosomes. *S. latifolia* possesses large heteromorphic sex chromosomes with extensive accumulations of repetitive elements. These sex chromosomes are similar in size to the whole *Arabidopsis* genome, making the identification of sex-linked genes very challenging even with cutting-edge sequencing methods. Nevertheless, by using chemical (epi)genetics, we were able to identify multiple sex-linked genes in *S. latifolia*. Here we present our current progress in the field of flower sex development by combining cytological and histological approaches with CRISPR/Cas9 genome editing to characterize sex-linked genes in *S. latifolia*. This will provide a solid foundation for the use of advanced methods, such as fluorescent activated cell sorting coupled with single-cell RNA-seq. This will further help us address the question of flower development and the role of sex chromosomes in one of the oldest plant genetics models.

This work was supported by GAČR project number 22-00364S

Mysterious biology of the B chromosome in wild Sorghum

Karafiátová Miroslava - Institute of Experiemental Botany of the Czech Academy of Sciences

Sorghum purpureosericeum is one of the five species of the genus carrying a supernumerary chromosome. Its distribution within the growing plant is rather variable and the permanent presence is limited to the reproductive tissues only. The B chromosome is excluded from the nuclei via mechanism of elimination, and its fate is determined early in developing embryo. The B chromosomes are totally absent in roots and leaves. However, many other tissues/organs show some level of chimerism. As in many other species, also sorghum B chromosome has also evolved its accumulation mechanisms. In addition to nondisjunction during the I. pollen division, there is a very peculiar mechanism of polymitosis in immature pollen. Those additional divisions take place between I. and II. pollen mitosis and result in multicellular pollen grain with several extra nuclei – all carrying the B chromosome.

Towards the understanding of the seed dormancy as the key domestication trait in legumes

Petr Smýkal - Department of Botany, Palacký University in Olomouc

The origin of agriculture was one of the key points in human history, and a central part of this was the evolution of new plant forms, domesticated crops. The transformation of wild plants into crop plants can be viewed as an accelerated evolution, the result of human and natural selection. These processes led to the so-called domestication syndrome, including changes in plant structure, plant defence and palatability. However, there are two traits considered crucial: reduced dispersal ability and eliminated seed dormancy. Particularly seed dormancy plays a significant role, as in the wild, many seeds will only germinate after certain conditions have passed, while crops tend to germinate as soon as they are wet and planted. In legumes, seed dormancy is executed by seed coat permeability, e.g. physical type.

We have used an integrated view combining comparative anatomy, metabolomics, genetic mapping, and transcriptome profiling of wild progenitor and respective crops in order to identify genes associated with loss of seed dormancy and pod dehiscence in pea, lentil, and chickpea. Genetic mapping identified 2 to 3 loci involved in seed dormancy and a single locus governing pod dehiscence. Using genome-wide analysis, we demonstrated that domesticated *Pisum sativum* and the Ethiopian pea (*P. abyssinicum*), were derived from different *P. elatius* genepool; therefore pea has at least two domestication events. Transcriptome profiling identified genes of the phenylpropanoid pathway significantly enriched and upregulated in dormant wild progenitors, supported also by metabolomics analysis. Notably, loss of seed pigmentation and non-functionality of polyphenol oxidase, have been selected during domestication. There is no clear picture if this is accidental, the result of direct selection due to the presence of anti-nutritional compounds affecting digestion and the palatability of the seeds, or the result of cultural behavior, favoring white color as a symbol of purity. We will discuss our findings in the context of various independently domesticated legume crops.

Acknowledgments: This work was supported by Grant Agency of Czech Republic (14-11782S, 19-07155S, 21-15856S) and Grant Agency of Palacky University (IGA-2022_002) projects.

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Saffron's progenitor species Crocus cartwrightianus has a high and chromosome-dependent haplotype diversity

Abdullah El-nagish[1,2], Susan Liedtke[1], Ludwig Mann[1] and Tony Heitkam[1] - [1] Faculty of Biology, Institute of Botany, Technische Universität Dresden, Dresden, Germany, [2] Botany and Microbiology Department, Faculty of Science, Sohag University, Sohag, Egypt

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 (Schmidt et al. 2019, Nemati et al. 2019, reviewed in Kazemi-Shahandashti et al. 2022). Comparative FISH analysis of saffron and closely related *Crocus* species revealed that *C. sativus* is an autotriploid species solely derived from heterogeneous cytotypes of *C. cartwrightianus. C. cartwrightianus* is an outcrossing species with numerous different cytotypes existing in the population with different phenotypes observed in flower colors and large differences in the repeat arrays on even homologous chromosomes. We assume that a cytotype of *C. cartwrightianus* produced unreduced pollen (n = 16) that fertilized a regular egg cell (n = 8) of another cytotype to produce the sterile triploid *C. sativus,* now vegetatively propagated as saffron crocus (Schmidt et al., 2019). Here, we build on our previous work and investigate the haplotype diversity within *C. cartwrightianus* stemming from different areas of Greece. We applied six-color FISH of CroSat satellite DNAs in combination with 18S-5.8S-25S rRNA and 5S rRNA genes and observed unique signal patterns at high resolution. Adding to our previous work, we investigated plants of six geographic *C. cartwrightianus* populations from Attica, Evia, Kea, Paros, Crete and Anafi and discovered high structural heterogeneity among all individuals, with each plant representing a different cytotype. We detected large differences in haplotype variability among chromosomes, with highest variability observed in chromosomes 5 and 6 and lowest in chromosomes 7 and 8. This suggests that some *C. cartwrightianus* chromosomes have obtained a higher genetic diversity across the Mediterranean than others. This haplotype diversity is also fixed in saffron's karyotype, a testimony to the genetic variability of *C. cartwrightianus* 5000 years ago.

What is the role of CENH3 encoded by maize B chromosome?

Bednářová Martina - Institute of Experimental Botany of the Czech Academy of Sciences, Centre of the Region Hana for Biotechnological and Agricultural Research

„Supernumerary“ B chromosomes are peculiar genetic elements, whose function and behavior in plants are still largely unknown. Their maintenance in plant populations is enabled by special accumulation mechanisms, which in the case of maize (Zea mays) involve nondisjunction (segregation failure) of the B chromosome(s) during pollen mitosis and preferential fertilization of an egg by B-containing sperm cells. As the nondisjunction is driven by B chromosome itself, we aimed to analyse gene expression and the degree of evolutionary conservation of genes encoded by maize B chromosome in order to reveal candidates that might be involved in this process. One of the strongest candidates appears to be B chromosome-specific variant of centromeric histone H3 (CENH3B), which slightly differs from the A chromosome variant (CENH3A). We have designed antibodies for CENH3A and CENH3B in order to find out whether CENH3B binds specifically to B chromosome centromere, but the results have been inconclusive so far. Therefore, we have constructed plasmids for the expression of fluorescently-labelled CENH3 variants and we have used them for Agrobacterium-mediated transformation to track the nuclear localization of CENH3A and CENH3B in vivo.

Extraordianry intraspecific variability of satellites on sex chromosomes of Rumex acetosa

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Satellites are fast evolving genomic repeats which show high variability in closely related species both in sequence and abundance. Intraspecific variability of satellites is, however, much less studied. We have decided to study intraspecific variability of satellites and chosen *Rumex acetosa* as our model for its high satellite content and XY^1Y^2 sex chromosomes. We have analyzed repetitive genomic content and genome sizes of males and females from 14 populations across Europe. The selected sites allow us to compare both geographically distant and close populations. We have discovered surprising satellite variability both in sequence and abundance. Satellites appear to be much more dynamic compared to other repeats such as LTR retrotransposons. The fastest evolution of satellites takes place on Y chromosomes, probably due to suppressed recombination on these heteromorphic chromosomes. The differences in Y-specific satellites abundance and sequence allows us to distinguish paternally inherited phylogenetic signal and 4 distinct Y haplotypes. This phylogenetic signal is partially reflected in the rest of the nuclear genome. On the other hand, maternally inherited chloroplast genome shows much less spacial mobility by grouping geographically close populations regardless of Y-haplotype or genome size.

Funding: This research was supported by the Czech Science Foundation (19-15609S and 22-00364S).

Keywords: Rumex acetosa, satellites, sex chromosomes

Spatial organization of Oryza sativa chromosomes in interphase nuclei

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The spatial organization of chromatin in the interphase nucleus and the interactions between chromosome territories (CTs) influence biological processes, such as DNA replication, transcription, and DNA repair. Understanding of the chromatin spatial organization in the nucleus has advanced in past decades due to two main technical approaches for 3D genomics studies (chromosome conformation capture) and three-dimensional fluorescence in situ hybridization (3D-FISH). However, chromatin studies in plants have lagged behind those in mammals, both 3D genomic approaches were successfully used in the model plant Arabidopsis and showed some discrepancies between results obtained by 3D genomics and 3D-FISH.

Our study focuses detailed 3D microscopical studies of chromatin organization in interphase nuclei of *Oryza sativa*, crop species with a small genome (1C ~ 450 Mb). Although Hi-C data are available, detailed microscopical studies of chromatin organization are missing. To assess whether the distribution of CTs in interphase is random or not chromosome oligo-painting was used to analyze the 3D distribution and association of chromosomes in flow-sorted nuclei of root and leave tissues. Six different positions of CTs were observed in G1 nuclei. CTs of chromosome 9 (acrocentric, bearing the NOR region, covering 8 % of the volume) was usually separated (92.54 %) in the root. In leaves, CT separations were less frequent (41,05 %). The shape of the nucleolus was almost the same, differences were observed in the size of the nucleolus and the number and size of 45s rDNA loci.

Unraveling the genomic past of Catolobus pendulus - a close relative of Arabidopsis

Perla Farhat, Terezie Mandáková, and Martin A. Lysak - CEITEC – Central European Institute of Technology, Masaryk University

Catolobus is a monospecific genus in the mustard family (Brassicaceae), assigned to the tribe Camelineae. Despite its phylogenetic closeness with *Arabidopsis*, the genome of *C. pendulus* remains unexplored. Only few reports presented *C. pendulus* as a diploid (2n = 16) and near-triploid (2n = 21) species. Here we aimed to get a very first insight into the genome structure and phylogenomic history of *C. pendulus.* To achieve these goals, we applied comparative chromosome painting and Hyb-seq phylogenomic analyses in 28 Catolobus populations throughout its Eurasian distribution range.

In contrast to the reported chromosome numbers, all investigated populations of C. pendulus were hypotetraploid (2n = 30) with highly conserved genome structure, harboring a genome size of ca. 326 Mbp/1C. Detailed cytogenomic analysis revealed that the post-polyploid descending dysploidy from 32 to 30 in *Catolobus* was mediated by complex rearrangements of five chromosomes. The Bayesian clustering of SNPs from Hyb-seq data suggested the existence of two highly differentiated genetic clusters at the East and West of the species distribution. In addition, populations presenting significant genetic admixture were detected to be randomly distributed throughout the Eurasian distribution range. Phylogenetic analysis distinguished the two identified clusters into two major well-supported clades that diverged simultaneously at around 5 Mya. The chloroplast genome of *Catolobus* showed a total length of ca. 154Kb, for which no intraspecific phylogenetic variation was detected.

Despite the conserved chromosome number and genome structure, our study revealed the inter-population genetic diversity in *Catolobus*. Taken together, this study uncovered the genomic and phylogeographical features of a promising wild model closely related to *Arabidopsis*, *Camelina*, and *Capsella*.

This work was supported by the Czech Science Foundation (grant no. 21-03909S).

Keywords: Brassicaceae, chromosome rearrangements, genome skimming, karyotype structure, phylogenomics, polyploidy, target enrichment.

Is cellulose synthesis linked to DNA damage stress in Arabidopsis?

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Cellulose is a common component of plant cell walls that has a role in the mechanical support of the cells and whole plant bodies. Furthermore, cellulose is isolating the inner content against external influence such as pathogens. Surprisingly, in a forward-directed genetic screen designed to identify new factors involved in DNA damage repair, we found several mutant alleles of a core component of the cellulose biosynthesis complex. This surprising finding may suggest that the synthesis of cellulose is required upon DNA damage. The exact function of this regulation is yet to be studied, but we hypothesize that cellulose may be needed to protect specific cells by enclosing plasmodesmata and decreasing permeability of the DNA damaging agents. Alternatively, it may be needed for the synthesis of the cell walls for newly forming cells that are replenishing the population of cells that underwent cell death. We will present the data concerning the mapping of our target gene and the future plans for the genetic, molecular and microscopic analyses. In general, this project has a strong potential to connect regulation of plant genome stability with the synthesis of an economically important compound – cellulose.

Genome diploidization associates with cladogenesis, trait disparity and gene coevolution in a crucifer tribe

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Angiosperm genome evolution was marked by many clade-specific whole-genome duplication (WGD) events. WGD is a trial-and-error process under natural selection, with subsequent genome diploidization proceeding with different intensities and being associated with speciation events. The Microlepidieae is a monophyletic tribe in the mustard family (Brassicaceae) formed after an ancient allotetraploidization, including c. 17 genera and 60 species endemic to Australia and New Zealand. Post-polyploid diploidization has resulted in the extant Microlepidieae genomes that differ in the extent of inter-subgenome reshuffling and the number of chromosomes (n=4 to n=12). To gain a deeper understanding of post-polyploid genome evolution in Microlepidieae, we analyzed phylogenetic relationships in this tribe using complete chloroplast sequences, entire 35S rDNA units, and abundant repetitive sequences. The four recovered intra-tribal clades mirror the varied diploidization of Microlepidieae genomes, suggesting that the intrinsic genomic features underlying the extent of diploidization are shared among genera and species within one clade. Nevertheless, even congeneric species may exert considerable morphological disparity (e.g., in fruit shape), whereas some species within different clades experience extensive morphological convergence despite the different pace of their genome diploidization. We showed that faster genome diploidization is positively correlated with mean morphological disparity and evolution of chloroplast genes (plastid-nuclear genome coevolution). Higher speciation rates in perennials than in annual species were observed. Altogether, the newly acquired results confirm the potential of Microlepidieae as a promising subject for the analysis of post-polyploid genome diploidization in Brassicaceae.

This work was supported by a research grant from the Czech Science Foundation (20-03419Y).

Mining data from sweet cherry resequencing

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Relatively cheap sequencing produces huge amount of sequencing data. Simultaneously, the available scripts, tools and programmes allow processing gained data sets in simple ways and obtain interesting results supporting fundamental and applied research as well as breeding. Thus, genome resequencing could be valuable resource for multiple analysis. However, the data processing is labouring and time consuming, extracting all available information is not possible.

In our study, we sequenced 235 genotypes from *Prunus avium* with minimal genome coverage 20X and called SNP markers. The main goal was to associate SNPs with phenotypes scored through nine years and design markers that will be used for marker-assisted selection in breeding. Besides, both the resequencing and the first basic analysis open the door for other research goals. With a basic tools we was able to detected genes responsible for particular phenotypes, find the mis-assembly in the reference genome, defined the deletion which take out MYB genes responsible for red colour of fruit, detected duplicated accessions on the basis of DNA fingerprint, designed the SSR and SNP markers for genotyping. How can this data be used more?

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Divergence of satellite DNA in the Cannabaceae family

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Satellite DNA (satDNA), organized into a long tandem array of monomer units, is a rapidly evolving part of the plant genome. Satellite families are typically shared among related species with a certain level of divergence that makes satDNA a powerful cytogenetic tool to study individual chromosomes and chromosomal rearrangements. Members of the *Cannabaceae* family differ in their genome size and sex chromosome system and display extensive chromosomal rearrangements. Despite species divergence, three dioecious species *Humulus lupulus*, *Humulus japonicus*, and *Cannabis sativa* share conserved monomer units of subtelomeric satellites HSR, HJSR, and CS-1 (previously described by several authors). Although, these satellites are important cytogenetic markers for differentiating sex chromosomes and autosomes, the origin, nature, and abundance of HSR, HJSR, and CS-1 satellites have not been described. In this study, we compare DNA sequence subunit similarity for all three shared subtelomeric satellites and identify their inter-species localization using fluorescent *in situ* hybridization (FISH) in *H. lupulus*, *H. japonicus*, and *C. sativa.* We demonstrate that satellites with higher similarity (HSR and HJSR) have the same pattern in subtelomeric regions on metaphase chromosomes of *H. lupulus* and *H. japonicus*. Interestingly, satellite CS-1 in *H. lupulus* is distributed also in perincentromeric regions as well as satellite HSR in *C. sativa*. Our findings provide new insight into the evolutionary processes of the *Cannabaceae* family.

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Evidence of novel epigenetic marks within Silene latifolia genome

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Silene latifolia is a model organism for the study of sex chromosome evolution in plants. Its sex chromosomes include large regions in which recombination became gradually suppressed (Hobza et al., 2018). These non-recombining regions are represented by accumulation of repetitive elements and degeneration of genes (Hobza et al., 2017). Epigenetics play a crucial role in *S. latifolia* sex chromosome evolution (Li et al., 2017; Rodríguez Lorenzo et al., 2020). In particular DNA methylation, which is involved in gene and repetitive element silencing (Kubat et al., 2014; Rodríguez Lorenzo, Hobza and Vyskot, 2018). Additionally, three recent cytosine modifications, 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC) and 5-carboxycytosine (5-caC) have become relevant in plants (Mahmood and Dunwell, 2019). Global quantification of cytosine modifications revealed no significant differences between male and female plants. However, cytogenetics showed differences in metaphasic chromosomes. By DNA immunoprecipitation coupled with qPCR we found differential regulation in genes of Y alleles by 5-hmC and X alleles by 5-fC. Transposable elements showed similar sex-dependent regulation, especially those with biased distribution in the sex chromosomes.

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Functional genomics in the genus Silene

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Being studied by some of the most prominent biologists, such as Charles Darwin or Gregor Johann Mendel, the genus *Silene* represents a classical model of genetics and evolutionary biology. Although numerous biological questions can be addressed using this model system (e.g., dioecy and sex chromosome evolution, plant speciation, sexual antagonism, epigenetic control of sex determination, sexually transmitted diseases, invasive species biology, heavy metal tolerance, etc.), this genus became rather neglected in past few decades. Until recently, the genus *Silene* remained neglected by the scientific community, mainly due to its large and repetitive genome as well as the unavailability of functional genetic techniques. As the recent methods are successfully overcoming the challenges associated with genome sequencing, assembly, and transcriptomic analyses, we have developed an approach to assess gene function for the genus *Silene*. We established an efficient *Agrobacterium*-mediated genetic transformation of *S. latifolia* and subsequently used this approach to demonstrate the possibility of CRISPR/Cas9 genome editing on plant sex chromosomes. Furthermore, we have adopted the RNAi machinery to decrease the mRNA level of endogenous genes. We present additional approaches to achieve functional analyses via gene delivery and transient assays to modulate gene expression in *S. latifolia*. We hope our efforts will contribute to the re-discovery of the genus *Silene* as a model system for studying interconnected questions in genetics, ecology, evolution, and developmental biology.

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Like a Story from Charles Dickens' Novels: Poor Fate of Orphans in Hybrid Environment

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Each genome is represented, besides others, by a set of protein-coding genes. While majority of the genes have an equivalent (ortholog) in others, usually closely related species, there is a certain number of genes, which are unique for a given species. These genes are called orphans and their proportion varies largely among phylogenetic tree of life. The first estimates usually referred to 10-20% of genes without any significant sequence similarity to genes of other species. Recent studies indicates that the proportion is probably somewhat lower, at least in plants. It is predicted that there are 1084 orphans, representing about 4% of all genes, in Arabidopsis thaliana. In some species, they may create various novelties beneficial for the organism, such as genes for cnidocytes and innate defense systems in Hydra and sometimes play a role in metabolic processes such as QQS in A. thaliana. Moreover, there is an evidence that orphans are preferentially expressed in reaction to abiotic stresses, which may indicate their beneficial role in plant adaptations.

The questionable is the retention and expression of the orphans in interspecific hybrids. In our work, we identified 1572 predicted genes, which were found in genome assembly of highly heterozygous diploid Italian ryegrass (Lolium multiflorum Lam.) containing 70668 predicted genes, which were not found in the genome of closely related meadow fescue (Festuca pratensis Huds.). We performed RNAseq of reciprocal hybrids and Lolium parent and estimate the expression of those 1572 genes (based on the comparisons of mean counts per million reads). Out of these, 87 and 30 genes (overlap of 17 genes) were expressed in Lolium parent, but not in Festuca x Lolium hybrids and Lolium x Festuca hybrids, respectively. On the other hand, 46 and 64 genes (overlap of 7 genes) were not expressed in Lolium parent, but display expression in Festuca x Lolium and Lolium x Festuca hybrids, respectively. Gene ontology analysis revealed that two of these genes are putative wall-associated receptor kinase (WAK), reported being expressed during pathogen exposure or wounding and DEAD-box helicase, which is known for its role in the recognition of foreign nucleic acids and the modulation of viral infection. Interestingly, in all 190 genes, which were expressed in Lolium parent and in its hybrids (Festuca x Lolium and Lolium x Festuca), the expression in Lolium parent and hybrids was either the same (122 genes) or lower in hybrids (68 genes), indicating higher rate of down-regulation and silencing compared to up-regulation and gene activation in hybrids. However, they may play a significant role in the evolution of hybrid genomes and have a critical effect on the adaptation towards the new optimum once changes in eco-climatic settings favor an individual carrying and expressing orphan genes.

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Mapping of a Race Non-specific Powdery Mildew Resistance Gene from Tetraploid Wheat

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Powdery mildew, caused by the fungus *Blumeria graminis* f. sp. *tritici*, is one of the most devastating diseases of wheat. Infection of susceptible varieties by powdery mildew leads to significant yield and grain quality losses. Control of powdery mildew disease relies, besides the application of fungicides, mostly on resistant genes (R-genes) and their implementation into elite cultivars. Unfortunately, R-genes are usually short-term and effective for only 3-5 years (Wolfe and McDermott, 1994; Dreiseitl, 2003). Therefore, it is necessary to search for new sources of resistance.

Recently, a major powdery mildew resistance locus *QPm.GZ1-2A*, conferring a full race non-specific resistance in homozygous recessive stage, was identified in emmer wheat landrace GZ1. QTL analysis located *QPm.GZ1-2A* in 16.1 cM interval on chromosome 2AL, which is syntenic with a 22.7 Mb long region of cv. Zavitan reference genome sequence (Avni et al., 2017). *QPm.GZ1-2A* locus was further saturated with 14 markers and narrowed down to 4.3 Mb. 55 candidate genes have been identified in this region; however, none of them is an apparent candidate for *QPm.GZ1-2A* resistance gene. Moreover, no recessive resistance gene or allele was located in a similar position, suggesting the presence of a new powdery mildew resistance gene in the GZ1.

Markers associated with the *QPm.GZ1-2A* locus could be used for trait selection in breeding programs. Mapping data are also an ideal base for cloning the gene and studying its function, regulation, and role in host-pathogen interaction.

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LTR retrotransposons in holocentric plants from the Cyperaceae and Juncaceae families

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LTR retrotransposons play a significant role in plant genome structure, organization, and evolutionary dynamics. Holocentricity represents an unusual and distinct genome organization type affecting many genomic processes and characteristics, nonetheless, the features of its interplay with LTR retrotransposons in holocentric genomes remain largely unexplored.

We present the analysis of LTR retrotransposons in several species of holocentric plants from the Cyperaceae and Juncaceae families. Holocentric and monocentric plant species were selected to represent a broad spectrum of genome sizes and chromosome numbers. Their genomic DNA was sequenced using a combination of long and short read sequencing platforms. These data were used for the analysis of the overall type and abundance of repetitive sequences and for the identification of transposable elements in partial genomic assemblies. Detected LTR retrotransposons were then investigated to characterize their lineage, age, completeness, and nested insertions.

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Reconstruction of phylogenies using repeatomefingerprinting and the genome composition of the basal land plants (Marchantiophyta)

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Marchantiophyta (liverworts) is a group of non-vascular early diverging land plants belonging to one of the three divisions of *Bryophyta*. Current liverwort phylogenies are based on chloroplast genes, eventually sequences of ribosomal DNA internal transcribed spacers (ITS) or morphological characters. Here we used a phylogenomic approach to infer the phylogenetic relationships in this group. It is based on repeatome fingerprints which considers similarities/dissimilarities in repeated DNA elements (transposons, ribosomal DNA, satellite repeats, chloroplasts) between the genomes. We used RepeatExplorer2 comparative clustering of Illumina reads from 50 species (11 newly sequenced and 39 published genomes) covering all major lineages. Similarities between reads in the clusters were converted into the Gower distances and the resulting matrix was used to construct NJ trees. As expected, two major groups, *Jungermaniopsida* and *Marchantiopsida* were clearly separated. Significantly, the leafy and thalloid species from *Jungermaniopsida* formed well-supported subclades, the latter being early diverging. The leafy *Jungermaniopsida* subclade was highly diversified and late diverging. Thus, morphological characteristics of liverworts seem to be well reflected in repeatome fingerprints. Bioinformatics analyses showed that most of the liverwort genomes were composed of class I and class II retroelements as in other plants. However, contrast to angiosperms only particular lineages of retroelements were represented (mostly Ty3_gypsy/Athila, Ty3_gypsy/chromovirus and Ty1_copia/Tork/). The abundance of satellite repeats is generally lower in liverworts and mosses compared to vascular plants which may be related to their haploid life and infrequent meiotic recombination. We conclude that the repeatome fingerprint approach is sufficiently robust allowing to infer phylogenetic relationships between evolutionary distant groups of liverworts. It could become a convenient phylogenomic tool not requiring genome assembly, deep sequencing or extraction of protein coding sequences.

Mapping of quantitative trait loci for agronomically important traits in einkorn wheat

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Wheat provides more than 20% of calories and proteins consumed by the human population. Due to the growing demand and changing climate conditions, it is necessary to improve its yield and resistance to biotic and abiotic stress factors. Modern breeding has limited wheat gene pool. The diploid einkorn wheat, *Triticum monococcum*, represents an attractive source of new genes and alleles. In the present study, a linkage map of einkorn was constructed using 81 F8 recombinant inbred lines derived from a cross of domesticated *T. monococcum* 'DV92' and wild *T. boeoticum* 'G3116'1. The map comprises 659 markers and spans 1033 cM divided into seven linkage groups with a marker density of 1.61 cM. Quantitative trait locus (QTL) analysis for twenty-three agronomic traits, including physiology and yield traits, identified 166 significant QTLs on all seven chromosomes. The percentage of explained variance for a trait ranged from 17.8% (the number of grains per spikelet) to 76.2% (rachis brittleness). 49 QTLs were overlapping with QTLs from other einkorn studies and were therefore verified. Out of 74 selected QTLs, 22 QTLs were verified using another two einkorn mapping populations developed for this study. The QTLs provide a valuable basis for the identification and study of genes responsible for the variability of the traits and for bread wheat gene pool enrichment.

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ECCsplorer: a pipeline to detect extrachromosomal circular DNA (eccDNA) from enriched next-generation sequencing data

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Extrachromosomal circular DNAs (eccDNAs) are DNA rings that may harbor extra gene copies and recently activated transposable elements. As eccDNAs occur in presumably all eukaryotes and are linked to stress, cancer, and aging, they have been prime targets in recent research – with their investigation limited by the scarcity of computational tools. The ECCsplorer is a bioinformatics pipeline to detect eccDNAs in any organism or tissue using next-generation sequencing of amplified circular DNA. The derived eccDNA targets are valuable for a wide range of downstream investigations – from cancer and organelle genomics to identification of active transposable elements.

The ECCsplorer is available online at https://github.com/crimBubble/ECCsplorer.

Horizontal gene transfer in grasses: on track of stolen genes in Hordeum

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Background
The pangenome is a set of all genes present in a given taxon. Besides core genes, a variable part of genes may be acquired from other taxa via horizontal gene transfer (HGT), potentially providing the recipient with an evolutionary benefit. The extent of acquisitions of foreign DNA, their stability and transmission to future generations, and the evolutionary consequences of HGT are almost unknown in plants. *Hordeum* (Pooideae) is a suitable model to investigate these processes. Species of sect. *Stenostachys* obtained foreign DNA, including protein-coding genes, from diverse panicoid grasses (Panicoideae) via at least nine independent HGTs.

Results
Initially, the presence of panicoid DNA in *Hordeum* was an accidental finding showing that *H. bogdanii* harbored a copy of ribosomal DNA (rDNA) corresponding to a Panicum species. A follow-up targeted screening involving all *Hordeum* diploid species identified panicoid rDNAs in all 16 species of sect. *Stenostachys*. Their origin was traced to five Panicoideae genera, namely *Panicum, Paspalum, Euclasta, Setaria,* and *Arundinella*. The transfer from *Panicum* is considered the oldest and the transfer from *Arundinella* the youngest.
The transfer from a *Panicum*-like donor was investigated in detail using sequenced BAC (bacterial artificial chromosomes) clones in two *Hordeum* species, representing Old World (Asian *H. bogdanii*) and New World (South American *H. pubiflorum*) *Hordeum* lineages. Both species possessed a *Panicum*-derived chromosomal segment, spanning over 440 kb in *H. bogdanii* and 219 kb in *H. pubiflorum*, which resided on a pair of NOR-bearing chromosomes. Conserved synteny and micro-colinearity of the segment in both species indicated a common origin of the segment and confirmed its acquisition by *Hordeum* before the diversification of sect. *Stenostachys* 5–1.7 million years ago. The chromosomal segment was hallmarked by several ~68 kb long repeated blocks containing five protein-coding genes in addition to rDNA and transposable elements. Two of the protein-coding genes, namely Ervatamin-C-like and Glutathione S-transferase T3-like, became new genes in the gene pool of wild *Hordeum* species. The former remained functional in some current *Hordeum* species.

Perspective
Horizontal transfer likely played a beneficial role in the evolution of the genus *Hordeum*. Yet, a full understanding of this role is far from being achieved. Currently, the research is ongoing under the frame of an international consortium aiming at the characterization of the genus-wide pangenome of *Hordeum*. The use of state-of-the-art technologies and bioinformatics approaches enables the full assembly and characterization of large *Hordeum* genomes, a task hardly achievable until recently. The genus-wide characterization of panicoid DNA in Hordeum will help to elucidate the mechanisms of HGT, the evolution and dynamics of foreign DNA in host genomes, and the role of foreign protein-coding genes in the evolution of sect. *Stenostachys*.

Identification and characterization of meiotic recombination hotspot in bread wheat within region introgressed from T. militinae

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Meiotic recombination is the most important process of evolution and adaptation to changing conditions. Even there is substantial knowledge about proteins involved in the process, targeting of specific DNA loci by the recombination machinery is not well understood. We identified the recombination hotspot in two mapping populations of wheat cv. Chinese Spring (with *Ph1* and *ph1* allele) and the wheat line 8.1 with *T. militinae* segment bearing *QPm.tut-4A*. The sequence, methylation pattern, and recombination frequency were determined for the hotspot (1586 bp) and compared with the “regular” CO site (2538 bp) localized nearby. Our results revealed that the recombination hotspot is characterized by a 5.94 times higher frequency of CO in comparison with adjacent recombination site. What is more, we found that the occurrence of recombination is directed to the specific regions, unalterable in sequence, and hypomethylated in all parental lines used for the creation of mapping populations. We determined also favorable maintenance of the *T. militinae* chromatin bearing *QPm.tut-4A* locus in the analyzed recombination lines. Further studies focused on the designation of hotspots in wheat and relative species would be useful for targeting crossovers to specific regions of this important crop.

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Wheat vernalization: still unsolved mystery

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Bread wheat (*Triticum aestivum* L., 2n=6x=42) is a temperate cereal that occurs in two main growth habits: a spring type and winter type (Snape et al., 2001). Winter wheat cultivars require a period of low non-freezing temperatures (vernalization) that triggers the transition from vegetative to generative stage, while spring cultivars flower safely without vernalization. The key player controlling vernalization requirement is *VRN-1* gene. Homoeologous *VRN-1* alleles designated *VRN-A1, VRN-B1* and *VRN-D1* can be present in their recessive or dominant form. All three recessive alleles confer winter habit, while at least one dominant allele confers spring growth habit. Recessive alleles have the gene body intact, whereas dominant ones carry several kb deletion in the first intron (*Vrn-B1, Vrn-D1*) or insertion of mutator DNA transposon in the promoter region (*Vrn-A1a*) (Yan et al., 2004; Fu et al., 2005).

Using TILLING population of winter wheat variety Hatcher, we have identified an early mutant (plant 8-1) flowering without vernalization. The mean heading time of 8-1 was 67 days in comparison to wild type Hatcher with heading time of 139 days (20°C/16°C, 16h light). Without vernalization, *VRN-1* expression analysis showed high levels in 8-1 plant comparing to low levels in Hatcher. Sequencing of *VRN-1* gene revealed no changes in the early mutant. The results suggest a novel gene involved in the vernalization mechanism. In another experiment, we identified winter varieties differing in heading when grown without vernalization (Dromos – 135 days, Patras – 191 days). These varieties carried the same set of *VRN-1* and *VRN-3* alleles.

Recently, we have reported novel *VRN1* alleles in bread wheat (Strejčková et al., 2021). We are also studying *VRN1* allelic variation and vernalization response in tetraploid wheat (Strejčková et al., in preparation). We have created mapping populations (8-1 x Hatcher, Dromos x Patras) to identify candidate genes for both phenotypes and to broaden our knowledge on wheat vernalization.

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Can stress exposure change the composition of extrachromosomal circular DNAs (eccDNAs) in plants?

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Extrachromosomal circular DNAs (eccDNAs) are polydispersed circular DNAs occurring in all eukaryotic cells investigated so far. They are diverse in size and may comprise sequences derived from genes, tandemly repeated sequences and transposable elements (TEs). As eccDNAs occur in addition to the nuclear genome, they add an additional level of flexibility to an organism's genome. Therefore, their involvement in transcriptional regulation, telomere protection, rDNA maintenance and aging has been shown repeatedly. In plants, eccDNAs may be even beneficial for the adaptation to new environmental situations. Here, we investigate the changes of the eccDNA composition as response to environmental stress, focusing on drought in two organisms:

In tobacco, we build on the finding that drought leads to the mobilization of Tnt1-type retrotransposons in the tobacco genome. We wish to reproduce the Tnt1 mobilization and observe the impact onto the eccDNA fraction in relation to transcriptomic and global DNA methylation changes.

In saffron crocus, the premise is slightly different. Despite saffron's adaptation to dry climates, we have previously observed widespread physiological changes under drought. However, if mobilization of transposable elements occurs, is still unknown. Here, we wish to identify drought-associated changes in the eccDNA fraction and use these results to investigate the relation of drought stress, TE mobilization and formation eccDNAs.

Overall, we aim to better understand how plant genomes may use eccDNAs to modulate or respond to environmental stresses.

Cap-analysis of gene expression defines features of barley promotoreome

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The promoterome is a set of all core promoter sequences in a given species. The eukaryotic core promoter is defined as the minimal sequence at the 5` of a gene, that is recognized by general transcription factors. Their binding is a prerequisite for the RNA polymerase pre-initiation complex (PIC) formation and subsequent events leading to firing off the transcription. Therefore, the core promoter region contains sequence of the 5` termini of all RNAs transcribed, which is important for a full-length transcript definition. A number of core promoter sequence elements, both evolutionarily conserved and lineage-specific have been characterized. The path to characterizing promoters can be taken from gene annotation or promoter prediction methods, however, direct proof of promoter position and distribution of TSSes provides methods such as Cap analysis of gene expression (CAGE) or TSS-seq. In plants, only Maize and Arabidopsis have been subjected to these assays, so far. Deciphering core promoter features in cereal crops is essential to our biotechnology ventures while relatively understudied. Here, we analyze the CAGE datasets from three early developmental stages of barley embryos by machine learning algorithm defining sequence features and integrating them with epigenetic features. This enables us to categorize barley promoters and relate them to specific groups of genes. Describing core promoter architectures contributes to our understanding of transcriptional regulation in this group of plants essential to the humanity.

Molecular cytogenetics tools for mango (Mangifera indica)

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Mango is an important fruit crop with high genetic diversity that encompasses many cultivars, varieties and closely related wild species. Due to its popular aromatic and nutritious fruits, it is cultivated almost all over world. However, the primary center of origin and diversity of mangoes lies on the Indian subcontinent. Despite recent advancements in mango genome research, the majority of the mango germplasm remains uncharacterized, hampering local breeding and conservation efforts. Genomically, mangos have rather small genomes (about 439 Mb) that are spread across 2n=2x=40 chromosomes.

Here, we develop cytogenetics for chromosome identification of mangos, which will support breeding and provide insights into mango evolution. Since repetitive DNAs are prime targets for the development of cytogenetics, we identified mango tandem repeats using publicly available short and long read sequences. We characterized them based on monomer lengths, sequence similarity, genomic abundance, and distribution along mango chromosomes and pseudochromosomes. Strikingly, most tandem repeats were embedded in other repetitive DNAs, such as the body of centromeric retrotransposons. This suggests a tightly linked tandem repeat-transposable element evolution, potentially a result of ongoing genome compaction processes in mango genomes.

Kinetochore proteins that have potential for the development of antibody-based universal centromeric markers

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The centromeres are chromosomal regions in which a protein complex known as kinetochore organizes to connect centromeric chromatin to microtubules and promote chromosome segregation. Their organization can vary from point centromeres with a single CENH3-containing nucleosome, to regional centromeres, to holocentromeres, extended along the entire length of chromosomes. Given the importance of centromeres in cell division, a marker for them that works in a wide range of species has always been sought to avoid the development of species-specific markers, which are time-consuming and expensive. Centromeres can be recognized morphologically as a constriction on condensed chromosomes, but this constriction is often not visible when chromosomes are small or when they have holocentromeres. An alternative and more efficient method for detecting centromeres is through the use of molecular markers such as specific DNA or proteins. Even if the chromosomes of a given species have a centromere-specific DNA repeat, which is often the case but not always, these repeats are frequently highly variable, even in closely related species. Centromeric proteins are generally more conserved. The most commonly used molecular marker for centromeres today is the centromere-specific histone CENH3, a variant that replaces the canonical H3 in centromeric nucleosomes. CENH3 is very similar to H3, except for the N-terminal tail, a region that has been selected for this reason for the production of antibodies to detect centromeres. Because this region is also highly variable between species, antibodies made for one species may only be used in closely related species, or they may even be species-specific. As an alternative, we present here antibodies against the two structural kinetochore proteins KNL1 and NDC80, which were originally raised for *Cuscuta* species, but that have relatively conserved sequence in phylogenetically distant species. Using these antibodies, particularly KNL1, we were able to detect centromeres in a wide range of higher plants, including monocot and dicot species with monocentric and holocentric centromere organization, indicating that they have great potential to be useful as centromere markers in higher plants in general.

The dynamics of chromatin changes during barley seed development

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Cereal grains are among the most important sources of energy for humans and domestic animals, owing to large and nutritious endosperm tissues. We study nuclear organization and chromatin in seeds of cereals using cultivated barley (*Hordeum vulgare subsp. vulgare*) - a diploid temperate zone model cereal used for both food and feed. Barley has a large genome (2n = 2x = 14, 1C = 5.1 Gbp) with interphase chromosomes organized in Rabl configuration with centromeres and telomeres clustering at opposite nuclear poles. Though considered to be typical for large cereal genomes, observations of Rabl configuration are based mostly on nuclei from meristematic tissues. We explored interphase chromosome organization in different tissues of developing barley grains and will show that Rabl chromosome organization is not a general rule in barley and it diminishes with the increasing nuclear DNA content and seed age. Furthermore, we performed transcriptome profiling of different barley grain tissues that allowed determining roles of different biological processes and also chromatin regulatory pathways during this critical stage of development. In summary, all these facets of our barley chromatin research create a fruitful environment for future functional analyses of nuclear organization and epigenetic regulation of gene expression in this agriculturally important species.

Post-polyploid diversification in the southern African tribe Heliophileae (Brassicaceae)

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The unigeneric tribe Heliophileae (Brassicaceae) includes c. 100 *Heliophila* species, all endemic to southern Africa. The tribe is morphologically the most diverse Brassicaceae lineage in every aspect of habit, foliage, flower, and fruit morphology. Despite this diversity, intra-tribal relationships and evolutionary history are virtually unknown. Our preliminary cytogenomic data suggested the existence of an octoploid event in the ancestry of the tribe. Post-polyploid genome diploidization associated with descending dysploidy towards modern quasi-diploid genomes seems to play an important role in diversification of Heliophileae. Here we addressed the evolutionary history of the tribe using comprehensive phylogenomic and cytogenomic analyses involving 400 *Heliophila* accessions and the sister genus *Chamira*. For an initial insight into the phylogenetic relationships in the Heliophileae, an extensive analysis using sequences of the nuclear ribosomal DNA internal transcribed spacers (ITS1 and ITS2) was conducted. Subsequently, a representative subset of Heliophileae accessions were included in the nuclear single-copy-based phylogenetic analysis with the aim to validate the topology of major ITS (sub)clades. Both phylogenies congruently demonstrated the presence of four major clades (A-D). The phylogenetic relationships in Heliophileae were correlated with chromosome numbers (2n = 16 to 80). Comparative chromosome painting unequivocally confirmed the meso-octoploid nature of the diploid-like Heliophileae species. Our data pointed to the existence of a shared diploidized meso-octoploid genome with 2n = 22 which underwent a clade-specific descending dysploidy towards 2n = 20, 18 or 16, and subsequent neopolyploidization(s). The phylo-cytogenomic observations were further put in the geographic context. The tribe Heliophileae represents an excellent model system demonstrating the key role of post-polyploid descending dysploidy in driving diversification and speciation.

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Centromere painting provides insight into the evolution of meta-polycentric chromosomes of Pisum sativum

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In most plant species, functional centromeres are marked by the presence of the histone variant CENH3. Depending on its distribution along the chromosome, two main types of centromere morphology have been described: monocentric and holocentric. Recently, chromosomes of the legume genera *Pisum and Lathyrus* were discovered to have a unique morphology characterized by multiple CENH3 domains located along an extended primary constriction. This meta-polycentric organization resembles a transition state between monocentric and holocentric chromosomes. Taking advantage of the assembly of Pisum sativum centromere 6 we designed a set of FISH painting probes consisting of oligo pools derived from single-copy regions. The probes were designed to cover 177.6 Mb region, including 81.6 Mb centromere and its adjacent chromosome arms. We used the probes to identify homeologous regions to pea CEN6 on chromosomes of selected Fabeae species, allowing us to elucidate the evolution of meta-polycentric chromosomes. Our results suggest that the spreading of CENH3 chromatin from the primary constriction into the adjacent chromosome arms can facilitate the expansion of the centromere region. Further growth could also occur by accumulating large arrays of satDNA along the primary constriction. However, what triggers this process and the molecular mechanisms involved remind to be elucidated.

First insights into the phylogenetic relationships and karyotype evolution in the Crocus serotinus group

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Phylogenetic relationships among the *Crocus serotinus* group, including *C. nudiflorus, C. clusii, C. cobbii, and C. salzmannii,* are still unclear. Confusing synonyms, little morphological differences of traditionally considered morphological characters, and variable genome sizes and chromosome numbers have contributed to this conundrum. The study aims to define the taxonomy and disentangle the karyotype evolution of the *C. serotinus* group by combining molecular, phylogenetic, and cytogenetic data. Using markers derived from chloroplast (*trnL-F, matK-trnQ*, and *ycf1* region) and nuclear ribosomal DNA (ITS1-5.8S-ITS2 and ETS), we analyzed the phylogenetic relationship of 52 *C. serotinus* group accessions and 15 outgroups using Bayesian phylogenetic inference. We estimated recent polyploidization based on genome size and SNP ratio from whole-genome and PCR-amplified sequences. We also investigated the abundance and composition of repetitive DNA using RepeatExplorer2 and then generated oligoprobes based on a comparative analysis of 6 accessions with whole-genome sequence reads. The phylogenetic trees revealed 16 clades, which likely represent individual species. Phylogenetic trees obtained using chloroplast and rDNA marker showed several incongruences, which we hope to resolve next using single-copy genes and genotyping-by-sequencing data. Furthermore, SNP ratio analysis revealed 38 diploid and 10 tetraploid accessions. However, the two accessions with the highest genome sizes, *C. salzmannii* and *C. clusii*, appeared to be diploid based on SNP ratio. This incongruence may likely be due to ancient polyploidization and subsequent diploidization. Alternatively, it could also be due to an accumulation of repetitive DNA, which seems to be the case for *C. salzmannii*. Currently, chromosome number validation and fluorescence in situ hybridization of rDNA and telomere probes are in progress.

Keywords: *Crocus serotinus*, polyploidization, SNP ratio, genome size, fluorescence in situ hybridization (FISH), dysploidy

Flow karyotyping of wheat-Aegilops additions facilitate dissecting the genomes of Ae. biuncialis and Ae. geniculata into individual chromosomes

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Breeding of wheat adapted to new climatic conditions and resistant to diseases and pests is hindered by a limited gene pool due to domestication and thousands of years of human selection. Annual goatgrasses (*Aegilops* spp.) with M and U genomes are potential sources of the missing genes and alleles. Development of alien introgression lines of wheat may be facilitated by the knowledge of DNA sequences of *Aegilops* chromosomes. As the *Aegilops* genomes are complex, sequencing relevant Aegilops chromosomes purified by flow cytometric sorting offers an attractive route forward. The present study extends the potential of chromosome genomics to allotetraploid *Ae. biuncialis* and *Ae. geniculata* by dissecting their M and U genomes into individual chromosomes. Hybridization of FITC-conjugated GAA oligonucleotide probe to chromosomes suspensions of the two species allowed the application of bivariate flow karyotyping and sorting some individual chromosomes. Bivariate flow karyotype FITC vs. DAPI of *Ae. biuncialis* consisted of nine chromosome-populations, but their chromosome content determined by microscopic analysis of flow sorted chromosomes indicated that only $7M^b$ and $1U^b$ could be sorted at high purity. In case of *Ae. geniculata*, fourteen chromosome-populations were discriminated allowing the separation of nine individual chromosomes ($1M^g$, $3M^g$, $4M^g$, $5M^g$, $6M^g$, $7M^g$, $1U^g$, $3U^g$, $6U^g$ and $7U^g$) out of the 14. To sort the remaining chromosomes, a partial set of wheat-*Ae. biuncialis* and a whole set of wheat-*Ae. geniculata* chromosome addition lines were also flow karyotyped revealing clear separation of the GAA-rich *Aegilops* chromosomes from GAA-poor A- and D-genome chromosomes of wheat. All of the alien chromosomes represented by individual addition lines could be isolated at purities ranging from 74.5 to 96.6% and from 87.8 to 97.7%, respectively. Differences in flow karyotypes between *Ae. biuncialis* and *Ae. geniculata* were analyzed and discussed. Chromosome-specific genomic resources will facilitate gene cloning and development of molecular tools to support alien introgression breeding of wheat.

ELIXIR Czech Republic: National infrastructure for biological data. Who we are, how we work, and what we offer to researchers

Anna Strachotová, Natália Pižemová - Institute of Organic Chemistry and Biochemistry of the CAS, ELIXIR CZ

ELIXIR Czech Republic National Infrastructure for Biological Data

Generating large amounts of data in current life-science research requires an extensive data and computing infrastructure and, at the same time, it places high demands on data curation and data management. ELIXIR is a robust European infrastructure that brings together life-science resources from across Europe. ELIXIR offers a wide range of bioinformatics services, such as tools for analysis and data processing, databases, or resources for data-intensive computing. There is also a lot of training material that describes how to use the offered tools the most effectively.

ELIXIR provides support with any aspect of so-called FAIR principles: findability, accessibility, interoperability, and reusability. Let's emphasise LS Login that enables researchers to use their home organisation credentials or community or other identities to sign in and access data and services they need.

ELIXIR CZ is one of the partner institutions of ELIXIR. ELIXIR CZ offers support to researchers in all phases of the data life cycle, especially at its very beginning. ELIXIR CZ can guide you through the process of a data management plan preparation. Funding bodies at both national and international level are increasingly recognizing the importance of data management plans. More importantly, proper data management planning can save researchers a lot of money and worries. ELIXIR CZ highly recommends the use of Data Stewardship Wizard, an online guide allowing you to efficiently compose data management plans for your research projects.

ELIXIR CZ participated in the creation of a platform with the most comprehensive information on research data management in life sciences called RDMkit. RDMkit takes into consideration specific requirements of individual research fields and provides good data management practices applicable to research projects from the beginning to the end.

ELIXIR Communities gather experts from specific scientific fields and domains. These Communities are open to new members; you can join the Plant Genomics ELIXIR Community and participate in preparation of new data standards or in specific data-related projects.

ELIXIR recognizes the utmost importance of sharing the knowledge, skills and best practices among scientists. The ELIXIR Staff Exchange Programme is a unique mobility programme that offers short and long-term visits to its partners' institutions or allows attending specific ELIXIR-related events with financial support to cover travel-related expenses.

Chromosome-specific dual-color Oligo-FISH reveals karyotype evolution in holocentric species of the genus Rhynchospora (Cyperaceae)

Yennifer Mata-Sucre and André Marques - Max Planck Institute for Plant Breeding Research

Advances in genome sequencing and assembly have allowed the design of oligo-specific probes for the individual identification of each chromosome of the karyotype, becoming a useful tool for the analysis of karyotypic and genome evolution. Here, we have developed the first set of oligo-based probes based on the reference genome of *Rhynchospora breviuscula,* a model species of the holocentric genus *Rhynchopora*. These two probes were composed of 27 392 (green) and 23 968 (red) oligos, respectively, and generated 15 distinct FISH signals as a unique barcode pattern for the identification of all 5 chromosome pairs of the *R. breviuscula* karyotype. Additionally, these probes were transferred to other species of the genus to better understand the evolutionary dynamics of their genome. Oligo-FISH analyses performed with these probes revealed different rearrangement events, such as fission, fusion, inversions and genomic duplication among the analyzed species. The application of oligo-based probes allowed us to demonstrate the presence of two rounds of whole chromosomes duplication, after chromosome fusion, that occurred during the evolution of the large genome karyotype of *R. pubera*. Considering the phylogenetic relationships and divergence time of the species, the specificity and synteny of the probes was maintained up to species with a divergence time of 40 Myr. However, more dynamic genomic rearrangements in more distant species made chromosome mapping difficult. This barcoding system will be a powerful tool to study chromosomal variations and genomic evolution of the genus *Rhynchospora*.

Chromatin dynamics during the cell cycle in barley

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Chromosomes undergo marked structural metamorphoses during the cell cycle. In most plant and animal genomes, the more relaxed interphase chromatin is organized in topologically associated domains (TADs) – chromatin loops that delimit genome segments with increased internal interaction frequency, which may be involved in regulating gene expression. The TADs disappear during mitosis, concurrently with chromosome condensation, essential for chromosome segregation. Novel molecular techniques based on chromatin conformation capture (3C), Hi-C in particular, enable studying these processes on the DNA level. The greatest amount of knowledge has been gathered in yeast and metazoans while information on plants has been limited.

Aiming to study chromatin dynamics during the cell cycle in barley, we coupled the Hi-C technique with flow sorting, which enabled purifying 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, which was confirmed by spatial structured illumination microscopy of large fluorescently labelled chromosome segments. Analysis of Hi-C data from G1, S and G2 revealed relatively small changes in chromatin condensation in nuclei from root tips. On the other hand, comparison of the G1 from the root tips with G0/G1 of a leaf tissue provided a significantly different profile of the chromatin interactions, suggesting distinct interphase chromatin arrangement between rapidly cycling and non-cycling cells, a feature not reported in animals. Studies conducted in plants also indicate different arrangement and, possibly, function of TADs compared to metazoans. Exploiting the barley Hi-C data, we carry out a detailed study of the TAD boundaries, aiming to reveal components of complexes involved in the chromatin looping. Our study will complement the missing information on the higher-order chromatin organisation in plants.

SMC5/6-mediated SUMOylation plays role in DNA-protein crosslink repair in Arabidopsis

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The ability to repair a broad spectrum of genomic lesions is an essential function needed by all living organisms. DNA-protein crosslinks (DPCs) are highly toxic lesions represented by covalently trapped proteins to DNA molecules. We recently demonstrated that cytidine analog zebularine is a potent inducer of Type 1 DPCs between DNA and CG DNA METHYLTRANSFERASE 1 (MET1) in specific heterochromatic regions of the Arabidopsis genome (Prochazkova et al., 2022).

To identify the molecular factors contributing to the mitigation of zebularine-induced DPCs in plants, we performed a forward-directed genetic screen using the Arabidopsis model system. The first characterized complementation group *HYPERSENSIVE TO ZEBULARINE 1 (HZE1)* corresponded to the SMC6B gene encoding for the core subunit of the *SMC5/6* complex. Genetic interaction and sensitivity assays revealed that the SMC5/6 complex is essential for plant DPC repair and works in both nucleolytic and proteolytic pathways. Moreover, we provide cytological evidence that the SMC5/6 complex is responsible for the SUMOylation of the MET1-DPCs. Altogether, we associate SMC5/6 complex functions with DPC repair and point towards its SUMOylation activity in this process.

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Dynamic activity of ribosomal RNA loci in cereals

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Ribosomal DNA (rDNA) is a general term for genes encoding several types of ribosomal RNA (rRNA), which are the structural components of ribosomes. 45S ribosomal rDNA loci are organized as long head-to-tail tandem arrays of nearly identical units, spanning over several megabases of sequence. Due to this structure, gene copy number, sequence composition and expression status of particular loci remain elusive, especially in complex genomes harbouring multiple loci. Combining cytogenomics and bioinformatics techniques, we reconstructed individual 45S rDNA arrays in bread wheat and barley, building an essential starting point for locus-specific analyses. The engagement of particular loci in rRNA synthesis was assessed across several tissues by analysing RNA-seq and Iso-Seq data and the locus-specific transcription was compared with cytosine methylation level in corresponding rDNA units. These analyses revealed that transcription activity of individual loci was not proportional to the array size. Subdominant loci with lower activity had shorter intergenic spacers and higher level of cytosine methylation in CHG context, and were subject to partial developmental silencing. Besides, the transcriptomics data pointed to several cases of posttranscription modifications in barley and wheat rRNAs, which may occur in distinct stages of the ribosome biogenesis.

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First Single-chromosome Sequence Analysis Using Microfluidic Platform

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Individual chromosomes of important grass crops cannot be identified and purified using DNA-based flow-sorting because the chromosomes usually cluster together within a flow karyotype, forming composite peaks. However, two options exist towards true single-chromosome sequencing: i) flow-sorting into individual wells of a PCR plate (or into tubes); and ii) chromosome enrichment using flow-sorting with subsequent microfluidic processing. The later approach enables high-throughput analysis, statistically improves sequence coverage, and reduces price and bias. Here, we describe a method for single-chromosome sequencing that uses a microfluidic platform to capture, amplify, and barcode the individual chromosomes. Chromosome encapsulation in “gel microbeads” allows lysis of chromosomes and tagmentation of DNA, while a “microfluidic double-merger" efficiently pairs each chromosome with a unique oligonucleotide barcode, allowing together single-chromosome sequencing. Prior to down-stream analyses, the sequencing data are de-multiplexed by barcode, resulting in subsets of reads originating from a single chromosome. This high-throughput and low-bias method will enable a wide range of (cyto)genomic studies, such as allocating of genes or scaffolds to individual chromosomes, precisely delimiting translocation events, and studying recombination. In the future, the method is foreseen to be utilized in organisms with large genomes (e.g., Triticeae crops) as well as organisms with numerous small chromosomes (e.g., sugarcane).

MAGNIFICENT PAINTINGS OF EPIGENETICS: Analysis of the ancient plant chromosomes in the Cycas revoluta, gymnosperm

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"*Cycads are to the vegetable kingdom what Dinosaurs are to the animal, each representing the culmination in Mesozoic times of the ruling Dynasties in the life of their age*." [1]. In a large Cycadaceae family comprising a single Cycas genus only diploid species are known - there is no evidence about polyploidization event within 200 MYRs [2]. The Cycas karyotypes are highly asymmetric with more than half of the chromosomes being telocentric. We found that the telomeric TTTAGGG repeats, and their variants are particularly abundant in these genomes reaching record copy number in plant kingdom. They accumulate not only at chromosome ends but also a large fraction is found in (peri) centromeric positions indicating numerous chromosome rearrangements [3].

To characterize the centromeric regions at the cytogenetic level we applied immunostaining of chromosomes using antibodies against the modified amino acids of histone H3 and 5-methylcytosine. In Cycas revoluta, the H3S28p antibody stained specifically centromeric but not (peri)centromeric regions. In contrast, the H3T3p and H3S10p antibodies showed strong signals at (peri)centromeric regions and not centromeric regions. Thus, these markers seem to efficiently distinguish between centromeres and (peri)centromeres. Both centromeres and (peri)centromeres were unusually large in telocentric chromosomes accumulating large amounts of 5-methylcytosine (DNA methylation). The euchromatic H3K4me3 marker showed a dispersed pattern along the chromosomes and no signals were detected on "*heads*" of telocentric chromosomes. In contrast, these "*heads*" were strongly stained with the heterochromatic H3K9me2 marker. In conclusion, the Cycas chromosomes are unique in many features reflecting the intricate evolutionary history of the genus.

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Comparative microsatellite analysis in Crocus revealed a hodgepodge of telomere repeat sequences

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Telomeres are indispensable chromosomal regions that ensure the physical integrity of chromosome ends. Microsatellite sequences such as the TTAGGG and TTTAGGG repeats make up the telomeres of a majority of vertebrate and plant (*Arabidopsis*-type) species, respectively, although non-canonical sequences have also been characterized in a few taxa. Disruptions in telomere function caused by double-strand breaks or telomere shortening may lead to catastrophic genomic consequences and even cell death. However, these same events can also trigger dysploidy pathways through chromosome fusion or fission. These mechanisms play important role in promoting biodiversity when reproductive barriers are established between new dysploid individuals and their euploid progenitors. The genus Crocus has ~240 species with diverse chromosome numbers (*2n* = 6–70). The high frequency of karyotype dysploidy in *Crocus* is unusual for plants with monocentric chromosomes. However, there is limited cytogenomic data to describe the genome structure and understand the high frequency of dysploidy in *Crocus*. Here, we first analyzed the microsatellite composition in 202 *Crocus* accessions to obtain a picture of the general microsatellite diversity in *Crocus*, particularly those of telomere-associated repeats. We used ~1x whole genome sequences to capture micro- and minisatellites with unit lengths ranging from 2 to 20 bp. We identified a combined 248 repeats in all 202 accessions with equal or more than 100 loci in each accession. Among these, we observed both the canonical vertebrate and *Arabidopsis*-type telomere sequences. The vertebrate type was present in all accessions whereas the *Arabidopsis*-type was detected in only 31 accessions. Moreover, different repeat variants were also abundant such as TTTGGC (101 accessions), TTAGGA (97), TTTAGG (33), and TTAGGC (30), However, exact copies of these repeats did not organize into >100bp arrays but longer arrays were formed by higher-order combinations of these repeats. This preliminary analysis shows the high sequence diversity of putative telomere-associated repeats in *Crocus*. FISH analysis should allow visualization of the chromosomal distribution of these repeats and hopefully allow to generate hypotheses on the karyotype evolution in *Crocus*.

Keywords: dysploidy, telomere, microsatellite, karyotype evolution

Using single-cell RNA sequencing of extracted pollen nuclei to study recombination dynamics in holocentric plant species

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Meiotic recombination across chromosomes is limited to certain hotspots that are typically distal from centromeres, i.e., crossover (CO) formation is suppressed around centromeric regions. Nevertheless, considering the fact that holocentric chromosomes are characterized by the interspersed centromere-like regions along the entire chromosome, whether recombination of holocentric species is also depleted at centromeric units would be an interesting question to address. Hence we constructed the first recombination map and genetic linkage map of holocentric species, *Rhynchospora breviuscula*, with repeat-based centromeres. CO events were observed in *R. breviuscula* by detecting haplotype conversion using 10X scRNA-seq data. Most COs were identified at one or both chromosomal ends. A further inspection of CO breakpoints showed that CO events neither occur within Tyba arrays, the centromeric repeat units specific in *Rhynchospora*, nor have any tendency off from Tyba arrays, which challenges the affection of centromeres to recombination patterning. Comparison of the recombination landscape to other genome-wide features, such as gene density, DNA methylation, and hetero- and euchromatin markers showed no correlation between them. This outcome also differs from most observations in monocentric species. A further mechanism that forms the recombination hotspot in *Rhynchopora* needs to be proposed and validated to interpret the observed pattern in *Rhynchospora*. Applying the same strategy to another holocentric plant *Rhynchospora* tenuis, no COs were observed suggesting that *R. tenuis* is achiasmatic which was consistent with our previous cytological observations.

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