

**PLANT MOLECULAR CYTOGENETICS  
IN GENOMIC AND POSTGENOMIC ERA**

23-24 SEPTEMBER 2014  
UNIVERSITY OF SILESIA IN KATOWICE  
POLAND

**ABSTRACT BOOK**

Organiser: Department of Plant Anatomy and Cytology,  
University of Silesia in Katowice

Co-organiser: The Committee on Cell Biology  
of the Polish Academy of Sciences

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## Welcome Message

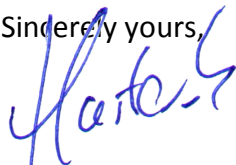
Dear Colleagues,

On behalf of the organising committee, I extend a warm welcome to the conference “Plant Molecular Cytogenetics in Genomic and Postgenomic Era” which will be held on 23 and 24 September in Katowice, Poland. This event is jointly organised by the Department of Plant Anatomy and Cytology, Faculty of Biology and Environmental Protection, University of Silesia in Katowice and the Committee on Cell Biology, Polish Academy of Sciences.

The objective of this conference is to gather the plant cytogenetics fraternity in Katowice and discuss in a friendly and productive atmosphere the most up-to-date and intriguing topics of plant molecular cytogenetics. It also provides an excellent opportunity to honour Professor Jolanta Maluszynska upon her recent retirement, who was the Head of the Department of Plant Anatomy and Cytology at the University of Silesia from 1992 to 2010, and is the founder of the ‘Polish School’ of plant molecular cytogenetics. Though retired, she continues to support us through her great scientific knowledge and experience.

Katowice, the venue of this conference, is a dynamically developing city at the heart of Upper Silesia – the region which is experiencing rapid changes in its post-industrial character, modern science, education and economy. This is the place where history meets modern and they make an interesting match. I hope it will be a rewarding trip for you and that you will enjoy the scientific activities.

Sincerely yours,



Robert Hasterok  
Head of organising committee

## Programme overview

**Venue:** Faculty of Law and Administration (FLA), University of Silesia in Katowice,  
11b Bankowa Street, 40-007 Katowice, Poland

### Day 1, Tuesday, September 23, 2014

7:30	9:00	Registration	Registration Desk, Main Foyer, Ground Floor, FLA
9:00	9:30	Opening Ceremony	Lecture Hall 8, 1 <sup>st</sup> Floor, FLA
9:30	11:10	Lecture Session 1	Lecture Hall 8, 1 <sup>st</sup> Floor, FLA
11:10	11:40	Coffee Break	Main Foyer, 2 <sup>nd</sup> Floor, FLA
11:40	13:30	Lecture Session 2	Lecture Hall 8, 1 <sup>st</sup> Floor, FLA
13:30	15:30	Lunch	Novotel Hotel Restaurant
15:30	16:45	Lecture Session 3	Lecture Hall 8, 1 <sup>st</sup> Floor, FLA
16:45	18:15	Poster Session	Main Foyer, 2 <sup>nd</sup> Floor, FLA
19:00	22:00	Social Event	‘Patio Park’ Restaurant

### Day 2, Wednesday, September 24, 2014

9:00	10:50	Lecture Session 4	Lecture Hall 8, 1 <sup>st</sup> Floor, FLA
10:50	11:20	Coffee Break	Main Foyer, 2 <sup>nd</sup> Floor, FLA
11:20	13:10	Lecture Session 5	Lecture Hall 8, 1 <sup>st</sup> Floor, FLA
13:10	15:10	Lunch	Novotel Hotel Restaurant
15:10	16:25	Lecture Session 6	Lecture Hall 8, 1 <sup>st</sup> Floor, FLA
16:25	16:55	Coffee Break	Main Foyer, 2 <sup>nd</sup> Floor, FLA
16:55	18:10	Lecture Session 7	Lecture Hall 8, 1 <sup>st</sup> Floor, FLA
18:10	18:40	Closing Address	Lecture Hall 8, 1 <sup>st</sup> Floor, FLA



## Programme

### Day 1, Tuesday, September 23, 2014

7:30-9:00      **Registration**

9:00-9:30      **Opening Ceremony**

#### Lecture Session 1

**Chairs: Ingo Schubert**                      (Leibnitz Institute of Plant Genetics and Crop Plant Research,  
Gatersleben, Germany)

**Trude Schwarzacher**                      (University of Leicester, UK)

09:30-09:35      **Chair's Introduction**

09:35-10:00      Endosperm cytology: a neglected field in epigenetics research

**Dieter Schweizer** (University of Vienna, Austria)

10:00-10:35      Chromosomes, crops and superdomestication

**J.S. Pat Heslop-Harrison** (University of Leicester, UK)

10:35-11:10      Plant Genetics in the Era of Modern Genomics

**Hans de Jong** (Wageningen University and Research Centre, the Netherlands)

11:10-11:40      Coffee Break

#### Lecture Session 2

**Chairs: Neil Jones**                      (Aberystwyth University, UK)

**Elwira Sliwinska**                      (University of Technology and Life Sciences, Bydgoszcz, Poland)

11:40-11:45      **Chair's Introduction**

11:45-12:20      Double-strand break repair – linking the molecular with the microscopic  
level

**Ingo Schubert** (Leibnitz Institute of Plant Genetics and Crop Plant Research,  
Gatersleben, Germany)

12:20-12:55      Centromere biology meets plant breeding

**Andreas Houben** (Leibnitz Institute of Plant Genetics and Crop Plant Research,  
Gatersleben, Germany)

12:55-13:30      Genetic and phylogenetic consequences of a unique paracentric  
inversion in *Arabidopsis*

**Paul Fransz** (University of Amsterdam, the Netherlands)

13:30-15:30      Lunch

### Lecture Session 3

**Chairs: Kesara Anamthawat – Jonsson** (University of Iceland, Reykjavík)  
**Jaroslav Dolezel** (Institute of Experimental Botany, Olomouc)

15:30-15:35 **Chair's Introduction**

15:35-16:10 More than meets the eye: numerical convergence, multiple cycles of hybridization, and contrasting evolutionary trajectories in polyploids of the *Prospero autumnale* complex

**Hanna Weiss-Schneeweiss** (University of Vienna, Austria)

16:10-16:45 Exploring plant genome by flow cytometry

**Elwira Sliwinska** (University of Technology and Life Sciences, Bydgoszcz, Poland)

16:45-18:15 Poster Session

19:00-22:00 Social Event 'Patio Park' Restaurant 101 Kosciuszki St. Katowice

### Day 2, Wednesday, September 24, 2014

### Lecture Session 4

**Chairs: Hans de Jong** (Wageningen University and Research Centre, the Netherlands)

**Hanna Weiss – Schneeweiss** (University of Vienna, Austria)

09:00-09:05 **Chair's Introduction**

09:05-09:40 'Optimising' the germplasm of barley by manipulating recombination

**Glyn Jenkins** (Aberystwyth University, UK)

09:40-10:15 More than the cabbage: chromosome and genome evolution in crucifers

**Martin Lysak** (Masaryk University, Czech Republic)

10:15-10:50 Hybridization, introgression and phylogeography of Icelandic birch

**Kesara Anamthawat-Jonsson** (University of Iceland, Reykjavík)

10:50-11:20 Coffee Break

## Lecture Session 5

**Chairs:** **Elzbieta Kuta** (Jagiellonian University, Cracow, Poland)  
**Dieter Schweizer** (Austrian Academy of Sciences, Vienna)

- 11:20-11:25 **Chair's Introduction**  
11:25-12:00 Cytomolecular analyses in genus *Lupinus*  
**Barbara Naganowska** (Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland)  
12:00-12:35 Cucumber genomes and chromosome polymorphism within the genus *Cucumis*  
**Wojciech Plader** (Warsaw University of Life Sciences, Poland)  
12:35-13:10 *Brachypodium* - a model genus to study grass genome organisation at the cytomolecular level  
**Robert Hasterok** (University of Silesia in Katowice, Poland)  
13:10-15:10 Lunch

## Lecture Session 6

**Chairs:** **Andreas Houben** (Leibnitz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany)  
**Andrea Pedrosa-Harand** (Federal University of Pernambuco, Recife, Brazil)

- 15:10-15:15 **Chair's Introduction**  
15:15-15:50 B chromosomes in plants: *quo vadis*  
**Neil Jones** (Aberystwyth University, UK)  
15:50-16:25 Holocentric chromosomes in monocotyledonous genus *Luzula* D.C. (Juncaceae) – from chromosome number to molecular structure  
**Elzbieta Kuta** (Jagiellonian University, Cracow, Poland)  
16:25-16:55 Coffee Break

## Lecture Session 7

**Chairs:** **J. S. (Pat) Heslop-Harrison** (University of Leicester, UK)  
**Barbara Naganowska** (Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland)

- 16:55-17:00 **Chair's Introduction**  
17:00-17:35 Chromosome-centric strategy for plant genomics  
**Jaroslav Dolezel** (Institute of Experimental Botany, Olomouc)  
17:35-18:10 Repetitive DNA in plant genomes  
**Trude Schwarzacher** (University of Leicester, UK)  
18:10-18:40 Closing Address



# Oral presentations

Organisers do not bear any responsibility for the content of the abstracts



### S1.1. **Endosperm cytology: a neglected field in epigenetics research**

#### **Dieter Schweizer**

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The endosperm is an ephemeral and rather undifferentiated tissue nourishing and protecting the developing F1 embryo. Formally, the endosperm, resulting from the double-fertilisation characteristic of flowering plants, can be considered as a secondary short-lived F1 embryo. The typical endosperm is triploid. It contains three sets of chromosomes: two maternal sets that are homozygous and homomorphic and one heterozygous paternal chromosome set. There are three different types of endosperm development known (nuclear, cellular, and helobial). Nuclear endosperm development is distinguished by consecutive waves of synchronous mitotic divisions. This makes this tissue an ideal material for cytogenetic and cytochemical studies. As an example, using chromosomal markers the cytological consequences of crossing over can be followed and the outcome of controlled genetic crosses can be conveniently and rapidly examined in endosperm instead of growing F1-plants. Developing endosperm has also been shown to provide favorable material in studies of nuclear architecture and on the spacial nuclear distribution of chromosomes. Maternal and paternal genome separation has been observed in *Anemone blanda* endosperm. More recently, epigenetic mechanisms governing seed and endosperm development became a strong focus of interest. Genomic imprinting during seed development has been demonstrated. In other studies, the DNA-methylation status of the endosperm and the embryo was analysed both at the nuclear/chromosomal and at the gene level. Histone-modification profiling was performed for different tissues of the developing seed. However, these latter studies using isolated nuclei of e. g. the embryo and of the endosperm were not able to discriminate between paternal and maternal genomes. Therefore, a strong plea is made for endosperm cytology using immunocytochemistry to complement epigenetic studies. Along these lines some preliminary data on the interdependence of DEMETER and the SMC protein DMS3 in *Arabidopsis* will be presented.

### S.1.2. **Chromosomes, Crops and Superdomestication**

#### **JS (Pat) Heslop-Harrison and Trude Schwarzacher**

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We need to know about the architecture of the genome to understand evolution, including speciation and the generation of diversity. Multiple processes are involved in generation of new types and combinations of diversity, including recombination, mutation, polyploidy, introgression, duplications, amplification and loss of sequences, and other types of chromosomal change. We also see consequences of interactions in the nucleus leading to epigenetic or sequence changes. Molecular cytogenetics enables the range of variation to be characterized, and we can study both the mechanisms and consequences of the various changes that occur. I will give a range of examples of these from cereals, other crops, and their wild relatives. Exploitation of evolutionary processes underpins species domestication and plant breeding: by considering what has already happened during evolution of wild species and through domestication processes, we can design approaches to exploit biodiversity in the development of new crop varieties.

Further information is available on our website at [www.molcyt.com](http://www.molcyt.com)

### S1.3. Plant Genetics in the Era of Modern Genomics

**Hans de Jong<sup>1</sup>, Sander Peters<sup>2</sup>, Saulo Alves Aflites<sup>2</sup>, José van de Belt<sup>1</sup>, Dóra Szinay<sup>1</sup>, Erik Wijnker<sup>1</sup>**

<sup>1</sup> Laboratory of Genetics, Wageningen University and Research Centre (WUR), Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands

<sup>2</sup> Applied Bioinformatics, WUR, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands

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In my presentation I will show some examples of recent studies that combine genetics, bioinformatics, genomics and chromosome research in crop species, with focus on tomato, potato and related species. The aims of these analyses were to confirm chromosomal positions for genetic markers and contigs on the cell complements using Fluorescent *in situ* Hybridization of BACs, plasmids and PCR fragments as probes on mitotic and pachytene cells, and naked DNA fibers. The studies demonstrated positions of annotated contigs and the size of the gaps in between them, and so supported directly the bioinformatics and *de novo* assembly of the pseudo-molecules. In addition, we were able to elucidate chromosome rearrangements, repeat dynamics, centromere and heterochromatin locations and were able to interpret linkage drag and other sources of problems in breeding programs. The existence of several inversions and translocations were demonstrated between tomato and potato, and several other species of the Solanaceae family, and helped to further support phylogenetic studies in the tomato and potato clades.

## S2.1. **Double-strand break repair – linking the molecular with the microscopic level**

**Giang TH Vu<sup>1</sup> and Ingo Schubert<sup>1,2</sup>**

<sup>1</sup> Department of Cytogenetics and Genome Analysis, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), D- 06466 Gatersleben.

<sup>2</sup> Faculty of Science and Central European Institute of Technology, Masaryk University, CZ-61137 Brno, CZ.

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DNA double-strand break (DSB) repair mechanisms differ in requirement of a homologous repair template and in the accuracy of the result. We aimed to quantify the outcome of repair of a single targeted DSB in somatic cells of young barley (*Hordeum vulgare* L.) plants. Amplicon sequencing of three reporter constructs revealed 47 to 58% of reads as repaired *via* non-homologous end-joining (NHEJ) with deletions and/or small (1-3 bp) insertions. Alternative NHEJ revealed 2 to 5 bp microhomology (15.7% of cases), or new replication-mediated short duplications at sealed breaks. Although deletions outweigh insertions in barley, this bias was less pronounced and deleted sequences were shorter than in Arabidopsis. Between 17 and 33% of reads likely represent restoration of the original sequence. Depending on the construct, 20 to 33% of reads arose *via* gene conversion (homologous recombination). Remarkably, <1 to >8% of reads apparently display synthesis-dependent strand annealing linked with NHEJ, inserting 4 to 61 bp, mostly originating from the surrounding of breakpoints. Positional coincidence of >81% of sister chromatid exchanges with target loci is unprecedented for higher eukaryotes and indicates that most repair events for staggered DSBs, at least in barley, involve the sister chromatid, and occur during S or G2 phase of the cell cycle. As expected, a single DSB did not increase the frequency of chromosome aberrations.

This work was supported by DFG(SCHU 951/17-1, by IPK, and by the European Social Fund (CZ.1.07/2.3.00/20.0189) to I.S.

## S2.2. Centromere biology meets plant breeding

**Andreas Houben, Takayoshi Ishii, Raheleh Karimi-Ashtiyani, Veit Schubert, Nils Stein, Stefan Heckmann, Jörg Fuchs, Jochen Kumlehn**

Department of Cytogenetics and Genome Analysis, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany

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Selective chromosome elimination has been reported in some wide crosses and frequently results in haploids of one of the parental species. The advantage of doubled haploids for breeders is that homozygosity can be achieved in the first generation, whereas in breeding systems such as pedigree or backcrossing, several selfed generations are needed to obtain high levels of homozygosity. Although this process has been exploited to produce doubled haploids, the mechanism behind is not well understood. I will report how the analysis of the *Hordeum vulgare* and *Arabidopsis thaliana* centromeres helped us to understand the mechanisms behind the process of selective elimination of chromosomes during early embryogenesis.

### S2.3. **Genetic and phylogenetic consequences of a unique paracentric inversion in *Arabidopsis***

**Paul Fransz**

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The short arm of chromosome 4 of the *Arabidopsis* ecotype Columbia contains a heterochromatic knob, hk4S, which is derived from the pericentric heterochromatin via a paracentric inversion. FISH experiments on pachytene chromosomes, extended DNA fibers and interphase nuclei narrowed down the breakpoint regions with an accuracy of 2 kb. Sequence comparison between Col and Ler fragments and subsequent BLAST searches revealed the exact map position of both breakpoints with basepair precision. The rearrangement was most likely induced by the activity of a Vandal transposon from the pericentric heterochromatin that inserted into an F-box protein-coding gene in the euchromatin. The novel heterochromatin–euchromatin border is demarcated by a sharp transition in epigenetic profiles, hinting at a recent occurrence of the inversion. Genetic analysis revealed a total lack of recombination in the region heterozygous for the inversion.

SNP analysis of the inversion region in 1200 accessions revealed extremely low polymorphism levels in 174 accessions compared to Col, suggesting many more ecotypes carrying the inversion. Indeed, sequence data from the 1001 genome project confirmed the inversion in at least 54 accessions. The sequence data indicate that the inversion has spread across large areas of the world through introgressive hybridization. The discovery of a paracentric inversion in different ecotypes distributed world-wide provides a unique system to examine the recent history and dynamics of *Arabidopsis* populations. Moreover, it enables to assess recent recombination events and the establishment of haplotypes across different ecotypes.



### S3.1. **More than meets the eye: numerical convergence, multiple cycles of hybridization, and contrasting evolutionary trajectories in polyploids of the *Prospero autumnale* complex**

**Hanna Weiss-Schneeweiss<sup>1</sup>, Tae-Soo Jang<sup>1</sup>, Khatere Emadzade<sup>1</sup>, John Parker<sup>2</sup>, Jiri Macas<sup>3</sup>, Petr Novak<sup>3</sup>, Andrew Leitch<sup>4</sup>, Franz Speta<sup>5</sup>**

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The circum-Mediterranean *Prospero autumnale* is karyotypically exceptionally dynamic exhibiting high levels of numerical (dysploidy, polyploidy, B-chromosomes) and structural variation. It comprises four evolutionary distinct diploid cytotypes (AA, B<sup>7</sup>B<sup>7</sup>, B<sup>6</sup>B<sup>6</sup>, and B<sup>5</sup>B<sup>5</sup>), each characterized by unique combination of basic chromosome number ( $x = 5, 6, \text{ and } 7$ ), genome size, karyotype structure, and repetitive DNA distribution. Basic chromosome number variation results from chromosomal fusions and inversions. Three diploid genomes (B<sup>6</sup>, B<sup>7</sup> and A) participate in hybridization and/or polyploidization giving rise to auto- and allopolyploids. Autopolyploids, only found in genome B<sup>7</sup>, are genomically stable and additive, while allopolyploids are variable, extent of the variation depending on the parental genomes divergence. Allotetraploids of B<sup>6</sup>/B<sup>7</sup> genomes exhibit exceptionally high levels of numerical (“compensating aneuploidy”;  $2n = 4x = 25\text{--}28$ ), structural, and repeat composition variation. This variation is structured when occurrence of four allotetraploid types rather than one is inferred involving repeated cycles of hybridization, accompanied by intervening genome restructuring. Numerical convergence of primary allotetraploids seems to be of little evolutionary importance, facilitating genome-wide modifications resulting in genetically balanced but unique genomes. Primary allotetraploids form secondary allotetraploids which are genomically stable and successfully colonize new areas. High incidence of chromosomal changes in all cytotypes of *P. autumnale* strongly contrasts with the relative stability of their genome-wide repeat composition (NGS), and with their morphological near-uniformity. All data suggest chromosomal restructuring both on diploid, and particularly on polyploids level to be a major mechanism of diversification and eventually speciation in *Prospero*.

The study is supported by Austrian Science Fund (FWF P21440) to HWS.

### S3.2. Exploring the plant genome by flow cytometry

**Elwira Sliwinska**

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Flow cytometry (FCM) is a fast and accurate method for the estimation of nuclear DNA content. It is commonly used in plant sciences, mostly for establishing ploidy and genome size. Since preparation of FCM sample is very simple and rapid, and DNA content can be established in several thousands of nuclei within a few minutes, in many laboratories the method has replaced classical microscopic chromosome counting or Feulgen densitometry. FCM is widely used in the breeding of polyploid crops, and when growing plants under stress conditions. It is especially convenient for *in-vitro*-produced plant material, which is usually characterized by a low frequency of mitotic cells. In tissue culture laboratories the method is applied to check the stability of the genome size of micropropagated plant material, to establish ploidy of (doubled)haploids, and to identify somatic hybrids obtained by protoplast fusion. Cells can be sorted by a flow sorter under sterile conditions and cultured *in vitro*. Using the sorting ability of a flow cytometer and cell-specific GFP lines, a global gene expression analyses within any cell or tissue type can be performed. FCM also enables establishment of the proportions of nuclei with different DNA contents and therefore is applied to study cell cycle activity and endoreduplication in different tissues/organs. This makes it suitable e.g. to follow seed development and maturation, to establish the progress of germination or seed treatment, and to screen for reproductive pathways.

#### S4.1. ‘Optimising’ the germplasm of barley by manipulating recombination

**Dylan Phillips<sup>1</sup>, Joanna Wnetrzak<sup>1</sup>, Candida Nibau<sup>1</sup>, Abdellah Barakate<sup>2</sup>, Luke Ramsey<sup>3</sup>, Frank Wright<sup>4</sup>, James D. Higgins<sup>5</sup>, Ruth M. Perry<sup>5</sup> and Glyn Jenkins<sup>1</sup>**

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A sizeable proportion of the genes of many agronomically important cereal and grass species is locked into recombination-poor regions of the genome because of pronounced distal localisation of chiasmata. This pattern of recombination caps genetic variation, reinforces linkage drag in introgression programmes, and reduces the effectiveness of map-based cloning approaches. Potentially, much is to be gained by shifting sites of crossing over to more proximal chromosomal locations in order to release novel genetic variation from which new phenotypes may be selected in advanced breeding programmes. This talk describes our strategies to alter the recombination landscape in barley (*Hordeum vulgare*), and highlights how we have gauged the success of our interventions by tracking the spatio-temporal expression of meiotic proteins using immunocytochemistry, and by high resolution analysis of crossing over by genetic linkage mapping.

JW was a recipient of an IBERS PhD studentship, and the work was supported by the Biotechnology and Biological Sciences Research Council (grant ref: BB/F018754/1)

#### S4.2. **More than the cabbage: chromosome and genome evolution in crucifers**

**Martin A. Lysak, Terezie Mandáková**

RG Plant Cytogenomics, Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic

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An American humorist and writer Mark Twain once said: Training is everything. The peach was once a bitter almond; cauliflower is nothing but cabbage with a college education. Since Twain's times our understanding of genome evolution in crucifers (Brassicaceae) made a quantum leap due to major advances in comparative genetic mapping, whole-genome sequencing, comparative chromosome painting and multi-gene phylogenetic studies.

The Brassicaceae family comprises 320 genera and over 3,600 species classified into some 50 bona fide monophyletic tribes belonging to three major phylogenetic lineages. The feasibility of comparative cytogenetic analyses and the available whole-genome sequences for a dozen of crucifer species (e.g., *Arabidopsis lyrata*, *Camelina sativa*, *Schrenkiella parvula*, *Brassica rapa*, *B. oleracea*) allowed us to reconstruct ancestral genomes and infer prevalent trends in genome evolution in the Brassicaceae. Two major ancestral genomes, ACK with  $n=8$  and PCK with  $n=7$ , were inferred for lineage I and II tribes, respectively. PCK seems to be derived from ACK through chromosome number reduction  $n=8 \rightarrow n=7$  (descending dysploidy) and multiple independent dysploidy events ( $n=8 \rightarrow n=7, 6$  and  $5$ ) marked the karyotype evolution across the family. While the ancestral karyotypes remained conserved in a number of extant species, in other groups they were reshuffled by translocations and inversions during descending dysploidy events. In tribes which experienced recent whole-genome duplication events, the extent and tempo of genome reshuffling towards diploidized genomes exceed that observed in true diploid genomes. Descending dysploidy events were mediated by reciprocal translocations accompanied by a centromere loss or through end-to-end translocations and centromere inactivation. Only one chromosome fission event was documented in crucifer species analyzed.

This work was supported by the European Social Fund (CZ.1.07/2.3.00/20.0189).

#### S4.3. Hybridisation, introgression and phylogeography of Icelandic birch

**Kesara Anamthawat-Jónsson, Ægir Thór Thórsson, Lilja Karlsdóttir, G. Benjamin Leduc**

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Birch woodland is an integral component of the Arctic and subarctic tundra. In natural woodland in Iceland two species of birch co-exist: diploid dwarf birch *Betula nana* and tetraploid tree birch *B. pubescens*. In this presentation an overview of our research on Icelandic birch over the past ten years will be presented. Our botanical, cytogenetic, palynological and molecular studies show that hybridisation between these two birch species is widespread in Iceland; the resulting gene flow via introgressive hybridisation is bi-directional; and that the process is dynamic through time and space. Qualitative and quantitative assessments of morphological variation of birch in natural woodlands clearly indicate introgressive hybridisation, and this, as also shown by the extensive sharing of cpDNA haplotypes across ploidy groups, is supported by the statistical analysis of introgression indices and the variation components. The molecular data also reveal a geographical structure of introgression and phylogeographical patterns, both within Iceland and in relation to Europe. Present-day birch in Iceland is most probably post-glacial in origin, migrating from Western Europe and colonizing Iceland in the early Holocene. Iceland is clearly a birch hybrid zone. Despite extensive gene flow, an integrated AFLP and chromosome analysis reveals strong species integrity and ploidy stability, with diploid and tetraploid species genetically separated. By using ploidy-based morphometric standards on pollen in peat sediments from different sites in Iceland, we have further shown that birch hybridisation occurred since early on, from the time birch vegetation began to develop soon after deglaciation. More importantly, we have found that such hybridisation occurred in waves associated with climate warming especially during the Holocene Thermal Maximum. A new wave could be expected in parallel to the ongoing global warming.

### S5.1. Cytomolecular analyses in the genus *Lupinus*

**Barbara Naganowska, Michał Książkiewicz, Katarzyna Wyrwa, Karolina Susek, Anna Szczepaniak, Sandra Rychel, Wojciech Bielski, Bogdan Wolko**

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The genus *Lupinus* is an evolutionary ancient legume taxon of paleopolyploid origin. In addition to wild species, it also contains environmentally-sustainable important crops. Genomics of *Lupinus* ssp. have until recently been lagging behind, because of scarce genomic resources. Our earlier work dealt with the analysis of genome size within the genus and with the development of basic molecular cytogenetics for lupins. Later studies have focused on the narrow-leaved lupin (*Lupinus angustifolius*), a reference species for lupin genomics and one of the main crops within this genus. Setting up the *L. angustifolius* nuclear genome BAC library was undoubtedly the milestone that accelerated research. It allowed us to show the genetic background of *L. angustifolius* resistance to two pathogenic fungi and to establish complete sequences for several genes involved in processes of vital importance for plants. We also characterized the genome regions carrying the genes in question. Comparative analysis of gene rich regions has led us to the identification of their syntenic links with six other legume species genomes. In the narrow-leaved lupin genomics we exploited molecular cytogenetics as tools to complement molecular work. Thus, the BAC clones were used for cytogenetic mapping (BAC-FISH), and the BAC-derived DNA markers for genetic mapping, which allowed the integration of the genome map. BAC-FISH also helped us to assemble and verify contigs, and to localize them in chromosomes. Currently, we use the molecular cytogenetic techniques described above in ongoing tracking lupin evolutionary chromosome rearrangements and determination of copy numbers of genes from crucial metabolic pathways.

## 55.2. Cucumber genomes and chromosome polymorphism within the genus *Cucumis*

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This work presents the analysis of chromosome polymorphism of genus *Cucumis*, based on *Cucumis sativus* L. Several researchers have reported cytogenetic work of *Cucumis sativus* L.; however, it was hard to identify each chromosome due to the small size and poor staining. The chromosomal identification of our inbred line 'Borszczagowski' has been successful with fluorescent banding methods which is reproducible among the other cultivars. Additionally, we could supply the data distribution of repetitive DNA (5S and 45S rDNA, telomere, Type III and Type IV), location of the genes (Xet, Gy (sex) and AOX), insertion of mitochondria DNA and five types of transgenic insertions using fluorescence *in situ* hybridization methods. The chromosome specific signals were also detected with BAC clone probes from Cucumber genome sequencing. Based on chromosome information, the comparative fluorescent banding among several cultivars indicated polymorphism in cucumber.

For understanding the chromosome polymorphism of *Cucumis* genus, we analyzed six wild African species. *C. heptadactylus* is the one of the dioecious plant in genus *Cucumis*, and the most of the others are monoecious. The relation among these African species is still unclear by phylogenetic research. Our results suggested that they have the common rDNA chromosomes, and *C. africanus* and/or *C. myriocarpus* might be related to the origin of *C. heptadactylus*. If it would be clear, it could contribute to understanding the sex system of *Cucumis* genus, and these data is able to feed back to the cucumber sex system in future.

This research was partially financed through a grant from the National Science Centre (no. 302083139).

### S5.3. ***Brachypodium* – a model genus to study grass genome organisation at the cytomolecular level**

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In contrast to animals, the organisation of plant genomes at the cytomolecular level is still relatively poorly studied and understood. However, the *Brachypodium* genus in general and *B. distachyon* in particular represent exceptionally good model systems for such study. This is due not only to their highly desirable ‘model’ biological features, such as small nuclear genome, low chromosome number and complex phylogenetic relations, but also to the rapidly and continuously growing repertoire of experimental tools, such as large collections of accessions, WGS information, large insert (BAC) libraries of genomic DNA, etc. Advanced cytomolecular techniques, such as fluorescence *in situ* hybridisation (FISH) with evermore sophisticated probes, empowered by cutting-edge microscope and digital image acquisition and processing systems, offer unprecedented insight into chromatin organisation at various phases of the cell cycle. A good example is chromosome painting which uses pools of chromosome-specific BAC clones, and enables the tracking of individual chromosomes not only during cell division but also during interphase. This presentation outlines the present status of molecular cytogenetic analyses of plant genome structure, dynamics and evolution using *B. distachyon* and some of its relatives. The current projects focus on important scientific questions, such as: What mechanisms shape the karyotypes? Is the distribution of individual chromosomes within an interphase nucleus determined? Are there hot spots of structural rearrangement in *Brachypodium* chromosomes? Which epigenetic processes play a crucial role in *B. distachyon* embryo development and selective silencing of rRNA genes in *Brachypodium* allopolyploids?

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## S6.1. **B chromosomes in plants: quo vadis**

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Early studies with B chromosomes dealt largely with their occurrence and distribution in natural populations, and with their various modes of transmission, effects on the phenotype and processes of drive. They were viewed as enigmatic entities of the genome. Their presence can only be detected by looking at chromosomes at mitosis, and especially at meiosis where by definition they fail to pair with the standard A chromosome set. Critical too is the fact they only occur in some individuals of a population, together with those having none. The discovery of mitotic and meiotic drive made it difficult to accept the idea that their existence is due to any adaptive properties, and the view that they are simply 'selfish' genetic elements gained widespread acceptance, although many species exist without any known drive mechanisms. Throughout the history of the story of Bs there has always been the desire to understand their molecular structure and organization and their origin, bearing in mind the failure to find genes, other than those for ribosomal RNA genes in some species. The molecular tool box we now have at our disposal has led to rapid advances in our understanding of the structure and origin of Bs, starting with FISH and GISH and through to the current use of 454 sequencing, largely done with rye and maize. These latest findings will be discussed.

## S6.2. **Holocentric chromosomes in monocotyledonous genus *Luzula* D.C. (Juncaceae) – from chromosome number to molecular structure**

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Holocentric (holokinetic) chromosomes are dispersed in animal and in plant kingdoms and are considered evolutionary parallelism. The differences between monocentric chromosomes are the distribution of centromeric DNA sequences and kinetochore proteins along the whole holocentric chromosome chromatids and lack of primary constriction.

The review of our long-term studies on holocentric chromosomes based on species from the genus *Luzula* focus on several aspects of their structure and evolution: 1) alteration in chromosome numbers *in vivo* and in culture conditions; 2) correlation between chromosome numbers and C-value; 3) distribution of spindle microtubules in mitotic metaphase and also telomeric/centromeric DNA and a Ty1-copia retroelements.

Diverse genomic mechanisms lead to the range of karyotypes arising *via* chromosome fission (agmatoploidy), chromosome fusion (symploidy) and/or polyploidy accompanied by the amplification or elimination of DNA. Different mechanisms of karyotype evolution operate in different clades of the genus *Luzula* viewed within phylogenetic framework, rather than one particular trend in karyotype or genome evolution throughout the genus (Bozek et al. 2012).

### REFERENCES

Bozek M, Leitch AR, Leitch IJ, Zaveska Drabkova L, Kuta E. 2012. Chromosome and genome size variation in *Luzula* (Juncaceae), a genus with holocentric chromosomes. *Botanical Journal of the Linnean Society* 170: 529–541.

### S7.1. **Chromosome-centric strategy for plant genomics**

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Nuclear genomes of plants are organized into subunits called chromosomes. The chromosome-centric strategy of genomics exploits this to overcome problems due to large genome size and DNA sequence redundancy. Purification of individual chromosomes by flow cytometric sorting reduces sample complexity and simplifies genome mapping and sequence assembly. This is particularly attractive when dealing with large genomes, which are typical for many important crops, including barley, rye and wheat. The chromosome-centric approach is most useful in polyploids, as it avoids problems due to homoeologous sequences. Due to the reduction of sample complexity, the chromosome-centric approach stimulated rapid advance in genomics of species, which were considered intractable using standard whole-genome strategies. In hexaploid wheat, BAC libraries were constructed from all chromosomes and their availability facilitates positional gene cloning, construction of physical maps and production of reference genome sequence. Apart from the primary aim of reducing the template complexity, taking a chromosome-based approach enables independent teams to work in parallel, each tasked with the analysis of a different chromosome. Next generation sequencing of DNA amplified from small numbers of flow-sorted chromosomes is a powerful approach to identify majority of genic sequences and develop virtual gene order maps. Recent results demonstrate the suitability of flow-sorted chromosomes for optical mapping, further expanding the rich toolbox of chromosome genomics.

This work was supported by the National Program of Sustainability I (grant award LO1204).

## S7.2. Repetitive DNA in plant genomes

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All eukaryotic genomes contain large amounts of repetitive DNA sequences, both tandemly repeated satellite sequences and dispersed transposable elements. While whole genome sequencing projects attempt to mask repeats deliberately to allow assembly and tandem repeats are difficult to find, many dispersed elements are present in large abundance. In this talk I will discuss the composition of transposable elements in *Petunia*, *Brassica* and other genomes using *in silico* DNA analysis and fluorescent *in situ* hybridization experiments to somatic and meiotic chromosomes. In *Brassica* pairwise analysis of BACs from corresponding regions in related genomes identified many DNA transposons and retroelements, including new families, and indicates recent movement of the elements. Within the *Solanaceae* species it is interesting to note that genome increase due to repetitive DNA increase is achieved by the amplification of different elements in different species. In *Petunia* large numbers of DNA transposons are found which in turn is reflected in the relatively low number of LTR retro-elements. The *Petunia* genome, as many other *Solanaceae*, harbours endogenous pararetroviruses including the *Petunia* vein clearing virus (PVCV) that in the hybrid *Petunia*, *P. hybrida*, is linked to disease outbreaks. In the parental genomes *P. axillaris* and *P. inflata* much lower amounts of PVCV were found as single copies or in arrays of several degenerate copies. Pararetroviruses are closely related to gypsy-like pseudoviridae retro-elements and are often found in physical proximity and we speculate about common silencing and amplification mechanisms.

We thank Hazara University, Pakistan and EU Framework V programme Paradigm. For references see <http://www.molcyt.com>

## Poster presentations

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**P1. Molecular cytogenetic studying of the novel hemp tandem repeat CS-237 that is linked with IGS 18S-26S rDNA**

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The novel chromosome specific tandem repeat CS-237 has been discovered in *Cannabis sativa* genome. FISH experiments on metaphase chromosomes revealed the location of CS-237 signal in pericentromeric region of chromosome pair (chromosome 5) and in the pericentromeric region of NOR bearing chromosome pair (chromosome 9) with extended distribution to NOR region. Multicolor FISH showed co-localization of distributed CS-237 signal on NOR bearing chromosome 9 and 45S rDNA probe. Two primer pairs for CS-237 amplification were developed. PCR experiments demonstrate the ladder-like patterns, which testified about tandem organization of repeat units. The comparative analysis of the CS-237 repeat unit structure showed two similar 120 bp and 117 bp subunits. One of these subunits was found in some IGS sequences of 18S-26S rDNA arrays by Repeat Explorer. *In silico* the location of this subunit in IGS was predicted. PCR analysis with CS-237 and 18S gene specific primers detects the amplification of expected length fragments. This result discussed in serve as an example towards understanding the initiation and the expansion of the satellite repeats in complex eukaryotic genome.

The work was supported by Russian Foundation of Basic Research No.13-04-01804.

## P2. Hybridisation of Icelandic birch in the Holocene reflected in pollen

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Introgressive hybridisation between downy birch (*Betula pubescens* Ehrh.) and dwarf birch (*B. nana* L.) has been confirmed in Iceland but limited knowledge on the extent or timing of such hybridisation exists. The present study focuses on birch hybridisation in the Holocene, its frequency and scope, and the environmental factors initiating hybridisation. The history of *Betula* in Iceland is reviewed and development of woodlands in the Holocene are discussed. On the methodology side, we first described the size and shape of *Betula* pollen, sampled from *B. nana*, *B. pubescens* and triploid hybrids from present-day woodlands (Karlsdóttir et al. 2007, 2008). The results were then used in the studies of Holocene birch presented here. The first two studies, from northern (Hella in Eyjafjörður) and south-western (Eyvík in Grímnes) regions of Iceland, revealed events of hybridization connected to *B. pubescens* colonization and early expansion (Karlsdóttir et al. 2009, 2012). The latest study is of a peat monolith from north-eastern region, Ytra-Áland from Thistilfjörður, covering the last ten thousand years of the Holocene in Iceland (Karlsdóttir et al. 2014). As found in all three studies, periods of hybridization were connected to the early expansion of *B. pubescens* woodlands, or from around 9 to 7.5 cal ka BP, but the whole Holocene profile from Thistilfjörður revealed another peak of hybridization in the later part of Holocene or around 3.3 cal ka BP. These distinct periods of hybridisation were found to be associated with warming climate and tree birch advances near the Holocene Thermal Maximum.



**P3. Evolution of tandem repeats in chromosomally extremely variable  
*Prospero autumnale* complex (Hyacinthaceae)**

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The *Prospero autumnale* complex (Hyacinthaceae) represents a unique system in which to address mechanisms and patterns of genome evolution. This complex exhibits a spectacular array of genomic and chromosomal variation, encompassing four basic diploid cytotypes (AA, B<sup>7</sup>B<sup>7</sup>, B<sup>6</sup>B<sup>6</sup>, B<sup>5</sup>B<sup>5</sup>) each with unique combination of chromosome base number ( $x = 5, 6$  or  $7$ ), genome size and karyotype structure, as well as their auto- and allopolyploids. High levels of structural variation are observed also on individual and populational levels, involving translocations, inversions, presence of supernumerary chromosomal segments and B-chromosomes. NGS analyses of all diploid *Prospero* species and cytotypes revealed that repetitive DNA fraction (up to 70%) is dominated by retrotransposons, with only one highly amplified satellite DNA shared by all cytotypes. Two other satellite DNAs, CL138 and CL147, were found to be specific for cytotype B<sup>6</sup>B<sup>6</sup> and AA, respectively.

The aim of this study is to analyze the chromosomal distribution patterns of CL138 and CL147 loci and copy number of their monomers in all *P. autumnale* cytotypes, as well as to assess the levels of their polymorphisms in diploid chromosomal races. Additionally, the evolution of these repeats in allopolyploids is assessed in comparison to their diploid progenitors. Each of these satellites is amplified in one specific cytotype, but they are also present in low copy numbers in related cytotypes. There is no evidence of intergenomic spreading of these two satellites in allopolyploid genomes, but their distribution patterns in polyploids differ from those observed in parental genomes.

This study was supported by Austrian Science Fund project P21440 to HWS.

**P4. Evolution of the S-genomes in diploid and polyploidy *Aegilops* and *Triticum* species**

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Alterations in the amount and distribution of highly repeated DNA sequences on the S genome chromosomes of diploid and polyploid *Aegilops* and *Triticum* species was studied using C-banding and fluorescence *in situ* hybridization with eight DNA probes. The S-genomes were subdivided into two groups: (1) *Ae. speltoides* and polyploid wheats and (2) diploid *Aegilops* of Emarginata section and polyploid *Aegilops* with US genome constitution (*Ae. peregrina* and *Ae. kotschyi*). The first group is characterized by prominent pericentromeric bands and interstitial localization of pSc119.2 sites; two major NOR are located on group 1 and 6 chromosomes. *Ae. speltoides* chromosomes are poorly labeled with pAs1 and Fat probes and possess sites of both Spelt-1 and Spelt-52 DNA sequences. The B/G genomes of polyploid wheat show the reduced number and size of Spelt-1 and Spelt-52 signals and smaller C-bands in subterminal position. Diploid *Aegilops* species of Emarginata section carry major NORs on group 5 and 6 chromosomes and have terminal pSc119 hybridization sites; C-bands are located interstitially, though four diploid species differ substantially in the amount of heterochromatin. *Ae. sharonensis* and *Ae. longissima* chromosomes hybridized with Spelt-52 probe (up to 15 signals per diploid genome), the amount of hybridization decrease significantly in the derived polyploids. Rather weak signals of pAs1 probe were concentrated in distal, while the Fat-probe in proximal chromosome regions. An artificial allopolyploid *Ae. umbellulata* x *Ae. sharonensis* is identical to the parental line of *Ae. sharonensis* in the distribution of all types of repetitive sequences.

This work was supported by grant from Russian State Foundation for Basic Research.

**P5. Exploring the mechanisms of nucleolar dominance – cytomolecular studies on allotetraploid *Brachypodium* representatives**

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Nucleolar dominance is a phenomenon which takes place in some plant and animal hybrids, and is characterised as preferential suppression of activity of the 35S rRNA gene set inherited from one parental species. Considerable attention has been paid to discover the molecular basics which are responsible for a particular state of ribosomal DNA-linked chromatin. Most experiments using bisulphite-mediated 5-methylcytosine mapping, DNA hypomethylating agents and RNAi knock down of the genes encoding for chromatin modifying enzymes proved that nucleolar dominance has an epigenetic origin (Preuss *et al.* 2008, *Molecular Cell* 32: 673–684). Although 80 years passed since the discovery of nucleolar dominance in *Crepis* hybrids, our understanding of exact mechanisms that determine this phenomenon is still fragmentary. Nucleolar dominance has been described in the *Brachypodium* genus. *B. hybridum* is a natural allotetraploid (2n=30) with putative parental genomes originating from two diploid species: *B. distachyon* (2n=10) and *B. stacei* (2n=20). Selective silencing of *B. stacei*-inherited ribosomal genes was confirmed in this hybrid. Here we aimed to investigate the epigenetic status of 35 rRNA gene loci in *B. hybridum* and its diploid putative progenitors. DNA methylation status and histone immunopatterns (e.g.: H3K9me2, H4K5ac, H3K9ac, H3K4me3) of 35S rDNA loci were determined. Moreover, we show the results of molecular characterisation of intergenic spacers (IGS) between 25S rDNA and 18S rDNA in *B. hybridum* and its ancestors as well as their physical localisation in both metaphase chromosomes and interphase nuclei of the hybrid.

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P6. **Distribution of chromosome domains in interphase nuclei of *Brachypodium distachyon***

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At interphase, chromosomes are highly decondensed and tend to occupy distinct three-dimensional areas of nuclear volume known as chromosome territories (CTs). This hypothesis was initially proposed in 1885 by Carl Rabl, and confirmed almost a century later in human cells using fluorescence *in situ* hybridisation method (FISH) with chromosome-specific probes. Since then, many studies on nuclear architecture have been conducted both in animal and plant species. However, plants are considerably more difficult to investigate at the cytogenetic level than animals because of large amounts of repetitive DNA in their nuclear genomes, limiting our understanding of CTs distribution in that group of organisms. The first and only complex model of CT arrangement in plants was proposed a few years ago for the model dicot *Arabidopsis thaliana*. Recently, chromosome painting which enables visualization of chromosome territories has also been feasible in the representative of monocots - *Brachypodium distachyon*, a model species for temperate cereals and forage grasses. In this work, we present the results of studies on different aspects of nucleus architecture in *B. distachyon*. The distribution of centromeres and telomeres in the interphase nuclei from different organs was analysed using FISH. Moreover, a chromosome painting approach was used to visualise the arrangement of chromosome territories and associations between homologous chromosomes.

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## P7. Genomic constitution of wheat genotypes with blue aleurone

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Anthocyanins are recognized as health-enhancing components for human due to their antioxidant and anti-inflammatory activities. They can be found in fruits, vegetables and some cereals. Recently, wheat with different grain colours (especially blue and purple) has been identified as a new source of anthocyanins and several lines with blue aleurone layer have been developed. The blue colorization has been observed after the introgression of chromatin from wild relatives into wheat. At least three different donors have been identified including *Thinopyrum ponticum*, *Triticum monococcum* and *Th. bessarabicum*. We employed GISH/FISH to detect the introgression of *Th. ponticum* and identified individual wheat chromosome(s) carrying the introgression. Our results clearly demonstrate that there are at least six different types of introgression, ranging from the addition of entire chromosome (cvs. Blue Baart and Blue Norco) to substitution of chromosome arm (cv. UC66049) and chromosome segment(s) (cv. Xiao Yan). These introgressions were located on the wheat chromosomes of homoeologous group 4 and/or are in the form of disomic additions. In some lines (i.e. cvs. Skorpion and Tschermaks Blaukörniger Sommerweizen), we were unable to detect introgressed chromatin of *Th. ponticum* indicating different source of blue aleurone trait (presumably *T. monococcum*). Currently, we are optimizing protocol for sorting individual chromosomes with introgression using FISHIS (FISH in suspension). Our next goal is to identify the origin of *Th. ponticum* introgressions in various genotypes.

This work has been supported by the European Regional Development Fund (Operational Programme Research and Development for Innovations No. CZ.1.05/2.1.00/01.0007).

## **P8. Usage of Tandem Repeats for Karyotyping in *Festuca-Lolium* Complex**

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Highly repeated sequences represent major part of nuclear genomes in most plant species. Based on their distribution in genome, repetitive DNA elements are classified into tandem organized repeats (TRs) and dispersed repeats. Repetitive DNA sequences are frequently used as cytogenetic markers that entirely replaced previous banding methods in many species. Fluorescent labeled probes such as pAs1, pSc119.2 or GAA microsatellite facilitate precise identification of individual chromosomes and cytogenetic mapping in cereals. Unfortunately, cytogenetics of forage and turf grasses lies far behind wheat, barley and rye. Only 5S and 45S rDNAs and a few BAC clones have been more or less successfully utilized for karyotyping of fescue and ryegrass species yet. In our previous research, we obtained Illumina sequences from flow sorted 4F chromosome of meadow fescue (*Festuca pratensis*) and identified 15 putative TRs. In recent study, we are focused on application of TRs for distinguishing of individual chromosomes and karyotyping in fescue and ryegrass species. Southern hybridization on genomic DNA isolated from different genotypes of both genera was performed to validate tandem character of the repeats. Based on hybridization pattern, five TRs were chosen for mapping on metaphase chromosomes of meadow fescue cv. Fure using multicolor FISH. Various combinations of fpTR4, fpTR6, fpTR7, fpTR12 and fpTR15 together with 5S and 45S rDNAs enable clear discrimination of seven meadow fescue chromosomes. We expect employment of these TRs for karyotyping in other species belonging to the *Festuca-Lolium* complex.

This work has been supported by the Czech Science Foundation (grant award P501/11/0504) and the National Program of Sustainability I No. LO1204.

**P9. Identification of rDNA-bearing chromosomes in *Festuca* × *Lolium* hybrids**

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The method based on 5S and 35S rDNA sequences (rDNA-FISH) combined with genomic *in situ* hybridization (GISH) enables identification of two chromosomes of *Festuca pratensis* (2F, 3F) and one chromosome of *Lolium perenne* (3L) and tracking them in their intergeneric *F. pratensis* × *L. perenne* hybrids. Our previous experiments revealed genomic differences in a number and distribution of 5S and 35S rDNA loci both in *Festuca* and *Lolium* species and their hybrids, showing structural chromosomal variation and rearrangements.

The aim of this study is to investigate genome and known *Festuca* and *Lolium* rDNA-bearing chromosome variation in the successive generation of *F. pratensis* × *L. perenne* hybrids. Our rDNA-FISH/GISH investigations of F<sub>2</sub> hybrids demonstrated various patterns of rDNA loci (gain and loss of rDNA sites) and *Festuca*-genome-like chromosomes seem to be the most frequently rearranged. Statistical calculations concerning cytological and molecular features (chromosomal markers, rDNA-bearing chromosome/arms rearrangements, PCR-based inter-simple sequence repeat markers) are being carried out.

**P10. Microspore genotyping – a tool towards unraveling an individual's recombination landscape?**

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Meiotic recombination and *de novo* mutation are primary sources of genetic diversity in plants. In human and livestock genetics, recombination analysis using meiotic gametophytes was developed more than 20 years ago. Recently, a high throughput method for genome wide analysis of intraindividual recombination sites and the rate of *de novo* mutations using single sperm cells was published. Despite the wide application of gametophytes in the field of animal breeding and genetics the use of microspores to measure genome wide recombination frequency and distribution lagged behind.

In this study, we aim to develop a method for rapid and efficient genotyping of single microspores to investigate an individual's recombination landscape. To achieve this, we are working on efficient ways to isolate single microspores, extract the DNA and amplify the whole genome to obtain a sufficient quantity and quality of DNA for downstream analyses.

In a plant breeding programme, genotyping of microspores combined with a segregating double haploid population might enable us to determine what proportion of genetic variability is carried into the next generation via microspore culture and furthermore, we might be able to assign genomic regions that promote plant development throughout the microspore culture. It is suggested that recombination frequency and distribution is affected by genotypic and environmental effects and thus only those genomic constitutions that ensure viable pollen development are propagated into the next generation. Using the microspore genotyping approach we aim to investigate those implications on recombination by looking at the full breadth of meiotic recombination present in microspores.



**P11. Cytogenetic and structural variability in the *Avena* amphiploid after 5-azaC treatment**

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Epigenetic regulation plays a major role in the expression of some eukaryotic genes. The aim of the research was to establish different relationships between parental species and the octoploid selection from their amphiploid. DNA methylation patterns of *Avena* chromosomes and their alteration with 5-azacytidine treatment were studied. Sequentially applied silver staining and FISH allowed the determination of active rDNA loci in two types of genomes (methylated and demethylated) in the amphiploid *Avena barbata* x *A. nuda*. It was observed that the demethylation induces a development of multiporate pollen grains, an increased number of nucleoli, inhibits the germination of seeds and weakens plants in stressful conditions. However, it was not documented changes in the spatial arrangement of chromosomes in mitotic nucleus domains and relationships between the genomes, and number of translocations also.

The research was supported by National Committee of Scientific Research, grant 4427/PB/IBR/10.

**P12. Up and down in the genome (size) evolution of *Genlisea* –  
Characterization of two species at both ends of the scale**

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The monophyletic carnivorous genus *Genlisea* (*Lentibulariaceae*) is characterized by a bi-directional genome size evolution resulting in a 25-fold difference in nuclear DNA content. This is the largest range found within a genus so far and makes *Genlisea* an interesting subject to study mechanisms of genome and karyotype evolution.

*G. nigrocaulis*, which is with 86 Mbp one of the smallest plant genomes, and the 18-fold larger genome of *G. hispidula* possess with 2n=40 identical chromosome numbers but differ severely in their chromatin organization. Interphase nuclei of *G. nigrocaulis* are hallmarked by intensely DAPI-stained chromocenters, carrying typical heterochromatin-associated methylation marks, while in *G. hispidula* the heterochromatin is more evenly distributed. Based on WGS sequence data differences in the centromere and, surprisingly, also telomere organization could be identified, supporting the fast genome evolution in this genus. In addition, newly identified tandem repetitive sequences together with rDNA probes allow the unequivocal discrimination of almost the half chromosome set in *G. hispidula*.

The obtained data not only provide first comprehensive cytological analyses but also support genomic approaches elucidating mechanisms responsible for shrinking and expanding of genomes within the genus *Genlisea*.

**P13. Evolution of protein transport systems in primary plastids and *Paulinella* chromatophores**

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Primary plastids are photosynthetic organelles characteristic of glaucophytes, red algae and green plants. They evolved 1.5 billion years ago, when a phagotrophic eukaryote enslaved an *Anabena*-like cyanobacterium and transformed it into a true cell organelle, in the process called primary endosymbiosis. During primary endosymbiosis, the cyanobacterium underwent a tremendous transformation involving gene transfer and evolution of protein import machinery for nuclear-encoded proteins. Interestingly primary endosymbiosis took place again, ~60 million years ago, between a *Synechococcus*-like cyanobacterium and an amoeba *Paulinella chromatophora*, resulting in two photosynthetic bodies called chromatophores. At present, almost all nuclear-encoded proteins are imported into primary plastids using N-terminal transit peptides and Toc-Tic translocons. Because the translocons are quite complex, a simpler pathway based on vesicular trafficking was hypothesized to operate in the ancestral primary plastid. The existence of several proteins with N-terminal signal peptides targeted to primary plastids in endomembrane vesicles is considered evidence for such primordial transport. To test this hypothesis, we performed thorough bioinformatics analyses. Our results indicate that vesicular trafficking evolved relatively late in primary plastids, contrary to the Tic-Toc translocons, possibly only in higher plants, to permit glycosylation and/or transport to more than one cellular compartment. Moreover, we investigated how proteins reach photosynthetic chromatophores of *Paulinella*. In contrast to primary plastids, *Paulinella* chromatophores possibly use the vesicular trafficking as the main protein import pathway.

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**P14. Comparative BAC-FISH mapping reveals rearrangements between potato and a wild relative (*S. commersonii* Dun) used in introgressive hybridization breeding**

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*Solanum commersonii* is one of the diverse genetic resources in the potato tertiary gene pool and carries bacterial wilt (*Ralstonia solanacearum*) resistance and frost tolerance. Our main objective is to explore the use of this species by reducing major bottlenecks in introgressive hybridisation breeding through advanced genetic and genomic approaches. Triploid hybrids were obtained by crossing *S. commersonii* with *S. tuberosum* Group Phureja and were crossed to *S. tuberosum* Group Tuberosum genotypes. The resulting pentaploid BC1 individuals were backcrossed to different *S. tuberosum* Group Tuberosum genotypes in an effort to introgress desirable traits into potato. Chromosome pairing and recombination at meiotic prophase I of the 3x hybrids were studied by FISH using single copy BAC DNA from tomato and potato as probes. Triploid hybrids showed nearly-autotriploid meiotic behaviour, forming up to 12 trivalents (most frequently 7 trivalents) indicating high levels of homeologous recombination. Chromosomes painted by the cross-species BAC-FISH painting were observed in III and II+I configurations, and various rearrangements between some homeologues were revealed. Comparative high-resolution BAC-FISH mapping between *S. commersonii* and *S. tuberosum* Group Phureja also displayed differences in the distance between BAC signals, indicating insertions/deletions or translocations, and inverted signals, which suggest an inversion. Our results anticipate linkage drag and other potential introgression problems caused by chromosome rearrangements between the homeologues. This issue will be looked at in more depth in whole genome sequence comparisons, coupled with optical mapping of stretched DNA in nanochannels to achieve accurate *de novo* assembly and detection of structural variations. This work was funded by CSIC UdelaR, ANII FMV and WUR.

**P15. The origin of polymorphic sex chromosome system XX/XY<sub>1</sub>Y<sub>2</sub> in *Rumex hastatulus* Baldw**

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Heteromorphic sex chromosomes have been only described in a small number of dioecious species belonging to six genera of Angiospermae. Most of these taxa were reported in the genus *Rumex*, in which two sex chromosome systems were described: XX/XY and XX/XY<sub>1</sub>Y<sub>2</sub>. Only one species from this genus, *Rumex hastatulus*, has two chromosomal races: the Texas (2n = 8 + XX/XY) and the North Carolina race (2n = 6 + XX/XY<sub>1</sub>Y<sub>2</sub>). The presence of two different chromosome systems in one species provides a unique opportunity to study the origin of multiple sex chromosomes in plants. Based on literature data, the XX/XY<sub>1</sub>Y<sub>2</sub> system has evolved from the standard XX/XY one by X/autosome translocation. For more detailed chromosome investigation, FISH with rDNA probes and C-banding/DAPI were applied. This approach showed differences in distribution of 5S rDNA loci and DAPI-positive segments within karyotype of analysed races. In the Texas race, 5S rDNA signals were detected on the 3<sup>rd</sup> pair of autosomes, whereas in the North Carolina one on X and Y<sub>2</sub> chromosomes. This FISH observation, along with the reduction in autosome number, seem to confirm the translocation hypothesis. Most probably, one of the autosomes possessing 5S rDNA was translocated to the X chromosome (forming neo-X) and the remaining one gave rise to the neo-Y chromosome. The original Y (Y<sub>1</sub>) and neo-Y (Y<sub>2</sub>) chromosomes underwent changes resulting in resizing and the accumulation of DAPI-positive heterochromatin.

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**P16. Comparative analysis of Miniature Inverted-repeat Transposable Elements (MITEs) in genomes of carrot (*Daucus carota* L.) cultivars**

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Transposable elements are DNA segments capable of changing their genomic localization. Miniature Inverted-repeat Transposable Elements (MITEs) are a heterogeneous group of small non-autonomous transposable elements related to and mobilized by DNA transposons using a 'cut-and-paste' mechanism of transposition. Some MITE families can reach thousands of copies in host genomes. Here, we focused on the comparative analysis of two MITE families, i.e. *Krak* and *DcSto1*, in 12 carrot cultivars of different origin, characterized by a high level of genetic and phenotypic diversity. We used a Transposon Display (TD) system for *Krak* and *DcSto1* elements to analyze MITE insertion site polymorphisms and triple color fluorescence *in situ* hybridization (FISH) with *Krak* or *DcSto1* sequences, Cent-Dc (*Daucus carota* centromeric sequence) and telomeric sequences as probes on enzymatically digested root-tip meristem cells to investigate chromosomal localization of these repetitive sequences. Both *Krak* and *DcSto1* TD revealed high levels of insertion polymorphisms, but no significant differences in estimated copy numbers were observed. The cultivars were divided into three distinct groups, partially reflecting their origin. As a result of FISH, hybridization signals for *Krak* and *DcSto1* elements were observed as small dots spread throughout all chromosomes for all analyzed cultivars. Furthermore, hybridization sites for *DcSto1* elements were much more pronounced, as compared to *Krak*, likely reflecting the difference in the copy number between the two families.

The research was supported by the Polish Ministry of Science and Higher Education fund for statutory activity of the University of Agriculture in Krakow.

**P17. Localization of carrot centromeric repeats on chromosomes of wild *Daucus* species**

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Centromeres represent crucial regions of eukaryotic chromosomes responsible for cohesion of sister chromatids and their equal segregation into daughter cells during cell division. Although the function of centromeres is conserved throughout eukaryotes, the profile of DNA in this region is almost species-specific. In plants centromeric DNA is mainly composed of megabase-sized arrays of 126-755 bp satellite repeats. In general, centromeric satellite DNAs represent rapidly evolving components eventually being specific to only closely related species. Recently, centromeric-associated repeat (Cent-Dc) with a length of ~159 bp was identified in cultivated carrot (*Daucus carota* L. subsp. *sativus* Hoffm.,  $2n = 2x = 18$ ; Iovene et al., 2011). In the present study we investigated Cent-Dc in genomes of wild *Daucus* species. As a probe for FISH experiments we used labeled with Cy5 subrepeat of Cent-Dc monomer sized of 35 bp (Nowicka et al., 2012). The probe was hybridized to mitotic preparations of three 22-chromosome *Daucus* species including *D. aureus*, *D. broteri*, and *D. pusillus*. Similar to carrot, the Cent-Dc repeats with different signal intensity were located at the centromeric regions of all chromosomes. Our results suggest, that Cent-Dc satellite repeat may be centromere-specific for *Daucus* species and may provide chromosomal evidence of their evolutionary relationship.

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**P18. The meiotic behavior of rye B chromosomes**

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Beside a set of standard chromosomes *Secale cereale* exhibits supernumerary chromosomes causing a numerical chromosome variation. These so called B chromosomes (B's) are dispensable, not essential to normal growth and development, have a detrimental effect on vigour and fertility at higher numbers, do not pair and recombine with standard chromosomes during meiosis and by this do not follow the mendelian inheritance pattern. Rye belongs to the higher plants owning a polymorphic B system. The most frequent variant is an acrocentric 'standard' B but in addition seven isoforms are described. Isoforms deficient for the terminal region of the long arm do not undergo directed nondisjunction at the first pollen mitosis and by this lacking the 'drive' mechanism. Despite being one of the best studied B chromosome model in the past 90 years less is known about the dynamics of rye B's at meiosis especially regarding the deficient B isoform and the lack of the nondisjunction control region on their meiotic behaviour. Newly available DNA probes for fluorescence in situ hybridisation (FISH) allow the tracking of B chromosomes throughout all stages of meiosis. By combination of FISH with immunohistochemistry we investigate the behaviour of different B isoforms at early stages of meiosis and subsequent compare between standard and B chromosomes. Immunostaining using antibodies specific for the synaptonemal complex proteins ASY1 (lateral element) and ZYP1 (axial element) revealed a delayed synapsis of rye B chromosomes. Rye plants carrying more than two B chromosomes show a clustering of B's during early meiotic stages.



**P19. Cytogenetic mapping of *Brachypodium mexicanum* chromosomes using the BAC-FISH method**

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Comparative cytogenetic studies are helpful in inferring the genus evolution at the chromosomal level. Genus *Brachypodium* provides an interesting subject to study the karyotype evolution and divergence of grasses due to the variation in genome size, chromosome number and ploidy level of the species it comprises. While the Eurasian *Brachypodium* species have become recently a target of extensive cytomolecular analyses, similar studies of the other members of the genus have been relatively scarce.

In this work we present the results of cytogenetic analysis of *B. mexicanum* ( $2n = 40$ ) karyotype using *B. distachyon* ( $2n = 10$ ), a model grass species, as a reference. The chromosomes of *B. mexicanum* were analysed by fluorescence *in situ* hybridisation of selected single-locus BAC clones derived from the *B. distachyon* genomic libraries. All clones used in the study belonged to chromosome Bd1 of *B. distachyon*, but mapped to several different chromosome pairs in *B. mexicanum*. The number of signals observed suggests polyploid origin of *B. mexicanum*. Moreover, the results imply that the component parental genomes lack several of chromosome fusions present in *B. distachyon* genome.

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P20. **Independent sex chromosome evolution from the same pair of autosomes in *Carica papaya* and *Vasconcellea parviflora***

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Sex chromosomes evolved from autosomes. The evolutionary events from autosomes to sex chromosomes appear to be relatively rare, which is reflected by the fact that all mammals share a homologous XY system and all birds have a homologous ZW system. However, recent studies in non-model eukaryotes have revealed multiple, independent sex chromosome evolutionary events. One question raised by these studies is whether some genes or chromosomes are better suited at triggering the first steps of sex chromosome evolution. However, there is a lack of well established cases of independent sex chromosome evolution from the exact same autosome among closely related species. Approximately 6% of angiosperms are strictly dioecious and among them a small portion contains sex chromosomes. Interestingly, most species in the Caricaceae family are dioecious. Papaya (*Carica papaya*), an important fruit crop in Caricaceae, contains a pair of young but heteromorphic X/Y chromosomes that emerged approximately 7 million years ago (MYA). The *Vasconcellea/Jacaratia* clade in Caricaceae split from the papaya clade ~27 MYA. Therefore, if sex chromosomes emerged in the dioecious *Vasconcellea/Jacaratia* species, they must have evolved independently from papaya. In this study, we mapped a set of bacterial artificial chromosome (BAC) clones associated with the papaya X/Y chromosomes in two Caricaceae species, *Vasconcellea parviflora* and *Jacaratia spinosa*. We demonstrate that *V. parviflora* contains a pair of heteromorphic X/Y chromosomes that are homologous to the papaya X/Y chromosomes, documenting a case of independent sex chromosome evolution from the same autosome pair in two related plant species.

## P21. Functional characterization of barley CENH3 variants

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The kinetochore is essential for the faithful segregation of chromosomes includes several proteins including the centromere-specific histone H3 variant CENH3. Barley (*Hordeum vulgare* L.) encodes two types of CENH3 ( $\alpha$  and  $\beta$ CENH3) which significantly differ in sequence and expression dynamics. Super high resolution microscopy reveals that  $\alpha$  and  $\beta$ CENH3 containing nucleosomes are loaded into distinct centromere subregions. Screening of a barley TILLING population resulted in the identification of functionally compromised  $\beta$ CENH3 mutant, which is not capable of  $\beta$ CENH3 loading to the centromere. Homozygous  $\beta$ CENH3 mutants do not show a severe growth phenotype or changes in ploidy level, but exhibit a reduced seed setting. The functional significance of the identified CENH3 mutation was tested in *Arabidopsis*. Mutated *Arabidopsis* CENH3 showed a reduced centromere loading compared to wild type CENH3 but complemented a homozygous *CENH3* null mutant of *Arabidopsis*. Crossing between complemented and wild type *Arabidopsis* plants resulted in haploids in 10% of F1 plants.

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**P22. Parental genome divergence and patterns of genome restructuring in hexaploids of the *Prospero autumnale* complex (Hyacinthaceae)**

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The Mediterranean *Prospero autumnale* complex comprises four evolutionary distinct diploid lineages – AA ( $x = 7$ ), B<sup>7</sup>B<sup>7</sup> ( $x = 7$ ; two sub-lineages), B<sup>6</sup>B<sup>6</sup> ( $x = 6$ ), and B<sup>5</sup>B<sup>5</sup> ( $x = 5$ ) – each having a unique combination of chromosomal and genomic features. Three of these cytotypes are involved in polyploidization, giving rise to autopolyploids (B<sup>7</sup> genome) and allopolyploids (combinations of A and B<sup>7</sup>, and B<sup>6</sup> and B<sup>7</sup> genomes). A combination of number and location of 5S and 35S rDNA and satellite *PaB6* give unique fluorescence *in situ* hybridization (FISH) patterns in each diploid genome, and, coupled with genomic *in situ* hybridization (GISH), have been used to identify the parental genomes in hexaploid lineages. The genomes of B<sup>7</sup> autohexaploids are largely additive, with minor variations due to single or duplicated 5S rDNA loci. The two allopolyploids analysed, however, are much more dynamic in their repetitive sequences and deviated from strictly additive patterns, each following a unique evolutionary trajectory. AAB<sup>7</sup>B<sup>7</sup>B<sup>7</sup>B<sup>7</sup> hexaploids have lost the A genome (paternal) 35S rDNA but are additive for 5S rDNA and *PaB6* loci. B<sup>6</sup>B<sup>6</sup>B<sup>7</sup>B<sup>7</sup>B<sup>7</sup>B<sup>7</sup> allohexaploids, by contrast, were highly variable in all GISH and FISH analyses. These data, interpreted in a phylogenetic context (derived from nrITS and plastid regions), allow correlations to be made between parental genome divergence and the genome dynamics in allopolyploids.

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**P23. *Luzula elegans* - a holocentric species with an inverted sequence of meiotic chromosome segregation events?**

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Meiosis in organisms with holocentric chromosomes illustrates that our views of meiotic organization and control of meiosis based on observations of monocentric chromosomes may not apply to all organisms. We have indication that in the holocentric plant species *Luzula elegans* the sequence of meiotic chromosome segregation events is inverted. Contrary to a monopolar centromere orientation in monocentrics, cenH3-positive sister kinetochores of *L. elegans* behave as two distinct functional units during meiosis I mediating bipolar attachment to microtubules. Whereas in monocentrics sister chromatid cohesion is released in a two-step way, in *L. elegans* sister chromatid cohesion is released along holocentric chromosomes with the possible exception at the end-to-end association between non-sister chromatids. The end-to-end association of non-sister chromatids during anaphase and telophase I seems to be chromatin-based mediated by satellite DNA in *L. elegans*. The analysis of *L. elegans* genes involved in meiotic recombination, crossover formation and sister chromatid cohesion/separation is in progress.

**P24. Dynamics of DNA synthesis in the root cap during seedling growth of *gfp*- transformed *Arabidopsis thaliana***

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The root cap is a dynamic system in which cell turnover can be observed. This makes it an excellent model in which to study cell division, endoreduplication and programmed cell death. A transformed line of *Arabidopsis thaliana* with NLS-GFP-GUS chimeric protein, which highlights nuclei, was used for confocal time-lapse imaging and flow cytometric analysis of DNA synthesis and mitosis in the root caps of growing seedlings. The aim of the study was to follow the cell cycle and endoreduplication and to localize nuclei with different DNA contents within the root cap. Images were collected using a Leica TCS SPE confocal microscope and 3D videos were processed using the LAS AF programme. Nuclear volumes were measured using Imaris (Bitplane) software. Flow cytometry revealed the presence of nuclei with 2C, 4C, and 8C DNA, which allowed us to correlate those with different volumes with these three ploidies. Numerous cell divisions occurred in the central part of the root cap, which enabled us to define the volume of 2C (directly after mitosis) and 4C (before mitosis) nuclei. Ultimately, a map showing the locations of nuclei with different DNA contents was created, which showed that 2C nuclei were most common in the apical part and 4C in the central part of the root cap. 8C nuclei were mostly located close to the root-cap border. The results allowed us to establish the time of the mitotic cycle and the endocycle and to visualize these two events.

P25. **Karyotype and genome characterization of *Brachypodium mexicanum***

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*Brachypodium* Beauv. is a small genus within the family Poaceae belonging to separate tribe Brachypodieae and comprising temperate grasses distributed worldwide. One representative of the genus, *B. distachyon*, has been accepted as a new model species for economically important cereals and forage grasses of the temperate zone. *Brachypodium* species are often polyploids with small genomes and variable chromosome base numbers of 5, 7, 8, 9 and 10. One of the most interesting species of *Brachypodium*, in terms of its cytogenetics and phylogeny is *B. mexicanum* ( $2n=40$ ). This species is currently one of the least-characterized representatives of the genus with its exact ploidy level to be still unclear.

In this study, using silver staining, CMA<sub>3</sub>/DAPI fluorescent staining and fluorescent *in situ* hybridization we demonstrated the number, localization and activity of 18S-5.8S-25S rRNA loci in *B. mexicanum*. We also demonstrated chromosomal distribution of selected centomere-specific sequences and constructed the preliminary karyogram. Moreover, we analyzed the relations between *B. mexicanum* and some other species of the genus using genomic *in situ* hybridization.

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**P26. Characterization of repetitive DNA and genes localized on sex chromosomes in *Rumex acetosa***

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Sex chromosomes are unique genomic regions that are shaped by specific evolutionary processes. Sex chromosomes are present in some dioecious plants where *Rumex acetosa* is a representative of the XY<sub>1</sub>Y<sub>2</sub> sex chromosomal system. In *R. acetosa*, both Y chromosomes are heterochromatic and are thought to be degenerated. We characterized the structure, copy number, transcription and chromosomal localization of all main types of transposable elements and tandem repeats (satellites) in *R. acetosa* based on 454 and Illumina sequencing followed by similarity-based clustering. We found that gypsy retrotransposons are dominant followed by copia elements. Some transposable elements had accumulated on Y<sub>1</sub> and Y<sub>2</sub> chromosomes while many retrotransposons were ubiquitous on autosomes and the X chromosome but absent on Y<sub>1</sub> and Y<sub>2</sub> chromosomes, and others were depleted from the X chromosome. Satellites occupied specific genomic regions, some of them were strongly accumulated on the Y chromosomes. We found that chloroplast DNA was strongly gathered on both Y chromosomes in *R. acetosa*, much strongly than in *S. latifolia* what also indicates the higher degeneration of *R. acetosa* Y chromosomes. We sequenced flow-sorted X and Y chromosomes by Illumina to get deeper insight into structure of repetitive DNA localized on sex chromosomes. Our genetic analysis of *R. acetosa* RNA-Seq data from parents and progenies allowed us to predict dozens of genes located on the X and Y chromosomes. Therefore, the Y chromosomes of *R. acetosa* are not as degenerated as was thought before despite they contain many repetitive DNA.

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## P27. **Genomic distribution of repetitive DNA and FISH-based karyotype of chickpea**

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A draft genome sequence of chickpea (*Cicer arietinum* L.) was obtained recently after whole genome shotgun sequencing CDC Frontier, a *kabuli* chickpea variety (Varshney et al., Nat. Biotechnol. 31:240, 2013). Despite the relatively small genome size (1C~882Mb), the pseudomolecules represent only about 40% of the genome with the remaining scaffolds un-anchored. Interestingly, pseudomolecules of the two longest chromosomes A and B represent only ~27% of their predicted size. In general, the quality of genome sequence assemblies is compromised by the presence of repeated DNA and due to the lack of genetic markers available for anchoring sequence contigs. In order to gain insights into to repeat landscape of the chickpea genome, we analyzed sequences obtained by shotgun sequencing flow-sorted chromosomes. We have identified a variety of DNA repeats and established the genomic distribution of selected repeats using fluorescence *in situ* hybridization (FISH) on mitotic chromosomes. While FISH with probes for transposable elements resulted in dispersed signals, indicating their distribution along the whole chromosome length, tandem organized repeats clustered to distinct loci. Interestingly, most of the newly identified tandem repeats localized to the longest chromosomes, A and B. These results contribute to develop a karyotype of chickpea employing the combination of tandem repeats and single copy BAC clones as probes for FISH. This work provides fundamental progress in the analysis of chickpea chromosome structure and sheds more light on chickpea genome organization.

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**P28. Molecular cytogenetics (GISH, FISH) of dioecious sea-buckthorn (*Hippophae rhamnoides*) with XY Chromosome Sex Determination System**

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Sex chromosomes are relatively rare in plants and morphologically distinct sex chromosomes were characterized in only a dozen species in five genera: *Cannabis*, *Humulus*, *Rumex*, *Silene* and *Coccinia*. The origin of sex chromosome dimorphism in plants seems to be recent, and it is believed that sex chromosomes arose in plants many times independently. Characterization of new species with distinct sex chromosomes provides an opportunity for the study of the origin of sex chromosomes and the pattern of their evolution in plants.

Dioecious sea-buckthorn ( $2n=22+XX$  – female;  $2n=22+XY$  – male) was karyotyped using chromosome measurements, fluorescence *in situ* hybridization (FISH) with pTa71 (45S rDNA), pCT4.2 (5S rDNA), telomeric repeats probes, C-banding/DAPI, and genomic *in situ* hybridization (GISH). The GISH with total genomic DNA as a probe revealed clear visualization of the Y chromosome. The strong GISH signal painted heterochromatic arm of the middle sized Y chromosome. The X chromosome was identified as one of the biggest chromosomes in the karyotype. The location of pseudoautosomal region (PAR) was established. To our knowledge the sea-buckthorn, addition to *Humulus lupulus*, is a second species with the much smaller Y chromosome compared to the X chromosome.

**P29. Cytological events during microsporogenesis in *Orobanche bartlingii* Griseb.**

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*Orobanche bartlingii* Griseb. member of family Orobanchaceae is lacking the chlorophyll holoparasite of *Libanotis pyrenaica*. *O. bartlingii* is distributed from central Europe to China. Genus *Orobanche* includes diploids ( $2n = 2x = 38$ ) and tetraploids ( $2n = 4x = 76$ ). The chromosome number of *O. bartlingii* has not been recorded yet. Due to lack of proper root and shoot meristems, metaphase plates suitable for chromosome counting occur mainly during pollen grains development. The aim of the study was to determine chromosome number and analyse meiosis abnormalities in *O. bartlingii* during microsporogenesis. Squashed anthers of young flower bulbs (< 5 mm) were stained in acetocarmine (4%) and analysed using standard light microscopy. The chromosome number of *O. bartlingii* was estimated ( $n = 19$ ). However, the quality of metaphase plates did not allow to make accurate chromosome counting. Some meiosis abnormalities were observed. In analysed *O. bartlingii* individuals meiosis were desynchronized. Stages from 1<sup>st</sup> prophase to tetrads were observed within one pollen sac. Some univalents during 1<sup>st</sup> metaphase were also noted. Causes of these meiotic abnormalities are still not well understood.

**P30. Integrating the recombination and cytogenetic maps in onion (*Allium cepa* L.) by single gene/marker *in situ* mapping**

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We have applied tyramide-FISH for visualization of genes or markers on the chromosomes of onion, a monocot plant with an extremely large genome. This technique combines the advantage of an enzymatic procedure with fluorescence-based detection. A collection of cDNA probes, gene sequences from public databases, and SNP markers from the transcriptome were used to reveal their physical location. We cloned, sequenced and located the alliinase (probe 1100 bp) and lacrymatory factor synthase (LFS, probe 550 bp) genes that encode enzymes important in the biochemical pathway responsible for the characteristic flavor of onion. Physical mapping the alliinase genes revealed a lack of collinearity among *Allium* species closely related to onion. SNP and indel markers closely linked to the nuclear locus (Ms) controlling male-fertility restoration were mapped on mitotic metaphase and pachytene chromosomes of onion. Through integration of recombinational and cytogenetic maps, the distribution of recombination along the length of a chromosome was determined by analysis of hundreds of individual plants within a mapping population for each marker. We found a higher frequency of recombination within the distal regions of chromosomes as compared to the proximal regions. These results are discussed in terms of chromosomal mapping sensitivity and resolution.

**P31. An easy “SteamDrop” method for high quality plant chromosome preparation**

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Chromosome preparation is the key step in all cytogenetic techniques. However, it remains a difficult task for many plant species and no robust and generally applicable method has been developed. Therefore, by studying all steps of plant chromosome preparation in detail, we developed a protocol “SteamDrop” for reliable preparation of mitotic and meiotic plant chromosomes. Our “SteamDrop” method was successfully applied for 28 species with different chromosome size and number, belonging to 13 monocot (20 species) and 7 dicot (8 species) genera. The by “SteamDrop” prepared chromosomes of onion allowed physical mapping of small DNA fragments using Tyramide-FISH as well as repetitive DNA using conventional FISH. These results demonstrate the effectiveness of our “SteamDrop” method for high quality chromosome preparation of plant species with small and large chromosomes. The method has many advantages: long storage of cell suspension, short time of chromosome preparation, slide preparation with low concentration of acetic acid and controlled spreading.

**P32. Molecular and cytogenetic evidence for an allotetraploid origin of *Chenopodium quinoa* and *C. berlandieri***

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Most of the cultivated chenopods are polyploids but the knowledge on their origin and evolutionary history is very limited. Andean *C. quinoa* and North American *C. berlandieri* are tetraploids ( $2n = 4x = 36$ ). Broader phylogenetic analyses of nrITS and plastid regions allowed in a group of related diploid and polyploid *Chenopodium* species indicated putative parental species of the two allotetraploids. Fast evolving 5S rDNA spacer region (NTS) was also applied for phylogenetic analysis further corroborated the results of the nrITS and plastid data. The phylogenetic analysis clearly indicated that both *C. quinoa* and *C. berlandieri* are of allotetraploid origin and that they might potentially share very similar if not identical diploid parental taxa. The inferred origins of the two allotetraploid species were further investigated using the genomic *in situ* hybridization (GISH). Several diploid *Chenopodium* species belonging to the groups identified in phylogenetic analyses were tested as a putative diploid genome donors. GISH differentiated sets of parental chromosomes and further corroborated the allotetraploid origin of *C. berlandieri* and *C. quinoa*. Uniparental loss of rDNA loci was revealed for both allotetraploids when GISH and FISH with rDNA sequences were used in succession.

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**P33. Epigenetic modifications in *Brachypodium distachyon* embryos during seed maturation, desiccation, imbibition and germination**

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Seed development can be divided into four distinct stages: maturation, desiccation, imbibition and germination. During maturation seed undergoes a period of embryo growth, reorganisation of metabolism and synthesis of storage compounds. Maturation ends with the desiccation phase after which the embryo enters into a quiescent state. This stage is associated with a major loss of water, which leads to a dry seed in preparation for the quiescent period, dormancy and thereafter germination. Seed development involves a plethora of spatially and temporally synchronised genetic and epigenetic processes. Although it has been shown that epigenetic mechanisms act on a large number of genes during seed development and germination, to date the global levels of histone modifications have not been studied in a tissue-specific manner in plant embryos. In this study we analysed the distribution of three epigenetic markers, i.e. H4K5ac, H3K4me2 and H3K4me1 in ‘matured’, ‘dry’, ‘imbibed’ and ‘germinating’ embryos of a model grass, *Brachypodium distachyon*. Our results indicate that the patterns of epigenetic modifications vary not only between particular tissues of the same embryo type but also between different types of embryos analysed. The scutellum, coleorhiza and coleoptiles are the most variable organs in terms of histone H4 acetylation and histone H3 methylation in all four types of embryos analysed. The distinct patterns of epigenetic modifications may be involved in the switch of the gene expression profiles in specific organs of the developing embryo and may be linked with the physiological changes that accompany seed maturation, desiccation, imbibition and germination.

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P34. **FISH/GISH technology to study genome constitution and recombination of *Festuca pratensis* × *Lolium perenne* hybrids**

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The *Festuca-Lolium* complex contains numerous species of high agronomic and scientific importance, which are also good models for studying the evolution of genomes through the use of chromosome- and genome-based markers. Physical mapping of rDNA loci enables to detect genomic variation, and based on literature data, various numbers of 5S and 35S rDNA loci has already been observed in species and intergeneric hybrids within the *Festuca-Lolium* complex. Recently, our FISH/GISH investigations of *Festuca* × *Lolium* hybrids have demonstrated extensive homoeologous recombination, substitution of whole *Festuca* chromosomes by whole *Lolium* chromosomes, and also various rDNA loci patterns in advanced generations developed from the allotetraploid ( $2n=4x=28$ ) hybrid of *F. pratensis* × *L. perenne*. In this work, we present the results on genome constitution and recombination profile as well as dynamics of rDNA loci patterns in unselected materials of F<sub>2</sub>-F<sub>4</sub> generations derived from the allotetraploid *F. pratensis* (4x) × *L. perenne* (4x). The frequency of numerical and structural changes in rDNA-bearing chromosomes increased from generation to generation, although the respective value of this character was higher for *Festuca* than for *Lolium*. Similar dynamics of *Festuca* chromosomes was observed in the recombination profile, which also increased from generation to generation. Statistically significant differences in a range and type of modification of *Festuca* rDNA-carrying chromosomes between successive generations were observed. Evaluation of stability/instability of meiotic chromosomes is in progress.

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**P35. Molecular cytogenetic identification of chromosomes in interspecific hybrids and mutants within the genus *Brassica*, with known resistance to clubroot**

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Amphidiploid rapeseed as a very important oil plant became a widely cultivated crop in many countries worldwide. Searching of forms with improved traits is highly desirable and from that point of view, interspecific crossing is a valuable tool for widening the variability of useful traits, e.g. seed quality and resistance to some diseases such as clubroot caused by soil-inhabiting protist *Plasmodiophora brassicae*, which is known as damaging to oilseed rape and vegetable brassicas. Main sources of resistance used up to date originate from different species of the genus *Brassica*, including *B. campestris* (A-genome), *B. oleracea* (C-genome) and *B. napus* (AC-genome).

We focused our attention on (i) analysis of types of rDNA-bearing chromosomes in individuals of F<sub>3</sub>-F<sub>6</sub> generations, which resulted from the interspecific crosses between *B. napus* and *B. campestris*, *B. napus* and *B. carinata*/*B. juncea* as well as in *B. napus* mutants (ii) determination the parental genomes using FISH with C-genome specific BAC-based probes (BAC-FISH) and B-genome specific sequence, and (iii) assignment of known *Brassica* chromosomal markers to their corresponding genomes. The use of *B. oleracea* BAC clone revealed chromosome re-arrangements between A- and C-genomes in the synthetic *B. napus* forms, which can be a rapid response to formation of the allotetraploid *B. napus* genome. In this work, the identification of chromosome identity and their re-arrangements in synthetic *B. napus* forms will be presented.

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**P36. Effective methods of DNA isolation from seeds**

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Polymerase chain reaction (PCR) has revolutionized genetic and molecular biology studies becoming one of the most popular techniques in functional genomics. Implementation of PCR requires DNA of sufficient purity which is often a limiting step. The main problem during DNA isolation from plant tissues is a contamination by polysaccharides, polyphenols and lipids. More protocols presented in the literature concern DNA isolation from leaves (fresh and mature) than from seeds. In this work we have adapted protocols which require simple, cheap and commonly used chemicals. We present the comparison of the quality of received DNA samples based on their A(260)/A(280) and A(260)/A(230) ratios and distinct bands on agarose gels. Our results show that DNA isolated using methods modified by us can be appropriate for molecular studies.

**P37. Analysis of the S phase and DNA damage after mutagenic treatment in *H. vulgare* cells**

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The genotoxic effect of mutagenic treatment on plant cells is commonly analysed as the DNA damage. As well mutagens could lead to the disturbances of S-phase of the cell cycle. This work presents the comparative analysis of the effects of three mutagens, characterised by different mechanisms of action: gamma radiation, maleic hydrazide (MH), and N-nitroso-N-methyl-urea (MNU) on *Hordeum vulgare* root meristematic cells. In order to measure the rate of active DNA synthesis the incorporation and detection of EdU (5-ethynyl-2-deoxyuridine), nucleoside analog of thymidine, a novel alternative for BrdU was used. TUNEL test for the detection of DNA fragmentation was used. The study of effects of mutagenic treatments includes the analysis of the frequency of cells with DNA damage and frequency of cells in S phase of the cell cycle. The quantitative acquisition and analysis of the fluorescence intensity of the damaged cells and DNA replicating cells using a high-content screening system (Scan'R, Olympus) based on a wide-field microscope Olympus IX81 was performed. An attempt was also made to determine whether the cells with DNA breaks can undergo the S phase of the cell cycle by using simultaneous application of incorporation and detection of EDU and TUNEL method.

**P38. Genetic diversity of *Aegilops* species used in triticales (*× Triticosecale* Witt.) improvement**

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Wild species of goatgrasses (*Aegilops* spp.) are closely related to hexaploid wheat (*Triticum aestivum* L.; AABBDD) and hexaploid triticales ( $\times$  *Triticosecale* Witt.; AABBRR), which is expressed in their taxonomic affinity to tribe *Triticeae*. *Aegilops* species are belong to the secondary gene pool of wheat, which means that at least one of their genomes is homologous with a cultivated wheat/triticales genome, allowing favourable traits to be transferred to cultivated species using conventional crossing and normal recombination methods. The aim of this research is to study the localization of repetitive sequences in chromosomes of diploid, tetraploid and hexaploid *Aegilops* species from different habitats. Fluorescence *in situ* hybridization (FISH) with pSc119.2, pAs1, pTa794 (5S rDNA), pTa71 (18S–5.8S–25S rDNA) and short repetitive oligonucleotide probes were used to differentiate particular chromosomes. Genomic *in situ* hybridisation (GISH) was applied to categorize the chromosomes of amphidiploid species.

FISH/GISH analyses allowed to identify the similarities and the differences in localization of repetitive sequences between analogue subgenomes in diploid and amphidiploid species of *Aegilops* genera. The ability to recognize the *Aegilops* chromosomes is used to identify the introgressed goatgrass chromatin in the (*Aegilops*  $\times$  *Secale cereale*)  $\times$  triticales hybrid forms.

We are grateful to Dr Marie Kubalakova (Institute of Experimental Botany, Czech Republic) for providing pSc119.2 clones, to Dr Tomasz Książczyk (Institute of Plant Genetics, PAS, Poland) for providing pTa794 and pTa71 clones and to Mrs Grażyna Cicha and Mrs Joanna Maszner for technical assistance.

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**P39. Anchoring linkage groups of the *Rosa* genetic map to physical chromosomes with Tyramide-FISH and EST-SNP markers**

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Genetic maps are already made for several *Rosa* species with indication of the position of desirable genes and QTLs. However, no linkage groups are yet anchored to the physical chromosomes. In our study, we optimized the Tyramide-FISH technology for roses (*Rosa wichurana*) in order to cytogenetically map single-copy genes. To be able to recognize *Rosa wichurana* chromosomes more clear, cytogenetic chromosome markers were developed. By this we could physically map 3 genes on 3 different chromosomes of *Rosa wichurana*. Using the HRM technology we were also able to connect the physical position of these 3 genes with their genetic position on the linkage groups of *Rosa wichurana*. Our results demonstrate that (1) Tyramide-FISH is a powerful tool for physical mapping of short DNA fragments on *Rosa* chromosomes and (2) by combining the opportunities of Tyramide-FISH and HRM, linkage groups can be anchored to physical chromosomes of *Rosa wichurana*.

P40. **Molecular cytogenetic characterization of pre-breeding material produced with perennial *Thinopyrum* species**

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Wild relatives of cultivated wheat represent a rich potential source of genetic variation for many agriculturally significant characteristics. Perennial *Thinopyrum* species includes diploid and polyploid ones, containing genomes that are non-homologous to those of wheat, are important as tertiary gene pools for wheat improvement.

Pre-breeding material was developed earlier in Martonvásár by a cross between BE-1 (a *T. aestivum* - *Thinopyrum ponticum* partial amphiploid) and *T.aestivum* cv.GK Öthalom (leaf rust susceptible genotype) and was maintained in nursery and greenhouse. After the 12 generations, new partial amphiploid lines with higher chromosome number (50-56) were isolated and *Thinopyrum* chromosomes were detected by GISH. Several stable pre-breeding lines with 42 chromosomes were selected; however, GISH did not reveal any rearrangement. Chromosome 3B of the LRMAS 10 line based on FISH using DNA repeats showed polymorphism compared to the 3B FISH chromosome pattern of the GK Öthalom and BE-1 parental genotypes. Their leaf rust resistance was evaluated in an artificially inoculated nursery. After artificial infection, LRMAS 10 line was selected as a leaf rust resistant genotype. Based on FISH polymorphism data, the LRMAS 10 3B chromosome was flow-sorted. Further experiments in order to detect alien DNA in the wheat background by GISH and molecular marker analysis is in progress.

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P41. **Comparative chromosome organization of repeated DNA in *Thinopyrum bessarabicum*, *Pseudoroegneria spicata* and *Thinopyrum intermedium***

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The wheat tribe Triticeae is a diverse group of grasses. Besides globally important cereals, there are also wild grasses which are of great practical value. *Thinopyrum bessarabicum* (2n=14, J), *Pseudoroegneria spicata* (2n=14, St) and *Th. intermedium* (2n=42, JJsSt) possess many desirable agronomic traits that make them an invaluable source of genetic material useful in wheat improvement. The grass genomes contain high amount of repetitive DNA (90-95%). The knowledge about chromosome organization of this DNA is important for our understanding of grass evolution.

In this study, the number of repeated DNA sequences was discovered by bioinformatics approach and cloned. The high copy number repeats were localized on *Th. bessarabicum*, *P. spicata* and *Th. intermedium* chromosomes by fluorescence *in situ* hybridization (FISH). The 295-bp tandem repeat showed subtelomeric signal localization in *Th. bessarabicum* and *P. spicata*, and subtelomeric and centromeric localization in *Th. intermedium*. The 53-bp tandem repeat showed dispersed signal on all chromosomes of *Th. bessarabicum* and subgenome J of *Th. intermedium*. The evolution of *Th. bessarabicum*, *P. spicata* and *Th. intermedium* is discussed.

P42. **Characterization of *KIF4A*-like and *SHOC1* genes of Rye A and B chromosomes**

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In rye (*Secale cereale*) plants, except the basic set of standard A chromosomes, they can also harbor supernumerary B chromosomes. This phenomenon also occurs in many other eukaryotic groups. Many studies reported that B chromosomes are different from the A chromosomes in inheritance. And the fact that B chromosomes are not required or even bad for the growth and development of the host organisms. Due to the dispensable nature of chromosome, they can be present or absent among individuals of the same population in a species.

Most studies showed that B chromosomes did not contain functional genes, genes are mostly fragmented in B chromosomes. In contrast, our recent sequencing data showed that rye B chromosomes are rich in gene-derived sequences after we compared them between sorted A and B chromosomes. Here, we selected two rye B-located genes (kinesin *KIF4A*-like and Shortage of chiasmata1 (*SHOC1*) gene) as candidates to conduct the research: a. We certified these two genes are located on both A and B chromosomes through single-copy FISH. b. We compared and found the differences between B-located sequences and their ancestral A-located counterparts in genomic and mRNA level. c. RT-PCR results showed these two genes have different transcription activity in different tissues when B chromosomes occur.

The work was supported by the DFG (HO1779/14-1, HO1779/10-1).



**P43. Cytogenetic studies of triticales hybrid forms with introgression of D - genome chromatin from *Aegilops tauschii* Coss.**

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Widening the genetic variability is important in the case of triticales, as it is a fully synthetic species with a narrow range of genetic variation. Therefore, the distant crossing is an excellent method which enable to generate new forms of triticales with chromatin introgression i.e. from wild species of the *Aegilops* sp. genus. The main aim of this research is identification and characterization of substitutions, additions and translocations of D- genome chromatin in triticales forms in purpose to transfer genes determining resistance to fungal diseases, especially leaf rust. In the experiments four highly repeated sequences were used as probes: pTa 794 (5S rDNA), pTa71 (35S rDNA), pSc119.2 and pAs1 to distinguish chromosomes. First FISH experiments were performed for the ancestral species of the hybrids: *Aegilops tauschii* Coss. ( $2n = 2x = 14$ , DD) and *X Triticosecale* cultivar "Bogo" ( $2n = 6x = 42$ , AABBRR) in case to facilitate the exact identification of D- genome chromatin introgression. GISH was used to differentiate D- genome chromatin from triticales and also to identify rearrangements among genomes of hybrid forms. The next step was to analyze given *Aegilops*/triticales hybrids and to select ones with D- chromatin introgression, that could enrich the triticales in valuable traits such as resistance to biotic and abiotic stresses. The results have shown that there is introgression of one or two chromosomes 2D carrying genes determining resistance to leaf rust which was confirmed with molecular markers. This approach will increase resistance to fungal infection and improve agronomic value of triticales.

We are grateful to Dr Marie Kubalakova (Institute of Experimental Botany, Czech Republic) for providing pSc119.2 clones, to Dr Tomasz Książczyk (Institute of Plant Genetics, PAS, Poland) for providing pTa794 and pTa71 clones and to Mrs Grażyna Cicha and Mrs Joanna Maszner for technical assistance.

P44. **Genome evolution and the origin of Australian crucifers**

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We showed previously that three endemic Australian crucifer species (Brassicaceae) have undergone a whole-genome duplication (WGD) followed by extensive and species/lineage-specific diploidization (Mandáková et al. 2010, *The Plant Cell* 22: 2277-2290). Some of the lowest chromosome numbers ( $n = 4-7$ ) known for crucifers can be found among the Australian endemics.

The present study aimed to analyze the genome structure in species from different Australian crucifer genera by comparative chromosome painting (CCP). Ancestral Crucifer Karyotype (ACK) with eight chromosomes served as a reference genome for CCP analyses. The structure of two ACK chromosomes was investigated in 12 species belonging to nine genera (*Arabidella*, *Ballantinia*, *Blennodia*, *Cuphonotus*, *Geococcus*, *Harmsiodoxa*, *Microlepidium*, *Phlegmatospermum*, and *Stenopetalum*) with variable chromosome numbers ( $n = 4, 5, 6$ , and  $7$ ). As all the inspected genomic blocks were found duplicated and rearranged within the analyzed genomes, we conclude that all the genera experienced presumably the same mesopolyploid WGD event, followed by massive karyotype reshuffling toward diploid-like genomes. Mechanisms of genome diploidization will be discussed.

The phylogeny of Australian Brassicaceae species was inferred using two nuclear (phytochrome A and LUMINIDEPENDENS) and two plastid (*ndhF* and *trnL-trnF*) single-copy genes. Two paralogous copies of nuclear genes identified in Australian mesopolyploids support the theory of their allopolyploid origin. The results of our phylogenetic study showed that putative ancestors of Australian crucifers were presumably closely related to tribes Crucihimalayae and Smelowskieae.

This work was supported by the European Social Fund (CZ.1.07/2.3.00/20.0189, CZ.1.07/2.3.00/30.0037).

## P45. The More the Merrier: Recent Hybridization and Polyploidy in *Cardamine*

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Inter-species hybridization and polyploidy are playing a crucial role in speciation of spermatophytes. Nevertheless, surprisingly little is known about the subgenome stability and interplay within hybrid and allopolyploid genomes. *Cardamine schulzii* is one of the classical examples of recent (<150 yrs) allopolyploidy. To investigate modes of reported hybridization and assumed polyploidization in *Cardamine* species at Urnerboden, Switzerland, comparative karyotype analysis on a population level was - for the first time - performed by combination of genomic *in situ* hybridization and comparative chromosome painting techniques (GISH and CCP). Parental chromosome complements were identified by GISH, whereas chromosome rearrangements were revealed by CCP. Urbanska and Landolt (1972, 1974) reported that hybridization between diploid species *Cardamine rivularis* (2n=16, RR) and *C. amara* (2n=16, AA) resulted in the origin of the triploid hybrid *C. ×insueta* (2n=3x=24, RRA) was followed by polyploidization towards the alloautohexaploid species *C. schulzii* (2n=6x=48, RRRRAA). We have reconstructed cytogenetic maps of many individuals across the population of the parental, triploid and bona fide hexaploid taxa. CCP analysis showed that the parental species share six ancestral chromosomes and differ by chromosome rearrangements on two chromosomes. The predominant chromosome stability and remained parental chromosome complements was revealed in the recent hybrid *C. ×insueta*. Only one subpopulation of *C. ×insueta* differed by a 2.4Mb paracentric inversion on chromosome C18 of *C. rivularis* homeogemone. Despite an extensive and in-depth population screening, we did not find a single autoallohexaploid *C. schulzii* with 48 chromosomes. Instead, all plants from the locus classicus locality possessed 46

chromosomes and tree individuals had 38 chromosomes. These hybrids contained only one copy of *C. amara* genome. GISH analysis, presence of chromosomes with terminal heterochromatic knobs which are specific for *C. pratensis* ( $2n=38$  and  $2n=30$ , respectively), and most importantly the *C. pratensis*-like fusion chromosome indicated that the allopolyploids have genome structure PPPPPA and PPPPA, respectively, and originated via hybridization of reduce gamete *C. amara* ( $n=8$ ) and unreduced gamete of *C. pratensis* ( $2n=5x=38$  and  $2n=4x=30$ , respectively) which was also found to occur at Urnerboden. Our unexpected data demonstrate how unpredictable is the genome evolution and how important is to revise the routinely repeated textbook examples using modern (molecular cytogenetic) techniques.

Urbanska K, Landolt E (1972) Ber. Geobot. Inst. ETH. Stiftung Rübel, Zürich 41: 88-100.

Urbanska KM, Landolt E (1974) Acta Soc. Helv. Sci. Nat. 1974: 89-90.

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P46. **Tribal-specific reciprocal translocation in the Cardamineae (Brassicaceae)**

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We used the Ancestral Crucifer Karyotype (ACK;  $n=8$ ) consisting of eight ancestral chromosomes and 24 conserved chromosomal blocks as a basis for the reconstruction of karyotype evolution in eight  $x=8$  genera from the tribe Cardamineae (Brassicaceae) (*A Armoratia, Barbarea, Cardamine, Dentaria, Leavenworthia, Nasturtium, Rorippa* and *Sisymbrella*). Comparative chromosome painting with Arabidopsis painting probes arranged according to the 24 ancestral chromosomal blocks revealed (i) high level of chromosome colinearity between analysed species and the ACK karyotype, and (ii) tribal-specific reciprocal translocation between AK6 and AK8 chromosomes shared by all analysed Cardamineae species. Two Cardamineae-specific translocation chromosomes AK6/8 and AK8/6 represent evolutionary significant karyotype alterations which should be used as phylogenetic demarcation of the tribe.

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P47. **Whole-genome triplication and species radiation in the Southern African tribe Heliophileae (Brassicaceae)**

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The unigeneric tribe Heliophileae includes c. 90 *Heliophila* species, all endemic to southern Africa. The tribe is morphologically the most diversified Brassicaceae lineage in every aspect of habit, foliage, flower, and fruit morphology. Despite this diversity, virtually nothing is known about its origin and genome evolution. Here we present the first in-depth information on chromosome numbers, rDNA *in situ* localization, genome structure, and phylogenetic relationship within the Heliophileae. Chromosome numbers determined in 27 *Heliophila* species range from  $2n = 16$  to  $2n = c. 88$ , but  $2n = 20$  and  $22$  prevail in 77% of species. Chromosome number variation largely follows three major lineages (A, B, and C) resolved in the ITS phylogeny. B-clade species possess mostly  $2n = 20$ , whereas  $2n = 22$  is the dominating number in C clade. The number and position of 5S and 45S rDNA loci vary between species and cannot be employed as phylogenetically informative characters. Seven species with different chromosome number and from the three ITS clades were analyzed by comparative chromosome painting. In all species analyzed, 90% of painting probes unveiled three homeologous chromosome regions in *Heliophila* haploid chromosome complements. These results suggest that all *Heliophila* species, and probably the entire tribe Heliophileae, experienced a whole-genome triplication (WGT) event. We hypothesize that the mesohexaploid ancestor arose through hybridization between genomes resembling the Ancestral Crucifer Karyotype with  $n = 8$ . The WGT has been followed by species-specific chromosome rearrangements (diploidization) resulting in descending dysploidy towards extant quasi-diploid genomes. More recent neopolyploidization events are reflected by higher chromosome numbers ( $2n = 32-88$ ). The WGT might have contributed to diversification and species radiation in the Heliophileae.

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**P48. Identification of the first centromere-specific tandem repeat in a holocentric species**

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Centromeres are the chromosomal region responsible for proper chromosome segregation during mitosis and meiosis. Monocentric chromosomes often show an interaction between centromeric CENH3-containing nucleosomes and tandem repeat sequences. Holocentric chromosomes differ from monocentric ones by the presence of the centromere throughout the entire chromosome length. As several previous studies failed to find any centromere-specific DNA (cenDNA) in holocentric organisms, holocentric chromosomes were supposed to be free of cenDNA. It was concluded that the organization of holokinetic centromeres does not allow the establishment of centromeric tandem arrays. Our analysis of the Cyperaceae *Rhynchospora pubera* is challenging this conclusion. We have identified a major satellite family (called Tyba) representing 3.3% of the genome. Genomic comparative analysis revealed that Tyba-like repeats are also found in the genome of related *Rhynchospora* species. Surprisingly, after *in situ* hybridization we observed that Tyba repeats label the extended centromere of all chromosomes of *R. pubera* and Fiber-FISH indicates an interspersed distribution of Tyba along the chromatin fiber. To demonstrate an interaction between CENH3 and Tyba repeats we have identified the CENH3 gene of *R. pubera* and generated a RpCENH3-specific antibody for subsequent immunoprecipitation experiments. Immunostaining with anti-CENH3 followed by sequential FISH indicates an interaction between Tyba repeats and CENH3 chromatin. Preliminary sequence analysis of Tyba repeats revealed evidences of a high order repeat structure typical of large tandem arrays. Based on our observations a centromeric chromatin organization model is proposed.

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**P49. The biphasic interphase-mitotic polarity of cell nuclei induced under DNA replication stress is dependent on Pin2 localization in root meristems of *Allium cepa***

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Long-term treatment of *Allium cepa* seedlings with low concentration of hydroxyurea (HU) results in the disruption of cell cycle checkpoints, leading root apex meristem (RAM) cells to an abnormal organization of nuclear structures forming interphase (I) and mitotic (M) domains of chromatin located at opposite poles of the nucleus. Thus far, both critical cell length and an uneven distribution of cyclin B-like proteins along the nuclear axis have been recognized as essential factors needed to facilitate the formation of interphase-mitotic (IM) cells. Another prerequisite consists in a distinct separation of centromeric and telomeric domains of the interphase nucleus, which appears to be a consequence of chromatin polarity acquired during mitotic division. Two aspects related to the control of IM polarity were investigated: the first concerns a possible relationship (positive or negative) between the polarity of increasing chromatin condensation (IM orientation) and the acropetal (base→apex; B→A) alignment of RAM cell files, while another involves the effects of auxin (IAA) on the frequency of IM cells. We provide evidence that there is a strong association between the advanced M-poles of the biphasic cell nuclei and the polarized accumulation sites of auxin efflux carriers (PIN2 proteins) and IAA. Furthermore, there are exclusion regions for PIN2 proteins in the microtubule-rich structures, such as preprophase band (PPB) and phragmoplast. Our current and previous studies have prompted us to formulate a hypothetical mechanism linking PIN2-mediated unilateral concentration of IAA and the induction of bipolar IM cells generated in HU-treated RAM cells of *A. cepa*.

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## P50. Allopolyploidy-associated changes of genome composition in genus *Melampodium*: does evolution repeat itself?

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Allopolyploidy has played an important role throughout the evolution of the flowering plants. Genome mergers have been shown to be accompanied by often significant and rapid alterations of genome size and structure via chromosomal rearrangements and altered dynamics of tandem and dispersed repetitive DNA families. Recent developments in sequencing technologies and computational methods have allowed for a comprehensive investigation of the repetitive component of the genome. Herein, we investigate the repeat dynamics in a group of closely related allopolyploids and their progenitor species in the genus *Melampodium*. The largest section of the genus (sect. *Melampodium*;  $x = 10$ ) encompasses six distinct lineages of diploids, two of which participated in the origin of four allopolyploid species ( $4x$  and  $6x$ ). While the two allotetraploid species originated from different parental taxa, both allohexaploids originated via same direction cross of two species, a diploid and one of the allotetraploids. The genomes of parental diploid taxa differ significantly in genome size and structure. Significant differences in genome composition in diploid parental taxa manifested in genome size variation have been investigated using low-pass NGS. The composition of genomes of parental taxa is further used as a basis for comparative analysis of repetitive DNA dynamics in polyploids. The ultimate goal of this study is to infer the patterns of genome dominance in allopolyploids with respect to their parental taxa and to test the hypothesis of evolution repeating itself in allopolyploids that share both maternal and paternal parents.

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**P51. Condensin dynamics in Arabidopsis interphase nuclei**

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Plants, due to their settledness, need a strict regulation of transcription to respond effectively to environmental changes. Transcription and its regulation occur during interphase, when chromatin is more relaxed and regulatory elements can access to it. Thus, analyzing the nuclear organization and the interactions between genes and regulatory elements during interphase is required.

Condensins are best known for their role shaping mitotic and meiotic chromosomes, but also other functions during interphase, as organization of interphase chromatin and transcriptional control, have been reported in yeast, *Caenorhabditis*, *Drosophila* and vertebrates. Yeast has one condensin complex, while higher eukaryotes have two of them (condensin I and II). Both, condensin I and II, are conserved in plants, but so far little is known about their function.

In this project we study the subunits CAP-D2 (condensin I), CAP-D3 (condensin II) and their potential regulatory elements to reveal their function during interphase chromatin organization and transcription. We selected *Arabidopsis thaliana* T-DNA insertion mutants impairing the condensin subunits and potential interacting proteins to check their cytological phenotypes. In addition, we use antibodies and GFP reporter lines in combination with Super Resolution Microscopy (SIM and PALM) to analyze the dynamics of the proteins of interest in interphase and during cell division.

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**P52. Investigation of CenH3 genes and centromeres in *Fabeae* species**

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Centromere is a chromosome domain that is essential for faithful chromosome segregation during cell division. In all eukaryotes studied so far, functional centromere domains are defined by presence of CenH3 histone which is centromere specific variant of histone H3. In most diploid species, including those that underwent whole genome duplication, CenH3 is encoded by a single copy gene. This is in contrast with canonical H3 genes, which exist in slightly different variants and multiple copies, and suggests that occasional duplication events are usually followed by degeneration of one copy.

We found that duplication of CenH3 gene occurred also early in the evolution of *Fabeae* tribe of *Fabaceae* family about 16 - 23 million years ago. While both CenH3 paralogs have been maintained until now in *Pisum* and *Lathyrus* spp., one of them was lost in the ancestor of *Vicia* and *Lens* spp. The evolutionary period since the duplication event was sufficient for noncoding intron sequences to change considerably due to frequent substitutions and indel mutations. On the other hand, coding sequences remained relatively conserved and were found to evolve under purifying selective pressure. Importantly, both variants of CenH3 histones fully colocalized in all centromeres. This strongly suggests that both CenH3 paralogs are required for the proper function of centromeres in *Pisum* and *Lathyrus* spp.. Strikingly, species of these two genera were found to have considerably longer centromeres than *Vicia* and *Lens* spp., in some cases exceeding 100 Mbp and displaying multiple domain structure that we refer to as metapolycentric.

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**P53. Karyotyping of *Daucus carota* L. chromosomes using multicolor-FISH**

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Carrot (*Daucus carota* L., 2n=18) chromosomes are small and poorly differentiated in size and morphology. Identification of individual chromosomes in a complement is usually a difficult task in the case of most plant species, especially for those with small, numerous, and morphologically uniform chromosomes. The present study focuses on the localization of various types of repetitive sequences on carrot chromosomes using fluorescence *in situ* hybridization (FISH). The chromosomal localization of repetitive sequences was carried out using triple color FISH with 45S rDNA and 5S rDNA (both DIG labeled), synthetic oligonucleotide Cent-Dc-Cy5 (*Daucus carota* centromeric sequence) and telomeric (Telo-Cy3) sequences on enzymatically digested root-tip meristem cells. A simultaneous use of these four probes, as well as chromosome measurements allowed for an easy and reliable identification of nine pairs of carrot homologous chromosomes and karyotype analysis. Subsequently, three carrot genome-specific DCRE (*Daucus carota* repeat elements) sequences, i.e. DCRE9, DCRE16, DCRE22. DCRE9 was evenly distributed throughout chromosomes. DCRE16 was accumulated around the pericentromeric regions. Hybridization sites for DCRE22 were prevailing in pericentromeric and interstitial localizations on chromosome arms. The localization and intensity of FISH signals for repetitive genomic DNA fragments was useful for identification of individual chromosomes.

The research was supported by the Polish Ministry of Science and Higher Education fund for statutory activity of the University of Agriculture in Krakow.

P54. **Genomic organisation of a 5S rDNA-related satellite repeat in *Phaseolus* L. (Fabaceae)**

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Satellite DNA repeats constitute one of the most dynamic fractions of eukaryotic genomes. They may arise *de novo* or from part of an already repetitive element. Here, based on a cytogenomic analysis, we describe a satellite DNA that is related to the NTS (Non Transcribed Spacer) of the 5S rDNA and differentially amplified in *Phaseolus* genomes. *Jumper*, as it was named, was identified close to a subtelomeric satellite repeat from *P. microcarpus* plasmid clone. BLAST searches against *P. vulgaris* complete genome sequence revealed two different *jumper* organisations: as clusters of tandem repeats or as part of the NTS of the 5S rDNA. PCR using genomic DNA from different *Phaseolus* species and related genera (*Cajanus*, *Macroptilium* and *Vigna*) revealed that *jumper* is present as a tandem repeat in all *Phaseolus* species analysed, while it showed different organisations in other genera. FISH signals were seen in *P. microcarpus*, but not in *P. vulgaris*, *P. leptostachyus*, *P. lunatus* nor related genera, even at lower stringency. All *P. microcarpus* chromosomes showed labelling either at pericentromeres, subtelomeres or both. A ladder-like pattern with bands starting ~170 bp was observed in Southern hybridization only for *P. microcarpus* and the related *P. oligospermus*. Phylogenetic analysis of *jumper* clones is in accordance with the library model of satellite DNA evolution. In summary, *jumper* probably emerged from or inserted into the 5S rDNA early in the diversification of the genus and became a high-copy sequence in a specific lineage at both subtelomeric and pericentromeric chromosome domains.

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P55. **GISH painting of the Y chromosomes in dioecious Cannabaceae species**

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The Cannabaceae family includes three dioecious species. Hemp (*Cannabis sativa*,  $2n=20$ ) and common hop (*Humulus lupulus*,  $2n=20$ ) use the XX/XY chromosome system, while Japanese hop (*H. japonicus*,  $2n=16$  for female and 17 for male) contains multiple sex chromosomes (XX/XY<sub>1</sub>Y<sub>2</sub>). The genome size of these three related species varies widely: *C. sativa* – 0.84-0.91 pg, *H. japonicus* – 1.7 pg and *H. lupulus* – 2.90 pg. Such differences provide good models to the study of the origin and evolution of different sex chromosome systems in plants.

In this work, GISH visualization of the Y chromosomes was successfully applied to *C. sativa*, *H. lupulus* and *H. japonicus* with best differentiation in *H. japonicus*. The use of male or female DNA as a probe for GISH gave the same result. Application of female blocking DNA with male or female DNA as a probe showed downgrade of the Y chromosomes differentiation from other chromosomes in GISH experiments. It suggests the presence of repeated DNA that was preferentially accumulated in the Y chromosomes. In addition, unpainted presumably pseudoautosomal region (PAR) was detected on the Y chromosomes. The size of PAR was covering about 10%, 20% and 40% of the Y chromosome in *H.japonicus*, *H.lupulus* and *C.sativa*, respectively.

This work was supported by Russian Foundation of Basic Research No.13-04-01804 and No.13-04-02116.

**P56. The subtelomeric repeat *hipu* is spread out along *Phaseolus* L. beans genome**

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The satellite DNA *hipu* is the main subtelomeric repeat identified in the common bean (*Phaseolus vulgaris*) genome. This sequence is present in several BAC clones mapped to subtelomeric chromosome regions, but seemed absent or present in very low amounts in *P. leptostachyus*, *P. lunatus* and *P. microcarpus*. Here, we have combined molecular and cytological approaches to investigate the dynamics of *hipu* in these divergent *Phaseolus* species. A mixture of *hipu* clones from *P. vulgaris* hybridized *in situ* at low stringency confirmed the presence of *hipu*-like sequences on subterminal chromosome regions in the analyzed species. Fragments of *hipu* amplified from genomic DNA using different sequence-specific primers were cloned and sequenced. Maximum likelihood analyses combining 30 sequences from *P. leptostachyus*, nine from *P. lunatus* and 21 from *P. microcarpus*, as well as 67 from the *P. vulgaris* genome, revealed closer relationship among *P. vulgaris* and *P. lunatus* units. However, higher similarity between some *P. vulgaris* and *P. leptostachyus* or *P. microcarpus* sequences was also observed. FISH at higher and lower stringencies using clones from different species showed differences in the number and intensity of signals, even though all signals were located at subterminal regions. These results agreed with those from Southern hybridization. Taken together, the data argue for *hipu* as a widespread subtelomeric repeat in the genus and for its intra- and interspecific variability, following the library model of satellite DNA evolution.

Financial support: CAPES and CNPq (Brazil) and INRA-DGAP (France)

**P57. Chromosomal fragility in the cell cycle of species of *Lolium* and *Festuca***

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Studies in *Lolium* showed that the occurrence of breaks and gaps in metaphasic chromosomes coincide with the 45S rDNA sites, that were characterized as fragile sites. Fragile sites are chromosomal regions which are susceptible to narrowing or constriction and constitute lesions or promote breaks in one or both chromatids. However, no reports about the occurrence of fragile sites in *Festuca* and its expression in the cell cycle of these species.

The aim of this study was to investigate the expression of fragile sites in the cell cycle of *Lolium perenne* (cv. Ellett (2x) and cv. Oro Verde (4x)), *Lolium multiflorum* (cv. 161 (2x) and cv. Barjumbo (4x)) and *Festuca arundinacea* (cv. Rizomat and cv. Quantum II, both 6x). In all species were observed chromosomal breaks in prophases, metaphases and anaphases, evidenced by the formation of fragments varying in number and size. The observation of abnormalities, such as bridges in anaphase and stickiness, can be resulted of expression of fragile sites in these species. The FISH analyzes confirmed that the breaks occur in of 45S rDNA sites, showing that it is a fragile site already observed in C-metaphases of *L. perenne* and *L. multiflorum*. In interphase, the number of signals of 45S rDNA was variable and different than expected, indicating that the breaks occur during the stage of DNA replication.

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P58. **Chromatin structure and epigenetic modification in two diploid *Brassica* species cells *in vivo* and *in vitro***

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Chromatin is the most dynamic structure in eukaryotic cell and is regulated mainly by DNA methylation and numerous post-translational modifications of core histones. During the life cycle, plants pass through the stages of epigenetic "reprogramming" in different cell types and at certain stages of development. Epigenetic regulation of gene expression during morphogenesis, unlike in animals, allows plant cells to maintain a high degree of developmental plasticity. In specific condition of *in vitro* culture differentiated plant cells have the ability to dedifferentiation and re-enter to cell cycle to form callus. This process is associated with structural, biochemical, cytological and epigenetic changes. The aim of the presented research was to identify changes in chromatin structure and pattern of DNA methylation and histone modification during callogenesis. The two diploid *Brassica* species – *B. rapa* and *B. oleracea* significantly differ in chromatin structure and pattern of epigenetic modification in differentiated tissues of cotyledons. During callogenesis from cotyledon explants chromatin underwent changes in condensation and organization. The process of cell dedifferentiation and callus proliferation was accompanied by relocation of some epigenetic modification and global increase in the level of histone H4 acetylation as well as methylation of histone H3K9. The smaller extent of these changes was found in *B. rapa*, while the larger in *B. oleracea*. The observed differences between *Brassica* species probably were associated with the capability to callogenesis and morphogenetic potential of callus tissue. These results demonstrate that during cell dedifferentiation and callus proliferation chromatin undergoes very dynamic rearrangements.

P59. **Molecular composition of B-chromosomes in *Aegilops speltoides* Tausch.**

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The B-chromosomes, or Bs exist in addition to the regular chromosome complement. They are also called “accessory” or “supernumerary” chromosomes because they are not essential for normal growth and development of host organisms. The main problem in studying Bs is that their presence in the species is not constant and even organisms from the same population can differ in the number of Bs. Bs exist in wild or semi-wild populations of plants, but they were never found in cultivated forms. *Aegilops speltoides* is a carrier of B-chromosomes. They are submetacentric and the length amounts to about 2/3 of the average A-chromosome length. A maximum eight Bs per cell was reported. The Bs are absent from the roots, but are present in other organs of the plant. The number of Bs is constant in different aerial tissues of the same individual. Despite the fact that *Ae. speltoides* is a well studied species, only little information is available about their B-chromosomes. We characterized DNA composition and the size of *Ae. speltoides* Bs. The chromosomal distribution of sequences along A- and B-chromosomes was analyzed by FISH method. The sequencing of genomic DNA of OB and +B plants and cluster analysis allowed us to identify the B-specific repeats, which were used as a FISH probe to confirm B-specificity. *In situ* hybridization with labelled organellar DNA showed B-specific accumulation of chloroplast- and mitochondria-derived sequences which suggests a reduced selection against the insertion of organellar DNA in supernumerary chromosomes.

The work was supported by the DFG Germany (HO 1779/14-1, <http://www.dfg.de/>).

**P60. Karyotype analysis in *Agropyron cristatum*****Mahmoud Said<sup>1</sup>, Adoración Cabrera<sup>2</sup> and Jaroslav Doležal<sup>1</sup>**<sup>1</sup> Institute of Experimental Botany AS CR, CZ-78371 Olomouc, Czech Republic<sup>2</sup> Genetics Department, ETSIAM, University of Cordoba, 14071, Spain

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The genus *Agropyron*, which belongs to tribe Triticeae, comprises a number of species that may provide novel genes for wheat improvement. *Agropyron cristatum* L. (Gaertn.) is a perennial species of economic importance as forage; it is facultatively allogamic and auto-compatible, showing high crossability with other members of Triticeae. A detailed knowledge of its karyotype is a prerequisite for the identification of chromosomes introgressed into wheat. We have analyzed the karyotype of a tetraploid *A. cristatum* ( $2n=4x=28$ , PPPP) accession PI222957 from Iran. Fluorescence *in situ* hybridization (FISH) with a set of probes showed specific patterns for the majority of homologous chromosome groups. However, variability in the number and position of FISH signals were observed for some homologues in different individuals. The probe pHvG39 containing GAA satellite sequence showed a large signal in the subterminal region of the long arm of one homologous pair, while the 5S rDNA probe showed two to four sites of hybridization. The repetitive sequence pAs1 showed signals mostly in the terminal regions of all chromosomes and it could be used to identify the individual homologous pairs. The terminal location of the pSc119.2 sequence was also detected on ten to fourteen chromosomes. *In situ* hybridization with the 45S rDNA probe revealed eight hybridization sites, located in the terminal position of the short arms of four chromosome pairs. The results of this work support the view that the tetraploid *A. cristatum* accession has a non-autoploid nature and that the four P genomes have differed by structural changes like reciprocal translocations. Hence this accession could be a segmental allopolyploid. Variability in the number and position of pSc119.2 and 5S signals, and pAs1 patterns, can help to identify chromosomes involved in the structural changes. More work is needed to characterize the four P genomes in the tetraploid accession, and the first step towards this goal is to establish the karyotype of a diploid *A. cristatum*.

**P61. Hydrogen peroxide is involved in Pb-induced chloroplast avoidance-like movements in *Lemna trisulca* L.**

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Lead ( $\text{Pb}^{2+}$ ) is one of the most important heavy metals polluting the natural environment due to human activity. Uptake of this metal may cause many destructive changes in plants, among others in the structure and function of both the nucleus and chloroplasts. However, little information exists concerning the effects of Pb on regulation of chloroplast redistribution. In this study we compared the effects of lead ions ( $15 \mu\text{M Pb}^{2+}$ ) and hydrogen peroxide ( $1 \text{ mM H}_2\text{O}_2$ ) – acting as a signaling molecule inducing chloroplast movements (Wen et al. 2008) – on their distribution patterns in mesophyll cells of water angiosperm *Lemna trisulca* L. (star duckweed). An analysis of confocal microscopy images of *L. trisulca* fronds treated with lead in darkness revealed an enhanced accumulation of chloroplasts in profile position at the anticlinal cell walls in comparison to untreated plants. Interestingly, a similar rearrangement of chloroplasts as in the fronds treated with lead ions in darkness was observed also in a strong light avoidance response. Furthermore, an exogenously applied hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) to *L. trisulca* cells exerted a similar effect to that of Pb-induced redistribution of chloroplasts in darkness. In addition, after treatment of plants by lead, we observed an enhanced accumulation of endogenous content of  $\text{H}_2\text{O}_2$ . Interestingly, the catalase – a  $\text{H}_2\text{O}_2$ -specific scavenger – partly abolished the Pb-induced chloroplast response. These results suggest that  $\text{H}_2\text{O}_2$  may be involved in avoidance-like movement of chloroplast induced by lead.

**Wen F. et al., J. Exp. Biol., 2008, 59(10): 2891–2901.**

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P62. **From “x” to phylogenies: Inference of chromosome number evolution in 2014**

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Since 1932, botanists have been proposing basic (or base) numbers,  $x$ , for entire groups, often in uncritical and idiosyncratic ways. Zoologists never warmed to the approach, which is of doubtful heuristic value. First, the reliability of the inferred number depends on the sampling density, that is, the percentage of individuals and species in a group for which there are chromosome counts. Second, the approach is a holdover from an era before reliable phylogenies and “tree thinking” and does not take advantage of insights and quantitative data that come from molecular phylogenies and molecular cytogenetics. To infer past changes in chromosome numbers in a reproducible manner and with measures of confidence, we combined model-based ancestral trait reconstruction and molecular cytogenetics in the Araceae genus *Typhonium*, which has 50-60 species. New and published chromosome counts were used to model evolutionary changes in chromosome complements along a phylogeny that includes a dense sample of ingroup and outgroup species. Ten species with  $2n = 8$  to 24 from different parts of the phylogeny were investigated by Fluorescence In Situ Hybridization with three probes (5S rDNA, 45S rDNA, and *Arabidopsis*-like telomeres). The FISH results supported three of the events inferred on the phylogeny: Two chromosome number reductions and one polyploidization event. Other inferred events, however, were not supported. This is the first time that phylogenetic trait reconstruction for chromosome numbers has been tested against physical (microscopy-based) evidence.

This work was supported by the German Science Foundation (DFG RE 603/7-1).

**P63. How *Coccinia grandis* (Cucurbitaceae) got its huge Y chromosome**

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In the flowering plants, heteromorphic sex chromosomes are known from only 19 species belonging to Cannabaceae, Caryophyllaceae, Cucurbitaceae, and Polygonaceae. Conspicuously neglected among the species with heteromorphic sex chromosomes is the Cucurbitaceae *Coccinia grandis*. Although important studies in the 1950s and 1970s had established the male-determining effect of the presence of the Y chromosome, prior to our work the size of the *C. grandis* genome and details of its karyotype were unknown. Towards a genomic understanding of the evolution of sex chromosomes in this species, we determined the C-values of male and female individuals of *Coccinia grandis* and performed Fluorescence In Situ Hybridization with 5S rDNA, 45S rDNA, and *Arabidopsis*-like telomeres probes, Genome In Situ Hybridization, and C-banding. Analysis of the C-values revealed a male/female genome difference of almost 0.1 pg of DNA or ca. 10% of the total genome, which is in the range of entire small plant genomes (e.g., *Genlisea margaretae*). The Y chromosome in *C. grandis* is heterochromatic, similar to the Y chromosomes of *Rumex acetosa*, and thus different from the euchromatic Y chromosome of *Silene latifolia*; it is more than two times larger than the largest other chromosome in the genome; and the type of repetitive DNA in the centromere of the *C. grandis* Y chromosome appears to be different from those in the centromeres of the autosomes/X chromosome. Our next step will be the micro-dissection of the *C. grandis* sex chromosomes (already tested) to determine their gene and repeat composition.

This work was supported by the German Science Foundation (DFG RE-603/6-1 and 6-2).

**P64. Development and molecular cytogenetic characterization of wheat-perennial rye (*Secale cereanum*) introgression lines**

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Perennial rye (*Secale cereanum*) is a stable artificial hybrid of the winter rye (*Secale cereale* L.) and the perennial wild rye (*Secale montanum* Guss.). This variety can be in culture 3-5 years, resistant to leaf rust, stem rust and powdery mildew, has good frost and drought tolerance, adapts well to the disadvantageous soil and weather conditions, and owing to its fast shooting and excellent stooling perennial rye protects the loose soils against erosion and deflation.

Progeny plants of a wheat×perennial rye hybrid backcrossed with wheat were studied using *in situ* hybridization technics (GISH és FISH) in order to select wheat-perennial rye introgression lines carrying useful traits of the perennial rye (e.g. resistance, drought tolerance and perennial growth habit). Based on their FISH hybridization patterns four disomic (1R, 3R, 4R, 6R) and one monosomic (7R) addition lines were identified from which the 4R addition was the most frequent. The disomic addition containing 1R<sup>m</sup> chromosomes from *S. montanum* can be an important genetic resource for resistance breeding. Numerous wheat-rye chromosome rearrangements were also detected. The 7RS.4BL centric fusion showing characteristic pSc119.2 pattern has been identified, the identification of the other translocation is in progress.

**P65. (Cyto)genetics for narrow-leaved lupin genome mining**

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*Lupinus angustifolius* (narrow-leaved lupin), is a valuable legume crop, and is considered to be the reference species for genomic studies within the genus *Lupinus*. The international research effort has resulted in comprehensive genetic resources for the species, including the draft genome sequence, linkage maps, nuclear DNA libraries and chromosome-specific landmarks. Our studies are focused on: 1) variation and duplication of narrow-leaved lupin genes, 2) genomic synteny in legumes, 3) integration of *L. angustifolius* linkage groups with cytogenetic chromosome maps and 4) lupin genome evolution at the chromosome level. Using quantitative PCR, digital PCR and fluorescent *in situ* hybridization (FISH), we have determined the copy number variation of genes playing crucial roles in nitrogen fixation and fatty acid synthesis. Cross-genera genomic and bioinformatic analyses have enabled us to identify strong and conserved syntenic links between *L. angustifolius* gene-rich regions and homologous *Medicago truncatula*, *Glycine max*, *Lotus japonicus*, *Phaseolus vulgaris* and *Cajanus cajan* chromosome segments. We have successfully assigned 20 linkage groups from the genetic map of *L. angustifolius* to respective chromosomes, applying BAC clones from the nuclear genome library as genetic and chromosome markers. These sets of markers also facilitated the survey of chromosome rearrangements and, therefore, the study of genome evolution in Old World lupins. The cross-species BAC-FISH provided information on genome organization, evolution and phylogenetic relationships in analysed species. Our results contribute to *L. angustifolius* genomics and phylogeny of legume plants. Moreover, selected BACs may constitute a cytogenetic platform to study the karyotype evolution of lupins.

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P66. **Genetic recombination in species and hybrids of *Brachiaria* (Poaceae)**

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RAD51 protein is highly conserved among eukaryotes and plays a key role in the search for chromosomal homology and in meiotic recombination by catalyzing the repair of DNA double-strand breaks (DSBs) and effecting the crossover. Genetic recombination is a process that allows the generation of new allelic combinations, with important consequences for genome evolution and genetic improvement. Through immunolocalization of the RAD51 protein, this study aimed to identify and compare the frequency of hotspots of homology and meiotic recombination in sexual diploid and tetraploid species, apomictic tetraploid and hybrids of *Brachiaria*.

The analysis of RAD51 in meiocytes revealed diffuse signals in the nucleus at leptotene. In zygotene, the mean of foci of RAD51 was similar for all species. In this phase, we identified individual RAD51 foci in unpaired or partially paired chromosomes and adjacent RAD51 foci in paired chromosomes, confirming its function in chromosomal homology. During pachytene, RAD51 foci were located between chromosome axes at recombination hotspots. The number of recombination foci was higher in apomictic *Brachiaria* species (*B. decumbens*, 85.80 and *B. brizantha*, 83.87) in comparison with hybrids and with *Brachiaria ruziziensis*.

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**P67. Histone H3 phosphorylation at serine 10 during mitosis and its relationship with hybridization and polyploidy in *Pennisetum* spp. Rich. (Poaceae)**

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Phosphorylation of histone H3 at serine 10 (H3S10ph) may play important role in chromosome condensation and sister chromatid cohesion. Studies have been shown that the role and distribution of H3S10ph may differ between species. The pattern of H3S10 phosphorylation was evaluated by immunodetection technique during mitosis in *Pennisetum purpureum*, *Pennisetum glaucum* and triploid and hexaploid interspecific hybrids. Roots were fixed in PFA 4%. We applied primary antibody (rabbit polyclonal IgG, Santa Cruz Biotechnology, USA) and detected with a secondary antibody (Goat anti-rabbit IgG-FITC, Santa Cruz Biotechnology, USA). The pattern of H3S10ph during mitosis showed coordination in space and time and it was similar in chromosomes from the parents and hybrids. The phosphorylation begins in prophase, proceeds in metaphase, where all chromosomes present H3 phosphorylation at the pericentromeric region, remains in anaphase and gradually disappears in telophase. Phosphorylation was coincident with cohesion restricted to pericentromeric region, which is crucial for the correct orientation of chromosomes during cell division, in addition to stabilize the connection between sister chromatids by the forces generated by the spindle fibers. In triploid and partial hexaploid hybrids, bridges in anaphases and telophases were found, which showed signs of phosphorylation in H3S10, suggesting loss of chromosomes fragments. In the hybrid hexaploid, metaphases with not oriented chromosomes and anaphases with delayed and lost chromosomes with and without immunosignal were found. This suggests elimination of chromosomes with active and inactive centromeres. These abnormalities occur as a result of genomic rearrangements that are frequent events in hybridization and polyploidy.

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**P68. Genome size distribution patterns in different Austrian vegetation types: testing the nucleotype hypothesis**

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Nuclear genome size varies strongly among species. This variation is almost 2400-fold in green land plants and does not correlate with organismic complexity and, moreover, is caused mostly by the non-coding, so-called “junk DNA”. In contrast, the number of coding sequences is relatively stable. Consequently, the presence of such high variation raises questions about a peculiar function and modulation of the nuclear DNA mass beyond genetic information genome size. Meristematic cell volume, minimum mitotic cell cycle duration, and duration of meiosis have been described as the key characters, which all have an impact on the plant development, and, therefore, influence the individual’s ability to adapt to ecological conditions. It has been proposed that plants occupying short-lived habitats possess limited monoploid genome size. In the 1970s the “nucleotype hypothesis” was proposed, which explains C-value diversification among species by natural selection on the mass of DNA. The present project focuses on analyses of genome size distribution in Austrian muddy river bank and snow bed communities using PI flow cytometry, Feulgen densitometry, and chromosome counting. These two types of communities follow contrasting life strategies (annual versus perennial), but in both vegetation periods are very short. Preliminary statistical analysis of genome size of these two communities points to a significant genome size difference between perennial snow bed community members and annual muddy river bank inhabitants ( $p < 0.01$ ). Ultimately, the data will allow direct testing of the nucleotype theory, as well as new C-values will also aid modern molecular studies, e.g., AFLPs or next generation sequencing.

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**P69. On the origin of polyploids in grasses: multi-approach insight into evolutionary history of European *Anthoxanthum***

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European members of the genus *Anthoxanthum* (Sweet vernal grass, Poaceae: Pooideae: Aveneae) represent an interesting diploid-polyploid complex with blurred evolutionary history. Our study was aimed at elucidating i) phylogenetic relationships among all European taxa (belonging to *Anthoxanthum odoratum* complex) and ii) evolutionary history of polyploids. A number of interacting techniques of plant biosystematics was used, including namely molecular approaches (single-copy nuclear loci sequencing, ITS, GISH, FISH) and flow cytometry. The gathered data provided consistent insight into evolutionary processes that shape European members of *Anthoxanthum*. Both auto- and allopolyploidy was soundly proved to take place in the evolution of *Anthoxanthum odoratum* complex. Nevertheless weak gene and taxa differentiation at diploid level caused unclear determination of parental taxa of allopolyploids. On the contrary, our data suggested that mountain taxon *A. alpinum* was undoubtedly involved in all polyploidization events within European members of *Anthoxanthum*.

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**P70. Determination of nuclear DNA content of colchicum species from Turkey by using flow cytometer**

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Genus *Colchicum* is a member of the *Colchicaceae* family and includes approximately 100 perennial flowering species which grow from bulb-like corms. It is native to West Asia, Europe, parts of the Mediterranean coast, down the East African coast to South Africa and the Western Cape. Fourty seven of the *Colchicum* species grow in Turkey naturally.

Because of its complexity, the genus remains as a poorly known group. Until today, no modern techniques have been used in classification of the genus *Colchicum*. In this study, we used flow cytometry to determine nuclear DNA content of the 47 species collected from Turkey. Based on the results of our study, nuclear DNA content of *Colchicum* species varied from 3.97 pg/2C (*C. boissieri* Orph) to 10.48 pg/2C (*C. chalce* Azn. subsp. *Punctatum* K.Perss). The results of nuclear DNA content analysis will be used to determine ploidy of the species and to study genomic relations within the genus.

**Key words:** *Colchicum*, flow cytometry, nuclear DNA content, ploidy, genomic relations

**P71. Exploring the huge genome size variation in *Oxalis* (Oxalidaceae) by next-generation sequencing**

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*Oxalis* L. is a cosmopolitan genus comprising about 500 species, most of them native to South America and South Africa. It displays great cytological variation, with chromosome numbers ranging from  $n = 5$  to 11, and different chromosome morphologies. Its range of nuclear DNA content is one of the widest known among Angiosperms (from 0.58 in *O. linarantha* to 41 pg in *O. psoraleoides*, ~70-fold variation). This uncommon genome variation makes *Oxalis* an excellent model for genome evolution studies among plants. In this work we characterized and compared the repetitive DNA genome fraction in five *Oxalis* species and determined its contribution to the genome size differences. We used next-generation sequencing and a graph-based clustering approach for the repeat sequence assembly as implemented in the RepeatExplorer pipeline. Repetitive sequences represented up to 70% of the nuclear genome (in *O. psoraleoides*) and LTR retrotransposons account for most of its DNA content variation. Striking differences in LTR superfamilies and lineages were observed among species. Some taxa possessed a higher proportion of Gypsy, others have equal amount of Gypsy and Copia, while in *O. refracta* Copia exceeds Gypsy. The gypsy lineages Chromovirus and Ogre account for most of the genome of *O. psoraleoides*, while for the rest of the species other Gypsy lineages, or even Copia, such as TAR, made more important contributions. Although there was no clear correlation between genome size and the proportion of certain LTR retrotransposons, there seems to be a phylogenetic pattern that should be further investigated.

MV was recipient of an IANAS seed grant award and a CSIC (Uruguay) scholarship

**P72. Enigmatic *Siliquamomum* (Zingiberaceae: Alpinioideae): an intergeneric hybrid or a result of rapid radiation of gingers?**

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Despite of several phylogenetic studies of Zingiberaceae, the enigmatic genus *Siliquamomum* (3 species, Zingiberaceae) still remains in unresolved position within Zingiberaceae, subfamily Alpinioideae. We have analysed all currently known species of *Siliquamomum* (*S. tonkinense*, *S. oreodoxa* and *S. allicorne*) and several closely related genera using three independent regions from nuclear DNA (*ITS*, *DCS*, *GAPDH*) and two non-coding cpDNA regions (*trnL-trnF* and *matK*). The resulting phylogenetic trees were incongruent in topologies. Such pattern might be consequence of two distinct processes – hybridization or deep coalescence. We have therefore aimed to test both hypothesis in order to resolve the evolutionary origin of this peculiar ginger genus. The hypothesis of gene tree incongruence due to deep coalescence and incomplete lineage sorting was tested using up-to-date approaches based on modeling of coalescence process and species tree reconstruction (\*BEAST, STEM). The alternative hypothesis that *Siliquamomum* originated *via* hybridization between species from two distantly related lineages was tested by modeling of coalescence process in presence of gene flow (JML, STEM-hy), and by the method of genomic *in situ* hybridization (GISH). Putting modern molecular-cytogenetic data into a molecular phylogenetic framework significantly expedited our understanding of the role of hybridization and polyploidy in the genome evolution of ginger family.

This work was supported by the Czech Science Foundation, GAČR (grant no. GA14-13541S). The work of last author is supported by National Parks Board, Singapore.

**P73. The role of Wings apart-like protein (Wapl) in *Arabidopsis thaliana***

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Wings apart-like protein (Wapl) is a key negative regulator of cohesion in mammalian and yeast cells. The typical X-shape chromosome structure is an effect of Wapl-mediated cohesin releasing on chromosome arms. During prophase pathway cohesin held sister chromatids together. Wapl interact with cohesin subunits as a result opens a cohesin ring and release sister chromatids. In centromere regions cohesin is protected by Sgo1 protein and cleaved by separase during anaphase. These distinct steps allow proper segregation of chromosomes. Interaction between Wapl-cohesin put a direct impact on chromosome dynamics and segregation errors protection. Wapl possesses a conserved C-terminal and diverged N-terminal domain. Its function seems to be conserved across species. However, it has not been analyzed in any plant. In order to address its function in plants, we intend to analyze the two *Wapl* homologs (*AT1G11060*, *AT1G61030*) of *Arabidopsis thaliana* in detail. Therefore we will employ T-DNA insertion line mutants targeted to *Wapl* genes, genomic modifications like overexpression and gene silencing to determine phenotypic effects and its role in plant development. The generation of specific antibodies and reporter lines will allow localization studies of Wapl proteins during cell division.



**P74. Expression of nuclear envelope SUN2-like protein in root meristem cells of *Allium cepa* during hydroxyurea-induced DNA replication stress**

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Regardless of the DNA replication stress induced by continuous treatment of *Allium cepa* seedlings with low concentration of hydroxyurea (HU), some of the root meristem cells override the cell cycle control mechanism (S-M checkpoint) and initiate either premature chromosome condensation (PCC) or an abnormal type of nuclear division, forming half interphase/half mitotic chromosomes (IM cells). A common ground for both types of M-phase cells relies on the enhanced transcription level (evidenced using 5-ethynyl uridine incorporation assay), which allows for sustained nuclear functions and persistent cell growth. We provide evidence that an intensified metabolic activity of HU-treated cells results in the increased number of nuclear bodies (NBs) and an enhanced level of proteins similar to SUN2, one of the highly conserved SUN domain proteins linking nuclear envelope and major components of the cyto- and nucleoskeletal structures. In addition to NE membranes, phragmoplast and cell plate, our immunofluorescence observations extend an array of subcellular compartments at which SUN2-like proteins (SUN2-LP) are localized. These include centromeric regions of condensed mitotic chromosomes and cortical preprophase band of microtubules (PPB). Except of nuclear domains occupied by the condensed chromatin and nucleolar regions, proteins equipped with epitopes recognized by anti-SUN2 antibodies are also localized in chromatin and NBs (distinct from Cajal bodies).

This work was supported by a grant (N N303 503038) from National Science Centre.

**P75. Endogenous pararetrovirus sequences associated with 24 nt small RNAs at the centromeres of *Fritillaria imperialis* L. (Liliaceae), a species with a giant genome**

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Endogenous pararetroviral sequences are the most commonly found virus sequences integrated into angiosperm genomes. We describe an endogenous pararetrovirus (EPRV) repeat in *Fritillaria imperialis*, a species under study for its exceptionally large genome (1C = 42 096 Mbp, approx. 240 times bigger than *Arabidopsis thaliana*). The repeat (FriEPRV) was identified from Illumina reads using the RepeatExplorer pipeline, and exists in a complex genomic organisation at the centromere of most, or all, chromosomes. The repeat was reconstructed into three consensus sequences which formed three interconnected loops, one of which carries sequence motifs expected of an EPRV (including the *gag* and *pol* domains). FriEPRV shows sequence similarity to members of the *Caulimoviridae* pararetrovirus family, with phylogenetic analysis indicating a close relationship to *Petuvirus*. Potentially no complete EPRV sequence exists, although our data suggests an abundance that exceeds the genome size of *Arabidopsis*. SNP analysis revealed elevated levels of C to T and G to A transitions, consistent with deamination of methylated cytosine. Bisulphite sequencing revealed high levels (up to 100%) of methylation at CG and CHG motifs, and on average 10-15% methylation at CHH motifs. FriEPRV's centromeric location may suggest targeted insertion, perhaps associated with meiotic drive. We observed an abundance of 24nt small RNAs (smRNAs) that specifically target FriEPRV, potentially providing a signature of RNA dependent DNA methylation. Such signatures of epigenetic regulation suggest that the huge genome of *F. imperialis* has not arisen as a consequence of a catastrophic breakdown in the regulation of repeat amplification.

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**P76. Chromosomal evolution and the organization of repetitive DNA sequences in diploid and polyploid *Brachiaria* forage grasses**

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*Brachiaria* is a genus of grasses including diploid and polyploid species with similar chromosomes and basic numbers of  $x=6$ ,  $x=7$  and  $x=9$ ; many are apomictic and may multiply vegetatively. We aimed to use rDNA, microsatellite, tandem repeats and transposable elements to characterize major components of the genome, and to identify chromosomes by *in situ* hybridization. As well as developing karyotypes and understanding processes of genome evolution and speciation, the results will assist breeders in characterization of interspecific hybrids. The materials included *B. decumbens*, D4 ( $2n=2x=18$ ), D62 ( $2n=4x=36$ ); *B. brizantha*, B72 ( $2n=4x=36$ ), B183 ( $2n=4x=45$ ); and *B. humidicola*, H112 ( $2n=9x=54$ ). The 45S rDNA probe (pTa71) was both highly polymorphic and showed heterozygosity. In the diploid accession D4, there were major 45S sites on the short arms of three chromosomes, as well as four (two pairs) interstitial sites of 5S rDNA (pTa794). A tetraploid accession of the same species, D62, also had three terminal 45S signals but six 5S sites in interstitial regions on short chromosome arms. H112 showed approximately 5S six signals. rDNA signals on the *B. brizantha* polyploids, also suggested heterozygosity and give evidence about genome constitution: accessions B72 and B183 had five 45S rDNA signals, ten and approximately twelve 5S sites respectively. The accession B72 had one chromosome with two sites. Condensation and expression of 45S rDNA in both interphases and prophase/early metaphases shows differences between chromosomes and accessions. Currently we are investigating additional repetitive DNA probes and more accessions to model chromosome evolution and inheritance of the rDNA sites where the preliminary data suggests the number of signals per genome varies. Allopolyploidy, deletions and structural alterations play a part in chromosome evolution in the *Brachiaria* genus.

Further information is at [www.molcyt.com](http://www.molcyt.com).

**P77. Extending chromosome genomics for wild wheat relatives: Isolation of individual chromosomes of S, U, M and C genomes from *Aegilops* by biparametric chromosome sorting**

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The S, U, M and C genomes are present in 20 out of 23 species of genus *Aegilops*. The species may provide new alleles for wheat breeding to sustain yield and quality under extreme conditions. However, the introgression of favorable traits from wild relatives to wheat is hampered by poor knowledge of their genomes and scarcity of molecular tools. The application of genomics tools to chromosomes simplifies genome analysis by reducing sample complexity and has been used in wheat to obtain shotgun sequences, to develop markers and to construct sequence-ready physical maps. Chromosome genomics relies on the ability to purify chromosomes via flow cytometric sorting. However, discrimination of individual chromosomes based on DNA fluorescence alone requires different size between chromosomes in a karyotype, a condition which is not frequent in plants. The discrimination could be improved after fluorescent labelling by *in situ* hybridization in suspension (FISHis) those DNA repeats, which are distributed unevenly among the chromosomes. Here we report on biparametric analysis and sorting chromosomes from diploid *Ae. speltoides*, *Ae. umbellulata*, *Ae. comosa* and *Ae. caudata*. Bivariate flow karyotypes were obtained after the analysis of DAPI-stained and FISHis-labelled chromosomes and the content of discriminated chromosome populations was determined. It is demonstrated that the S, U, M and C genomes could be dissected into pure fractions of individual chromosomes (1S-7S, 84.4-99.2%; 1U-7U, 88.7-98.9%; 1M-7M, 62.6-98.4%; 1C-7C, 80.09-97.9%). Purified chromosome fractions provide an attractive resource to investigate the structure and evolution of *Aegilops* genomes and deliver molecular tools for wheat introgression breeding.

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## **p78. J and St genomes for wheatgrass chromosome discrimination in wheat × *Agropyron glael* hybrid progenies using mcGISH**

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Wheatgrasses are important grasses in the temperate regions. These species are known as tertiary gene pools for wheat improvement. Species belonging to the *Thinopyrum* genus (formerly *Agropyron*) possess genes for leaf and stem rusts resistance etc., making these species suitable for improving the disease resistance of wheat. *Agropyron glael* (developed by Cicin in the 1930's) is a synthetic hybrid of two *Thinopyrum* species: *Th. intermedium* (J<sup>S</sup>St, 2n=6x=42) and *Th. ponticum* (JJJJ<sup>S</sup>J<sup>S</sup>, 2n=10x=70). Its genome composition is not known. The chromosomes of the parent species are belonging to the J, St, and J<sup>S</sup> genomes. Wheat (*Triticum aestivum* L.) line Mv9kr1 was crossed with *A. glael* in order to incorporate its biotic and abiotic stress resistance into wheat. The hybrids were backcrossed with wheat. McGISH analyses of the parent lines of *A. glael* were required for detection of alien chromosomes together with the optimization of mcGISH technique. DNA from diploid wheatgrasses (*Th. bessarabium*, J genome; *Pseudoroegneria spicata*, St genome) were used for probe labeling, and wheat DNA were used as a block during the hybridization process. The reduction of alien chromosome number was observed in the BC<sub>1</sub>-BC<sub>2</sub> progenies. A leaf rust and yellow rust resistant partial amphiploid was selected among the progenies. Nine pair of *Thinopyrum* chromosomes belonging to the J, J<sup>S</sup> and St genomes were detected in the amphiploid. Wheat/*Thinopyrum* translocations and 2-4 *Thinopyrum* chromosomes were identified in some lines. The resistant lines could be excellent genetic material for improving the disease resistance of bread wheat.

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