International Conference on Polyploidy, Hybridization and Biodiversity
Scientific committee

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Jonathan F. Wendel   Iowa State University, USA
Aleš Kovařík   Institute of Biophysics ASCR, Czech Republic
Pamela Soltis   Florida Museum of Natural History, USA
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Eric Schranz   Wageningen University, Netherlands
Miroslav Plohl   Institute Rudjer Biskovic, Zagreb, Croatia
Višnja Besendorfer   Faculty of Science, University of Zagreb, Croatia

Organizing Committee

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Vedrana Vičić   Faculty of Science, University of Zagreb
Bojan Hamer   Centre for Marine Research, Ruder Bošković Institute, Rovinj
## Program overview ICPHB2016

**Wednesday, May 11**
17:00 - 20:00 Registration and poster set-up (Hotel Lone, Conference centre)
19:00 - 22:00 Welcome reception (Hotel Lone)

### Thursday, May 12

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### Friday, May 13

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**Program**

**Wednesday, 11th May 2016**

17:00 - 20:00  Registration and poster set-up (Hotel Lone, Conference centre)
19:00 - 22:00  Welcome reception (Hotel Lone)

15:00 - 15:30  Coffee break

14:30 - 15:00  
14:00 - 14:30  
12:45 - 14:00  Lunch break

12:05 - 12:20  
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10:00 - 10:30  Coffee break

10:30 - 10:50  Dirk Albach: Is genome downsizing correlated with diversification of polyploid lineages?
10:50 - 11:10  Alexandre Péglé: The poor lonesome Brassica napus: A subgenome may not survive without its mate
11:10 - 11:30  Hanna Weiss-Schneeweiss: More than meets the eye: contrasting evolutionary trajectories in polyploids of the *Prospero annulare* complex (Hyacinthaceae)
11:30 - 11:50  Blaine Marchant: How polyploidy, transposable elements, and life history traits shape fern genome evolution
11:50 - 12:05  Itay Mayrose: PloiDB: a phylogenetic framework for investigating the evolutionary consequences of polyploidy
12:05 - 12:20  Amnel Salomon: How to detect duplicated sequences within highly polyploid species without any reference? An introduction to the use of the Pyro- and Illumina-based pipelines for *Spartina* genomics
12:20 - 12:35  Sarah Marburger: Causes of genome size expansion in neotropical catfish - unravelling the evolutionary history of the Corydoradinae
12:45 - 14:00  Lunch break

**Thursday 12th May 2016**

From 8:00  Registration (Conference centre)
8:30 - 9:00  Welcome addresses and organizational information

**Session 1: Polyploidy in deep time (long-term consequences of polyploidy)**
Chairperson: Aleš Kovarik

9:00 - 9:30  Jonathan F. Wendel: The Wondrous Cycles of Polyploidy in Plants
9:30 - 10:00  M. Eric Schranz: Ancient angiosperm MADS-Box transcription factor duplications revealed by Synteny-Network (SyntNet) phylogenomic analysis
10:00 - 10:30  Coffee break

10:30 - 10:50  Dirk Albach: Is genome downsizing correlated with diversification of polyploid lineages?
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12:45 - 14:00  Lunch break

**Session 2: Polyploidy at shallower time-scales (direct responses to polyploidy)**
Chairperson: Pamela Soltis

13:30 - 13:55  Mahaila Ainouche: Genome merger as evolutionary springboard: Insights from recurrent hybridization and polyploidy in *Spartina*
13:55 - 14:20  Natasha Glover: Genomics: Evolutionary responses and consequences of polyploidy
14:20 - 14:45  James T. Clarkson: Polyploidization through time: comparing neopolyploids and established polyploids
14:45 - 15:10  Yoav Ben Shlomo: Perturbations of meiotic recombination in neopolyploid maize
15:10 - 15:35  Natasha Glover: Genomics: Evolutionary responses and consequences of polyploidy
15:35 - 15:50  James T. Clarkson: Polyploidization through time: comparing neopolyploids and established polyploids

15:30 - 15:45  Yves Van de Peer: Of dupes and dinos: evolution at the K/Pg boundary
15:45 - 16:00  Keith Adams: Reshaped patterns of alternative splicing after allopolyploidy in *Brassica napus*
16:00 - 16:15  Natasha Glover: Genomics: Evolutionary responses and consequences of polyploidy
16:15 - 16:40  James T. Clarkson: Polyploidization through time: comparing neopolyploids and established polyploids
16:40 - 16:55  Annalesse S. Mason: Multi-genome meiosis in synthetic *Brassica* hybrids and polyploids
16:55 - 17:10  Antoine Fort: Maternal parent hypermethylation overcomes inter-ploidy and inter-species F1 seed abortion blocks in *Arabidopsis thaliana*
17:10 - 17:25  Mischa A. Olson: Perturbations of meiotic recombination in neopolyploid maize
17:30 - 18:30  Poster sessions 1 and 2 (PS1-1/PS1-25; PS2-1/PS2-19)

**Friday 13 May 2016**

**Session 1: Polyploidy in deep time (long-term consequences of polyploidy)**
Chairperson: Ovidiu Paun

9:00 - 9:25  Ovidiu Paun: Epigenetic impacts of recent allopolyploidy on ribosomal RNA genes in *Tragopogon minius* and its interpopulation hybrids
9:25 - 9:45  Terezie Mandáková: Multiple patterns of genome evolution in the *Brassicaceae*: a lesson from the polyploid-rich genus *Cardamine*
9:45 - 10:00  Elvira Horandt: The evolution of apomixis in angiosperms: a consequence of hybridity, polyploidy, or of environmental influence?
10:00 - 10:30  Coffee break

**Session 1: Polyploidy in deep time (long-term consequences of polyploidy)**
Chairperson: Hanna Weiss-Schneeweiss

10:30 - 10:50  Mario Vallejo-Marín: Interfertility and phenotype of independently originated populations of the neo-allopolyploid *Mimulus peregrinus* (Phrymaceae)
10:50 - 11:05  Thomas Wolfe: The impact of allopolyploidy on gene expression in *Dactylorhiza*
11:05 - 11:20  Clayton J. Wigler: Using synthetic spike-in RNAs to quantify expression level divergence following autopolyploidy
11:20 - 11:35  Ovidiu Paun: Molecular basis of adaptive diffusion after recurrent allopolyploidization in *Dactylorhiza*
11:35 - 11:50  Boulos Chalhoub: Deciphering the post-neolithic *Brassica napus* oilseed genome reveals the fascinating diversifying force of polyploidy
11:50 - 12:05  David Kopecký: Unexpected gene expression changes in newly developed *Festuca × Lolium* hybrids
12:05 - 12:20  Jasna Puizina: Triparental origin of triploid onion *Allium × comutum* (Clementi ex Visiani, 1842) (2n = 3x = 24)
12:20 - 12:35  Juraj Paule: Polyploidy and range expansion in the South American genus *Fosterella* (Brome-liaeae)
12:45 - 14:00  Lunch break

**Session 1: Polyploidy in deep time (long-term consequences of polyploidy)**
Chairperson: Maurine Neiman

13:55 - 14:20  James T. Clarkson: Polyploidization through time: comparing neopolyploids and established polyploids
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17:10 - 17:25  Mischa A. Olson: Perturbations of meiotic recombination in neopolyploid maize
17:30 - 18:30  Poster sessions 1 and 2 (PS1-1/PS1-25; PS2-1/PS2-19)
15:00 - 15:30  **Anna Selmecki**: Polyploidy can drive rapid adaptation in yeast

16:00  Bus departure to Bale (Histria aromatica - autochthonous aromatic herb plantation) and Svetvinčenat (conference dinner)

**Saturday 14th May 2016**

**Session 2: Polyploidy at shallower time-scales (immediate responses to polyploidy)**

**Session 3. Polyploidy in light of ecological genetics**

Chairperson: Malika Ainouche

9:00 - 9:25  **Andrew R. Leitch**: Genome size and chromosomal ploidal level influence angiosperm species biomass under nitrogen and phosphorus limitation

9:25 - 9:45  **Kirsten Bomblies**: Adaptive evolution of meiosis in response to whole genome duplication and habitat

9:45 - 10:00  **Kentaro Shimizu**: Advantages and tradeoffs of “general purpose genotype”: zinc accumulation, cold response and genome-wide homeolog expression in the self-compatible allopolyploid Arabidopsis kamchatka

10:00 - 10:30  Coffee break

**Session 3. Polyploidy in light of ecological genetics**

Chairperson: Jonathan Wendel

10:30 - 10:50  **Petr Šmarda**: The worldwide distribution of polyploid plants

10:50 - 11:05  **Magdalena Holcová**: Adaptive evolution of meiosis in diploid and polyploid Arabidopsis arenosa across its native range

11:05 - 11:20  **Julie Ferreira de Carvalho**: Dead-end trajectory of young triploid apomicts: Can transposable elements improve their adaptive potential?

11:20 - 11:35  **Thomas Dejaco**: Diploidization without reduction of genome size in an Alpine jumping bristletail

11:35 - 11:50  **Peter Schönswetter**: Evolutionary patterns, contact zones and ecological segregation in an alpine autopolyploid complex

11:50 - 12:05  **Jun Sese**: Genome-wide statistical detection of hyper-biased homeologs in allopolyploid and their changes after hybridization

12:05 - 12:20  **Warren Albertin**: Hybridization in yeast is associated with phenotypic novelty for life-history, metabolic and proteomic traits

12:20 - 12:35  **Jeanette Whitton**: Disentangling the causes of differences in distribution of diploid sexual and autopolyploid apomictic Easter daisies (Townsendia hookeri: Asteraceae)

12:45 - 14:00  Lunch break

14:00 - 14:30  **Maurine Neiman**: Sex, phosphorus & polyploidy: Can nutrient costs of nucleic acids contribute to ploidy and sex polymorphism in nature?

14:30 - 15:00  **Levi Yant**: Borrowed alleles and convergence: serpentine adaptation in the face of inter- and intraspecific gene flow

15:00 - 15:30  Coffee break

15:30 - 15:45  **Laura Bankers**: Influences of ploidy level and reproductive mode on patterns of adaptive molecular evolution in a New Zealand freshwater snail

15:45 - 16:00  **Kyle McElroy**: Evaluating the dynamics of transposable element evolution in non-hybrid polyploids

16:00 - 16:15  **Veit Herklotz**: The fate of ribosomal RNA genes in spontaneous dogrose hybrids (Rosa L. sect. Caninae (DC.)

16:15 - 16:30  **Helene Rousseau**: Polyploidy and phenotypic novelty: Phylogenetic context of DMSP (dimethylsulfiniopropionate) biosynthesis in Spartina (Poaceae, Chloridoideae)

16:30 - 16:45  **Leen Leus**: Are tetraploid roses better resistant to stress compared to diploids?

17:00 - 18:00  **Poster session 3 (PS3-1/PS3-35)**

18:00  Poster awards and closing ceremony
ABSTRACTS
INVITED LECTURES
The Wondrous Cycles of Polyploidy in Plants

Jonathan F. Wendel
Department of Ecology, Evolution, & Organismal Biology, Iowa State University Ames, IA 50011, USA (jfw@iastate.edu)

One of the signal realizations of the genomics era is that all flowering plants are multiply polyploid, varying only in the number and relative antiquity of whole genome doubling events. Gossypium, the cotton genus, exemplifies this recurrent, episodic polyploidization, and even includes neoallopolyploids that originated following a biological reunion 1-2 MYA of divergent diploids from different hemispheres. This serendipitous merger generated a spectrum of genomic responses, which serve as illustrative models for understanding evolutionary genomic processes following polyploidy. These include gene silencing, intergenomic gene conversion, and genome-wide disruption and modification of ancestral expression patterns. Allopolyploid formation induces massive alteration in gene expression and complex transcriptomic responses, and novel cytonuclear interactions. Duplicate gene expression changes are temporally partitioned into alterations arising immediately as a consequence of genomic merger and secondarily as a result of long-term evolutionary transformations in duplicate gene expression, the latter reflecting long-term evolutionary forces such as duplicate gene neofunctionalization and subfunctionalization. Recurrent polyploidy in plants is followed by many diverse genomic processes, occurring over time scales ranging from several generations to millions of years, that collectively lead to genome downsizing, genomic fractionation, and full chromosomal diploidization. A major challenge is to connect these long and short-term processes to adaptive evolution and the generation of biodiversity.

Keywords: Gossypium, genomic responses, microevolution and macroevolution, allopolyploidy, genome evolution

Ancient angiosperm MADS-Box transcription factor duplications revealed by Synteny-Network (SynNet) phylogenomic analysis

Tao Zhao and M. Eric Schranz
Biosystematics Group, Plant Sciences, Wageningen University, P.O. Box 16, 6700AP The Netherlands (eric.schranz@wur.nl)

Conserved genomic context, or synteny, can provide valuable information about the evolution of genes and genomes. However, frequent polyploidy and chromosomal rearrangements in plants complicate such analyses, particularly when analyzing large multi-gene families across broad phylogenetic groups. We have developed a novel approach for interpreting phylogenomic data called Synteny-Networks (Syn-Nets) that takes genomic synteny between and within target genomes and uses network analysis tools for visualization. We use the completed genomes of fifty-one plant species to visualize genes by syntenic links allowing us to infer gene duplications resulting from polyploidy, ancient tandem-arrangements and transpositions. We present our analysis for the important MADS-box transcriptional regulator gene family of plants. By doing so, we can identify patterns not inferred by phylogenetic analyses including several very ancient Type II tandem-gene clusters (e.g. SEP-SQUA/TM8/FLC) that predate the diversification of angiosperms, lineage specific gene transpositions (such as AP3, PI and FLC homologs in Brassicales) and highly conserved Type I and MIKC* clusters involved in embryo and pollen development. These new insights provide new hypotheses about the function and evolution of MADS developmental regulatory genes and thus the evolution of plant phenotypes.

Keywords: synteny, gene duplication, polyploidy, MADS-Box, network analysis
Polyploidy and evolutionary novelty across microevolutionary and macroevolutionary timescales

Pamela S. Soltis and Douglas E. Soltis

Museum of Natural History, University of Florida, USA (psoltis@flmnh.ufl.edu)

Polyploidy has long been recognized as a source of genetic, biochemical, and evolutionary novelty. Duplicate gene pairs in allopolyploids result in novel enzyme phenotypes, which may in turn have downstream phenotypic effects, relative to the diploid progenitors. Beyond the novel phenotypes conferred by gene duplication, nucleotypic effects due merely to the increased size of the nucleus may be manifested as novelty in physiological, phenological, anatomical, morphological, and other traits in both auto- and allopolyploids. Extensive variation in gene content, gene expression, and karyotype within and among populations of naturally occurring and recently formed (within the past 80 years) allopolyploids in Tragopogon (Asteraceae) produces mosaics of genetically and phenotypically variable traits in novel combinations. This novelty that arises on a microevolutionary scale may ultimately lead to a unique trait that drives evolutionary diversification at the macroevolutionary level. Recent evidence indicates that many WGDs in angiosperms are associated with increased rates of diversification and seem to be associated with one or more key innovations. We suggest that the novel genotypes generated in the early stages of polyploid evolution may eventually be manifested as key innovations that drive diversification associated with ancient WGD.

Keywords: polyploidy, novelty, phenotypes, key innovations, diversification

Genome merger as evolutionary springboard: Insights from recurrent hybridization and polyploidy in Spartina

Malika Ainouche1, H. Chelaifa1, Mathieu Rousseau-Gueutin1, Julien Boutte1, Hélène Rousseau1, Julie Ferreira de Carvalho1, A. El-Amrani1, Armand Cavet-Radet1, Aleš Kovarík2, Ilija Leitch1, Andrew Leitch1, Abdelkader Ainouche1 and Armel Salmon1

1University of Rennes1, UMR CNRS 653 Ecobio, Rennes, France (malika.ainouche@univ-rennes1.fr); 2UMR 1349 IGEP, INRA Le Rheu, France; 3Current address: Dept. Terrestrial Ecology, Netherlands Institute of Ecology, Wageningen, The Netherlands; 4Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno; 5Jodrell Laboratory, Royal Botanic Gardens, Kew, UK; 6Queen Mary University of London, UK

Reticulate evolution has long attracted the interest of evolutionary biologists who documented the widespread and significant impact of genetic exchanges and the associated conceptual implications. In the recent years, data accumulated, revealing the vast array of responses to genome merger in terms of diverged genome interactions, gene expression regulation, and physiological, morphological or ecological traits. As datasets accumulate from different models, different historical and experimental genetic contexts, a key question is whether certain rules govern the evolutionary changes entailed by hybridization and allopolyploidy, and what is actually happening in natural populations. The polyploid genus Spartina (Poaceae, Chloridoideae) offers many opportunities to explore such questions, thanks to recurrent hybridization and historically well-documented allopolyploid speciation events. Of particular interest is the recent and independent formation of two natural hybrids between the East-American hexaploid Spartina alterniflora (introduced to Europe) and the Euro-African hexaploid Spartina maritima. These two sterile hybrids, formed during the 19th century in England (S. x townsendii) and in France (S. x neyrautii) respectively, have the same maternal (S. alterniflora) and paternal (S. maritima) parents, and exhibit marked morphological differences. Genome duplication in S. x townsendii resulted in the fertile allododecaploid species that has now colonized coastal saltmarshes of several continents. This system is then particularly suited to explore the “replayed tape” of reticulate evolution. We will examine (1) whether repeated genome merger from the same parental species had similar genomic, transcriptomic and physiological consequences in the two independently formed hybrids and (2) the respective impacts of genome merger and hybrid genome duplication in the evolutionary dynamics of the new allopolyploid species S. anglica. Results will be considered in the context of superimposed genome duplications in Spartina, and the ecological species abilities.

Keywords: Recent hybridization, genome shock, transcriptome evolution, phenotypic changes, Spartina
Epigenetic impacts of recent allopolyploidy on ribosomal RNA genes in *Tragopogon mirus* and its interpopulation hybrids

Ales Kovarik1, Roman Matyasek2, Eva Dobešová2, Andrew R Leitch1, Douglas E. Soltis2 and Pamela S. Soltis4

1Laboratory of Molecular Epigenetics, Academy of Sciences of the Czech Republic, v.v.i., Institute of Biophysics, CZ-61265 Brno, Czech Republic (kovarik@ibp.cz; matyasek@ibp.cz; dobesova@ibp.cz); 2School of Biological Sciences, Queen Mary University of London, E1 4NS, UK (a.r.leitch@qmul.ac.uk); 4Department of Biology, University of Florida, Gainesville, FL 32611, USA (psoltis@flmnh.ufl.edu).

Short term consequences of hybridisation and allopolyploidy are best studied in species that formed recently within past several hundred years. It is generally accepted that over time, allopolyploid populations of independent origin evolve their own expression and epigenetic patterns. One classic example of epigenetic reprogramming in allopolyploids is the occurrence of nuclear dominance among interspecific hybrids including allopolyploids. During nuclear dominance one parental array of tandemly arranged genes is expressed while the other(s) are frequently silenced by epigenetic mechanisms. Recently (~80 years ago) and independently formed populations of *Tragopogon mirus* (2n=4x=24) allotetraploid composed of parental *T. dubius* (2n=2x=12) and *T. porrifolius* (2n=2x=12) genomes show variable population-specific patterns of rDNA expression that appear to be stably inherited over several generations of individual lineages. However, interpopulation crosses resulted in an immediate reprogramming of expression states mostly towards the *T. dubius* alleles. These results explain why the *T. dubius* dominance is more frequent than the *T. porrifolius* dominance or codominance among natural populations. Newly formed rDNA epialleles showed correlation between expression, hypomethylation of RNA Pol I promoters and chromatin decondensation. In synthetic lines, nuclear dominance was stable in leaf but not root tissue. Such developmental instability is only rarely seen in natural populations indicating that several generations might be needed to fix epigenetic states in individual tissues of a recently formed allopolyploid species.

Keywords: Tragopogon, rDNA, population epigenetics, nuclear dominance

Multiple patterns of genome evolution in the Brassicaceae: a lesson from the polyploid-rich genus Cardamine

Terezie Mandáková1, Ales Kovarík1, Karol Marhold3 and Martin A. Lysak1

1CEITEC - Central European Institute of Technology, Masaryk University, Brno, Czech Republic (terezie.mandakova@ceitec.muni.cz); 3Institute of Biophysics, AS CR, Brno, Czech Republic; 4Institute of Botany, SAS, Bratislava, Slovakia

Cardamine (bittercress) is one of the largest Brassicaceae genera (200 spp.). The genus exhibits a large karyological diversity (2n = 16 to c. 256). Due to the feasibility of comparative chromosome painting and genomic in situ hybridization in the Brassicaceae, we documented both recurrent and deviating patterns of genome evolution in Cardamine polyploids:

(i) The North American *C. cordifolia* with a triploid-like chromosome number (2n = 24) is a diploidized tetraploid. The ancestral tetraploid chromosome number (2n = 32) was reduced to a triploid-like number through four terminal chromosome translocations (“chromosome fusions”). The *C. cordifolia* genome provides valuable insights into mechanisms of post-polyploidy rediploidization in plants.

(ii) In Europe, some tetraploid (2n = 32) populations of *C. pratensis* are on the way to decrease their chromosome number by “chromosome fusions” (2n = 30 and 28). On the contrary, some diploid (2n = 16) populations of *C. pratensis* contain hyperdiploid plants with one to four additional chromosomes (2n = 17, 18, 19, and 20).

(iii) We elucidated independent origins of several European and Asian tetra- and octoploid (2n = 64) species of the *C. flexuosa* complex through hybridization events involving three diploid progenitor species.

(iv) In Cardamine, hybridization and polyploidization is ongoing. We reconstructed the origin of the triploid hybrid *C. ×insueta* (2n = 24, RRA) through hybridization between *C. amara* (2n = 16, AA) and *C. rivularis* (2n = 16, RR) c. 100 years ago. Hybridization involving *C. ×insueta* and the hypotetraploid *C. pratensis* (2n = 30, PPPA) resulted in the origin of the hypohexaploid *C. schultii* (2n = 46, PPPPPRA); This shows how a semifertile triploid hybrid can promote the origin of trigeneric allopolyploids.

Keywords: polyploidy, inter-species hybridization, chromosome rearrangements, genomic in situ hybridization, comparative chromosome painting, cytogenetics, molecular phylogenetics, Brassicaceae
**Polyploid Ambystoma salamanders at the interface of environment, genomics, and ecology**

Rob Denton1,2, Katherine Greenwald3 and Lisle Gibbs1,2

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Unisexual Ambystoma salamanders are an all-female vertebrate lineage with the potential to add and exchange genomes from males of diploid, sexual salamander species. The result of this reproductive mode is a staggering diversity of genome compositions within unisexual salamanders: from triploid to pentaploid with genomes potentially representing five different species. While many unisexual animal lineages are considered “evolutionary weeds”, this unisexual lineage is currently the oldest recognized in vertebrates (~5 mya) and unisexuals are abundant across a large area of eastern North America. This presentation summarizes this unique vertebrate system and presents several ongoing projects that examine differential gene expression in unisexual genomes, compare dispersal abilities on agricultural landscapes, and quantify the niche overlap between unisexuals with different genome composition.

Keywords: amphibian, mitonuclear mismatch, ecological niche modelling, gene flow, dispersal

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**Multiple whole genome duplications during the evolution of hexapods**

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Polyploidy or whole genome duplication (WGD) is a major contributor to genome evolution and diversification. Although polyploidy is recognized as an important component of plant evolution, it is generally considered to play a relatively minor role in animal evolution. Ancient polyploidy is found in the ancestry of some animals, especially fishes, but there is little evidence for ancient WGDs in other metazoan lineages. Here we use recently published transcriptomes and genomes from more than 150 species across the insect phylogeny to investigate whether ancient WGD occurred during the evolution of Hexapoda, the most diverse clade of animals. Using gene age distributions and a recently developed phylogenomic approach, we find evidence for 31 ancient WGDs during insect evolution. Similar to flowering plants, our analyses place WGDs near the origin of many major clades, such as polyneopterous insects, Coleoptera and Lepidoptera. Together with recent research on plant evolution, our hexapod results suggest that repeated rounds of WGD contributed to the conquest of terrestrial ecosystems by two of the most diverse lineages of eukaryotes on Earth.

Keywords: paleopolyploidy, insects, phylogenomics
**Polyploidy can drive rapid adaptation in yeast**

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Many pathogenic and non-pathogenic fungal species exhibit a variety of ploidy states, including polyploidy. Polyploid fungi often arise through parasexual mating or genome duplication followed by cytokinesis failure. Environmental factors also drive the formation of polyploid fungi in vitro and in vivo, including treatment with the antifungal fluconazole. We used in vitro evolution and mathematical modeling to determine the impact of polyploidy on adaptation. We generated an isogenic ploidy series in the budding yeast *Saccharomyces cerevisiae* and found that polyploid (tetraploid) cells adapt faster than haploid or diploid cells in the poor carbon source raffinose (Selmecki et al. Nature 2015). Our mathematical modelling indicated that this rapid tetraploid adaptation was driven by higher rates of beneficial mutations and increased fitness effects of the acquired mutations. We performed extensive whole genome sequencing, comparative genomics and phenotypic analyses to test whether these theoretical estimates could be upheld. Indeed, we identified more mutations and a greater diversity of mutations in the evolved tetraploid clones compared to the haploid and diploid evolved clones. Furthermore, some of the mutations were shown to provide tetraploid-specific fitness gains, including chromosome aneuploidy, concerted chromosome loss, and specific point mutations. Increased levels of genome instability were evident: only tetraploid-evolved clones underwent ploidy changes, acquired aneuploidy, and acquired segmental aneuploidies at transposable elements. These results provide quantitative evidence that in some environments polyploidy can accelerate evolutionary adaptation, and that the increased genome instability may be important to this process. We are currently using in vitro evolution to elucidate what mechanisms cause high chromosomal instability in polyploid cells. Our preliminary data suggest that many polyploid evolved cells acquire genome-stabilizing mutations during long-term evolution experiments. The mechanistic role of these non-synonymous, gain-of-function mutations on polyploid genome stability and adaptation will be discussed.

*Keywords: polyploid yeast, in vitro evolution, aneuploidy, genome instability*

**Genome size and chromosomal ploidal level influence angiosperm species biomass under nitrogen and phosphorus limitation**

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Angiosperm genome sizes (GS) range c. 2,400-fold, but typically have small values despite multiple rounds of polyploidy in the ancestry of many lineages. Potentially there is selection against large genomes. We explore whether a source of selection is nutrient availability, particularly phosphorous (P) and nitrogen (N), since large genomes are costly to build in terms of N and P needed to make nucleic acids. To test the hypothesis that plant biomass production is dependent on interactions between GS, chromosomal ploidal level and N and P availability, we analysed the impact of different nutrient regimes on plant growth at the world’s longest continuously running ecological experiment, Park Grass (Rothamsted, UK), established 1856. We also compared our data with Grime’s plant C-S-R strategies. We show that biomass is indeed influenced by ploidal level and C-value, and that when N and/or P are in abundance, there is a selection for species that are polyploid, have a larger C-value and are competitors (C-strategy). These results are consistent with the long-term Rengen Grassland experiment published by Šmarda et al. (2013).

We have extended these analyses to consider the Silwood Park (UK) field experiment, set up over the last 24 years. These plots were also established to see the effects of fertilizer on species composition, but with a design that allows the effects of rabbit, insect and mollusc herbivory on biomass to be taken into account. Here we show that rabbits cause the relative abundance of species with large genomes to increase.

*Keywords: ecology, polyploidy, C-value, nitrogen, phosphate, herbivory, selection, competition*
Adaptive evolution of meiosis in response to whole genome duplication and habitat

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Meiosis is essential for fertility of sexual eukaryotes and its core structures and progression are conserved across kingdoms. Nevertheless, meiotic proteins are often less conserved in primary sequence than we might expect, and sometimes show evidence of having experienced directional selection. Why? What challenges does meiosis face that might cause it to evolve adaptively and how does this alter the system? Evidence from a range of studies shows that two important factors can challenge the stability of meiosis and drive evolutionary responses: whole genome duplication and environmental factors, especially temperature. Our group seeks to understand how meiosis evolves in response to challenges, that is, what its evolutionary plasticity is within the constraints of being an essential and complex structural progression. We use Arabidopsis arenosa, which occurs naturally as an autotetraploid and a diploid, and where both cytotypes have colonized a range of habitats. In a genome scan for adaptation to whole genome duplication, we found that eight interacting meiotic proteins critical for axis formation and synapsis show strong evidence of having been under selection in the tetraploid arenosa. This is associated with a reduction in crossover number, and a greater tendency for terminal localization of chiasmata. More recently, we found that two of the same genes under selection in tetraploids, also show strong evidence of having been under selection in a diploid A. arenosa lineage. This lineage colonized a warmer lowland habitat (the ancestral form is found in cooler mountain environments), and we have evidence that this lineage evolved greater temperature tolerance of meiosis. Distinct alleles of the same genes were under selection after both whole genome duplication and habitat colonization, and thus have twice come under selection for apparently distinct reasons; does this suggest evolutionary constraint on the system? Does it indicate that the same processes are challenged by distinct stresses? The finding that the genes that came under selection in both lineages are known to interact also highlights the possible need for co-evolution of interacting partners in meiotic evolution, which may be more broadly relevant to the evolution of proteins that participate in large complexes.

Keywords: polyploidy, adaptation, evolution, genomics, meiosis, co-evolution

Sex, phosphorus & polyploidy: Can nutrient costs of nucleic acids contribute to ploidy and sex polymorphism in nature?

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Sexual reproduction is both extremely common and very costly, suggesting that there are benefits associated with sex that counter these costs. Here, we address whether phenotypic costs associated with polyploidy, itself very often linked to asexuality, might contribute to the maintenance of sex and ploidy polymorphism in nature. In particular, we will focus on using a New Zealand freshwater snail system to evaluate connections between costs of polyploidy associated with building additional chromosomes when the environmental availability of the nutrients (e.g., phosphorus) of which nucleic acids are comprised is low and the maintenance/distribution of diploid sexual vs. polyploid asexual snails.

Keywords: Potamopyrgus antipodarum, asexual reproduction, triploidy, tetraploidy
Borrowed alleles and convergence: serpentine adaptation in the face of inter- and intraspecific gene flow

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Serpentine barrens represent extreme hazards for plant colonists, being characterized by a perfect storm of high porosity leading to drought, lack of essential mineral nutrients, and phytotoxic levels of metals. Nevertheless, nature forged populations adapted to these challenges. Here, we use a population-based evolutionary genomic approach coupled with elemental profiling to assess how an autotetraploid lineage of Arabidopsis arenosa adapted to a multi-hazard serpentine habitat in the Austrian Alps. We first demonstrate that serpentine-adapted plants exhibit dramatically altered elemental accumulation levels in common conditions and then resequence 24 autotetraploid individuals from three populations to perform a genome scan. We find evidence for highly localized selective sweeps that point to a polygenic, multi-trait basis for serpentine adaptation. Comparing our results to a previous study of independent serpentine colonizations in the closely related diploid A. lyrata in the UK and US, we find the highest levels of differentiation in 11 of the same loci, providing candidate alleles for mediating convergent evolution. This overlap between independent colonizations in different species suggests a limited number of evolutionary strategies are suited to overcome the multiple challenges of serpentine adaptation. Interestingly, we detect footprints of selection in A. arenosa in the context of substantial gene flow from nearby off-serpentine autotetraploid populations of A. arenosa as well as from autotetraploid A. lyrata. In several cases alleles exhibiting the strongest selective sweep signatures appear to have been introgressed from A. lyrata. This suggests that migrant alleles may have been shared freely between these autotetraploids and thus facilitated adaptation of A. arenosa to a very harsh, multi-hazard environment.

Keywords: polyploidy, gene flow, hybridisation, repeated evolution
LECTURES
Is genome downsizing correlated with diversification of polyploid lineages?

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Polyploidy is known to be one of the major mechanisms of speciation in angiosperms. However, a number of analyses have demonstrated that the extinction rate of polyploids is higher than that of diploids, leaving us with the question of what factors influence the long-term success of a polyploid lineage. Polyploidy principally should lead to a genome containing the sum of the DNA of its parents. However, the loss of DNA in polyploids is known to occur from the first generation, a phenomenon called genome downsizing. Genome size changes can affect the phenotype and, thus, be selected for, although a direct link with diversification has not been demonstrated, yet. Here, we use phylogenetic analyses of 128 species of the genus Veronica (ca. 30% of the genus) and 259 species of the genus Rhododendron (ca. 25% of the genus) to infer the evolution of genome size and patterns of diversification. Using phylogenetic analyses of genome size evolution and diversification analyses with the software BAMM in these genera, we demonstrate that genome downsizing precedes diversification of polyploid lineages. Comparison with data from other genera and theoretical considerations give credit to the idea that this may be a more general phenomenon. Whereas the evolution of key innovations may be a non-exclusive alternative explanation for diversification success, genome downsizing may be a prerequisite for diversification. We discuss possible explanations for this relationship and outline future studies required to investigate the underlying reason for the success of lineages exhibiting genome downsizing.

Keywords: BAMM, diversification, genome downsizing, species radiation

The poor lonesome Brassica napus A subgenome may not survive without its mate

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Whole genome duplication (WGD) is recognized as a major force in plant evolution and speciation, resulting from both structural and/or functional alterations. These modifications occur in the first few generations following WGD events and continue during the lifespan of the polyploid species. To study the long-term evolutionary impact of allopolyploidy, previous studies have almost exclusively compared a present allotetraploid species with its current diploid progenitors. However, the diploid progenitors have independently evolved since the allotetraploid formation, preventing to fully understand its evolution. To circumvent this problem, another approach corresponding to the subgenome extraction, offers the unique opportunity to elucidate the long-term evolution in an allopolyploid context. Presently, one of the best model to determine the role of allopolyploidy in genome evolution corresponds to oilseed rape (Brassica napus, AAC, 2n=38), formed about 8,500 years ago from a cross between B. rapa (AA, 2n=20) and B. oleracea (CC, 2n=18). To identify the structural rearrangements that occurred since this allotetraploid formation, we performed two different strategies to extract its diploid AA component. For the 1st strategy, a cross between B. napus var. Darmor and B. rapa was realized to produce a triploid AAC F1 interspecific hybrid. This triploid was then backcrossed three times to B. napus and the AAC progeny were selected at each generation in order to obtain an almost pure B. napus A subgenome. We finally attempted to separate the AA and C components by selfing the last AAC plant but no AA plant was obtained. For the 2nd strategy, a cross between the initial AAC F1 hybrid and B. rapa was performed to generate AA plants. After four cycles of selfed and also backcrossed to B. napus, AA plants mainly containing the B. napus A subgenome were obtained. Using the 60K SNP Illumina microarray and the genome sequence of B. napus var. Darmor, we assessed the genomic structure of the last AA plants produced. They presented a lower proportion of B. napus A subgenome extracted than expected and some introgressions from the C subgenome. Our analyses revealed that the genomic regions from B. rapa conserved in diploid AA plants were not randomly distributed along the A subgenome, suggesting that the A subgenome may not survive without the C subgenome, most presumably due to the loss of one homoeologous copy since the formation of the allotetraploid B. napus.

Keywords: Brassica napus, subgenome extraction, evolution, structural rearrangements
More than meets the eye: contrasting evolutionary trajectories in polyploids of the _Prospero autumnale_ complex (Hyacinthaceae)

Hanna Weiss-Schneeweiss, Tae-Soo Jang, Khatere Emadzade, Jiri Macas, Andrew R. Leitch and John Parker

Comparative data on the evolution of polyploid genomes suggest that genomic evolution in polyploids is more dynamic than that of their diploid counterparts. The circum-Mediterranean _Prospero autumnale_ complex exhibits high levels of all types of numerical and structural variation. It comprises at least four distinct diploid cytotypes (AA, B^1B^1, B^2B^2, and B^1B^2), each with a unique combination of basic chromosome number (x = 5, 6, and 7), genome size, karyotype structure, and repetitive DNAs distribution. Three diploid genomes (B^1, B^2, and A) gave rise to several polyploid lineages exhibiting vastly different rates of genomic evolution. _Prospero_, thus, presents an exceptional system to study the evolutionary trajectories and fates of genomes following polyploidisation. Autopolyploids, only found in genome B^1, are genomically stable, similarly to allopolyploids of A/B^1 origin. Allotetraploids of B^2/B^2 genomes encompass exceptionally high levels of numerical variation ("compensating aneuploidy"); 2n = 4x = 25-28), accompanied by structural and repeat composition variation. This seemingly chaotic variation can be explained by four allotetraploid types being involved successive cycles of hybridization. Specifically, numerical convergence of primary allotetraploids facilitates genome-wide modifications resulting in genetically balanced but unique genomes. These, in turn, form genomically stable secondary allotetraploids that can partake in further hybridizations. In contrast to structured evolution in diploids and tetraploids, hexaploid genomes experience intra- and inter-individual aneuploidy, chromosome copy number imbalances and a myriad of other genomic rearrangements. B-chromosomes and supernumerary chromosomal segments (SCSs), frequent in _Prospero_, exhibit different evolutionary patterns at varying ploidy levels. High incidence of chromosomal changes in all cytotypes of _P. autumnale_ strongly contrasts with the relative stability of their genome-wide repeat composition and with the morphological uniformity of _Prospero_. The data suggest chromosomal restructurings both on the diploid and particularly on the polyploid level to be a major mechanism of diversification in _Prospero_. The study was supported by Austrian Science Fund (FWF P21440) to HWS.

**Keywords:** chromosomal restructuring, cytotypes, genome evolution, origin, repetitive DNAs

How polyploidy, transposable elements, and life history traits shape fern genome evolution

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Our understanding of plant genomes has increased immensely within the last decade. Sequenced genomes are now available for every major green plant clade, from chlorophytic and streptophytic algae to a vast array of flowering plants - that is, every clade except the monilophytes (ferns). Ferns are the most biodiverse clade of land plants after the angiosperms and sister group to the economically significant seed plants. Notorious for large genomes (~12 Gb) and numerous chromosomes (~3x more than the average angiosperm), fern genomes are largely unexplored despite their evolutionarily significant position as a reference group for analyzing ancestral versus derived genomic and genetic characters in the seed plants. Using transcriptomic, genic, and chromosome fluorescent in situ hybridization techniques, we are delving into the genome of _Ceratopteris richardii_, a fast-growing tropical aquatic homosporous fern with a haploid genome size of 11.26 Gb and chromosome number of n = 39. We will present our results regarding gene specificity in the gametophytic and sporophytic life stages, gene density, and the role of ancient polyploidy, transposable elements, and small-scale duplications in the evolutionary genomics of this species. This research will provide novel perspectives on the characteristics and dynamics of genomes from this major clade, while also providing crucial resources for broader comparative genomic, phylogenetic, and developmental studies between ferns and seed plants. Such broad genomic comparisons will also yield new insights into the genome evolution of euphyllophytes as a whole.

**Keywords:** ferns, genomics, paleopolyploidy, transcriptomics
**PloiDB: a phylogenetic framework for investigating the evolutionary consequences of polyploidy**

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Polyploidy is widely recognized as a key feature in plant organismal diversity. A surge of research over the last decade has revealed an extensive history of polyploidization events in the genomes of most plant lineages. However, owing, in part, to the absence of large comparative data, most empirical studies are confined to particular geographic regions and/or narrow taxonomic space. As such, treatment of central theoretical hypotheses regarding various aspects of polyploidy evolution within a statistically robust phylogenetic framework is generally absent. Here, I will describe PloiDB, the plant ploidy-level database. PloiDB aims to provide the dated history of ploidy transitions for each plant species having cytological and/or sequence data. This phylogenetic benchmark should provide researchers with the ability to distinguish broad convergent trends, if such exists, from species-specific idiosyncrasies. Polyploidy transitions are inferred based on variations in chromosome number using chromEvol, a probabilistic method for inferring the patterns of chromosome-number change along a phylogeny, combined with novel automatic procedures for phylogeny reconstruction. In its current implementation, PloiDB contains inferences across 2,000 plant genera, encompassing more than 50,000 species, and concentrate on ploidy transitions occurring relatively recently (i.e., those occurring since divergence from the common ancestor of each genus examined). I will exemplify the use of this database with several ongoing studies.

**Keywords:** database, chromEvol, phylogenetic methods

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**How to detect duplicated sequences within highly polyploid species without any reference? An introduction to the use of the Pyro- and Illu-haplotyper pipelines for Spartina genomics**

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Discriminating duplicated genes within polyploid species is critical to understand the evolution of orthologous genes. While recent advances in genome sequencing and assembling allowed the accumulation of genomic and genetic data in both model and non-model species, detecting genes duplicated by polyploidy (homeologs) remains challenging for many polyploid species. This will be explored in genus Spartina, where hybridization and genome duplication are particularly well-illustrated. The most complex genome (namely the invasive allo-dodecaploid *Spartina anglica* arose recently in Europe c.a. 150 years ago, by genome duplication of the homoploid hybrid *S. x townsendii* resulting from an interspecific cross between the introduced *S. alterniflora* (2n=6x=62) and the European native *S. maritima* (2n=6x=60). In the perspective of understanding the role of reticulate evolution and whole genome duplications in the phenotypic plasticity and adaptation of the recently formed *Spartina anglica*, we developed bioinformatic approaches and tools for detecting the different putative orthologous copies originating its parents (duplicated homeologs). Our approach is relying on the detection of homologous sequences (or reads) for each species. In this prospect, after aligning (mapping) each species’ NGS reads to reference sequences, polymorphic sites are first detected and reads sharing the same polymorphisms are assembled as haplotypes. Parental and hybrid species haplotypes are then aligned, parental haplotypes serving as reference sequences to assign hybrid haplotypes a parental origin. This method is applicable for detecting any duplicated copies within and between genomes from sets of (genomic or transcriptomic) NGS reads and was translated as Galaxy modules including dedicated parameters (for mapping, SNP discovery, haplotype assembly) for fitting to the NGS technology used, the read depth obtained, and the investigated species of interest. These approaches will allow exploring the level of homeologous gene retention following the successive genome duplication events, their relative contribution to the transcriptome and proteome and to link polyploid genome dynamics to ecologically relevant traits in the recently formed invasive polyploid *Spartina* species.

**Keywords:** polyploidy, NGS, duplicates, transcriptome, bioinformatics
Causes of genome size expansion in neotropical catfish - unravelling the evolutionary history of the Corydoradinae

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Corydoras catfish are a diverse sub-family of neotropical catfish with more than 170 species described to date. The Corydoradinae display a wide range of different colour patterns. Many species co-occur in sympatric communities and participate in multiple instances of Müllerian mimicry. In addition, the family is characterized by an impressive range of genome sizes, with species containing between 0.5 pg and 4.8 pg of DNA. Generally, more derived species have larger genome sizes than basal species. The increase of DNA along the phylogeny could be explained by three Whole Genome Duplication (WGD) events, which could have facilitated the radiation and evolution of this family. However, other mechanisms such as tandem duplications or Transposable Elements (TEs) could also be responsible for an increase in genome size. In the absence of reference genomes, we selected a reduced representation sequencing approach to undertake a genome-wide analysis to test a diverse range of hypotheses. Here, we present a Restriction Site Associated DNA (RAD) sequencing data set for eleven species encompassing all lineages of Corydoras including an outgroup species. The RAD data set confirmed genome-wide shifts in duplicate, multi-haplotype regions across the Corydoradinae, and indicates that several species from higher lineages are functionally polyploid, whereas species that underwent earlier WGDs have largely rediploidized and are likely paleopolyploids. An increase in paralogous genes was noted, with Gene Ontology suggesting that gene retention in the Corydoradinae mirrors previously described retention in Tetraodon following the fish-specific genome duplication in the Teleostei. Intriguingly, the RAD data also identified a substantial expansion of Transposable Elements (TEs), driven by a DNA TE superfamily (Tc1-Mariner). This expansion significantly contributed to the genome size variation, though to a lesser degree than the WGD events identified.

Keywords: catfish, transposable elements, paleopolyploidy, genome expansion

Of dups and dinos: evolution at the K/Pg boundary

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Fifteen years into sequencing entire plant genomes, more than 30 paleopolyploidy events could be mapped on the tree of flowering plants (and many more when also transcriptome data sets are considered). While some genome duplications are very old and have occurred early in the evolution of dicots and monocots, or even before, others are more recent and seem to have occurred independently in many different plant lineages. Strikingly, a majority of these duplications date somewhere between 55 and 75 million years ago (mya), and thus likely correlate with the K/Pg boundary. If true, this would suggest that plants that had their genome duplicated at that time, had an increased chance to survive the most recent mass extinction event, at 66 mya, which wiped out a majority of plant and animal life, including all non-avian dinosaurs. I will review several processes, both neutral and adaptive, that might explain the establishment of polyploid plants, following the K/Pg mass extinction.

Keywords: whole genome duplication, K/Pg boundary, angiosperm evolution, adaptation
Reshaped patterns of alternative splicing after allopolyploidy in *Brassica napus*

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In allopolyploids, when two genomes are amalgamated into a common nucleus, the native cis-architectures of genes are placed in a new hybrid and polyploid trans-environment, releasing novel expression profiles with respect to parental patterns. In conjunction with changes in transcript levels, transcript splicing patterns undergo changes to create novel forms. We studied the degree to which these changes differ from the parental splicing patterns and the degree to which they are reciprocated between individual instances of allopolyploidy. Using RNA-seq, we analyzed the transcriptomes of four *Brassica napus* lines and from the *B. rapa* and *B. oleracea* parents. The results revealed myriad qualitative and quantitative differences in alternative splicing events among the genotypes. Several thousand alternatively spliced transcript forms were gained or lost in the polyploids. We show that allopolyploidy generates a variety of splicing landscapes from the same genetic merger, revealing both plastic and static changes in splicing, and our results complement studies of gene expression levels and patterns in allopolyploids. Our results show that changes in splicing patterns are a common occurrence after allopolyploidy and they contribute to the “transcriptome shock” experienced by newly formed allopolyploids.

Keywords: gene expression, alternative splicing, *Brassica napus*

Homoeologs: What are they? How to infer them? What are they useful for?

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Many species, particularly plants, have undergone at least one round of polyploidization in their evolutionary history. Generally, genes duplicated by allopolyploidy (whole genome duplication via hybridization) are commonly referred to as “homoeologs”, though this term has not always been used precisely or consistently in the literature. With several allopolyploid genomes sequencing projects underway, there is a pressing need for computational methods for homoeology inference. Here, we review the definition of homoeology in historical and modern contexts and propose a unifying, evolutionarily precise and testable definition. We then highlight the connection between homoeologs and orthologs with, homoeologs suffering from the same common misconceptions that afflict orthologs: the notion that homoeologs are necessarily at a one-to-one relationship, or that they have remained strictly in their ancestral positions since speciation. Next, we survey experimental and computational methods of homoeolog inference, considering the strengths and limitations of each approach. Establishing a precise and evolutionarily meaningful definition of homoeology is essential for understanding the evolutionary consequences of polyploidization.

Keywords: homoeology definition, homoeology review, homoeology inference
Diploidisation through time: comparing neopolyploids and established polyploids

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A sequence of genomic events, occurring over millions of years, is becoming evident in the diploidisation of polyploid plants. These events include: genomic shock, epigenetic effects, homogenisation of repetitive DNA, intergenomic translocations, loss of loci, specification at the polyploid level and chromosome number reductions. The results from two polyploid projects being undertaken at the Jodrell Laboratory (Royal Botanic Gardens Kew) will be compared and contrasted. Firstly, the study of neopolyploids in Dactylorhiza (Orchidaceae) and secondly the study of ancient polyploids in Nicotiana (Solanaceae). Both groups contain species of conservation interest that are difficult to classify due to their complex reticulate histories. Three allotetraploid marsh orchid species (Dactylorhiza majalis, D. traunsteineri and D. ebulensis) have been investigated. They are sibling species that were formed from the same progenitor diploid lineages at different times and all were formed less than 12,000 years old. Studies have shown they are genetically very similar but are morphologically distinct and occupy specific ecological niches. Epigenetic changes have been documented that modify the expression of genes in these young sibling taxa. These epigenetic effects are not encoded in the primary DNA sequence (e.g. methylation and histone modifications) and seem to act directly in response to environmental drivers. This type of modification can be seen as one of the early steps in the adaptation of allopolyploids and might in the future lead us to consider ‘population epigenetics’ as an important discipline. In contrast, Nicotiana section Suaveolentes is an Australian group of 26 ancient polyploid species. The group was formed from a single polyploid event followed by speciation at the polyploid level. Molecular clocks have shown the group to be just under 10 million years old. There is evidence of loci loss in nuclear genes, ribosomal regions and repetitive elements. Species in the group have 15-24 chromosomes, and evidence suggests that chromosome number reductions have occurred, as species inhabit areas closer to the arid south/centre of Australia. Diploidisation is at an advanced stage in these ancient polyploids of Nicotiana but it seems likely that this particular stage was preceded by much earlier processes such as the ones documented in the neopolyploids of Dactylorhiza. Thus studying polyploids of different ages opens up a window into evolutionary time.

Keywords: diploidisation, epigenetics, chromosomesal-fusion, Nicotiana, Dactylorhiza

Multi-genome meiosis in synthetic Brassica hybrids and polyploids

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Six agriculturally important Brassica crop species share a well-characterised genomic relationship, whereby three diploid (palaeopolyploid) species (A, B and C genomes) hybridized to form three recent allotetraploid species (AB, AC and BC genomes). Recurrent polyploidy in this genus and the close relationship of these genomes has created an intriguing question: how is non-homologous chromosome pairing prevented during meiosis? Although the three established allotetraploid species show effectively diploidized meiosis, newly resynthesized allotetraploids tend to show varying degrees of genomic instability. Understanding the mechanisms underlying prevention of non-homologous chromosome pairing is critical for production of stable allohexaploid Brassica, which may have potential as a new crop species with increased hybrid vigour and broader adaptation. In order to investigate factors affecting meiotic behaviour in trigenomic Brassica, we produced different hybrid combinations (AABC, BBAC, CCAB and AABBC) from crosses between the allotetraploid species. Allele and chromosome inheritance and meiotic behavior in these hybrids were assessed using a combination of high-throughput genotyping, and fluorescent and classical cytogenetics. Non-homologous recombination between the A, B and C genomes was frequent when these genomes were haploid (single copy, no matching homologue). However, the presence of homologous pairing partners resulted in relatively normal assortment of homologous chromosomes, even at higher ploidy levels. Interestingly, fragmented chromosome regions resulting from the ancestral palaeopolyploidy event still initiated non-homologous chromosome recombination at low frequencies, even in the presence of primary homoeologues. Preliminary evidence suggests that alleles from the allotetraploid species are more effective at preventing non-homologous pairing than alleles from the diploid Brassica species in the synthetic allohexaploids. Our results suggest that selection for improved regularity of homologous pairing from the plentiful allelic variation present in lower ploidy species may be an effective strategy to produce stable, higher ploidy lines in the Brassica genus. Genetic factors and availability of homologous pairing partners, rather than sequence similarity, are predicted to be the main determinants of stable meiosis in Brassica allotetraploids.

Keywords: Brassica, non-homologous recombination, genome stability, synthetic allopolyploids, interspecific hybridisation
Maternal parent hypermethylation overcomes inter-ploidy and inter-species F1 seed abortion blocks in Arabidopsis thaliana

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When diploid and tetraploid parental plants are crossed, the development of viable F1 seeds can be impeded by a post-zygotic block. This phenomenon is called the “triploid block”, where unbalanced ploidy crosses lead to seed endosperm failure and ultimately to seed abortion. We demonstrate that mutations in three DNA demethylases (REPRESSOR OF SILENCING 1, DEMETER-LIKE 2 and DEMETER-LIKE 3) overcomes the triploid block. When triple mutants carrying knockout mutations in each of the three DNA demethylase genes (i.e. the rdd triple mutant) are used as diploid females in rdd X 4x crosses, a high rate of viable F1 triploid seed formation occurs. Moreover, endosperm cellularisation, which is disrupted in 2x X 4x crosses, appears normal in rdd X 4x developing seeds. This indicates a role for the rdd mutation in rescuing endosperm development in incompatible inter-ploidy crosses. To unravel the mechanistic basis of rdd-dependent bypass of the triploid block, we performed whole genome bisulfite sequencing of seed endosperm nuclei. We discovered widespread rdd-dependent hypermethylation, indicating a profound effect of the rdd mutation on the epigenome of developing seeds. Amongst the hypermethylated genes, we identified endosperm development regulators such as FIS2 and members of the IKU pathway. Hypermethylation of these genes was found to correlate with diploid-like expression levels in rdd X 4x developing seeds, while strongly up-regulated in incompatible 2x X 4x seeds. Furthermore, knock-out mutations in the IKU pathway genes partially rescue F1 seed viability, indicating that rdd-dependent bypass of the triploid block could act through its control of the IKU and FIS2 pathways. In contrast, rdd knockout mutations in a tetraploid male donor leads to an aggravation of the triploid block, indicating that the effect of rdd mutation acts in a parent-of-origin specific manner in Arabidopsis thaliana. We finally demonstrate that rdd can also bypass interspecific hybridization barriers, through the generation of a large amount of viable seeds when crossing a female tetraploid rdd with a male tetraploid Arabidopsis arenosa. We have determined that both the triploid block and the interspecies block can be bypassed by hypermethylation of the maternal parent (through mutations in DNA demethylases).

Keywords: Triploid block, inter-species hybridisation, seed development, epigenetics

Perturbations of meiotic recombination in neopolyploid maize

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Meiotic recombination is a major source of genetic variation and a determinant of genome architecture. The genomes of newly formed polyploids are often extremely dynamic. There is evidence for large chromosomal rearrangements following polyploidization in many species, including translocations, duplications, inversions, and deletions. The mechanism by which these rearrangements occur is meiotic recombination, though little is known about how the recombination pathway itself is affected by polyploidization. Additionally, a myriad of genetic and epigenetic changes occur which might modulate recombination interactions. In order to investigate the immediate impact of polyploidization on meiotic recombination, we compared three synthetic tetraploid maize lines with their diploid progenitors over the course of the recombination pathway. Recombination is initiated by the formation of double-strand breaks (DSBs) in chromosomal DNA early in prophase I of meiosis, prior to pairing of homologous chromosomes. By the end of prophase I, DSBs are repaired, either as crossovers (COs) or non-crossovers. We observed that in polyploid lines, large numbers of DSBs remain unrepaird late in prophase I. Intriguingly, diploid mutants defective in homologous chromosome pairing also show this persistence of DSBs, which suggests that the homology search process is disrupted in neopolyploids. In normal diploid meiosis, COs are formed only between homologous chromosomes. However, in the synthetic tetraploid lines we observed COs between non-homologous chromosomes, sometimes resulting in the formation of multivalents. This result also indicates errors in the pairing process. Additionally, we found differences in meiotic chromosome behavior among the neopolyploid lines. Tetraploid lines created from diploid homozygous lines behaved like autotetraploids (lower bivalent frequency). These findings will lead to further understanding of how polyploidization impacts meiotic recombination mechanisms.

Keywords: meiotic recombination, maize, cytogenetics, chromosomes
The evolution of apomixis in angiosperms: a consequence of hybridity, polyploidy, or of environmental influence?

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Apomixis, the asexual reproduction via seed, is taxonomically widespread in angiosperms, and almost exclusively found in hybrids or polyploids (auto and allopolyploids). Apomixis represents a deregulation of the sexual developmental pathway, and is caused by changes in expression patterns of genes regulating meiotic sex. Traditionally, the genomic changes associated with polyploidy (with or without hybridity) were thought to trigger the shift in mode of reproduction. Recent studies on the *Ranunculus auricomus* polyploid complex showed that the first steps of apomictic development (apospory) can already appear in diploid sexual synthetic hybrids, but functional apomictic seed formation was only found in polyploid hybrids. Frequencies of apospory increase in second generation hybrids, suggesting an establishment process over generations. In the diploid/tetraploid model system of the alpine species *Ranunculus kuepferi*, we detected via flow cytometric seed screening occasional apomictic seed formation in diploid populations, without any indications of hybridity. We exposed diploid and tetraploid plants to cold shocks in climate growth chambers, and detected spontaneous appearance of apomictic seed formation in diploid, previously sexual plants. Tetraploid facultative apomicts reduced proportions of sexual seed formation after cold treatments. Natural tetraploid populations of *R. kuepferi* showed a clear preference of higher altitudes and colder climates. Results suggest that also cold temperatures can directly induce apomixis, and that polyploids originated out of diploid facultative apomicts. Apomixis can be caused by different kinds of disturbances of the meiotic pathway. We discuss the novel view that apomixis is possibly a pathway and transition period to polyploidization, rather than a consequence of polyploidy.

Keywords: apomixis, crossing experiments, temperature effects, polyploidization, *Ranunculus*

Interfertility and phenotype of independently originated populations of the neo-allopolyploid *Mimulus peregrinus* (Phrymaceae)

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Whole genome duplication, or polyploidisation, has played a fundamental role in the evolutionary history of plants. It has long been recognised that genome doubling can be responsible of triggering morphological diversification, and may increase the ecological tolerance of polyploid lineages. Yet, for a polyploid lineage to become evolutionarily successful, it must first overcome the dramatic loss of genetic diversity that is often associated with its origin. A principal mechanism that may rescue polyploids from a fate of depauperate evolutionary potential is the fact that polyploid species tend to be originated in multiple occasions. Although each instance of polyploid formation may result in limited genetic diversity, mating between independently generated polyploids may introduce genetic variation into the nascent lineage. In turn, increased genetic diversity may confer polyploid lineages with the required variability in ecologically important traits necessary to respond to environmental heterogeneity. Here, we addressed the overall question: Can multiple origins increase the evolutionary potential of a nascent polyploid? Specifically, we used the recently evolved allopolyploid *Mimulus peregrinus* to address the following questions: (1) Are independently originated allopolyploids inter-fertile? (2) Does mating between independently originated lineages increase genetic variation and phenotypic diversity? Our results indicate that independently originated polyploids are interfertile. However, the fertility and phenotype of inter- and intra-population allopolyploids is highly variable, suggesting considerable heterogeneity and potential instability of phenotypes during the early generations of a nascent polyploid. Studies of the phenotype, ecology and performance on neo-allopolyploids have the potential to help explaining the puzzling evolutionary success of polyploids.

Keywords: evolutionary success, fertility, hybridisation, phenotypic diversity, speciation
The impact of alloploidy on gene expression in Dactylorhiza

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Recurrent origins are the rule for most polyploids. This can create an array of genetically, ecologically, morphologically and physiologically distinct genotypes/populations, among which subsequent gene flow, independent assortment and recombination may produce additional variation. As these multiple origins provide natural replicates, sibling alloploids are excellent models to uncover mechanisms of adaptation to divergent environments, which are assumed to lead to evolutionary diversification and biodiversity increase. Our study focuses on three ecologically divergent, sibling alloploid orchids of different moderate ages (Dactylorhiza majalis, D. traunsteineri and D. ebudensis). These species have been formed through unidirectional hybridization between diploids D. fuchsii (always the maternal parent) and D. incarnata (always the paternal parent). Given the working hypothesis that in the short evolutionary times relevant to our study system, phenotypic divergence relies on quantitative differential gene expression rather than differences in the coding DNA sequence, we are specifically searching for genes that are differentially expressed between our alloploids. To show this, we are using RNA-seq data obtained for 30 individuals representing the three sibling alloploids and their diploid parents. Our results suggest that, although morphologically and ecologically divergent, the Alpine and certain Scandinavian D. traunsteineri resemble more D. majalis than the British D. traunsteineri. Furthermore, the gene expression patterns of the Scottish endemic D. ebudensis closely resemble those of the British D. traunsteineri. These results suggest that an ongoing gene flow between sympatric alloploids broadly homogenizes their gene expression, whereas their phenotypic divergence is controlled by a restricted set of genes. Indeed, an enrichment for certain ecologically important functional pathways, for example the response to abiotic stress, has been found within the differentially expressed genes between D. traunsteineri and D. majalis. In addition, the results of a recently developed method (HylITE) point to homoeolog expression bias, expression level dominance and the possible link of these effects on the divergent adaptation of our naturally occurring alloploid species.

Keywords: RNA-seq, differential gene expression, homeolog expression bias, expression level dominance

Using synthetic spike-in RNAs to quantify expression level divergence following autopolyploidy

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Allopolyploidy has historically been the focal point of researchers interested in the effects of whole-genome duplication. This focus has come at the near exclusion of autopolyploidy and reflects the traditional view of autopolyploidy being evolutionarily unimportant and rare in nature. Recently, the ever-broadening interest in the evolutionary implications of polyploidy has led to the discovery that autopolyploidy is frequent and perhaps a major evolutionary force, particularly in certain clades. Unfortunately, we know little about the ecological, physiological, and genomic implications of speciation by autopolyploidy. The angiosperm genus Tolmiea (Saxifragaceae) is an emerging evolutionary system for the study of speciation by autopolyploidy, comprising two species, the autotetraploid T. menziesii (2n = 28) and its diploid progenitor, T. diplomenziesii (2n = 14). We present the results of our study of transcriptome-wide divergence in gene expression following autopolyploidy in Tolmiea. We discuss our methods for normalizing expression count data of different ploidal levels through the use of spike-in RNA standards. Using this approach, we report on the relative differences per-transcriptome, per-cell, and per-biomass, and patterning with respect to ontology.

Keywords: autopolyploidy, gene expression, RNA standards

Keywords: RNA-seq, differential gene expression, homeolog expression bias, expression level dominance
Molecular basis of adaptive diffusion after recurrent allopolyploidization in *Dactylorhiza*

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Early-generation allopolyploids need to quickly accommodate divergent genomes into one nucleus by adjusting organization and function, thereby altering their ecological properties and adaptive success. To identify the drivers of adaptation to distinct environments after iterative whole genome doubling, we investigate the morphologically- and ecologically-divergent sibling allopolyploids *Dactylorhiza majalis* and *D. traunsteineri* (Orchidaceae), together with representatives of their diploid parents. With RADseq we document a genome-wide absence of genetic differentiation between the allopolyploids, despite their phenotypic divergence. In addition, we bring evidence of frequent gene flow between the polyploids in sympathy, which points toward a strong divergent selection required in order to maintain the observed phenotypic divergence. By using the sibling allopolyploids of different ages we investigate with RNAseq and smRNAseq the progression through time of alterations in gene expression and regulation after allopolyploidization, and their importance to the adaptation to distinct environments. We observe a general trend of increased overexpression of genes in the younger polyploid, whereas the transcriptome of the older resembles more closely those of the diploid parents. The phenotypic divergence between the polyploids appears mediated by a general parental dominance in opposite directions in the sister polyploids, a patterns retained partly also at the level of transgressively expressed transcripts. The differential expression between the polyploids affects several genes related to metabolic processes and responses to stimuli, with a putative ecological function. The differential posttranscriptional regulation via smRNAs is less significant, and affects a different population of transcripts, processes and functions, such as ion binding and nitrogen metabolism. We conclude that the major transcriptomic difference among the diploid parental species of these polyploids became reconciled in different ways in the sibling *Dactylorhiza* polyploids, most probably also as a result of distinct selection pressures specific for their characteristic environments. We finally discuss the importance of qualitative versus quantitative expression alterations in allopolyploid genomes, and their role for diversification in general.

Keywords: adaptation, RADseq, RNAseq, recurrent origins, smRNAseq

Deciphering the post-neolithic *Brassica napus* oilseed genome reveals the fascinating diversifying force of polyploidy

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Oilseed rape (*Brassica napus* L.), formed less than 7500 years ago by allopolyploidy between *B. rapa* and *B.oleracea*, rapidly became a successful crop. Together with more ancient and recurrent polyploidizations, this conferred an aggregate 72× genome multiplication since the origin of angiosperms and high gene content. We examined the *B. napus* genome and the consequences of its recent duplication in comparison with parental genomes. The most paleo-duplicated and youngest polyploid genome yet sequenced, *B. napus* has the highest gene density yet known. Recently joined in a common nucleus, its constituent A_1 and C_2 subgenomes are engaged in subtle structural, functional and epigenetic crosstalks, with abundant homoeologous exchanges playing a pivotal role in *B. napus* diversification, as suggested when comparing different *B. napus* morphotypes including winter, spring and Asian oilseed types, rutabaga and kale vegetables. Consistent with relatively short time to diploidize, most progenitor orthologous genes remain conserved as duplicates in *B. napus*, with both copies contributing to gene expression. Yet gene loss and expression divergence of duplicated genes are just beginning. In time, most (88-96%) asymmetric transposon proliferation between the A_1 and C_2 subgenomes is inherited from the progenitors, with no activation bursts following recent allopolyploidy. We showed that selection in *B. napus* oilseed types has accelerated loss of undesirable glucosinolate genes, while preserving the greatest known expansion of oil biosynthesis genes. These processes provide unique insights into early allopolyploid evolution and its interactions with crop domestication and improvement.

Keywords: Brassica, diversity, neo-polyploid, paleoduplications
Unexpected gene expression changes in newly developed *Festuca × Lolium* hybrids

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Allopolyploidization is one of the key processes leading to the origin of plant species. Merging two different genomes followed by the doubling of hybrid genome results in extensive changes in gene expression and thus, in the development of new phenotypes. The benefit of allopolyploids is evidenced by a substantial of crops (such as wheat, maize and cotton) being ancient allopolyploids. Recently, the use of interspecific hybridization in breeding programs has enabled the development of new forms of plants. For example, Festulolium, a hybrid of ryegrass (*Lolium* sp.) and fescue (*Festuca* sp.) species became popular among grass breeders and farmers. The genome constitution of Festulolium varies from cultivar to cultivar. However, almost nothing is known about the gene expression of parental alleles in Festulolium hybrids. The aim of our work was to analyse gene expression changes in reciprocal F1 and F2 hybrids of *Festuca × Lolium* allele and only between 55-70 genes with overexpression of *F. pratensis* allele in reciprocal F2 hybrids. The tendency to shift back to the equal contribution of both alleles on gene expression has been found in F2 hybrids.

Keywords: gene expression, reciprocal hybrids, RNAseq, Festuca, transcriptome

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Triparental origin of triploid onion *Allium × cornutum* (Clementi ex Visiani, 1842) (2n = 3x = 24)

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Triploid onion *Allium × cornutum* (Clementi ex Visiani, 1842) (2n = 3x = 24), is an established minor garden crop, widespread in southeastern Asia and Europe. Combining molecular, phylogenetic and cytogenetic data we provide evidence for the origin of its unique triparental genome. The phylogenetic analyses of the internal transcribed spacers ITS1-5.8S-ITS2 of 35S rDNA and the non-transcribed spacer (NTS) region of 5S rDNA of *A. × cornutum* and its wild relatives of the section Cepa showed clustering of the obtained sequences into the three main clades, each with high sequence homology to one of three other species of section Cepa: *A. cepa*, *A. roylei*, and unexpectedly, the wild Asian species *Allium pskemense*. By application of double fluorescent in situ hybridisation (FISH) we localised the 35S and 5S rRNA genes on *A. × cornutum* chromosomes and their position largely corresponded to their respective positions in the three putative parental species. GISH (genomic in situ hybridisation) using DNAs of the three putative parental diploids enabled simultaneous visualization of the three genomes on somatic and meiotic chromosomes of the triploid onion. In order to identify the female parent of *A. × cornutum* three different regions of the chloroplast genome (*trnH-psbA, atpB-rbcL*, and *matK*) were cloned and phylogenetically analyzed in *A. × cornutum* and its parental species. cpDNA sequences of the triploid showed the highest similarity to the *A. cepa* cpDNA indicating this or some of other closely related wild *Allium* species as a female progenitor of the triploid. PCR amplification and RFLP analysis of the selected genes from the chloroplast genome of *A. × cornutum* (*accD, atpF and petB*) allowed us to identify SNPs and indel typical for a unique male-sterile cytoplasm of onion, thus confirming that tripeoloid hybrid onions harbour this unique type of cytoplasm, which is important for hybrid onion seed production.

Keywords: triploid onions, ribosomal genes, chloroplast DNA, cpDNA, fluorescent in situ hybridisation FISH, genomic in situ hybridisation GISH
Polyplody and range expansion in the South American genus *Fosterella* (Bromeliaceae)

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The role of polyplody along a relatively steep Andean elevation and climatic gradient was studied using the genus *Fosterella* L.B. Sm. (Bromeliaceae) as a model system. Ecological differentiation of cytotypes and the link of polyplody with historical biogeographic processes such as dispersal events and range expansion were assessed. DAPI staining and flow cytometry were used to estimate the ploidy levels of 161 plants from 22 species sampled throughout the distribution range of the genus. Ecological differentiation among ploidy levels was tested by comparing the sets of climatic variables and ancestral chromosome number reconstruction was carried out on the basis of a previously generated phylogeographic framework. The occurrence of polyplody was limited to the phylogenetically isolated “penduliflora” and “rusbyi” groups. Cytotypes were found to be ecologically differentiated, showing that polyploids preferentially occupy colder habitats with high annual temperature variability (seasonality). The results provide indirect evidence for both adaptive ecological and non-adaptive historical processes jointly influenced the cytotype distribution. The results also exemplify the role of polyplody as an important driver of speciation in a topographically highly structured and thus climatically diverse landscape.

Keywords: Fosterella, Bromeliaceae, climate, allopatry, Andes

Advantages and tradeoffs of “general purpose genotype”: zinc accumulation, cold response and genome-wide homeolog expression in the self-compatible allopolyploid *Arabidopsis kamchatcica*

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The advantages and disadvantages of allopolyploidization have long been discussed. Currently, little is known about how transcriptomic patterns of parental species underlying phenotypes are inherited in allopolyploid hybrids because of the complexity of polyploid genomes. To study phenotypes and gene expression levels quantitatively, we focused on zinc hyperaccumulation and cold responses in the allotetraploid *Arabidopsis kamchatcica*, which has a broad distribution range. One of the parental species, *A. halleri*, is a well-known hyperaccumulator of zinc and cadmium, while the other parent, *A. lyrata*, is distributed at higher latitudes but not a hyperaccumulator. Plants grown hydroponically under zinc stress demonstrated that the zinc concentration of *A. kamchatcica* was as high as about half of *A. halleri*, indicating the inheritance of this trait from *A. halleri*. The soil of some of the habitats of *A. kamchatcica* and *A. halleri* had high levels of zinc, suggesting that *A. kamchatcica* took advantage of hyperaccumulation to broaden its habitat range. We quantified the level of expression of duplicated homeologous pairs using a recently developed Illumina read sorting pipeline (HomeoRoq), and validated expression ratios by pyrosequencing. In *A. kamchatcica*, homeologs derived from *A. halleri* had significantly higher levels of expression of *HEAVY METAL ATPASE4 (HMA4)*, *METAL TRANSPORTER PROTEIN1 (MTP1)* and other metal-related genes than those derived from *A. lyrata*. As a result, *A. kamchatcica* has on average about half the level of expression of these genes compared with *A. halleri*, consistent with fixed heterozygosity inherent in allopolyploids. We suggest that the half level of expression contributes to the reduced levels of hyperaccumulation compared with the diploid specialist *A. halleri*, and thus confers a tradeoff for allopolyploids. Resequencing data showed a significant reduction in genetic diversity near the HMA4 locus derived from *A. halleri*. Similarly, cold treatment induced a homeolog derived from *A. lyrata* in many cold response genes such as COR15 and RD29B. Either of Zinc or cold treatment significantly changed the ratio of roughly 1% of homeologous pairs. Our combined results of metal and cold responses provide molecular evidence for the “general purpose genotypes” proposed by Stebbins about half a century ago, stating that polyploids have a tolerance to a wide range of environmental conditions and thus a broad distribution range.

Keywords: transcriptome, Arabidopsis, soil adaptation, self-compatibility
The worldwide distribution of polyploid plants

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Understanding the distributional patterns of polyploids is of key importance in understanding the biological relevance of polyploidy. For over a century, revealing such patterns has been an important goal of polyploidy research, motivating botanists to collect and assemble wide karyological data. Pioneering studies of polyploid distribution concentrated on the comparison of polyploid frequencies in local floras and used limited data to relate these with underlying climatic factors or species traits. However, with current data availability, spanning both karyological and distributional data, revising these questions has the potential to reveal new aspects regarding the conditions in which polyploids tend to establish and expand. Here, we tackle this challenge by performing a thorough mapping of worldwide distribution of polyploidy based on an unparalleled taxonomic and geographic breadth. To this end, we used phylogenetic approaches to infer the ploidy levels for tens of thousands of plant species. For each species, we assembled its distributional data as obtained through the Global Biodiversity Information Facility (GBIF). This allowed us to display the worldwide distribution of polyploidy abundance across the globe. We further extracted for each distributional point all available environmental data (climate, paleoclimate, soil nutrients, biomass productivity, etc.) as well as individual species traits, such as the plant life form. This allowed us to examine whether the distributional pattern is linked to specific eco-climatic conditions. In the talk we will present results of our mapping, identify the major factors responsible for the observed worldwide patterns of polyploid distribution, and revisit traditional hypotheses.

Keywords: geographical distribution, ecological limitation, angiosperms, chromosome number database

Adaptive evolution of meiosis in diploid and polyploid Arabidopsis arenosa across its native range

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Understanding the way molecular variation over space and time facilitates adaptation via the structure and function of genes is an important question in evolutionary biology. Here we study a highly conserved evolutionary process - meiosis - and specifically explore how it adapts to whole genome duplication. Using Arabidopsis arenosa, which exhibits natural ploidy level variation, we take advantage of the fact that each ploidy splits into several lineages to explore how the genes that control meiosis evolve as lineages diverge. Specifically, we will ask whether different tetraploid lineages, which diverged from a single ancestor, exhibit distinct ways of molecular adaptation to polyploidy, and if the patterns of molecular adaptation correlate with highly variable habitat preferences of A. arenosa populations of both ploidy levels. Using genomic resequencing data, we assess if there may be meiotic pre-adaptation to polyploidy in particular extant diploid lineages and ask whether there is a tendency for particular evolutionary changes to lead to selection on meiosis-related genes in populations varying in geographical and historical context. We complement the analysis of ~300 resequenced A. arenosa individuals from natural populations with cytological investigations of meiotic adaptation (chromosome pairing and segregation during prophase I) and with assessment of pollen fertility across a temperature continuum, which we use as a proxy for meiosis success.

Keywords: Arabidopsis arenosa, genome duplication, meiosis, molecular adaptation, pollen fertility
Dead-end trajectory of young triploid apomicts: Can transposable elements improve their adaptive potential?

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Apomixis is defined as the asexual formation of a seed from maternal tissues, circumventing normal meiosis and fertilization process. This form of asexuality is relatively prevalent in higher plants. However, just like other forms of asexual reproduction, apomixis is thought to have limited evolutionary potential leading apomicts to early extinction. Indeed, mutations, transposable elements (TEs) and other repetitive DNA can accumulate in non-recombining genomic regions. Nonetheless, TE dynamics may be particularly relevant within asexual lineages. While most transpositions are deleterious, increasing evidence is hinting at TE-induced mutations with potentially beneficial gene regulatory effects in non-recombining genomes. Thus far, limited evidence corroborates the accumulation of TEs in asexually reproducing organisms. This is likely due to contrasting effects of asexual reproduction on TE dynamics. Indeed, in the short term, transposition may be increased because epigenetic silencing mechanisms can be compromised following ploidy change and hybridization often accompanying transition from sexual to apomorphic reproduction. In the long term conversely, clonal lineage selection may maintain benign TEs with low transposition rates. To gain better insight into TE dynamics under apomixis, we use the model system Taraxacum officinale: Derived from sexual diploid ancestors (2n=2x=16) in South-Central Europe, dandelions from Northern Europe are usually triploid apomicts (2n=3x=24). In previous studies, we observed transgression divergence driven mainly by TEs and TE-related genes within triploid apomorphic lineages; and detected heritable methylation variation within and between lineages. In this new study, we use low coverage Illumina genomic sequencing to compare TE content and dynamics within and between sexual populations from France and Czech Republic against 7 apomict populations sampled around Eastern Europe and Sweden. We also integrate phenotypic traits and climatic data to better comprehend the drivers of apomict evolution. While we explore the dynamic of transposable elements following hybridization and change of ploidy levels in triploid apomict genomes, we propose hypotheses as how TEs can trigger additional genetic variation in young apomorphic lineages. We also discuss the ecological relevance of TEs in contributing to adaptive potential of natural populations facing changing environments.

Keywords: apomixis, evolutionary dead-end, hybridization, transposable elements, genome evolution.

Diploidization without reduction of genome size in an Alpine jumping bristletail

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Whole genome duplications have boosted organismal diversification in several eukaryotic lineages and are considered a powerful driver of evolution. In extant organisms, however, old duplication events are hard to discern due to a general tendency towards diploidization. Relatively young polyploid species are thus valuable models for studying the interrelation of genomic rearrangements and speciation. In a recent study, the relative genome size of Machilis glacialis (Insecta: Archeognatha) was determined to be twice that of closely related species, indicating a young tetraploid. Interestingly, its chromosome number (2n=56) was close to the chromosome numbers of congenic species (2n=52 to 2n=56). While the M. glacialis karyotype mostly consisted of metacentric chromosomes, acrocentric chromosomes prevailed in the other species. We compared karyotypes and genome sizes of two geographically distant populations of M. glacialis with samples of M. montana, M. mesolcinensis, and M. fuscistylis. Preliminary results corroborated the doubled DNA content but also uncovered varying chromosome numbers in both populations of M. glacialis, ranging from 2n=56 to 2n=60. Moreover, fluorescence-in-situ-hybridization experiments revealed considerable variation in copy number of ribosomal (18S) and histone (H3) clusters among species, which was not consistent with the scenario of a whole genome duplication followed by simple fusion of homologous chromosomes. We speculate that M. glacialis, after initial genome duplication, underwent diploidization via extensive chromosomal rearrangements while retaining its doubled genome size. Alpine jumping bristle-tails are confirmed as an excellent model for studying polyploidy and chromosomal rearrangement.

Keywords: Chromosomal rearrangements, FISH, Archaegnatha
Evolutionary patterns, contact zones and ecological segregation in an alpine autopolyploid complex

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The evolution and ecology of autopolyploids has received little attention as compared to allopolyploids. Our model plant Senecio carnioicus (Asteraceae) is common in alpine to subalpine vegetation in the Eastern Alps and the Carpathians. It exhibits large ploidy level variation, and stable cytotype mixtures were found in many populations. Using an array of genetic markers, ecological in situ as well as common garden experiments, germination experiments and observational field data collected on different spatial scales, we investigated the interplay of polyploid evolution and ecology in an autopolyploid complex. We focussed on the origin of the cytotype mixture (primary or secondary hybrid zones), the stability of hybrid zones as well as on patterns of gene flow from the local level up to whole-range patterns. Two strongly divergent diploid lineages exhibit a vicariant distribution. Tetraploid and hexaploid cytotypes, though being relatively distinct morphologically, are genetically similar and - as shown by a novel in silico modelling approach - originated from the eastern diploid lineage by autopolyploidisation, whereas the western diploid lineage has not taken part in the origin of the polyploid cytotypes. Field studies suggested ecological niche differentiation, small-scale spatial segregation of all three cytotypes and niche shifts in heteropolyploid as compared to homoploid sites, which are indicative of ecological displacement. Artificial crosses between di- and polyploids produced low seed sets, whereas there is no reproductive barrier between the - ecologically strongly segregated - polyploid cytotypes. Hybrids between di- and polyploids germinate poorly if at all, whereas germination and survival rates of pentaploid hybrids are similar to those of their tetra- and hexaploid parents. Interestingly, pentaploid individuals produce viable, 2.5-ploid pollen, which is producing viable offspring when crossed with tetra- and hexaploids, suggesting that pentaploids successfully mediate gene flow across ploidy levels.

Keywords: alpine plants, autopolyploidy, contact zones, crossing experiments, niche differentiation

LS3-7

Genome-wide statistical detection of hyper-biased homeologs in allopolyploid and their changes after hybridization

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Allopolyploidization, the poliploidization between two different species, is a common process during the evolution of eukaryotes, especially plants, prompting adaptation to broader and novel environmental niches. While the allopolyploidization causes drastic expression changes, called “transcriptome shock” creating the adaptation capability, few studies observed how the expression changes happen. This study first revealed that gene expression ratio patterns in recently synthesized polyploids were similar to each other, and introduce an arbitrary-threshold-free detection algorithm of hyper-biased (HB) homeologs named SIGN. Then, the changes of HBs in allopolyploidization were analyzed with Arabidopsis kamchatcica, closely related allotetraploid species to the model species Arabidopsis thaliana. The analysis results in synthetic allopolyploids were, furthermore, compared with two natural allopolyploids. These analyses implied that allopolyploidization causes that a lot of HB homeologs become expressed in both homeologs, but gradually many genes become HB in adaptation process. HB homeologs were not stochastically selected and have high parental effects. The commonly HB genes include key genes in parental species function including cold stress and metal tolerance related genes. However, the expressions of quite a few genes are highly divergent among independently crossed lines. Careful investigation of the genes in fatty acid metabolism pathway shows pathway specific HB/de-HB in one of natural lines. HB gene analysis could uncover adaptation mechanisms after hybridization.

Keywords: Hyper-biased expression, RNA-seq, gene expression analysis, silencing
Hybridization in yeast is associated with phenotypic novelty for life-history, metabolic and proteomic traits

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The most apparent consequence of hybridization is usually hybrid vigor, also called heterosis, a phenomenon defined as the superiority of the hybrid over its progenitors. Hybrid vigor has long been exploited by humans for the genetic improvement of cultivated plants or livestock. However, the molecular mechanisms underlying heterosis are still debated. In this work, we investigated hybrid vigor in a yeast model under winemaking conditions. A collection of four Saccharomyces uvarum and seven S. cerevisiae parental strains and their 55 possible hybrids (28 inter- and 27 intraspecific hybrids) was phenotyped at two temperatures (18°C and 26°C). The experimental design included 396 fermentation assays, for which we measured 35 traits including life-history traits (viability, population size, etc.), and metabolic traits (fermentation products, wine aromas, etc.). We also applied high-throughput shotgun LC-MS/MS technique to measure the abundance of around 1400 proteins. Our results showed that both intra- and interspecific hybridization were associated with an increased range of phenotypic values for most life-history and metabolic traits. The extent of phenotypic variation depended on the type of traits, and also on genetic and environmental conditions. In addition, the hybrids were able to maintain more stable phenotypes with respect to temperature change than their parents. This phenomenon, called homeostasis, may allow the hybrids to buffer the effects of external perturbations and thus to maintain fitness in diverse habitats. At the protein level, hybridization was associated with drastic remodeling of the proteome, which was larger for interspecific hybrids. For intraspecific hybrids, the proteomic changes were larger at non-optimal temperature. The remodeling of the proteome predominantly affected highly abundant proteins involved in cell viability and/or metabolism. Moreover, we found that heterotic proteins were globally encoded by genes with higher number of putative transcription factors. The complex biological dataset produced by this work is far from being fully exploited, and is currently integrated using mathematical and statistical models for predicting heterosis.

Keywords: hybridization, heterosis, yeast, proteomics, winemaking.

Disentangling the causes of differences in distribution of diploid sexual and autoploid apomictic Easter daisies (Townsendia hookeri: Asteraceae)

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Ecological divergence between sexual polyploids and their diploid progenitors permits new polyploids to avoid minority cytotype interactions that would limit their establishment. These dynamics are potentially different when polyploids are apomictic, because apomixis can protect nascent polyploids from hybridization. Further, apomicts that retain male function can reduce recruitment of sexual diploids by siring interploidy hybrids with sexual diploid mothers, suggesting that sexual diploids are threatened by the presence of apomicts, even when apomictic polyploids are the minority cytotype. Townsendia hookeri includes autoploid (triploid and tetraploid) apomicts and sexual diploids. The ranges of the two cytotypes are distinct, but overlap in southern Wyoming and Northern Colorado, with the sexuals extending to southern Colorado, and the apomicts extending north to southern Alberta and British Columbia. Species distribution models reveal evidence of differentiation in climatic niches of the cytotypes, but suggest that diploids may have the capacity to spread further into the polyploid range. Crossing experiments in the field indicate that pollen from apomicts can readily fertilize oovules of sexual diploids, producing viable offspring of varying DNA content, mostly above diploid levels. Along with the results of niche modeling, our crossing results suggest that sexual expansion could be limited by reproductive interference rather than ecological factors. In order to test the role of ecological divergence in limiting the distribution of apomicts and sexuals, we set up a largescale experiment that includes common gardens that span allopatric and sympatric parts of the range of each cytotype. Eight transplant gardens were established in the summer of 2014, and are being monitored for survival, growth and reproductive traits, with a separate experiment to look at germination success. Our predictions, that apomictic polyploids would have low fitness in sexual diploid sites, is not supported by first year survival data. The study of ecoevolutionary interactions between cytotypes in an apomict such as Townsendia hookeri, in addition to providing insights into sexual-apomictic systems, emphasizes the need to consider the selective pressures that act on diploids, during and after the establishment of polyploids.

Keywords: apomixis, ecological divergence, reproductive interference
Influences of ploidy level and reproductive mode on patterns of adaptive molecular evolution in a New Zealand freshwater snail

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Polyploidy has the potential to facilitate adaptive evolution by providing redundant genome copies that are free to evolve new functions. By contrast, asexuality, with which polyploidy is often associated, is expected to restrict adaptive evolution by decreasing the efficacy of natural selection and access to new genetic variation. How polyploidy and reproductive mode actually shape molecular evolution and the interplay between potential evolutionary benefits of polyploidy and expected negative consequences of asexuality remain largely unresolved, particularly in animals. Here, we evaluate how ploidy level and reproductive mode influence patterns of adaptive molecular evolution in the New Zealand freshwater snail Potamopyrgus antipodarum to assess 1) the potential evolutionary genomic benefits of recent polyploidy, and 2) how patterns of adaptive molecular evolution in asexuals are influenced by polyploidy. Potamopyrgus antipodarum is well suited to study variation in reproductive mode and ploidy level because obligately sexual diploid individuals often coexist and compete with triploid and tetraploid asexual counterparts, enabling direct comparisons between otherwise similar individuals. Because genes involved in host-parasite interactions are among the most rapidly evolving genes in the genome, they comprise excellent candidates for studying patterns of adaptive molecular evolution. Accordingly, we used RNA-Seq to identify genes involved in response to parasite infection in P. antipodarum and then used the inprogress P. antipodarum genome project to obtain DNA sequences from 51 candidate genes, including several immune genes, from 27 P. antipodarum lineages (10 diploid sexuals, 12 triploid asexuals, 5 tetraploid asexuals) and a diploid sexual outgroup, Potamopyrgus estuarinus. We are now using these data to evaluate patterns of adaptive molecular evolution (e.g. positive selection) across these lineages to determine the relative roles of polyploidy and reproductive mode as facilitators of adaptive evolution in P. antipodarum.

Keywords: asexuality, adaptive molecular evolution, positive selection

Evaluating the dynamics of transposable element evolution in non-hybrid polyploids

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Changes in ploidy level are expected to have major effects on evolutionary trajectories and genome architecture. The overwhelming predominance of diploidy in eukaryotes thus suggests that this trait is maintained at least in part via selection, though the specific evolutionary mechanisms involved remain unclear. One possibility is that polyploidy is associated with a higher genomic burden of mildly deleterious mutations. The insertion of transposable elements (TEs), mobile DNA sequences that replicate and insert themselves throughout a host genome, constitutes a mutational event of particular evolutionary significance. TEs, despite their often deleterious consequences, are ubiquitous across nearly all organisms and are one of the main drivers of the immense variation in eukaryotic genome size. Understanding the conditions that influence the spread and accumulation of TEs is thus of central importance to understanding the evolution of genome architecture. Ploidy elevation is expected to result in bursts of TE proliferation for several reasons, ranging from the masking of harmful recessive insertions to an increase in the availability of “neutral” genomic regions for insertion. Most empirical support for this hypothesis has come from allopolyploids, meaning that the relative importance of the roles of hybridation vs. polyploidy per se remain unclear. Here, we use Potamopyrgus antipodarum, a New Zealand freshwater snail characterized by frequent coexistence of closely related and ecologically similar diploid, autotriploid, and autotetraploid lineages to evaluate how changes in ploidy level affect the accumulation of TEs in non-hybrid polyploids. We have identified and classified several hundred families of TEs representing the major eukaryotic TE groups in the P. antipodarum genome through de novo bioinformatic techniques. We are now using this TE catalog and whole-genome resequencing data from 27 P. antipodarum lineages collected from natural populations to conduct a rigorous evaluation of the relationship between ploidy elevation and TE abundance. While these analyses are still in progress, our data do suggest a recent burst of TE activity in P. antipodarum and extensive variation in TE abundance across ploidy levels. Regardless of outcome, this research will provide a novel window into the processes of TE evolution in non-hybrid polyploids.

Keywords: mobile elements, Potamopyrgus antipodarum, genome evolution, repeat landscape
The fate of ribosomal RNA genes in spontaneous dogrose hybrids (Rosa L. sect. Caninae (DC.))

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Dogroses (Rosa L. sect. Caninae (DC.) Ser.) originating by complex hybridisation evolved a unique imbalanced meiosis system allowing for sexual reproduction of these odd ploidy level species. In pentaploid dogroses only two sets of chromosomes pair during meiosis: one set is provided by the haploid sperm cell the other by the tetraploid egg cell. The remaining non-pairing univalents are exclusively transmitted by the egg cell. Spontaneous interspecific hybrids are most frequently hexaploids derived from the fusion of unreduced (pentaploid) egg cells and haploid sperm cells of another. We investigated the evolutionary fate of nrDNA loci in these hybrids compared to their parental species by performing FISH, traditional sequencing, NGS amplicon sequencing and transcription analyses of the nrITS region. Additionally, we estimated genome sizes by flow cytometry. Since rDNA-loci were stably inherited and sequence homogenization between sub-genomes was nearly absent hybrids preserve the rDNA variation of parents. The transcription was dominated by one nrITS-type.

Keywords: ribosomal DNA, expression, hybridization, Rosa

Polyplody and phenotypic novelty: Phylogenetic context of DMSP (dimethylsulfoniopropionate) biosynthesis in Spartina (Poaceae, Chloridoideae)

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Polyplody has pronounced effects on phenotypes. Here we explored the ability to produce DMSP (dimethylsulfoniopropionate), a tertiary sulfonium compound with major ecological importance in the global sulfur cycle, in the polyploid genus Spartina (Poaceae). DMSP is commonly produced by marine algae but is very rare in Angiosperms (restricted to a few Poaceae or Asteraceae species) where its anti-stress and osmoregulation role was proposed. In these different lineages, synthesis of DMSP has evolved independently and it has been shown to involve different enzymatic steps. However, little is known about the genetic and regulatory mechanisms involved in the DMSP biosynthesis pathway. DMSP production was examined in Spartina using 1H Nuclear Magnetic Resonance and UPLC-MS on plants maintained in controlled conditions. Species from various ploidy levels (ranging from tetraploid to dodecaploid) were examined. Our analyses revealed that this new phenotypic trait has a monophyletic origin and arose during the emergence of the hexaploid Spartina lineage (S. maritima, S. alterniflora, S. foliosa) and was inherited by its derived hybrids and allopolyploid species. All these species colonize low marsh zones and are able to tolerate several hours of immersion under sea water. The tetraploid high marsh or inland Spartina species do not produce DMSP. According to molecular dating based on nuclear and chloroplast DNA, the ability to produce DMSP arose between 2 and 10 million years ago in Spartina. S. alterniflora leaves exhibit more DMSP than S. maritima. Their two recently formed hybrids S. x townsendii (in England) and S. x neyrautii (in France) display different amounts, S. x neyrautii being intermediate with respect to its parents, whereas S. x townsendii patterns are more similar to those of S. maritima. The allododecaploid S. anglica exhibits similar DMSP amounts than S. x townsendii in spite of genome doubling. Comparative approaches between DMSP producing and non-producing species are being performed including enzyme assays, candidate gene analyses in order to understand the molecular mechanisms involved in the emergence of this ecologically important functional innovation inherited from the hexaploid Spartina ancestor.

Keywords: Spartina, biosynthesis pathway, DMSP, evolution
Are tetraploid roses better resistant to stress compared to diploids?

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Ploidy levels in rose species vary from diploid to octoploid. Examples of diploid species are R. arvensis, R. banskiae, R. bracteata, R. nitita, R. rugosa and R. wichurana. Tetraploids are for example R. foetida, R. gallica and R. spinosissima. A special case is found in the pentaploid dogroses (section Caninae) with their very specific asymmetric meiosis. In most rose species only one ploidy level is observed. However, some can have different ploidy levels. For example R. chinensis can be di-, tri- or tetraploid, while R. acicularis can be tetra-, hexa- or octoploid. Interspecific hybrids between different ploidy levels have occurred spontaneously in wild species, but also in the breeding history of roses different ploidy levels were used. Now, most garden roses are diploid, triploid or tetraploid. Typically cut roses are tetraploid. In this study we aimed to compare biotic and abiotic stress resistance in diploid versus tetraploid roses. Therefore ten diploid rose genotypes were artificially chromosome doubled in tissue culture using the antimitotic agent oryzalin. Morphological differences could be observed between plants of the two ploidy levels. In general the tetraploids grew slower and had lower dry matter content compared to the diploids. Four diploid rose genotypes and their polyploidized counterparts were evaluated for biotic stress resistance towards the fungal pathogen, powdery mildew (Podosphaera pannosa). Two pathotypes of rose powdery mildew were used for artificial inoculation and disease indexes were calculated for the diploids and tetraploids. For all four genotypes the tetraploids exhibited slightly higher resistance towards powdery mildew compared to the diploids. Ten diploid rose genotypes and their tetraploid counterparts were submitted to drought as abiotic stress inducer. Biomass, relative water content, stem diameter, leaf water potential, stomatal conductance, carbohydrates etc. were measured. Results were genotype dependent, on the tetraploid level three genotypes became more tolerant to drought stress, while five were less tolerant. In two genotypes there was no difference in drought stress tolerance between the two ploidy levels. Nevertheless, strong drought stress has led to metabolic adaptations, both in the level of stress related hormones (abscisic acid) as osmotic adaptation (soluble sugars and proline content). Also the type of adaptation was genotype depended.

Keywords: drought, mitotic polyploidisation, powdery mildew, Rosa, stress resistance
POSTERS
Coevolution of homoeologous vernalization genes $VRN\text{-}1$ in polyploid wheats: a new possibilities for wheat adaptation

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Adaptability of polyploid wheat to a wide range of environments has been at least partially facilitated by allelic diversity in $VRN\text{-}1$ genes regulating growth habit and flowering time. Changes in the growth habit of winter wheat to spring wheat as well as modulation of flowering time are primarily due to polymorphisms in regulatory regions (promoter or intron 1) of $VRN\text{-}1$. We showed that almost all known $VRN\text{-}1$ alleles characteristic of polyploid wheat species probably arose during different stages of polyploid evolution and were selected during domestication. Still $T.\text{ dicoccoides}$, the wild form of all domesticated emmer wheats had a distinct set of $VRN\text{-}1$ alleles compared to the diploid progenitors, indicating an independent origin of spring tetraploid forms. Our data confirmed that each homoeologous $VRN\text{-}1$ gene has specific polymorphisms preferentially affecting one of the regulatory $VRN\text{-}1$ regions. The transcript levels from $VRN\text{-}1$ homoeologs vary considerably depending on the allelic combination at homoeologous loci. This implies the coordinated transcription of homoeoalleles with a wide range of productivity. A higher variability of $VRN\text{-}1$ loci in polyploid species in comparison with diploids may be explained by the hypothesis that stresses due to allopolyploidization may provoke genome instability. This variability gives polyploid wheat an advantage in conferring adaptation to a broader range of environments.

Keywords: vernalization gene, flowering time, growth habit, wheat

To the problem of the genetic variability of Mentha × verticillata L.

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We have studied a hybrid species Mentha×verticillata L. (M. arvensis L. × M. aquatica L) widespread in the European territory of Russia. Formation of hybrid offspring is highly probable when populations of M. arvensis and M. aquatica come into contact. The hybrid species is characterized by variable combinations of parental characters and is sometimes virtually indistinguishable from one of the parents phenotypically, but Inter Simple Sequence Repeat (ISSR) marker profiles enable identification of hybrids. ISSR markers were generated with 12 different primers for 46 samples of M.xverticillata and parental species from 6 geographically distant localities. Principal coordinates analysis (PCoA) of the ISSR data reveals the majority of M.xverticillata samples forming a loose cloud together with those of M. arvensis, while some of them are located close to an independent cloud of M. aquaticD samples. The key diagnostic character of inflorescence structure in M.xverticillata seems to poorly separate it from both parents when tested statistically. The hybrid species may often occupy the same ecological niche as one of the parents. In our study plants identified as M.xverticillata occurred in all populations of M. arvensis growing in contact with M. aquaticD. Bayesian analyses using the Structure and the New Hybrids programs show that all the samples identified morphologically as M.xverticillata demonstrate admixed genetic nature, with the exception of the plants from Kaluga and Penza regions of Russia. The analyses of the ISSR data in the New Hybrids program identified most of the morphologically typical M. arvensis and M. aquaticD samples as the parental species, while all the other samples were represented by F1 and F2 hybrids with rare backcrosses notwithstanding their morphology. Only samples of M.xverticillata from Belgorod region appear to be both morphologically and genetically identified as typical representatives of this hybrid.

Keywords: Mentha×verticillata, hybridization, DNA, PCR, ISSR
Cross species comparison of genome-wide time-course expression after submergence with two diploid *Cardamine* species and their allotriploid offspring

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Polyploidization is one of significant speciation events in plant evolution because the polyploidization, especially allotriploidization, contributes to environmental adaptation. To understand the transcriptome mechanisms, we observed submerged leaf expression profiles of genus *Cardamine*, which is closely related species to *Arabidopsis thaliana*. We used two diploids *C. amara* (2n=2x=16, AA) and *C. rivularis* (2n=2x=16, RR) and their allotriploid offspring *C. insueta* (2n=3x=24, RRA), whose hybridization happened ~100 years ago [Mandáková et al., 2013]. *C. amara* grows in wetland habitats while *C. rivularis* prefers dry fields. *C. insueta* lives in the middle niche of the two species. We measured genome-wide gene expressions from the three species after submergence using RNA-seq. Leaf samples were collected at the nine timepoints (between 0 to 96 hours) from each species. From *C. insueta*, homeolog-specific gene expression levels were using HomeoRoa [Akama et al., 2014]. An average number of expressed genes over all samples is 11,607. We compared numbers of up-regulated genes after submergence among three species. Software edgeR detected up- or down-regulated genes by comparing expressions of 0h with another timepoint on each species. The number of up-regulated genes in *C. amara* achieves the maximum at 2h after submergence, while that in *C. rivularis* is maximized at 24h, indicating the difference in gene regulation responses between two diploid species. Interestingly, the expression change in *C. insueta* has two peaks at 2h and 24h, suggesting that transcriptome in *C. insueta* have a capability of both parental species. Surprisingly, homeolog-specific expression profile showed that both parental origins were equally up-regulated at both timepoints, implying that trans-regulation between two different species in the allotriploid has a significant role for the stimulus response.

Keywords: RNA-seq, homeolog-specific expression, gene expression analysis, differentially expressed genes, submergence

Hybridization and polyploidization in *Chenopodium album agg.* (Chenopodiaceae): allotriploid origin of hexaploid *C. album s. str.*

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Reticulate evolution is characterized by occasional hybridization between two species, creating a network of closely related taxa below and at the species level. In the present research, we aimed to verify the hypothesis of the allotriploid origin of hexaploid *C. album s. str.*, identify its putative parents and estimate the frequency of allotriploidization events. We sampled representatives of *C. album agg.* covering most of the species distribution range in Eurasia. In addition we performed a detailed sampling of five populations in Czech Republic. Our samples included putative progenitors of *C. album s. str.* of both ploidy levels, i.e. diploids (*C. ficifolium, C. suessicum*) and tetraploids (*C. striatiforme, C. strictum*). To fulfil these objectives, we analyzed variation in genome size, sequence variation in the nrDNA ITS region and the cp32-trnL, intergenic spacer of cpDNA, performed genomic in-situ hybridization (GISH) and analyzed the variation at 10 nuclear microsatellite loci. We confirmed the allohexaploid origin of *C. album s. str.* According to the results of genome size and GISH analyses it originated via hybridization between a diploid and tetraploid species. Analysis of cpDNA revealed tetraploid as the maternal species. In most accessions of *C. album s. str.*, ITS sequences were completely or nearly completely homogenized towards the tetraploid maternal ribotype. The particular parental species of most hexaploid accessions could not be identified unambiguously. However higher homology in GISH and higher similarity based on analysis of microsatellites indicates that *C. striatiforme* rather than *C. strictum* is the tetraploid species involved in the origin of *C. album s. str.* The patterns nrDNA ITS variation at continental scale indicate that *C. album s. str.* have originated multiple times. The co-occurrence of genetically uniform individuals with those showing a certain degree of genetic admixture in respect of microsatellites, or those with completely homogenized ITS sequences together with individuals harboring both parental sequences, within a single population suggest that recently growing populations of *C. album s. str.* may in fact represent a mixture of ancient and recent hybrids.

Keywords: cytogenetics, hybridization, intraspecific variation, molecular phylogeny, polyphyletic origin
The presence of foreign ITS ribotypes in barleys (Hordeum, Triticeae) suggests horizontal transfer from panicoid grasses

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Recently, we discovered in some members of Triticeae (Pooidae) unexpected foreign genetic material from a different grass subfamily, Panicoideae. In particular, allohexaploid Elymus repens and its likely progenitor Hordeum bogdanii harboured small amounts of ITS ribotypes corresponding to Panicum. A follow-up detailed analysis of the distribution of panicoid ribotypes across diploid Triticeae revealed a spectacular diversity of foreign ITS ribotypes within the genus Hordeum. They corresponded in phylogenetic analysis with up to five present-day genera of panicoid grasses, namely Psammopogon, Panicum, Euclasta, Setaria and Arundinella. Distribution patterns differed between species and even between accessions within species. While Central Asian and North American species harboured only a single foreign ribotype (corresponding to Panicum or Psammopogon, respectively), the highest ribotype diversity was found in South American taxa, in which individual plants harboured up to five ribotypes with high sequence similarities to species from across the Panicoideae subfamily. Substitution patterns and preliminary expression studies carried out on the panicoid ribotypes suggested that they may be pseudogenes. So far, the time and mode of ribotype transfer between the subfamilies are unknown. Their distribution across Hordeum and panicoid phylogenies along with their putative pseudogene status suggest that the transfer(s) occurred before and/or during the rapid diversification of genome Hordeum species, some 1.4 my ago and/or later. Sequencing of the BAC clone, possessing the foreign ITS ribotypes in Hordeum bogdanii, revealed the presence of LTR-retrotransposons, enclosing the arrays of foreign ribotypes and suggesting a possible role of the TEs in the gene transfer. BAC-FISH showed that while the foreign ITS arrays in H. bogdanii reside on the NOR-bearing chromosome, they reside on the opposite chromosome arm. Although the patterns are not readily explicable, due to the overall phylogenetic distance of and the presence of crossing barriers between current pooid and panicoid grasses we consider horizontal gene transfer(s) via unknown vector as the most plausible mode of the ribotypes acquisition. These findings represent an enigmatic example of multiple foreign genetic material transfers between members of different grass subfamilies.

Keywords: Hordeum, ITS ribotypes, horizontal transfer, LTR-retrotransposon

Evolutionary history and genetic variation of Dianthus arenarius

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Dianthus (Caryophyllaceae) is a very polymorphic genus with a high degree of endemism. Its diversity is significantly shaped by polyploidy, hybridization, edaphic speciation and geographic vicariance (splitting in the range of a taxon). This is also true for European Dianthus sect. Plumaria species. We studied the most widespread member of this group - white flowered Dianthus arenarius. There are five subspecies traditionally recognized, occurring on sandy soils from Central Europe to Russia and Scandinavia. Real distribution, relationship between subspecies, as well as their morphological differentiation is not properly known. Special attention was paid to the Czech stenoendemic D. arenarius subsp. bohemicus, occurring on a single site in the Czech Republic. We employed flow cytometry (absolute genome size), cpDNA sequencing, microsatellite analysis and multivariate morphometrics to evaluate the relationships between Dianthus arenarius subspecies and among other species from D. sect. Plumaria. Within Dianthus arenarius, subsp. bohemicus significantly differs in genome size (1C value) from all other subspecies, but is almost similar to other sect. Plumaria species (e.g. D. tunnolitieri, D. plumarius, D. serotinus). The microsatellites pattern of subsp. bohemicus is closer as well to other D. sect. Plumaria species than to D. arenarius. The morphological variation of the studied D. sect. Plumaria species is highly continuous and D. arenarius subspecies and related species are very close. The differences of subsp. bohemicus compared to the rest of D. arenarius may be caused by 1) hybridization with other species (e.g. from D. sect. Plumaria) or most probably by 2) taxonomic non-homogeneity of D. arenarius (i.e. subsp. bohemicus may evolved from other species independently).

Keywords: Dianthus arenarius, flow cytometry, genome size, microsatellites, morphometrics
Genus *Allium* in North-Africa: Phylogeny, polyploidy and endemism

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The genus *Allium* L. is one of the most species-rich genera of Amaryllidaceae. In the Mediterranean region, this genus exhibits high taxonomic diversity accompanied by the occurrence of disyploidy (x = 7, 8, 9, 11) and high ploidy levels (4x, 5x, 6x, 8x). Cytotype distribution, karyotype evolution and phylogenetic relationships were investigated in North-African taxa. Analyses were performed on natural populations belonging to the polyploid complexes *A. ampeloprasum*, *A. cupanii* and the endemic *A. fontanesii*, *A. odoratissimum*, *A. trichocnemis* and *A. seirotrichum*. Phylogeny was based on nuclear rDNA (ITS region) and plastid DNA sequences, and comparative analyses were performed with *Allium* sequence databases available from Genbank. This allowed us to situate the North-African *Allium* samples, including previously unexplored endemics, in the global phylogenetic context of the genus. Karyological investigation within the *A. ampeloprasum* complex indicated an unexpected higher frequency of diploid populations (2n=16) than tetraploids (2n=32). *A. cupanii*, *A. odoratissimum*, *A. fontanesii*, *A. seirotrichum* and *A. trichocnemis* are all tetraploids (2n=32). Concerning, *A. ampeloprasum*, diploid and tetraploid representatives from North Africa were grouped in the same clade in both cpDNA and ITS trees suggesting that autoploidization operated as a major process among these taxa. In addition, our results clearly indicate that the North-African *A. ampeloprasum* diploids, most likely contributed to the formation of other euro-Mediterranean allopolyploid *Allium*. For instance, these diploids appear as progenitors of the cultivated 6x 8x Great-headed Garlic and 4x Leek group. The tetraploid endemic taxa are divided into three distinct well-supported clades. The narrow endemics, *A. seirotrichum* and *A. trichocnemis*, occurring in scattered stands of the Tellian Atlas, are grouped in the same clade and are phylogenetically isolated from the *A. cupanii* group, despite their karyological and ecological similarities. *A. fontanesii* and *A. odoratissimum* endemic to NW and NE Africa respectively, are separated into two distinct clades. Finally, the high occurrence of diploid populations of *A. ampeloprasum* complex in northern Africa suggests a recent radiation that is at the origin of the Mediterranean polyploid lineages, whereas the endemics could represent relict polyploids in this area.

Keywords: *Allium*, endemism, taxonomy, karyotype, molecular phylogeny.

Karyotype evolution and diversification in *Pulsatilla* Mill. (Ranunculaceae)

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Karyotypic changes such as polyploidy, disyploidy and chromosomal rearrangements are amongst the major drivers in plant evolution and speciation. Their role is investigated in the North hemisphere genus *Pulsatilla* Mill. (Ranunculaceae). Chromosome numbers, karyotype morphology, banding pattern, 5S and 35S rDNA localization in the species were investigated and interpreted in a phylogenetic context drawn from sequences of the nuclear ribosomal internal transcribed spacer (ITS) and plastid *accD-psy*, *psbM-trnD* and *trnL-trnF* intergenic spacers. The phylogenetic analysis of concatenated sequences identified the main phylogenetic lineages within the genus, which mostly corresponds to the classic series-level classification. The cytogenetic data obtained in this study agree with the molecular phylogeny of the *Pulsatilla* obtained here for the first time; the lineages in the phylogenetic tree correspond with the number and distribution of rDNA loci. All species had a chromosome base number of x=8. The karyotype showed a significant variation among diploids with one or two 35S rDNA loci to one to three 5S rDNA loci. Hemizygous 5S rDNA sites have been widely observed in many *Pulsatilla* species. The position of the 35S rDNA loci is relatively conservative, as the 35S rDNA loci are located terminally on the short arm of acrocentric chromosomes. 5S rDNA sites showed much more variability both in number and physical location than did 35S rDNA sites. For 5S rDNA there is no preferential chromosomal position as it exhibits terminal, subterminal, interstitial or pericentromeric position, and is located either on acrocentric or metacentric chromosomes. Greater variation in the number and position of 35S and 5S rDNA loci is found in the polyploid taxa analysed. Tetraploids exhibit changes such as rDNA loss/gain. The dispersed 35S rDNA signals occurred at centromeric positions which are hotspots for chromosomal breakpoints and are also enriched for transposable elements. Therefore we postulate that the seeding of rDNA repeats to ectopic locations in the genome could be the result of transposable element activity in polyploids. Genome restructuring, especially involving 5S rDNA characterizes the evolution of *Pulsatilla*. Ancestral state reconstruction of number of rDNA signals brings evidence that these characters have followed increases and decreases during evolution in *Pulsatilla*.

Keywords: karyotype evolution, phylogeny, *Pulsatilla*, rDNA
Comparative analysis of repetitive DNA in eight representatives of fescues and ryegrasses

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Fescues (Festuca sp.) and ryegrasses (Lolium sp.) are economically important grass species widely distributed over all continents except Antarctica. Fescues are used for turf and high quality forage for livestock, while ryegrasses are the most important forage and turf grass species. Both genera include diploid and polyploid species and their artificial hybrids called Festuloliurns, which may differ in the contribution of parental genomes, combine superior agronomical traits from both parents. Fescues and ryegrasses have large nuclear genomes (1C ranging from about 3,000 Mb to 10,000 Mb), which consist mainly from repetitive DNA. This class of DNA sequences evolves more rapidly than coding sequences and can be used to analyze genetic diversity and study processes accompanying speciation and genome evolution. The present work aims to characterize repetitive part of nuclear genomes of seven species of ryegrass and fescue (L. multiflorum, L. perenne, F. arundinaceae, F. gigantea, F. glaucescens, F. mairei and two cultivars of F. pratensis) and identify the contribution of DNA repeats to their genome diversity. Partial genome sequence data were obtained by illumina sequencing and used for all-to-all comparisons. The RepeatExplorer pipeline was used for genome-wide comparative analyses, to estimate genomic abundance, identify orthologous repeat families and to estimate inter-genomic differences. Retrotransposons were the most abundant repeat type identified in all species. Out of them, Ty3-Gypsy elements were the most frequent (more than 3.5 and 6 times more abundant than Ty1/Copia in fescues and ryegrasses, respectively). DNA transposons and LINE elements were less frequent, but relatively high number of different tandem repeats was identified in nuclear genomes of all species. In general, high number of similarity hits was found between the species of Festuca and Lolium. Within the fescues, F. mairei and F. glaucescens showed the lowest similarity of DNA repeats as compared to other species of Festuca. This work provides detailed insights into the genome composition and its variation in fescues and ryegrasses; it will be a useful resource for repeat masking during genome sequence analyses and facilitate development of new cytogenetic markers suitable for studying of genome organization by FISH.

Keywords: Festuca, Lolium, repetitive DNA, illumina sequencing

Hybridization processes in wild rose populations of South-East Russia and Ukraine

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It is often difficult to identify species of Rosa because of their morphological variability, ability to hybridize with each other, and balanced heterogamy characteristic of the section Caninae. However, there are only a few studies showing that hybridization indeed occurs in nature while the frequency and consequences of these hybridization events are unknown. We used ISSR fingerprinting and cpDNA intergenic spacer trnV-ndhC sequencing along with morphological analyses and field artificial hybridization experiments to confirm hybridization in wild rose populations from 3 distant localities in the Eastern Europe.

Locality 1. SE European Russia, Volgograd Prov., a wide gulley N of Volgograd. Here we have studied 38 samples from a sympatric growth of R. canina, R. caesia and R. cinnamomea. R. canina hybridizes with R. cinnamomea acting as a pollen donor. The hybrids morphologically correspond to R. caesia, some morphologically intermediate plants appear to be backcrosses to R. canina. Locality 2. SE Ukraine, Donetzk Prov., “Khomutovskaya steppe” Reserve. We have analysed 47 plants of 15 rose species listed for the Reserve. It appeared, that all the morphological diversity of local roses was represented by hybrids between R. rubiginosa (pollen parent) and R. grossheimii (seed parent), a local endemic of SE Ukraine from the same Caninae section. Locality 3. S European Russia, Belgorod Prov., “Belgorje” Reserve, “Stenki Izgorja” protected area. We analysed 32 samples of R. villosa, R. rubiginosa, and a local endemic R. oskolenis. It appeared that R. oskolenis and a few unidentified plants are triple hybrids between R. villosa and R. rubiginosa, and a third species related to R. grossheimii, which served as a seed parent. Our results confirm frequent hybridization between wild roses of Caninae and Cinnamomeae sections with quite different results, from introgressive hybridization, to strong morphological segregation in hybrid populations without backcrossing to parental species.

Keywords: Rosa, hybridization, morphological segregation.
Consequences of hybridisation processes in the intergenic spacer of ribosomal DNA in Armeria (Plumbaginaceae)

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In a series of studies going back fifteen years, an active evolutionary role of hybridisation, including introgression (also known as introgressive hybridisation), has been well documented in genus Armeria (Plumbaginaceae). The present research is focused on Armeria pungens, the most distinct species of the genus in morphological terms. It grows on coastal sand dunes along SW Iberia, from Setúbal to Cádiz, and disjuntly, in Corsica, Sardinia and the Cíes Islands (Galicia). We focus on the southernmost population of Punta Camarinal (Cádiz) as previous phylogeographic studies revealed introgression with A. macrophylla, recognised as the cpDNA donor. A. macrophylla occurs on pine understory of subcoastal areas on sandy but more developed soils as compared to A. pungens. The introgressed population of A. pungens occurs on a fossil dune colonised by a shrub community instead of the specific dune vegetation, typical of the species. We have sampled the introgressed A. pungens population, the cp-DNA donor population A. macrophylla (lying next to the former) and the closest non-introgressed population from A. pungens (Cabo Trafalar, Cádiz). In the framework of a wider project on the activity of transposons (TE) in non-model plants, we are here interested in the genomic consequences of TE activation after hybridisation, a genomic stress that could cause burst of TE activity. In particular, we assess the occurrence of TE in the intergenic spacer (IGS) of the ribosomal DNA (rDNA). Here we present their complete sequences, ranging from 3.7 kb (non-introgressed A. pungens), 4.3 kb (A. macrophylla) to 4.8 kb (introgressed A. pungens). Our findings show that the three IGS may be functional, as regulatory sequences are present. There are also several subrepeated regions, as typically found in the IGS of angiosperms. Remarkably, the sequence similarity is higher between A. macrophylla and the introgressed A. pungens (86.93%) than between introgressed and non-introgressed A. pungens (60.81%). Also, up to six fragments with high homology with TE (including LTRs and superfamily CACTA transposons) were found, suggesting that rDNA may have been frequently focus of TE activity. Certain TE fragments are detected in the introgressed population of A. pungens that are not present, neither in A. macrophylla, nor in the non-introgressed A. pungens. However data on more individuals is needed to support the role of TE in introgression processes within genus Armeria.

Keywords: intergenic spacer, introgression, ribosomal DNA, subrepeats, transposable elements

Does introgression to Salvia nemorosa threaten relict Salvia nutans?—a study using additive polymorphism and flow cytometry

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Salvia nutans is a strictly protected stepetic relict in Hungary, which can only be found on two locations. On these sites Salvia nemorosa is also present and hybridisation between the two species is well documented. In some cases it is difficult to identify the hybrid Salvia × betonicifolia, because some of the species are morphologically close to S. nutans, and observations suggest introgressive hybridisation. For nature conservation it is very important to identify the hybrid specimens. Tissue samples were collected from the field, we were able to analyse four Salvia × betonicifolia, four S. nutans, one S. nemorosa samples from ‘Kondors’, and three Salvia × betonicifolia, three S. nutans and one S. nemorosa from ‘Tatársánc’. Identification of one specimen from Tatársánc and four from Kondors were uncertain. In addition three more samples were included from Ukraine and Russia, which were not hybrids. We screened low-copy nuclear genes (LCNG) to use as molecular markers to identify F1 hybrids. We also used flow-cytometry to study the DNA-content of the parents and the hybrid. Seven LCNG regions were tested, which were originally designed in Salvia miltiorrhiza and Mentha × piperita. Although several gave specific band at the PCR-amplification step, only one of the seven markers was not length-polymorphic. The region was sequenced on the whole dataset yielding 402 bp. The sequences separated the parents by several SNPs. Although several double-peaks were found in the sequence, if we focused only on additive polymorphic sites (APS - sequential differences that separates the two species), they could be taken as evidence of hybridisation, since both bases could be found in the hybrids. Additive sites were coded following IUPAC nucleotide ambiguity codes. In total nine APS could be identified, eight SNPs and an indel at the position 325. We confidently identified F1 hybrids based on the sequences, but none of the possible backcrossed hybrids gave hybrid genotype. Although we cannot exclude the possibility of fast elimination of alleles from the other species, our DNA-content investigation makes introgression unlikely. Flow cytometry indicated intermediate value for S. × betonicifolia, and, together with the published chromosome numbers (S. nutans: 2n=22, S. nemorosa: 2n=14), this would imply an uneven chromosome number for the hybrid (2n=17). Based on our results, the Hungarian populations are not threatened by introgression.

Keywords: additive polymorphic site, introgression, homoploid hybridisation, low-copy nuclear gene, flow cytometry
Mesopolyploid evolution in the Australian and New Zealand crucifers

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We showed previously that three endemic Australian crucifer species (tribe Microlepidieae, Brassicaceae) have undergone a whole-genome duplication (AUS-WGD) followed by lineage-specific diploidization, generating some of the lowest chromosome numbers (n = 4-7) known for the crucifers (Mandáková et al. 2010a). The New Zealand genus Pachycladon has undergone either the AUS-WGD or an independent WGD followed by less extensive genome reshuffling towards n = 10 genomes (Mandáková et al. 2010b). To further elucidate the origin and fate of Australian/New Zealand mesopolyploid genomes, we analyzed 12 species (9 genera) with variable chromosome numbers (n = 4, 5, 6, and 7) by comparative chromosome painting and gene sequencing. We concluded that:

(i) All members of the Microlepidieae tribe had the same allopolyploid origin through AUS-WGD. This WGD most likely spurred the diversification of the group on the Australian continent.

(ii) Cytogenetic signatures and multi-gene phylogenies suggest that the ancestral allopolyploid genome had most likely 30 chromosomes (n = 15) and was formed through an inter-tribal hybridization (tribe Crucihimalayeae x Smelowskieae/Descurainieae), followed by a long-distance dispersal from Asia to Australia.

(iii) The AUS-WGD was followed by independent and massive genome rediploidizations towards the extant diversity of quasi-diploid genomes (n = 4-7) of the Australian Microlepidieae taxa.

(iv) The New Zealand genus Pachycladon was formed by an independent WGD event.

Keywords: Brassicaceae, mesopolyploidy, whole-genome duplications, karyotype evolution, chromosomes, ancestral genome, cytogenetics

Incidence of polyploidy in aquatic members of genus Ranunculus (formerly Batrachium)

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Genome duplication is a driving force especially in the evolution of angiosperms affecting not only their genetic and genomic structure but also the phenotype, therefore influencing their ecology and geography. Despite that, the incidence of polyploidy in aquatic flowering plants has scarcely been studied. The aim of this study is to analyse the frequency of polyploidization within the aquatic members of the genus Ranunculus in Austria. To this end ploidy levels were established in populational samples of all aquatic Ranunculus species. Individuals were collected in rivers, back water, streams, lakes and ponds in all but western Austria (Vorarlberg and Tyrol), from low- to highland ecosystems. Different ploidy levels (2x, 3x and 4x) were found using DNA flow cytometry in several species indicating both intra- and interspecific polyploidization. Sequencing of several plastid DNA regions allowed relationships among the species to be inferred and the mode of polyploid origin to be determined. Chromosome numbers have been established for selected individuals of all species. In addition to genetic and genomic analyses aiming to elucidate the frequency and mode of polyploid formation plant sociology and selected chemical parameters of the water were examined. No correlation has been found between the human impact on waterways (assessed through plant sociology and water chemistry) or the chemical characteristics of the water and the distribution of diploid and polyploidy cytotypes of analysed Ranunculus species. These results indicate that incidence and level of polyploidization are not influenced by water chemistry.

Keywords: aquatic Ranunculus species, ploidy levels, distribution, phylogeny, water quality
Comparative analysis of wheat chromosome arms 3BS and 3DS

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Bread wheat (Triticum aestivum) is one of the most important crop plants. It is allohexaploid species with genome composed from three closely related subgenomes (2n=6x=42; AABBDD). Bread wheat arose from two recent hybridization events. The first one took place between diploid progenitors of A genome (T. urartu) and B genome (species related to Aegilops speltoides) about half million year ago. The other one occurred between tetraploid wheat that had arisen from the first hybridization (T. dicoccoides; AABB) and diploid progenitor of D genome (Ae. tauschii) about ten thousand years ago. As a consequence, B and D subgenomes of bread wheat have stayed in a polyploid nature together with similar genome(s) for different time. Hence, we expect extended structural fractionation of the B genome compared to the D genome. In this work, we established physical map of bread wheat chromosome arm 3DS (321 Mbp). The map was developed using 3DS-specific BAC library and High-Informative Content Fingerprinting technique. In total, 870 contigs were built in FingerPrinted Contigs software in several steps with successively decreasing stringency. Cumulative length of the physical map is 275 Mbp, which corresponds to 85% of expected size of the arm. Majority of contigs is anchored to at least one marker/sequence representing 268 Mbp (97% of the physical map). We further focused on integration of genes homoeologous to genes found on chromosome arm 3BS. Up to now, we identified homoeologues of 618 genes located on 3BS and determine their position on chromosome arm 3DS. Sequencing of complete physical map is in progress with aim to identify as complete set of genes on 3DS as possible. Finally, gene content of the two chromosome arms will be compared in detail. (This work was supported by Czech Science Foundation award 13-8786S.)

Keywords: physical mapping; homoeologous chromosomes; sub-genome comparison; bread wheat

Polyploidy and hybridization within the Genus Spiraea L. (Rosaceae Juss.)

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Polyploidy is a common phenomenon in the plant kingdom. Ploidy levels from 2x up to 8x have been reported from the about 30 species of the Spiraea genus. But in natural populations diploids and tetraploids occur more frequently. The nucleotide variability in the ITS region has been studied in 10 Russian species of Spiraea. The species-specific single nucleotide polymorphisms and insertions / deletions of 2-8 nucleotides in length have been found. The hybrids between closely related taxa were identified in the Asian part of Russia (north of the Amur region, south-east of the Yakutia and Zabaikalskii Region). Spiraea salicifolia x S. humilis and S. media x S. dahurica have three transitions. Newly established mutations may have an adaptive role. (The study was supported by a grant RFBR 15-04-03093.)

Keywords: Spiraea, ITS, SNP, hybrid
Introduction to the animal database of ribosomal DNA loci

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Eukaryotic ribosomal DNA (rDNA) loci vary both in positions and numbers. rDNA encodes the four critical genes needed for ribosome function: the 5S, 5.8S, 18S and 26S rRNA. In animals, the 18S-5.8S-28S genes (45S) are always linked in a single 45S rDNA unit. The 5S genes occur as separate tandems or linked to the 45S units. Here, we describe a database containing information about the chromosomal position, number of 5S and 45S and their relative position (linked /co-localized/ separated) in more than 790 animal species. The data based on in situ hybridization of metaphase chromosomes have been collected from more than 320 research articles. Species representation is descending in the following order: Fishes (45% of species), arthropods (31%), mammals, mollusks, reptiles, amphibians, birds, annelids, cartilaginous fish and flatworm. Fishes also have the greatest variability in the number of rDNA loci (1 to 21 5S sites and 1 to 23 45S sites per haploid genome) which may be explained by overrepresentation of these genera in the database, but other factors such as ploidy, karyotypic divergence and genome plasticity should also be considered. Average numbers of rDNA loci are 2.6 5S and 2.7 45S per haploid genome. The 5S and 45S loci are located on different chromosomes in most of the genomes (81%). Thirty five percent of species have 5S rDNA in a single locus, whereas 51% of species have 45S rDNA in a single locus. The animal database will soon become accessible via a web-page as a mirror to recently issued Plant rDNA database (http://www.plantrdnadatabase.com/).

Keywords: rDNA, FISH, animal, chromosome, evolution, database

Inferring Paleopolyploidy using a MultitAxon Paleopolyploidy Search (MAPS) Analysis

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Paleopolyploidy or ancient whole genome duplication (WGD) is common among many plants and some animals. Turnover in genome content following WGDs and saturation of substitutions over time make ancient WGDs, especially very old WGDs, challenging to detect. Multiple methods for detecting paleopolyploidy have been developed, but many methods provide limited information on the phylogenetic placement of ancient WGDs. We present a novel MultitAxon Paleopolyploidy Search (MAPS) algorithm with high power to detect and accurately place ancient WGDs in a phylogeny. Using a comprehensive set of gene family simulations and empirical data, we demonstrate the power of MAPS to infer the well documented At α and At α WGDs in the Brassicales. We also use MAPS to explore the evidence for paleopolyploidy in two lineages where polyploidy is not thought to be important: gymnosperms and insects. Using MAPS and other genomic approaches, we found evidence for three ancient genome duplications during the evolution of gymnosperms, two in the ancestry of major conifer clades (Pinaceae and cupressophyte conifers) and one in Welwitschia (Gnetales). We also confirm that a WGD hypothesized to be restricted to seed plants is indeed not shared with ferns and relatives (monilophytes). In animals, ancient polyploidy is found in the ancestry of some groups, especially fishes, but there is little evidence for ancient WGDs in other metazoans. We used transcriptomes and genomes from more than 150 species across the insect phylogeny to investigate whether ancient WGD occurred during the evolution of Hexapoda. Using MAPS and gene age distributions analyses, we find evidence for 31 ancient WGDs during insect evolution. This is the first evidence that ancient WGDs contributed to the evolution of insects, the most diverse lineage of eukaryotes. Together with other recent studies, our results suggest polyploidy likely to play an important role in the evolution of two most diverse eukaryotic lineages, plants and insects.

Keywords: phylogenomics, paleopolyploidy inference, gymnosperms, insects
Inferring the age of origin of allopolyploids and parental species in the genus *Melampodium* (Asteraceae) and evolutionary dynamics of their repetitive DNA fractions

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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 bioinformatic methods have allowed for a comprehensive investigation of the repetitive component of the genome. Herein, we investigate repetitive DNA fraction dynamics in the group of six closely related diploid and their allopolyploid derivative species belonging to the largest section of the genus *Melampodium*. Allotetraploid *M. strigoum*, the product of hybridization between diploid *M. glabracteatum* and *M. americanum*, hybridized further with yet another diploid species, *M. linealubum*, and gave rise to two allohexaploid species, *M. pringlei* and *M. sericeum* (both 2n = 6x = 60). These two share both the parental taxa and the same direction of the cross. Genomic constitution of each of the three allopolyploids has been confirmed using bi- and tricolor formamide-free genomic in situ hybridization. Two novel satellite repeats, CL189 and CL123, recently identified in NGS data sets of diploid species of *Melampodium* are employed here to elucidate the patterns and dynamics of the evolution of tandem repeats in the allopolyploids. The satellite CL189 (180 bp monomers) has been mapped to subtelomeric regions of all chromosomes in diploid *M. americanum* and to some chromosomes in *M. linealubum*, but was absent in *M. glabracteatum*. All three allopolyploids (4x and 6x) experienced loss of CL189 some loci from *M. americanum* subgenome, but also spread of this repeat to subgenome of *M. glabracteatum*. The IGS-derived satellite CL123, carrying 155 bp monomers, was localized in pericentromeric regions of all chromosome pairs in diploid *M. glabracteatum* and *M. linealubum*, but only in one pair of *M. americanum* chromosomes. Allopolyploids (4x and 6x) experienced some reduction of copy numbers of this repeat, coinciding with loss of some of the loci from *M. glabracteatum* subgenome in allotetraploid and from *M. linealubum* subgenome in both allohexaploids. (The study is supported by FWF project P25131 to HWS)

**Keywords:** Melampodium, tandem repeats, hybridization, FISH, FIGISH

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Dynamics of novel tandem repeats following stepwise allopolyploidization in *Melampodium* (Asteraceae)

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Chromosomal change, both numerical and structural, is major force accompanying plant diversification and speciation. Here, we demonstrate the origin and evolution of major tandem repeats of allotetraploid and two allohexaploid species resulting from complex hybridization of three closely related diploids of American daisies (*Melampodium*, Asteraceae). The genus *Melampodium*, endemic to Mexico and adjacent territories, includes 40 species with five different basic chromosome numbers (x = 9, 10, 11, 12, and 14). The largest section, *Melampodium* (all x = 10), encompasses number of tetra- and hexaploids. Allotetraploid *M. strigoum* (2n = 4x = 40) originating from cross of diploid *M. americanum* and *M. glabracteatum*, subsequently participated in another round of hybridization with diploid *M. linealubum* and gave rise to two allohexaploids, *M. pringlei* and *M. sericeum* (both 2n = 6x = 60). These two share both the parental taxa and the same direction of the cross. Genomic constitution of each of the three allopolyploids has been confirmed using bi- and tricolor formamide-free genomic in situ hybridization. Two novel satellite repeats, CL189 and CL123, recently identified in NGS data sets of diploid species of *Melampodium* are employed here to elucidate the patterns and dynamics of the evolution of tandem repeats in the allopolyploids. The satellite CL189 (180 bp monomers) has been mapped to subtelomeric regions of all chromosomes in diploid *M. americanum* and to some chromosomes in *M. linealubum*, but was absent in *M. glabracteatum*. All three allopolyploids (4x and 6x) experienced loss of CL189 some loci from *M. americanum* subgenome, but also spread of this repeat to subgenome of *M. glabracteatum*. The IGS-derived satellite CL123, carrying 155 bp monomers, was localized in pericentromeric regions of all chromosome pairs in diploid *M. glabracteatum* and *M. linealubum*, but only in one pair of *M. americanum* chromosomes. Allopolyploids (4x and 6x) experienced some reduction of copy numbers of this repeat, coinciding with loss of some of the loci from *M. glabracteatum* subgenome in allotetraploid and from *M. linealubum* subgenome in both allohexaploids. (The study is supported by FWF project P25131 to HWS)

**Keywords:** Melampodium, tandem repeats, hybridization, FISH, FIGISH

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Keywords: molecular dating, repetitive DNA, RepeatExplorer
Phylogeography of European Rosa sect. Rosa and a hybrid origin of the Caucasian R. oxyodon: glacial survival or glacial expansion?

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The section Rosa is the largest section of the genus Rosa distributed mostly in Asia. In Europe it is represented by 4 species: W Siberian-European R. cinnamomea, Central and W European R. pendulina, Caucasian R. oxyodon, and Circumboreal R. acicularis. However, only R. pendulina has been studied as to its phylogeography so far using chloroplast trnL-trnF (Fer et al., 2007) and AFLP (Daneck et al., 2015) markers. We studied R. cinnamomea, R. oxyodon, and R. pendulina from the whole area of their distribution as to their phylogeography and phylogeography with trnL-trnF and ITS1 markers. The results confirmed a westward expansion of R. cinnamomea from Siberia to E Europe in glacial to postglacial time. At the same time, it appeared that Caucasian R. oxyodon bears chloroplast and nuclear haplotypes both from R. cinnamomea and R. pendulina, what implies a hybrid origin of this species. Both species served as seed and pollen parents in reciprocal crosses that finally led to formation of R. oxyodon, with subsequent extinction of both the parental species from the Caucasus. It is striking, that only plants bearing the Carpathian chloroplast haplotype of R. pendulina took part in R. oxyodon formation. This enables us to suppose, that R. pendulina not only survived in the Carpathians during the last glacial maximum, as Daneck et al. (2015) hypothesized from the AFLP markers geographic pattern, but rather expanded its area eastwards up to the Caucasus, evidently through the steppe area N to the Black Sea. The remnants of R. pendulina and R. cinnamomea glacial migrations survived as R. donetzica, a local endemic to Donetz area in E Ukraine, which bears haplotypes both from R. pendulina and R. cinnamomea, too.

Keywords: Rosa cinnamomea, R. pendulina, R. oxyodon, trnL-trnF, ITS1, phylogeography.

Analysis of Plant rDNA Database: cytogenetic features of rRNA genes across land plants

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The online resource www.plantarndanadatabase.com, storing information on number, position in chromosomes and structure of the 5S and 18S-5.8S-26S (35S) ribosomal DNA loci in plants, was exploited to study distribution patterns of these sites. Relationships between both loci number, linked (L-type) or separated (S-type) arrangement of rDNA genes, chromosome number, genome size and ploidy level were assessed. By statistical analysis of 2839 records corresponding to 1609 species and 84 plant families, modal karyotypes have been described. These have two to four terminal 35S loci and two interstitial 5S loci for angiosperms (2n=18) and 12 interstitial 35S loci and two SS interstitial loci for gymnosperms (2n=24), the latter strongly biased by the prevalence of Pinaceae in the gymnosperms sample. To overcome this problem, ancestral karyotypes were also reconstructed, considering phylogenetic relationships between taxa. As a result, the ancestral karyotype has one terminal 35S locus and one interstitial 5S locus. Non-terminal position of 35S was found in 20% of species with a single locus, suggesting that such a preference may not be necessarily linked to 35S functionality and expression. We hypothesise that concerted and/or birth-and-death evolutionary mechanisms are strongly related with both the position in chromosomes and the abundance of 5S and 35S rDNA loci in karyotypes. Almost one third of karyotypes show chromosomes with both 5S and 35S rDNAs, in most cases in the same arm, indicating some tendency for these genes to occur in close proximity. While most species present the S-type organisation, reconstructed as the ancestral condition in land plants, a minority of species have evolved an L-type arrangement, acquired independently several times during evolution. The analyses here presented summarise current knowledge on rDNA loci numbers and distribution in plants, providing groundwork for comparisons for further rDNA-based cytogenetic approaches.

Keywords: birth-and-death evolution, concerted evolution, fluorescence in situ hybridisation, karyotype, ribosomal DNA
Sweet vernal grass (*Anthoxanthum* L.) in Europe: evolutionary history and phylogenetic relationships

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The genus *Anthoxanthum* (sweet vernal grass, Poaceae) represents a taxonomically intricate polyploid complex with a large phenotypic variation and still poorly resolved evolutionary relationships. We used a combination of molecular and cytogenetic techniques (DNA flow cytometry, sequencing of single-copy nuclear loci (*GBSSI*, *ITS* and cpDNA, FISH, and GISH) in order to elucidate: (i) ploidy and genome size variation in a representative set of samples collected across Europe and covering all currently recognized taxa, (ii) phylogenetic relationships among the detected cyto- and morpho-types, and (iii) the evolutionary history of polyploids. Eight taxonomic groups that partly corresponded to traditionally recognized species were delimited on the basis of genome size values and phenotypic variation. While our data supported the merger of *A. aristatum* and *A. ovatum* (with 0-6 B-chromosomes in their karyotypes), eastern Mediterranean populations traditionally referred to as diploid *A. odoratum* were shown to be cytologically distinct and may represent a new taxon. The evolution largely proceeded at diploid level; polyploids were detected in three morphological species (tetraploids in *A. alpinum* and *A. odoratum*, and high polyploids in *A. amarum*). Autopolyploid origin was suggested for 4x *A. alpinum*. In contrast, the origin of tetraploids morphologically matching *A. odoratum* is more complex and the available evidence supports multiple allopolyploidization events from different diploid ancestors.

Keywords: *Anthoxanthum*, B-chromosomes, flow cytometry, genome size, GISH

New insights on the evolution and taxonomy of the diploid-polyploid complex *Veronica* subsection *Pentasepalae*

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The diploid-polyploid complex *Veronica* subsection *Pentasepalae* is a good model to study the role of hybridization and polyploidy in the origin and evolution of plant species given that these events have played an important role in the recent diversification of the group. The complex is composed of c. 22 closely related perennial species distributed in Eurasia and North Africa, with an important diversification center in the Balkan Peninsula. We used amplified fragment polymorphisms (AFLPs) on more than 200 individuals to try to shed light on the taxonomy of this intricate complex and to elucidate the role that these evolutionary processes have played in the history of the group. Overall, the comparison of our AFLP data with the previous studies based on morphological, molecular and cytological data showed high degree of congruence. Thus, while the monophyly of most of the previously described taxa was confirmed, the existence of some previously undetected divergent lineages suggests that cryptic speciation is taking place in the group. The lack of genetic divergence between taxa of different ploidy level suggests that autopolyploid events are also occurring in the group. Our results confirm that AFLPs are a useful tool to clarify the evolutionary mechanisms, and the phyllogenetical and phylogeographical history of the diploid-polyploid complex *Veronica* subsection *Pentasepalae*.

Keywords: AFLPs, species delimitation, autopolyploidy, *Veronica*
Incomplete gene fractionation after paleopolyploidy: the first study case in flowering plants revealed by comparison of the *Petunia axillaris* N. and *Solanum lycopersicum* genomes

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Genomic investigations have found that polyploidy is ubiquitous among angiosperms and have identified independent lineage-specific ancient polyploidy events. While most paleopolyploidy events are ancient genome doublings, there are few important examples of ancient triplications. Among these we can cite the gamma polyploidy event near the origin of Eudicots. Traces of these ancient polyploidy events, or paleopolyploidy doublings, there are few important examples of ancient triplications. Among these we can cite the gamma polyploidy event near the origin of Eudicots. Traces of these ancient polyploidy events, or paleopolyploidy events, can still be identified although duplication events are followed by massive gene loss (fractionation) and polyploidy event near the origin of Eudicots. Traces of these ancient polyploidy events, or paleopolyploidy events, can still be identified although duplication events are followed by massive gene loss (fractionation) and chromosomal structural rearrangements. In this study we took advantage of the sequencing of the genome of *Petunia axillaris* N to study paleopolyploidy and gene fractionation in the evolutionary context of radiation due to the unique phylogenetic location of Petunia in the Solanaceae family. We used the comparative genomic platform, CoGe, to perform whole genome collinearity analysis and microsynteny analysis. Our study confirms the previously inferred Solanaceae paleohexaploidy event. We also demonstrate that the Petunia lineage has experienced at least two rounds of paleohexaploidyization, the older gamma hexaploidy event, which is shared with other Eudicots, and the more recent Solanaceae paleohexaploidy event that is shared with tomato and other Solanaceae species. Despite the shared paleohexaploidy event, we found that the process of gene fractionation is less profound in Petunia compared to tomato. This indicates that fractionation of gene content was not complete when these lineages diverged and independent gene loss events may have contributed to the speciation of the lineages, similar to what has been observed in Saccharomyces yeasts but so far not shown in flowering plants.

Keywords: gene fractionation, radiation, Solanaceae

Ecological and evolutionary drivers of genome-wide differentiation in *Arabidopsis arenosa* diploid-tetraploid complex

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Wild members of *Arabidopsis* genus got recently into focus of evolutionary biology and experimental botany. Interpretation of the findings in a broad evolutionary framework is, however, still limited by incomplete information on their evolutionary history and range-wide genetic variation patterns. The *Arabidopsis arenosa* group features a diploid-autotetraploid complex unique within the entire genus by co-occurrence of both diploid and their close polyploid derivatives. In order to elucidate unclear internal relationships within A. arenosa, we conducted a large population-level field sampling across its whole range and evaluated patterns of cytotype and genetic diversity of 140 populations using flow cytometry, RAD sequencing, and nuclear microsatellites. We detected a largely parapatic distribution of the diploid and tetraploid cytotype with several diffuse contact zones in central and southeastern Europe. The diploid cytotype splits into five genetically very distinct groups that are geographically segregated but do not reflect the most obvious morphological and ecological differentiation between lowland vs. alpine areas. Niche differentiation along altitude-related bioclimatic gradients was the main trend in the phylogeny of the diploid cytotype but it does not play role in segregation of the two cytotypes. The most prominent niche shifts, however, characterized genetically only slightly divergent diploid populations that expanded into narrowly defined alpine and northern coastal postglacial environment. Presence of marked internal ploidy-level, morphological, ecological and genetic variation highlights A. arenosa as very promising model for addressing general evolutionary questions on the origins and consequences of genome duplication in plants.

Keywords: glacial refugia, niche differentiation, phylogeography, RADseq
Evolutionary consequences of polyploidization: The example of *Vicia cracca*

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In this study, we investigate the evolutionary consequences of polyploidization, using *Vicia cracca* as a model system. For this purpose we use natural diploid and tetraploid populations of *Vicia cracca* and synthetic neo-tetraploids derived from natural diploid population by using colchicine. All the tested samples originated from 3 mixed-ploidy populations in the Czech Republic. The first step was to ensure that we compare plants from the same lineage. Using microsatellites markers we therefore identified the natural tetraploid most closely related to the diploids and the neopolyploids derived from the diploid. With the advent of high throughput sequencing it is possible to examine the genome-wide consequences of polyploidization at different levels. Four main characteristics and their interactions will be studied: (1) genomes (WGS), (2) transcriptomes (RNA-seq), (3) methylomes (BS-seq), (4) small RNAs (siRNA-seq). Genome analysis will include genome annotation, comparison with close relatives, SNPs, indels and larger structural variations identification. We will perform differential gene expression analysis and analysis of alternatively spliced genes. We will generate cytosine methylation profiles and we will correlate siRNAs and epimutation clusters to describe de novo methylation by RNA directed-DNA methylation (RdDM). As the result of this project we will be able to determine and distinguish impact of polyploidization process itself (synthetic neo-tetraploids) and impact of evolution (natural tetraploids). We will be able to compare which genome regions are more affected, impact of these changes on gene expression and describe how siRNAs contribute to gene expression regulation in first stable generation of synthetic neo-tetraploids and natural tetraploids. These data will be complemented with data comparing growth and physiology of these cytotypes in a wide range of conditions. Combination of genome-wide data and phenotypic characteristics will provide unique and complex view on evolution of polyploids.

**Keywords:** comparative genomics and epigenomics, transcriptome, regulation of gene expression

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Polyploidy and changes in the sexual system in annual *Mercurialis* species

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This poster will present details of ongoing and planned work in our lab to establish a firmer basis for phylogenetic and evolutionary inference concerning the evolution of polyploidy and associated sexual systems in the European annual clade of the plant genus *Mercurialis*. Widespread *M. annua* and its sister species *M. huetti*, which has a limited distribution in southern France and northern Spain, are diploid and dioecious. In contrast, hexaploid *M. annua* populations in the Iberian Peninsula are either monoecious or, in some populations, androdioecious, with populations containing a mixture of males and hermaphrodites. Furthermore, monoecious tetraploid populations occur in Morocco, and monoecious populations with higher ploidy levels (octaploid to dodecaploid) are found in Tunisia, Sardinia and Corsica. *Mercurialis canariensis* on the Canary Islands is tetraploid but dioecious. We are currently working on different projects related to the connection between polyploidy and changes in the sexual system in this group. Colchicine-induced artificial autotetraploids of diploid *M. annua* have been shown to partly produce monoecious offspring. By analyzing gene dosage of Y-linked SNPs, we aim to determine whether these novel hermaphrodites are genotypic females that show a partial male phenotype, or whether they are genotypic males with a different number of Y chromosomes than dioecious males. Furthermore, we hope to establish a more solid basis for our understanding of the phylogenetic relationships between polyploid *Mercurialis* species in relation to the sexual system. Tetraploid and hexaploid *M. annua* as well as *M. canariensis* are assumed to be allopolyploids, but so far the phylogeny of annual *Mercurialis* is poorly resolved. We plan to create an improved phylogeny for all annual *Mercurialis* species and ploidy levels that resolves the origins of individual homeologs and that allows to determine, e.g., whether species with similar sexual systems have a common ancestry.

**Keywords:** sexual system, artificial neopolyploids, phylogenetics, *Mercurialis*
Interpopulation hybridization induced formation of novel meiotically stable rDNA epigenetic variants in allotetraploid Tragopogon mirus

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Uniparental epigenetic silencing of 35S rRNA genes (rDNA), known as nucleolar dominance (ND), is common in interspecific hybrids. Allotetraploid plant Tragopogon mirus Ownbey (2n = 2x = 24) composed of Tragopogon dubius Scop. (d, 2n = 2x = 12) and Tragopogon porrifolius L. (p, 2n = 2x =12) genomes shows highly variable ND. To examine the molecular basis of such variation, we studied the genetic and epigenetic features of rDNA homeologs in several lines derived from recently and independently formed natural populations. Inbred lines derived from T. mirus with a dominant dDNA homeolog transmitted this expression pattern over generations, which may explain why it is prevalent among natural populations. In contrast, lines derived from the prDNA dominant progenitor were meiotically unstable, frequently switching to co-dominance. Interpopulation crosses between progenitors displaying reciprocal ND resulted in dDNA dominance, indicating immediate suppression of homeologs in F1 hybrids. Original prDNA dominance was not restored in later generations (F2-F3), even in those segregants that inherited the corresponding parental rDNA genotype, thus indicating the generation of novel prDNA and dDNA epigenetic variants. Despite preserved intergenic spacer (IGS) structure, they showed altered cytosine methylation and chromatin condensation patterns, and a correlation between expression hypomethylation of RNA Pol I promoters and chromatin decondensation was apparent. Reversion of such epigenetic variants occurred rarely, resulting in co-dominance maintained in individuals with distinct genotypes. Generally, interpopulation crosses may generate epialleles that are not present in natural populations, underlying epigenetic dynamics in young allotriploids. We hypothesize that highly expressed variants with distinct IGS features may induce heritable epigenetic reprogramming of the partner rDNA arrays, harmonizing the expression of thousands of genes in allotriploids.

Keywords: ribosomal DNA, nucleolar dominance, interpopulation hybridization

Diversification and taxonomy of heteroploid Knautia drymeia

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Knautia drymeia is a morphologically variable, diploid and tetraploid temperate forest understory species distributed in southeastern Europe and adjacent areas. Diploids are disjunctly distributed, with one group ranging from the northern Apenine Peninsula over the southern margins of the Alps to the north-western-most Balkan Peninsula and the second one being restricted to the south-eastern Balkan Peninsula and the southern Carpathians. Tetraploid occur in intermittent areas on the Balkan Peninsula and extend into the Alps, the Pannonian plain and the areas north-east of the Alps. Tetraploid populations from the central Apenines have mostly been treated as independent endemic species, K. gussonei, however the relationships between the two taxa remain unclear. The aim of this study was to explore the genetic structure within the diploids, to provide insights into the origin of the tetraploids and to contrast morphology and genetic groups with current taxonomy in order to evaluate the status of K. gussonei and the intraspecific taxa of K. drymeia. Additionally, spatiotemporal diversification was compared with forest refugia identified based on palynological evidence. Amplified fragment length polymorphism fingerprinting and multivariate analyses of morphological characters were performed on 57 populations spanning the distribution area of K. drymeia. K-means clustering, comparison of in-silico tetraploids and observed tetraploids, and a phylogeographic analysis using relaxed random walks were used to infer genetic structure and spatiotemporal evolution. The genetic structure was strongly geographically correlated and yielded four genetic groups; K. gussonei was inseparable from K. drymeia. Distributions of diploid lineages are suggestive of glacial refugia in the north-western-most and south-eastern Balkan Peninsula. Polyploids originated at least two times, as autoploids within North-eastern and South-eastern Groups and probably as allotriploids from North-eastern and South-eastern Groups. Morphological divergence did neither correspond to the genetic groups nor to current taxonomy. Therefore, genetic and morphometric data did neither confirm recognition of K. gussonei as distinct species nor support recognition of subspecies within K. drymeia. Hence, we propose treating K. drymeia as morphologically and genetically variable species without infraspecific taxa.

Keywords: forest understory plant; glacial refugia; Knautia; continuous geographic analysis; taxonomy
Gene conversion events and variable degree of homogenisation of rDNA loci in cultivated varieties of *Brassica napus*

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*Brassica napus* (MCC, 2n = 38, oilseed rape) is a relatively recent allotetraploid species derived from the parental diploid species *Brassica rapa* (AA, 2n = 20) and *Brassica oleracea* (CC, 2n = 18). To determine the influence of intensive breeding conditions on evolution of its genome, we analysed structure and copy number of rDNA in twenty one cultivated varieties of *B. napus*, representative of genetic diversity. We used NGS genomic approaches, Southern blot hybridization, expression analysis and fluorescent *in situ* hybridisation (FISH). A subgenome-specific sequence from the intergenic spacer (IGS) of the Ageneome was isolated and used for identification of genes origin on chromosomes. The *B. napus* varieties showed overall disbalanced A/C homeolog ratios ranging 0.85-6.14. Most varieties (18/21, 86%) had more Agenome than the C-genome copies. Deep sequencing revealed high homogeneity of arrays mostly composed of Agenome variants in *B. napus* ‘Darmor’. The Agenome-specific probe hybridised to loci on both A and C chromosomes indicating that the loss of C-genome genes was accompanied by their replacement with the A-genome units. Such pattern was not observed in *B. napus* ‘Yudal’ that displayed large intragenomic heterogeneity, additive inheritance of rDNA variants and intactness of parental loci. At the expression level, most varieties showed stable A-genome was not observed in the loss of C-genome genes was accompanied by their replacement with the A-genome units. Such pattern occurred in cultivated varieties of *Galium pusillum* group in central Europe.

Speciation complex of *Galium pusillum* includes approx. 27 polymorphic taxa encompassing up to five cytotypes distributed from lowland grasslands and rocky sites up to subalpine habitats. Our study tries to resolve the evolutionary history (hybridization, recurrent polyploidization) of diploids and tetraploids represented by species widespread in central European mountains (the Alps and the Carpathians, *G. anisophyllum*), rare lowland species of relict rocky areas including serpentina (*G. austriacum, G. valdepilosum*) and rare locally distributed species growing in spatially isolated mountain and serpentine habitats (*G. sudeticum*). Our morphological and molecular data (AFLP, plastid DNA sequences) suggest different history of the two *G. sudeticum* lineages occupying the isolated areas. The populations in Krkonose Mts. are putative relics of past hybridization between mountain and foothill species (*G. anisophyllum* and *G. valdepilosum*) which likely took part during postglacial vegetation shifts. On the contrary, most of the serpentine populations of *G. sudeticum* in western Bohemia are indistinguishable from tetraploid cytotype of *G. valdepilosum*. Additionally, one serpentine population exhibit AFLP profile similar to *G. valdepilosum* cytotypes, suggesting autopolyploid origin. These plants may represent a rest of probably older migration and illustrates the role of edaphically distinct sites as refugia of intraspecific cryptic diversity. On the other hand, close genetic position of tetraploid *G. anisophyllum* and *G. austriacum* suggests hybridization and/or possible allopolyploidy, supporting theory from previous study that tetraploid *G. anisophyllum* originated through its diploid progenitor and tetraploid *G. austriacum*. Although the whole studied group is little diversified and several of its species are restricted to isolated Holocene refugia, it exhibits overall high genetic variability within populations. CpDNA data shows high number of haplotypes shared among studied species, but somehow geographically restricted, indicating ancestral polymorphism or frequent hybridization in the past.

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Keywords: *central Europe, Galium pusillum, postglacial, endemism*
**Increased fertility in later generations of allohexaploid Brassica suggests karyotype stabilization**

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The genus Brassica includes some of the most important vegetable and oil seed crops in the world. The diploid species B. rapa (2n = AA; Chinese cabbage/turnip), B. nigra (2n = BB; black mustard) and B. oleracea (2n = CC; cabbage) formed interspecific hybrids resulting in the development of B. napus (AACC; rapeseed), B. juncea (AABB; Indian mustard) and B. carinata (BBCC; Ethiopian mustard). Polyploidy and interspecific hybridization processes in nature contribute to new, diverse and successful species and may be useful in breeding new allohexaploid crop types with increased hybrid vigour in Brassica. However, in early generations allohexaploid hybrids have shown low fertility due to meiotic instability, largely caused by homeologous recombination and resulting in aneuploid or rearranged karyotypes in the following generation. We produced a number of allohexaploid hybrids by crossing B. napus (A′A′C′C′) with B. carinata (B′B′C′C′) and crossing the F1 Hybrids (2n=A′A′B′B′C′C′) to B. juncea (AABB). Many of the trigeneric tetraploid F3 hybrids produced unreduced gametes (n = ABC) that led to the successful production of hybrids of the desired karyotype (2n=nA/A/B/B/C/C). The allotetraploid Brassica species were exclusively used as parents, in the hope that since these species have established mechanisms to prevent homeologous recombination between their two subgenomes, the alleles responsible will also potentially enhance meiotic stability in their allohexaploid progeny. We measured seed set and pollen viability as a proxy for meiotic/genomic stability in the subsequent generations. Over the generations we see increased seed set in the allohexaploid inbred lines, with some lines displaying seed set close to that of the parental species. This suggests that these lines have developed mechanisms for better control of meiosis, and may have established stable karyotypes. Additionally, we hypothesize that control of homeologous recombination is enhanced by alleles from the allotetraploid species. New stable allohexaploid hybrid lines are promising step forward towards establishing allohexaploid hybrids as crop species. These may be used as starting material to breed for increased agronomic properties themselves or as crossing partners to confer meiotic stability. Validation of the meiotic phenotypes and karyotypes and identification of the genetic and genomic factors linked to meiotic stability in our hybrid lines will be the next step in future research.

**Keywords:** Brassica, non-homologous recombination, genome stability, synthetic allohexaploids, interspecific hybridisation

**Campanula baumgartenii Becker: taxonomic complexity resulting from reticulation**

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The taxonomic status and evolutionary origin of Campanula baumgartenii, a rare endemic species of Campanula section Heterophylla, was studied using morphometry, flow cytometry, AFLPs, as well as chloroplast and nuclear DNA sequence markers. Flow cytometry revealed that C. baumgartenii is tetra- or hexaploid with presumed hybrid origin. While the ITS data suggest close relationship of C. baumgartenii and C. rotundifolia, the AFLP data differentiate these taxa and the cpDNA trnL-rpl32 fragment proposes a maternal ancestor related to the alpine C. scheuchzeri. Additionally, the hexaploid C. baumgartenii hybridizes in one locality with co-occurring tetraploid C. rotundifolia resulting in pentaploid hybrids, for which C. baumgartenii served as both seed and pollen donor. Seeds collected from the pentaploid hybrid were viable and relative DNA content of the seedlings was in the range of tetraploid C. baumgartenii. Based on the molecular differences and in spite of the lack of clear morphological discriminating characters we propose to keep C. baumgartenii as a separate species.

**Keywords:** Campanula sect. Heterophylla, hybridization, endemic species
The development of a *Brachypodium* polyploid model

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We have generated synthetic allopolyploids of *Brachypodium*, resembling the natural *B. hybridum*, by interspecific hybridizations between *B. distachyon* and *B. stacei*. The parental species, *B. distachyon*, has similar genome size to *B. stacei*, but twice lower basic chromosome number (2n=10 and 2n=20, respectively) whereas its individual chromosome size is approximately two times larger. Several lines from each species were hybridized. F1 interspecific dihaploid hybrids were obtained at very low frequencies (0.15% and 0.122%, respectively), all of which were totally sterile. The fertility was restored after genome doubling by colchicine treatment to produce S1 generations. Synthetic allopolyploids were shown to be relatively stable as characterized and compared with parental species and natural *B. hybridum* allopolyploid at the phenotype, karyotype and genetic levels. Our results illustrate the strong dependence of interspecific hybridization and allopolyploid synthesis based on genotypes of parental species. The successful synthesis of this allopolyploid offers the possibility to investigate various allopolyploidy-related changes at the genomic, epigenetic, gene expression and chromosome levels at the early stages of evolution as well as in comparison with later stages using natural allopolyploid *B. hybridum*.

Keywords: *Brachypodium*, interspecific-hybridization, polyploidy, speciation

Contrasting patterns of phenotypic and genetic variation in hybrid offspring: Causes and consequences

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The outcome of natural hybridization in plants is difficult to predict. The symmetry of the hybridization depends on numerous elements, such as the viability and fertility of hybrid offspring, the initial sizes of the parental populations, and pollinator preferences. If hybridization is asymmetrical, it is expected that the inheritance will be oriented towards the same parental taxon on both molecular and morphological levels. Using molecular and morphological markers, we analysed the natural hybridization between *Salvia officinalis*, an indigenous species well adapted to the local environment, and *S. fruticosa*, a non-native species struggling with marginal environmental conditions. Results reveal that hybridization has an asymmetrical pattern and that gene flow is oriented towards *S. fruticosa*. However, the hybrid individuals resulting from back-crossing with *S. fruticosa* are more morphologically similar to *S. officinalis*. We believe that environmental pressure is responsible for such a hybridization outcome. To achieve the phenotypic optimum previously achieved by *S. officinalis*, hybrids use the genetic potential gained from the hybridization to become morphologically more like the native species. Our research demonstrates that in the processes of hybridization and speciation, ecological factors play a crucial role, and inheritance of genetic and phenetic traits by hybrid offspring does not need to exhibit a strong correlation (if any).

Keywords: hybrid, hybridization, phenotype, genotype, inheritance, *Salvia*
Cryptic speciation, polyploidy and hybridisation in \textit{Medicago prostrata} (Fabaceae)

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Although hybridization through genome duplication (allopolyploidization) has been widely explored, hybridization without genome duplication (homoploid hybrid speciation, HHS) is less well understood. There have been few well-documented cases reported so far. A possible case of HHS involving \textit{Medicago prostrata} Jacq. was indicated in an earlier study, but using only two genes and a single sample from this species. We tested the hypothesis that this species is the product of HHS by sampling eight nuclear loci and 22 individuals, with an additional 27 individuals from related species, using gene capture and illumina sequencing. We inferred both uncalibrated and time-calibrated gene trees using Bayesian methods and tested whether gene tree differences could be explained by incomplete lineage sorting (ILS) alone in a coalescent framework. We found evidence for the presence of three and four alleles in several loci of particular individuals and thus were able to infer that these individuals are polyploids, probably tetraploids. These results were checked with chromosome counts for one individual each of a diploid and tetraploid. We found that tetraploid \textit{M. prostrata} individuals contained alleles found in two clades: closely related to \textit{M. sativa} alleles, and/or in the core \textit{M. prostrata} clade that includes all diploid individuals. The coalescent-based test rejected the null hypothesis of ILS alone to explain the difference among the gene trees with tetraploid \textit{M. prostrata} alleles from the \textit{M. sativa} clade included, but not with only diploid \textit{M. prostrata} alleles or both diploid and tetraploid alleles from the diploid clade. We found no evidence to support the hypothesis that phylogenetic differences among \textit{M. prostrata} individuals is the result of homoploid hybrid speciation. Instead, the observations are consistent with an autopolyploid origin of the tetraploid individuals with subsequent introgression from the \textit{M. sativa} complex, presumably tetraploids. We argue that the tetraploid \textit{M. prostrata} individuals constitute a new species, characterized by a partially non-overlapping distribution and the presence of distinctive alleles (those from the \textit{M. sativa} complex). Furthermore, the observations indicate a lack of gene flow from tetraploid to diploid \textit{M. prostrata}, so at least partial reproductive isolation is also apparent.

Keywords: introgression, autopolyploidy, coalescent simulation, next-generation sequencing

Low genetic differentiation between cytotypes of \textit{Vicia cracca} L. and absence of any geographic structure in Europe

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\textit{Vicia cracca} L. (Fabaceae), a perennial herb native in Eurasia, is a polyploid complex comprising two major cytotypes: diploid and autotetraploid, and rarely observed triploids. Based on allozyme data and performance of cytotypes cultivated in a common garden we suppose that tetraploids might have arisen several times in Europe. Highly parapatric distribution of cytotypes in Europe with central European, South-western and South-eastern contact zones and low frequency of mixed-ploidy populations suggest the secondary contacts of cytotypes, whilst high rate of cytoype intermingling within mixed populations and low frequency of tetraploids suggest their local origin there. Hence, to clarify the history of cytotypes in Europe, we sequence a chloroplast marker atpH from plants originating throughout Europe and analyse in detail microsatellite data within several mixed-ploidy populations including the only two diploid populations comprising triploid individuals. Results obtained from different molecular markers are highly incongruent, however. As indicated by chloroplast data, tetraploids arose at least twice, perhaps 3 times (once in Asia with rapid spreading of these tetraploids towards west) and contact zones seem to be secondary. Nevertheless, the individual haplotypes are not geographically distinct. In contrast, microsatellite data suggest single polyploid event in Europe. Regarding private SSR alleles, triploids arise locally from diploids. Nonetheless, we have not recorded in the only populations comprising triploids any tetraploid individual indicating that these triploids are fertile and facilitate the origin of tetraploids. The absence of any genetic structure in Europe has two non-exclusive explanations. First, tetraploids have arisen recently or have been arising recurrently and quite frequently. Second, genetic structure has been erased by great seed dispersal by humans or extant gene flow between cytotypes via unreduced gametes. The recurrent formation of tetraploids and current intercytotype gene flow are both probable as confirmed by several detected cases of tetraploid offspring from diploid mother plant. Moreover, the extent of introgression of diploids into tetraploids by unreduced pollen is unknown for us, but we are sure it happens. Anyway, the absence of any genetic pattern might be quite common, but it is bad publishable, so we cannot assess how frequent it really is.

Keywords: atpH-atpH, contact zones, microsatellites, mixed-ploidy population, triploid
Is hybridization impacting the diversification rates of land plant polyploids?

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We assess the evolutionary significance of hybridization by comparing the extinction and speciation rates of species with a hybrid ancestry to those of non-hybrids. We focus on land plants, and more particularly on polyploids because their high prevalence allows the comparison of allopolyploids (i.e. stabilized hybrids) to autopolyploids (i.e. non-hybrids). The study is based on 38 comprehensive genus-level phylogenies (built from publicly available DNA sequences), and on over 3,500 species for which we reviewed the polyploidy origin based on the current literature. Using this survey, we first show that allo- and autopolyploids are equally abundant in the majority of the inspected genera. State-specific diversification rates (i.e. the polyploidization, speciation and extinction rates of diploids, allo- and autopolyploids) are then estimated using the Multiple State Speciation and Extinction model (FitzJohn et al., 2009). We show that the extinction rates of allopolyploids are significantly lower than those of the autopolyploids. No differences are however observed in speciation rates, resulting in a significantly increased net diversification rate for the allopolyploids. These results are discussed in light of the classic hypotheses about the importance of hybridization in evolution.

Keywords: local adaptation, allopolyploidy, autopolyploidy, extinction, speciation, minority cytotype disadvantage, ecological novelty, competition

Genotype specific effects of Whole Genome Duplication in Arabidopsis thaliana

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Whole Genome Duplication (WGD), resulting in the formation of polyploid organisms is widely seen during the course of plant evolution and is assumed to be an adaptive mechanism due to its repeated occurrence. Studies on newly generated polyploids show that genome duplication also has immediate effects on phenotypic expression of plant traits and abiotic stress tolerance. However, the reason why chromosome doubling affects and potentially improves plant traits is unclear since there is no change in the actual genetic information, only the dosage and regulation of gene expression can immediately change. We are interested in determining whether these immediate effects of ploidy on phenotype are genotype specific, and/or affected by the process of their artificial creation. For this, we use Arabidopsis thaliana as a model to study these immediate effects by artificially inducing tetraploidy via the action of colchicine. This study investigates the response of multiple naturally occurring genotypes of A. thaliana to increased ploidy. Three independent tetraploid lines are generated for each genotype to determine whether the effect of colchicine treatment is reproducible and to ensure that the observed response is due to genotype by ploidy interaction only. Preliminary results from phenotypic characterisation, show that there are genotype specific effects in response to increased ploidy, with the extent and direction of response varying for different genotypes. Further studies on understanding this variation in response to chromosome doubling will provide the necessary tools to determine the genetic mechanisms underlying changes in phenotype in response to chromosome doubling.

Keywords: natural variation, immediate effects, induced tetraploidy
Usefulness of synthetic polyploids in studies exploring consequences of polyploidization: between population variation and occurrence of aneuploids

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Comparison of natural and synthetic polyploids is a useful methodology for understanding the consequences of polyploidization for plant performance and for separating the effects of genome duplication per se from the effects of subsequent evolution. In spite of its usefulness, this methodology was used only in a limited number of systems in ecological studies and we still know very little about its possible limits. In this study, we optimized methodology to produce synthetic polyploids in our model species, Vicia cracca, and explored the difference in plant germination, plant growth, plant morphology as well as in content of photosynthetic pigments between the synthetic and natural polyploids and natural diploids (plant type) originating from 4 different mixed ploidy populations. Using plants from multiple populations enabled us to assess the possible effects of genetic identity on all the traits. In addition, we explored the variation in relative DNA amount and chromosome number of these synthetic polyploids and attempted to understand the consequences of this variation for plant performance. Plant type, population and their interactions have significant effect on plant performance with synthetic polyploids being comparable to diploids in some populations while being comparable to natural tetraploids in other populations. For photosynthetic pigments, the effect of plant type was rarely significantly with strong interaction between plant type and population. This strongly suggests that the effects of polyploidization are largely population specific. While in some populations a trait seems to be driven primarily by polyploidization, the same trait may seem driven by subsequent evolution in other populations. Considering the origin of the material used to create synthetic polyploids and using multiple source populations should thus be a key prerequisite for future studies exploring the consequences of polyploidization. The relative DNA amount of the synthetic polyploids fell within the range of natural polyploids. However, 25% of the synthetic polyploids turned out to be aneuploid. The differences in chromosome number are reflected in the performance of the synthetic polyploids. Detailed exploration of chromosome numbers of the synthetic polyploids should thus be included also in future studies attempting to understand the ecological and evolutionary consequences of polyploidization.

Keywords: aneuploidy, plant performance, plant metabolites, trait evolution

Ploidy-dependent parental and grand-parental effects on F2 seeds generated from isogenic reciprocal F1 triploids generated from inter-ploidy crosses in Arabidopsis thaliana

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The success or failure of interbreeding between plant populations of differing ploidy level is an important factor in flowering plant evolution. However, parental effects of inter-ploidy crosses on offspring fitness remain poorly understood. We previously determined that hybridization between diploid and tetraploid Arabidopsis thaliana parents produces viable reciprocal F1 triploids which when self-fertilized display differential ovule fertility between F1 genotypes. The genetically identical reciprocal F1 triploid pairs that we generate either contain two maternally derived chromosome sets or two paternally derived chromosome sets, and provide a system for testing of genome-dosage dependent parental or grandparental effects. To determine whether there are genome-dosage dependent grandparent-of-origin effects on F2 seeds obtained from selfed reciprocal F1 triploids, we analysed the viability, ploidy and abortion levels in the F2 seeds obtained from 178 self-fertilized triploid reciprocal F1 hybrids. We determined that the proportions of aborted or normally-developed F2 seeds differed depending on whether the F1 triploid contains a maternal excess or paternal excess of chromosomes. Single-seed ploidy analysis demonstrated that embryo ploidy in F2 seeds is also affected by these "grandparent-of-origin" effects. As we found with ovule fertility, significant heritable effects of natural variation for the F2 seed traits were also identified. To identify causal loci we performed genome-wide association mapping (GWAS) and identified a locus associated with normal F2 seed development in paternal-excess F1 triploids. Mutation of a plant-specific gene at this locus can recapitulate the parent of origin-specific effect on triploid fertility. These post-meiotic seed phenotypes vary between genotypically identical F1 triploids which have either two maternal or two paternal sets of chromosomes. Hence, we conclude that parental genome dosage (ploidy) effects can trans-generationally alter the fitness of F1 and F2 offspring. Our use of natural variation to investigate offspring fitness from interploidy crosses has identified a locus causal for inter-ploidy reproductive success.

Keywords: triploid, fertility, inheritance, parent-of-origin effect, Arabidopsis
Progressive heterosis and allelic contributions to heterosis in *Arabidopsis* diploids and polyploids

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Progressive heterosis can occur when an F1 polyploid with a more diverse set of chromosomes (e.g. from four different parents) displays higher levels of heterosis than an F1 polyploid with a less diverse set of chromosomes (e.g. from two different parents). We are investigating both conventional and progressive heterosis in diploid and tetraploid *Arabidopsis* accessions by examining progeny of crosses between F1 hybrid plants and either parental accessions (back-cross) or progressive genetic backgrounds (out-cross to either a third genotype or another F1 hybrid). One example is the F1 progeny we generate from crosses between the C24 accession and the F1 hybrid of a cross between Col and Ler accessions (annotated as; C24 X (Col X Ler)), where all progeny carry a C24 allele and segregate alleles from either Col or Ler. Our experiments aim to test the relevance of existing models of heterosis to *Arabidopsis* and to assess the potential of contemporary strategies to map or identify contributing genetic and/or epigenetic factors. Due to the complex interactions being tested, high-throughput phenotyping is required to effectively sample a representative set of interactions and to confidently assess the distribution of phenotypic outcomes from the segregating progeny of these crosses. We have developed a low-cost and scalable phenotyping platform to facilitate the continuous measurement of leaf area in standard laboratory growth chambers. In our facility, this system currently provides accurate measures of leaf area during early growth for over 1400 plants at a time. Our experiments build on the concept of progressive heterosis in polyploids which may also be relevant to diploid organisms through the stable inheritance of epialleles.

Keywords: ploidy, progressive heterosis, *Arabidopsis*, phenotyping, epigenetics

Disentangling the contribution of polyploidization versus hybridization to the phenotype and fertility in a new allopolyploid species

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Interspecific hybridization accompanied by polyploidization (allopolyploidy) can lead to an entirely new (fertile) species that is reproductively isolated from its parental species. Hybridization between evolutionary divergent parental genomes often produces sterile hybrids due to genic incompatibilities, erroneous meiotic pairing and/or odd ploidy number, whereas established allopolyploid species often show increased fertility in the range of fertilities found in the parental species. The rate at which fertility is restored in allopolyploids depends on the mechanism underlying sterility of interspecific hybrids which could be either genetical (e.g., Dobzhansky-Muller incompatibilities) or cytological (erroneous meiotic pairing). In case of meiotic pairing abnormalities in hybrids, polyploidization is expected to restore fertility by balancing chromosomes during meiosis. Instead, if fertility has a genetic basis, then fertility is not expected to be increased by polyploidization. To study the contribution of hybridization versus polyploidization to allopolyploid phenotype and fertility we used synthetically generated allohexaploids to reconstruct the early stages in the speciation event of one of the youngest examples of allopolyploid speciation in plants, i.e. *Mimulus peregrinus*, a new fertile species that arose after recent polyploidization of a highly sterile hybrid plant. Morphology and pollen fertility measurements in parental species, triploid and polyploid hybrids show that polyploidization increases germination rate, floral size, changes leaf shape and the relative investment in sexual reproduction in neoallohexaploids. As expected under the meiotic pairing hypothesis, a significant increase in fertility was observed after genome duplication. However, we also detected significant genetic variation in the fertility of allohexaploids derived from different crosses, consistent with the presence of segregating genic incompatibilities. These results indicate that reinstating chromosome-number balance is important in the restoration of fertility, but also that genic incompatibilities can modulate the extent of fertility of allopolyploids. Our findings show that the sexual fertility of recently formed hybrids and polyploids (and potentially their evolutionary success) depends not only on the effects of genome doubling, but also on the genetic compatibility of the parents.

Keywords: Dobzhansky-Muller incompatibilities, fertility restoration, hybridization, speciation, synthetic lines
What does the name *Bidens connata* stand for in Eastern Europe?

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*Bidens connata* Muehl. ex Willd. is distributed over vast area in Northern America, and is likely a new invasive taxon in Eastern Europe. Few populations identified as *B. connata* are scattered far from each other and from transportation routes. Phenotypic types from Europe and from Northern America differ from each other in many characters. Previous morphological studies showed that *B. connata* combines characters of the native taxon *B. cernua* L. and alien taxon *B. frondosa* from Northern American L. Also, some characters of *B. connata* are similar to those of native *B. tripartita*. Thus, we suggested that *B. connata* is a taxon of a hybrid origin. The hypothesis was tested by ISSR-PCR method. DNA was extracted according to CTAB method from herbarium material sampled in the Middle Russia and Belarus (10 specimens of *B. connata*, 5 of *B. cernua*, 6 of *B. frondosa*, 7 of *B. tripartita*). PCR was conducted using the 4 ISSR primers ((GA)8YG, (AG)8YA, (CAG)5, DBD(AC)7 and DDD-DDD-DDD-DDD-DDD-D), and for each reaction the same primer was used as the forward and reverse. The results were analyzed in Cross Checker, Structure 2.2, PAST 2.0 and NewHybrids software packages. According to the results of cluster analysis, three clusters emerged. The first cluster was comprised of *B. frondosa* individuals, the second of *B. tripartita* individuals, and in the third cluster all populations of *B. cernua* and *B. connata* were joined. Analysis of the results in New Hybrids program confirmed the hybrid origin of *B. connata* and the fact that *B. cernua* and *B. frondosa* are its parental species. The results showed that with a high probability (more than 50% for the majority of specimens) *B. connata* is a result of back-cross. We also rejected the hypothesis that *B. connata* represents a hybrid between *B. cernua* × *B. tripartita*. Thus, the name “*B. connata*** is attributed in Eastern Europe to the complex of hybrids and back-crosses derived hybrids from hybridization of the invasive species of North American origin *B. frondosa* and the native *B. cernua*.

**Keywords:** Bidens connata, back-cross, invasive species

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Is hybridization driving the evolution of climatic niche in *Alyssum montanum* (Brassicaceae)?

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After decades of interest, the contribution of hybridization to ecological diversification remains unclear. Hybridization is a potent source of novelty, but nascent hybrid lineages must overcome reproductive and ecological competition from their parental species. Here, we assess whether hybrid speciation is advantageous over alternative modes of speciation, by comparing the geographical, ecological ranges and niche evolutionary rates of stabilized allopolyploid versus autopolyploids in the *Alyssum montanum* species complex. As expected by theory, allopolyploids occur mainly along contact zones and are generally spatially overlapping with their diploid counterparts. However, they demonstrate higher rates of niche evolution and expand into different climatic conditions than those of their diploid congeners. In contrast, autopolyploids show lower rates of niche evolution, occupy ecological niches similar to their ancestors and are restricted to less competitive and peripheral geographic areas. Hybridization thus seems advantageous by promoting ecological niche evolution and more readily allowing escape from competitive exclusion.

**Keywords:** local adaptation, allopolyploidy, autopolyploidy, transgressive segregation, diversification, minority cytotype disadvantage, ecological novelty, competition
Cytogeography of *Stellaria graminea* L. (*Caryophylaceae*) from the Carpathians

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*Stellaria graminea* L. (*Caryophylaceae*) is karyologically variable taxon possessing several different ploidy levels including aneuploids. Only little is however known about cytotype demography at the population level, the geographic distribution of cytotypes as well as correlation between karyological and morphological variation in this species. The study presented herein is focused on the karyological and morphological variation of *S. graminea* from the Carpathians. Multivariate morphometric approach was used to determine morphological variation while direct chromosome counting and DAPI flow cytometry was employed to elucidate variation in chromosome numbers, ploidy levels and relative DNA content. Altogether, we analysed 103 populations (1012 individuals) originated from entire Carpathian arc. Three ploidy levels/ cytotypes were confirmed, diploid - 2n=2x=26, triploid - 2n=3x=39 and tetraploid - 2n=4x=52. Further, 21 populations were comprised exclusively of diploids, 43 harboured tetraploids and single population was triploid. Others 34 populations were heteroploid and involved mixture of diploid and polyploid cytotypes. The geographic pattern of cytotypes showed conspicuous north-southern gradient; diploids prevailed in the South-eastern Carpathians while polyploids were accumulated in the Western Carpathians. Although multivariate morphometric analyses indicated that diploids showed some trend of differentiation from polyploids, it has not been possible to clearly defined particular cytotype morphologically.

Keywords: flow cytometry, multivariate morphometrics, cytogeographic pattern

Ecogeography of sexual and apomictic ploidy cytotypes in *Potentilla puberula* (Rosaceae) - the importance of migration, habitat preferences and co-occurrence

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It is well known that migration history, ecological preferences and reproductive interactions among close relatives significantly affect the distribution of species. However, the relative importance of each of these factors is not well investigated. Cytotypes of a heteroploid species are a perfect model system to investigate these factors since they are likely to be differentiated in terms of ecological preferences, migration abilities and reproductive behaviour, but presumably still maintain strong interactions between each other. As a result lower ploid populations of plant species in the European Alps are frequently located in glacial refugia while their higher ploid congeners also occur in formerly glaciated areas. *Potentilla puberula* Krašan (Rosaceae) exhibits a continuous series of cytotpes ranging from tetra- to octoploidy in the European Alps. Sexual tetraploids occupy more natural habitats compared to apomictic penta- to octoploids growing in more intensively used ones. Importantly, ecological conditions suitable for tetraploids are typically encountered at the Southern edge of the Alps, supporting a “Southern refugium hypothesis” of Ice Age survival. This study aims to explain the current distribution of cytotypes, quantifying the relative effects of post-glacial migration, habitat preferences and co-occurrence of cytotypes using variance partitioning. Ploidy level and reproductive mode were established for 2000 individuals from 145 populations. Ecological site conditions, including bioclimatic data and soil parameters, were collected for each population. Representatives of each cytotype and population were chosen for AFLPs analysis and cpDNA-based haplotype reconstruction, to test recurrent formations of apomictic polyploids and the “Southern refugium hypothesis”.

Keywords: apomixis, phylogeography, ice age refugium, niche segregation
Strong differences in the composition of herbivore communities and seed damage in diploid and autotetraploid species

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Herbivory is one of the key interactions affecting plant fitness. Despite the large amount of data available on the effects of herbivores on various plant groups, we still know very little about the interactions between herbivores and plant taxa of different ploidy levels. We studied the relationship between diploid and autotetraploid Centaurea phrygia and the community of predispersal seed predators. In addition, we collected a set of data on flowering phenology, flower head morphology and chemistry to investigate potential mechanisms underlying the differences among cytotypes. The two cytotypes were strongly differentiated in their flower head morphology, chemistry and phenology, as flower heads of diploids were larger, contained more secondary metabolites and were characterized by later flowering time. Also, the two cytotypes strongly differed in the composition of insect communities in the flower heads as tetraploids suffered from higher seed damage. The diversity and composition of insect communities, however, strongly varied between years and environments. Flowering phenology could explain part, but not all of the differences observed between cytotypes, indicating that other factors such as flower head morphology or chemistry could also play a role. A subsequent study revealed both direct and indirect effects of ploidy level on seed production. The indirect effects were mediated by seed damage and range of traits such as flowering phenology, flower head size, flower head height above ground and number of seed predators in the flower heads. While the direct effects indicate higher seed production in diploids, indirect effects indicate the opposite. Such finding implies that prediction of the effect of ploidy level on seed production is mediated by a range of parameters and the outcome of their interaction may depend on slight changes in the environmental conditions. The differences between the two cytotypes are important determinant of the plant-herbivore interactions. However, the knowledge of the structure of interactions is more important that the overall effects of cytotype on the fitness trait. Future studies exploring determinants of fitness in different cytotypes should be oriented towards the disentanglement of the different drivers and their relationships.

Keywords: pre-dispersal seed predation, structural equation modelling, flowering phenology, secondary metabolites, herbivory

Effects of genetic diversity on colonisation, cytotype distribution and community composition of glasswort (Salicornia)

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Salicornia (Amaranthaceae) is one of the dominant genera of halophytic plants of the Wadden Sea. Its succulent and reduced morphology serve as adaption allowing these plants to grow in the lower salt marsh as well as the pioneer zone, which are flooded twice a day by sea water. This morphology impedes clear taxonomic differentiation of species. Phenotypic plasticity as well as inbreeding are additional reasons that make the circumscription of species difficult. While it is usually easy to differentiate between diploid and tetraploid cytotypes, neither morphology nor genetic markers allow further separation of species. Since growth is influenced by many different factors, individuals of one species can show a completely different form, even when they grow next to each other. This project mainly focusses on the German island Spiekeroog to gain an understanding of the correlation of Salicornia occurrence, cytotype, morphology and the colonisation by endophytic fungi, which, like mycorrhiza, can improve the performance of taxa that occur in extreme habitats. The genetic comparison of plants from the Salt Marsh of Spiekeroog and from Artificial Islands will provide information about the dispersal of Salicornia, whereas greenhouse experiments are planned to analyse growth of different cytotypes of Salicornia on mudflat soil as well as lower salt marsh soil with and without the addition of fungi. Dyeing of roots demonstrated that fungi occur in many individuals of Salicornia and 454-pyrosequencing identified them as Pleosporales (Pezizomycetes), Cladosporium (Dothidiomycetes) and Fusarium (Sordariomycetes). Cryptococcus (Basidiomycetes) were found as well. An ongoing analysis aims to gain knowledge if genetic markers like RADs offer a way to analyse population structure, since ITS, ETS and microsatellites offer no satisfying separation of species that have been described in the past.

Keywords: Salicornia, BEFmate, salt marsh, genetic diversity, endophytic fungi
Phylogeny, genome size evolution and chromosome counts in the (sub) tropical genus *Globba* (Zingiberaceae)

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Nuclear DNA content (i.e., absolute genome size) usually varies among species and genera, which makes it a powerful tool in biosystematics. There is still limited knowledge of genome size evolution as well as its mode and tempo; in particular, studies involving tropical material are extremely rare. The genus *Globba* (ca. 100 species) from the economically important family Zingiberaceae is widely distributed in both the monsoonal and temperate regions of Asia, with its centre of diversity in the Indochinese floristic region. There are three subgenera and seven sections recognized in the infrageneric classification. *Globba* is a polyploid complex with several different chromosome counts observed. The genus is still poorly understood in terms of nomenclature and taxonomy. We sequenced nuclear (ITS) and chloroplast (matK) DNA regions of more than 100 accessions. The dataset was supplemented by ca. 50 GenBank accessions to cover ca. 70 species. Previous classification was largely confirmed, but additional section needs to be recognised around *G. nipbetiana* where even mixed-ploidy populations were observed and genome size varied more than four-fold, ranging from 1.1 pg in *G. nuda* to 4.4 pg in *G. marantina*. Apparent differences among sections were observed and genome size evolution more or less followed the phylogeny. Species from the evergreen section *G. nuda* tend to have the highest genome sizes. Eight different chromosome counts were detected (2n = 20, 22, 24, 28, 32, 34, 48, and ca. 96). Putative polyploidy was observed in several sections. This was especially true for the section *Globba* where even mixed-ploidy populations were observed and very close relationships among described species were confirmed.

Keywords: *Globba*, phylogeny, genome size, chromosome counts

Hybridization success is largely limited to homoploid *Prunus* hybrids - a multidisciplinary approach

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Although interspecific hybridization is considered to be a major mechanism generating evolutionary novelties and consequently plant diversity, it can also blur species genetic integrity via backcrossing (also referred as genetic erosion). Backcrossing smears out differences between species, and introgression may even lead to genetic assimilation or extinction of parental species. An example of genetic erosion is interspecific hybridization of endangered *Prunus fruticosa* with other *Prunus* species. *Prunus fruticosa* frequently hybridizes with naturalized/cultivated *Prunus cerasus* (forming tetraploid hybrid *Prunus xemensina*) and native/cultivated *Prunus avium* (constituting triploid hybrid *Prunus xmohacysana*). Propidium iodide flow cytometry (FCM), distance-based morphometrics, elliptic Fourier analysis and embryology were used to evaluate the extent of hybridization in region of Central Europe. Three ploidy levels were detected by FCM: diploid (*P. avium*), triploid (*P. xmohacysana*) and tetraploid (*P. fruticosa, P. xemensina and *P. cerasus*). Moreover, *P. fruticosa* and *P. cerasus*, at the tetraploid level, differ in absolute genome size. Results from embryological analysis revealed the existence of a triploid block in *P. xmohacysana* and significant potential for hybridization among tetraploid taxa (indicated also by a continuous genome size and further reflected by morphometrics). Both hybrids significantly differ in ploidy level and embryological characteristics, but studied morphological characters are not able to distinguish them. Thus hybridization of *P. fruticosa* with naturalized, often cultivated and also alien *P. cerasus* should not be underestimated because of significant threat to wild populations of *P. fruticosa* through genetic erosion. On the other hand *P. avium* does not represent marked risk because the backcrossing results in sterile plants.

Keywords: *Prunus fruticosa*, interspecific hybridization, FCM, morphometrics, embryology
The value of flow cytometry in plant cytogeography: novel insights into ploidy distribution of the Campanula rotundifolia complex in central Europe

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One of the prerequisites for polyploid research in natural systems is knowledge of the geographic distribution of cytotypes. Distributional data provide useful insights into the evolutionary history of diploid-polyploid groups and can aid in interpretation of phylogenetic relationships. In addition, they serve as a foundation for exploring ecological preferences of individual cytotypes and allow for assessment of the historical development of modern distribution patterns. The field of cytogeography has been revolutionized by the advent of DNA flow cytometry (FCM). This high-throughput technique has allowed gaining detailed insight into ploidy variation at different spatial and temporal scales, and is changing our perception of the magnitude of ploidy variation and its dynamics in the wild. In contrast to other cytogenetic techniques, FCM can easily process large population samples and therefore provides a much more accurate picture of ploidy variation. Consequently, the biogeographic and evolutionary processes that shape cytotype distribution patterns can be reliably assessed, as can the interactions, ecological preferences, and pre- and postzygotic breeding barriers of the individual cytotypes. As a part of the biosystematic study, we used FCM to assess ploidy distribution of the Campanula rotundifolia agg. in Central Europe. The study group is a highly polymorphic polyploid complex, which splits into several taxa with diploid (2n = 2x = 34) to hexaploid (2n = 6x = 102) ploidy levels. Our large-scale ploidy screening revealed distinct cytogeographic patterns in some species and considerably revised published ploidy distribution data. The present work serves as a foundation for subsequent investigations into the phylogenetic relationships and the dynamics of genome duplication.

Keywords: Campanula, flow cytometry, cytotype distribution, cytotype coexistence, aneuploidy

Polyploidy in Odontarrhena (Brassicaceae): a driver of phenotypic, genetic and ecological diversity?

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The recently resurrected genus Odontarrhena consists of 87 perennial species (see www.alysseae.sav.sk) with native distribution predominantly in Eurasia and the center of diversity in the Mediterranean region. The genus includes several widespread species and many regional and local endemics. They occupy xeric and rocky habitats across a large altitudinal span, including very specific environments such as serpentine rocks and coastal sandy dunes. Several species have successfully spread into man-made habitats in their native area and few of them have become extremely invasive in North America where they were introduced two decades ago. Four different ploidy levels have been reported within the genus: diploids, tetraploids and much rarer hexaploids and octoploids. Published chromosome number records and ploidy level data are still very scarce for most of the species. Available data and our preliminary ploidy level investigations suggest that several traditional taxa include both diploids and tetraploids. However, little is known about the evolutionary history of particular species, the origin of polyploids and the level of differentiation between cytotypes. The main goals of our planned studies are to reveal the origin of polyploids, to reassess their taxonomic assignments, and to explore diversification associated to auto- and allopolyploidization events. The latter will be addressed by comparing diploid vs polyploid phenotypic and genetic variation, ecological niches, and distribution patterns. The studies introduced here will focus mostly on the broadly conceived Odontarrhena muralis s.l. and O. tortuosa s.l. species complexes within the area of the Balkan Peninsula.

Keywords: Odontarrhena, Balkan Peninsula, genetic variation, ecological niches
Polyploidy and ecological adaptation in *Cochlearia* L. (Brassicaceae) in Northern Norway

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Polyploidy and ecological adaptations are important aspects of plant speciation. In this project we study the two in combination using the genus Cochlearia, with focus on populations in Northern Scandinavia. Different taxa, varying in ploidy level (diploid to octoploid) and with adaptation to different habitats, are distributed across Europe, probably all recently evolved during and after the Pleistocene glaciations. The tetraploid *C. officinalis* is typically a coastal (halophytic) plant found all along the North Atlantic coast. In Northern Norway however, three eco-geographic races (also considered subspecies) are recognised. All three are separable on a few morphological and physiological criteria, and previous studies have shown that the phenotypic variation is stable when grown in common conditions. They rarely grow close together even if their general ranges are sympatric and intermediates occur. The coastal (halophytic) race grows in exposed gravel shores and salt marshes and in crevices in bird cliffs; the estuary race grows in inundated (brackish) habitats near river outlets at the fjord ends; and the spring race grows in cold and more or less eutrophic springs at inland localities up to 600 m a.s.l. In the first stage of this project, we explore whether and to what degree the eco-geographic races are genetically differentiated (applying SNP data obtained from RAD-sequencing). Comparison of populations of all three races from different geographical regions of Northern Norway will further enable discussion on whether the variation that we see today are the result of parallel evolution and adaptation to different habitats.

In the next step of the project, the evolution of the eco-geographic races of *C. officinalis* from Northern Norway will be considered in a larger context including taxa with comparable ecological affinity, but different ploidy level, from across Northern and Central Europe.

Keywords: ecological adaptation, plant speciation, parallel evolution, eco-geographic races, RAD-seq

The pipeline development of allopolyploid cis-trans regulation detection on SUSHI

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Gene expression in allopolyploid hybrid species depends on both cis and trans regulatory factors that influence parentally inherited gene copies. In hybrids, cis-expression divergence must evolve by selection on surrounding non-coding regulatory regions to enhance homeolog specific expression. We have integrated a statistical framework for detecting cis-trans regulation in polyploid species using SUSHI, a web-interface platform available for users of the Functional Genomics Center Zurich sequencing facility. The SUSHI is a pipeline framework of Supporting User for SHell-script Integration developed on the Ruby on Rails Web-application framework. The cis-trans regulation is detected in several steps: 1. Mapping polyploid RNAseq data to parental genome reference(s), 2. Mapped reads classification into either parental homeolog, 3. Quantifying differential homeolog expression ratio genes relative to parental expression using HomeoRoq, 4. Detecting differentially expressed genes between polyploid homeologs using EdgeR, and 5. Classifying genes into cis- or trans-regulated genes. Using Arabidopsis allopolyploid species and its parental diploid species, we detected 3868 cis and 395 trans genes in synthetic F1€™s and 8950 cis and 408 trans in a natural allopolyploid. The nearly twice difference in cis genes detected in F1€™s compared to the natural allopolyploid suggests strong cis-regulatory divergence among homeologs over evolutionary time. Using silent substitutions in coding sequences and non-coding 5â€™ and 3â€™ flanking regions, we will estimate the strength of selection on cis-regulatory regions using a MacDonald-Kreitman framework.

Keywords: cis-trans regulation, Arabidopsis, SUSHI, Ruby on Rails
Genome size variation in Onosma arenaria - O. pseudoarenaria species group.

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Onosma arenaria s.l. and O. pseudoarenaria s.l. form a hybrid species group. Onosma arenaria s.l. is triploid with 2n=12L+14S (L- large and small chromosomes respectively), while O. pseudoarenaria is tetraploid with 2n=12L+14S chromosomes. Due to slight morphological differences and unresolved evolutionary relationships within this species group, recent taxonomic treatments have had difficulty delineating the species. Both taxa can be recognized based on karyology (3x=20 vs. 4x=26). However, genome size data has proven to be unsuitable for this task. A limited number of studies have suggested that considerable variation in genome size exists within O. arenaria s.l. and O. pseudoarenaria s.l. In the present study, we investigate the genome sizes of selected populations of both O. arenaria and O. pseudoarenaria, using both leaf and seed material, based on fluorescence intensity measurement of propidium iodide (PI) stained nuclei. Incubation tests suggest that PI intercalation is affected in samples of simultaneously processed and stained nuclei in some of the investigated populations. Our data suggest that leaf material is less suitable than seed material in terms of incubation test results, with leaf material showing smaller genome size values than seed material. Moreover, our repeated measurements demonstrate considerable variation as a result of measurement error of the cytometer. Based on the seed material analyses, we provide evidence of genome size variation within and among populations. Our data show that flow cytometry genome size measurement in Onosma can be the result of actual genome size variation, although it is necessary to account for the effect of the cytosolic compound and measurement error of the flow cytometer.

Keywords: DNA amount, flow cytometry, inhibition effect, karyotype bimodality, measurement error

Variation and interspecific hybridization of Elymus repens and E. hispidus

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The study is focused on hybridization, potential introgression, aneuploidy and other microevolutionary mechanisms in economically important species Elymus hispidus and E. repens in Central Europe, as well as members of tribus Triticeae. While Elymus hispidus and E. repens are able to hybridize with each other, E. hispidus can even hybridize with Triticum species. In extreme cases they tend to create complex hybrid swarms which are taxonomically unsolvable. Several taxonomic approaches assessed complex morphological variation at different levels, having a description of numerous microspecies and subspecies for a result. Overall, 95 populations were sampled (41 populations of E. repens, 25 of E. hispidus, 6 of E. caninus and 23 of mixed stands) in Czech Republic, Slovakia and Poland (298 individuals, three to ten per population). The parallel case study of model hybrid swarm in southeast part of the Czech Republic (Certeryje - Bílé Karpaty Mts.) was more detailed (1.4 transsects/116 individuals). Flow cytometry (DAPI and PI staining), elliptic Fourier analysis and distance based morphometrics (generative and vegetative characters) were adopted to evaluate the processes generating the variation of involved taxa. Flow cytometry (extensive study - Central Europe) revealed that both involved species significantly differ in absolute genome size (Elymus repens C= 24.82 ± 0.69 pg, Elymus hispidus C=28.40 ± 0.87 pg; standard Pisum sativum 'Cítrad'). On contrary, absolute genome size of case study individuals reflects complex influence of hybridization (Elymus repens C= 27.33 ± 2.65 pg, Elymus hispidus C=28.92 ± 1.18 pg). Besides the anticipated correlation between absolute and relative genome size, in several cases significant differences that may indicate diverse genome constitution (especially in case of aneuploids) were revealed. The detected variation in absolute genome size (continuum from 24.6 pg to 35.7 pg) suggests different ploidy levels of species or various interspecific hybrids. Putative nonaploid plant (absolute genome size 35.71 pg) probably originated from Elymus repens unreduced gamete. Elliptic Fourier analysis and distance based morphometrics confirmed clear separation of involved species. However, the group of hybrids overlapped groups of putative parental species (mirroring potential introgression).

Keywords: Elymus repens, Elymus hispidus, interspecific hybridization, introgression, flow cytometry
Polyploid evolution of *Urtica dioica* agg.

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Although the European flora belongs to the best explored at the global scale, still there are several largely neglected plant groups, which may surprise by marked variation. One of these groups is *Urtica dioica* agg., consisting of vaguely described taxa (microspecies/subspecies) with indefinite distribution. The most important source of variation in *U. dioica* agg. is probably polyploidy (2x, 4x; x = 13). The complex comprises an ubiquitous tetraploid cytotype (*U. dioica* s.str.) and several obscurely defined relict 2x taxa. The cytogeographical analysis through Europe revealed marked distribution pattern of *U. dioica* agg. cytotypes (360 populations / over 1100 individuals). We identified 56 diploid populations (nearly 16%) and 304 tetraploid populations. For the first time we detected triploid and pentaploid level (from four mixed populations). Prevailing tetraploid *U. dioica* s.str. is predominantly synanthropic, whereas diploid taxa strictly occur in primary and relict habitats (e. g. alluvial forests, tundra, Mediterranean ranges). The analysis of the absolute genome size of *U. dioica* agg. and other closely related taxa showed different values of 2x *U. kiovensis* (19 % larger than 2x *U. dioica*) and 2x *U. atrovirens* (6% less than 2x *U. dioica*). The morphometric analysis (PCA and DA) of diploid (*U. d. subsp. subinermis* and *U. d. subsp. pubescens*) and ubiquitous tetraploid (*U. dioica* s.str.) revealed partial separation of involved taxa.

Keywords: *Urtica dioica* agg., flow cytometry, morphometrics

European populations of the steppe species *Astragalus onobrychis* contain two distantly related polyploid lineages

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Inner-Asian steppes are by far the largest continuous steppes in the world, extending from Mongolia westwards to the Pannonian steppes of Central Europe. Disjoint patches of steppe vegetation, however, can also be found in Western Europe and highly insular steppe areas are situated in interior parts of large mountain chains such as the European Alps. Among others, we explored the phylogeography of *Astragalus onobrychis* (Fabaceae), with the broad distribution from southern France to the Altai, to shed the light on the origin of European steppe vegetation. As different chromosome numbers (2n=16, 32, 64, 72) have been reported, we were also interested in the role of polyploidy shaping the distribution of *A. onobrychis* in space and time. Our main questions are: 1) What are the biogeographic connections of populations from Central Europe with other areas of steppe vegetation in Eurasia? 2) Are polyploidy events connected to the colonization of particular geographic regions? We sampled ca 100 populations of *A. onobrychis* from the entire distribution area and ca 20 populations of closely related species. We examined genome size and ploidy level variation. Genetic structure was explored by sequencing of the ITS region and by genotyping using Radseq. Biallelic SNP data were then used for population tree reconstruction using SNAPP that was subsequently used for phylogeography reconstruction via testing several biogeographical models using BioGeoBEARS. The ITS analysis suggested the Central European populations are not monophyletic and that *A. onobrychis*, as is currently defined, originated from two distantly related lineages within sect. *Onobrychidium*. Moreover, the pattern of ploidy level variation differs among these two lineages - the first lineage occurring in the Balkans and Inner-Alpine dry valleys includes two different ploidy levels (most probably 2n=16 and 64), whereas the second lineage occurring in Central Europe and Far East exhibits only a single ploidy level (most probably 2n=64). Preliminary RadSeq analyses corroborate the pattern discovered by ITS; more detailed analyses are underway and will elucidate the independent colonization of Central Europe by two unrelated lineages of *A. onobrychis*.

Keywords: *Astragalus*, steppe, RadSeq, speciation
Geographic and ecological patterns of polyploidy in the Cardamine pratensis complex (Brassicaceae)

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Polyploidy has played an important role in the evolution of the genus Cardamine, which is underlined by the fact that the majority of the species are polyploid, and intraspecific ploidy variation is also common. The C. pratensis group is a textbook example of the polyploid complex. Recent studies revealed its specific structure, with a few distinct diploid taxa occurring in the Mediterranean, and less differentiated diploids and polyploids, distributed mainly in Central and Northern Europe (C. dentata, C. majovskii, C. matthiolii, C. nymanii, and the highly polymorphic diploid to heptaploid C. pratensis s.s.). Current knowledge on the Central European diploids and polyploids is still rather limited despite that extensive research on the C. pratensis complex has been carried out since the 1950’s. A complete pattern of its cytotype distribution, however, has not been assembled up to now. Little is also known about the within-population ploidy level variation and intercytotype gene flow. Similarly, ecological factors affecting the incidence and spread of polyploid lineages are still insufficiently known. Moreover, C. pratensis s.s. most likely consists of a series of cryptic species, which might be delimited by specific cytotype variation, ecological niches and geographic range. The present study focuses on the C. pratensis complex in Central Europe. To reveal a cytogeographic pattern, DNA-ploidy level and/or chromosome number were identified for almost 200 populations (ca. 2100 plants) using flow cytometry and/or chromosome counting. In addition, previously published karyological records were revised (ca. 1500 records). Environmental data were gathered and analysed to assess ecological differentiation between the taxa/cytotypes of the C. pratensis group. For 80 of here investigated populations we found substantial intrapopulation variation (up to 58%) in the amount of nuclear DNA connected with variation in chromosome number. This can be mostly attributed to incidence of aneuploidy; only 13 are mixed-ploidy populations. The remaining populations consisted of a single cytotype (intrapopulation variation less than 6%). Cytotype variation within the C. pratensis complex is geographically structured and different climatic requirements of the cytotypes were revealed. We assume that these patterns reflect diversification of this species complex which are tightly connected to several independent polyploidization events and subsequent niche shifts.

Keywords: Central Europe, cytotype distribution, flow cytometry, niche differences

Sources of Sorbus aria agg. variation

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Interspecific hybridisation and polyploidy are accepted as key evolutionary features of the genus Sorbus. The fate of new hybrid and polyploid cytotype is determined by their mode of reproduction. Especially apomixis could be very advantageous in establishing of a new lineage. The S. aria agg. (subg. Aria) plays an important evolutionary role within the genus since its members are involved in all hybridisation events and thereby is responsible for the substantial part of variation of the genus. Flow cytometry, molecular markers and morphologic analyses were employed to evaluate the processes generating the variation in the S. aria group. Three ploidy levels (2x, 3x, 4x) were detected within 7 taxa belonging to the subg. Aria in the Czech Republic. Flow cytometry seed screen revealed 7 modes of reproduction. Breeding variation covers a wide range of sexual and apomictic (pseudogamous) types of reproduction including reduced and/or unreduced gametes. Diploid cytotypes are exclusively sexual, whereas among polyploid taxa prevails obligate apomixis (with certain degree of residual sexuality) and only two species reproduce both sexually and asexually. An extremely rare mode of reproduction in plants, haploid parthenogenesis, was detected for the first time in genus Sorbus (in 4x S. danubialis). All taxa/lineages were defined using microsatellite markers. Genetic variation was determined for each group. According to expectations, the most variable species is diploid and sexual S. aria s.str. However, there is no correlation between the rate of residual sexuality and genetic variation among apomictic polyploids. Possible parental species of hybrids were identified based on multilocus genotyping. Basically, it is possible to distinguish all studied groups using morphological characters, however, the characters on leaves tend to overlap due to the exceptional phenotypic plasticity of each species and even of the same genotypes.

Keywords: Sorbus, apomixis, hybridization, flow cytometry, microsatellites
Geographic distribution, origin and postglacial history of the two cytotypes of *Alnus glutinosa* in Europe

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Polyplody in plants has been studied extensively. In many groups, two or more cytotypes represent separate biological entities with distinct distributions, histories and ecology. This study examined the distribution, origins and postglacial history of *Alnus glutinosa* cytotypes in Europe, North Africa and Western Asia. A combined approach was used involving flow cytometry, microsatellite and cpDNA analyses with species distribution modelling using MIROC and CCSM climatic models, in order to analyse (1) ploidy and genetic variation, (2) the origin of tetraploid *A. glutinosa*, considering *A. incana* as a putative parent, and (3) past distribution changes of the species. The occurrence of tetraploid populations of *A. glutinosa* in Europe is determined for the first time. The distribution of tetraploid species is far from random, forming two geographically well-delimited clusters located in the Iberian Peninsula and the Dinaric Alps. Based on microsatellite analysis, both tetraploid clusters are probably of autoploidy origin, with no indication that *A. incana* was involved in their evolutionary history. A projection of the MIROC distribution model into the Last Glacial Maximum (LGM) showed that (1) populations occurring in the Iberian Peninsula and North Africa were probably interconnected during the LGM and (2) populations occurring in the Dinaric Alps did not exist throughout the last glacial periods, having retreated southwards into lowland areas of the Balkan Peninsula. Analysis of microsatellites revealed three main directions of postglacial expansion of diploid cytotype: (a) from the northern part of the Iberian Peninsula to Western and Central Europe and subsequently to the British Isles, (b) from the Apennine Peninsula to the Alps and (c) from the eastern part of the Balkan Peninsula to the Carpathians followed by expansion towards the Northern European plains. Newly discovered tetraploid populations are situated in the putative main glacial refugia, and based on cpDNA neither of them was likely to have been involved in the colonization of central and northern Europe after glacial withdrawal.

**Keywords:** autoploidy, ecological niche models, flow cytometry, glacial refugia, microsatellites

How genome size variation is linked with evolution within *Chenopodium* sensu lato

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We performed a comprehensive analysis of genome size in the genus *Chenopodium* by including diploid and polyploid species in a phylogenetic framework and constructed a new phylogeny covering most Eurasian members of *Chenopodium s.l.*. The tree is highly congruent with the one published previously, only with some new subclades. The results highlight the necessity to construct a tree that will include species from previously poorly sampled areas like South America and Australia. As concerns genome size and its relationship to phylogeny, we show that variation in 1Cx DNA size is not affected when a phylogenetic correction is applied. Polyploid species are not among those with the smallest 1Cx DNA values, suggesting no genome downsizing after polyploidization and indicating that genome size in polyploids might equal the sum of parental genome sizes. The strong phylogenetic signal detected in the genome size of the genus *Chenopodium* indicates that genome size variation is significantly associated with phylogenetic divergence and follows the random walk model. We also suggest a gradual mode of evolution with increased rates of evolution in longer branches rather than a punctual one. Moreover, there is a pattern of species-specific adaptation in the evolution of *Chenopodium* genome size, indicating an increase in the rate of genome size change in recent phases in the evolution of the genus. In our view, the modality of *Chenopodium* speciation is underpinned by species-specific adaptations to various habitats. We detected a positive relationship between genome size and life cycle duration, maximum plant height, average plant height and fruit diameter. The observed pattern might be understood as a result of nucleotypic effects increasing the size, mass and rate of development of multicellular structures and organs, leading to more robust plants with higher ploidy levels. Moreover, we tested the stability of genome size in some representatives of *Chenopodium s. str.* across a broad geographic range (from Portugal to eastern Russia) in Eurasia. The detected variation in genome size is extremely narrow in *Chenopodium s. str.* compared to other species. This makes it a useful tool for determining morphologically similar species of this group. For most of them, relative genome size may serve as an alternative marker for identifying morphologically extremely plastic species of *Chenopodium s. str.*

**Keywords:** *Chenopodium*, chromosome numbers, flow cytometry, genome size evolution, ITS, nuclear DNA content, phylogeny
Coexistence of diploid and triploid populations in the wine spoilage yeast species Brettanomyces bruxellensis

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The environmental conditions of wine are unfavourable for growth of many microorganisms, however Brettanomyces bruxellensis (B. bruxellensis) is highly adapted to the winemaking process which implies resistance to sulphur dioxide, high ethanol tolerance, growth on limited nitrogen sources and low pH. This yeast metabolism results in an alteration of the wine’s flavour profile (unpleasant leathery and/or mousey smell), thus leading to economic losses. B. bruxellensis is also associated with other industrial fermentations such as beer, cider, kombucha (fermented tea), kefir, bioethanol and others. In those last cases, the desirability/undesirability of this yeast is unclear and still debated. The industrial importance of B. bruxellensis is also associated with other industrial fermentations such as beer, cider, kombucha (fermented tea), kefir, bioethanol and others. In those last cases, the desirability/undesirability of this yeast is unclear and still debated. The industrial importance of B. bruxellensis has led to the study of its genome and population structure. Previous studies revealed a high genotypic diversity at intraspecies level and that phenotypic characteristics are strain-dependent. Furthermore, a comparison of genome assemblies revealed the coexistence of diploid and triploid populations and high dissemination of a triploid population in wine fermentations in Australia. We led a genotyping study of a large population of B. bruxellensis isolates from five continents and different substrates using microsatellite markers. Our study suggests that B. bruxellensis species is structured according to ploidy level and substrate. The potential contribution of the triploid state to the adaptation to industrial fermentations and to the dissemination of the species B. bruxellensis is discussed.

Keywords: Brettanomyces bruxellensis, fermentation, population structure

AlyBase - a research infrastructure for study polyploidy in the tribe Alysseae

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The Brassicaceae family is well known for the common occurrence of polyploids and considerable variation in chromosome numbers. It appears that chromosome and ploidy variation is substantial also in its third largest tribe Alysseae. However, the origin of polyploids and various base chromosome numbers in the Alysseae, as well as their evolutionary significance, have not yet been sufficiently understood. To address these questions, a detailed summary of the current knowledge on karyological variation of the tribe across all genera and the whole distribution area is an essential starting point. For this purpose, chromosome number and ploidy-level database is the most convenient information tool. Therefore, we presented to a scientific public a research platform for Alysseae taxa consisting of the database of published chromosome number/ploidy-level data. This database (AlyBase) is available on-line at www.alysseae.sav.sk. The tribe Alysseae encompasses 24 genera and 277 species. The AlyBase covers currently available chromosome numbers and/or ploidy levels for 171 genera and 277 species. The AlyBase provides also complete information on the origin of analysed material, voucher specimens and revision of the identification of plant material according to the collection place or voucher specimens (if available). Furthermore, it is possible to display respective records on a map.

Keywords: Alyssum, chromosome counts, Cruciferae, online database, ploidy levels
Karyological and cytological variation in Iranian representatives of Chenopodium album aggregate

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Genome size variation has been recognized as a useful source of information in different taxonomic groups in angiosperms. While tremendous interspecific genome size variation is a well-known phenomenon, intraspecific genome size variation is supposed to be exceptionally stable and thus useful as an alternative taxonomic marker. Using DAPI flow cytometry, we tested the stability of genome size in all Iranian representatives of the Chenopodium album aggregate. We also sampled and determined ploidy level in 297 individuals from 121 populations. Each ploidy level was confirmed by chromosome counting. We determined 3 different ploidy levels in the species studied, i.e. 2n = 2x = 18 (C. vulvaria), 2n = 4x = 36 (C. novopokrovskyanum, C. strictum and C. sosnowskyi), 2n = 6x = 54 (C. album subsp. album, C. album subsp. iranicum and C. opulifoilum). Mean relative genome size ranged from 0.918 pg (C. vulvaria) to 4.423 pg (C. opulifoilum). Intraspecific relative genome size variation was exceptionally low, ranging from 0.696% in C. strictum to 3.407% in C. album. Due to immense phenotype plasticity of individual Chenopodium species, genome size should be useful taxonomical marker for distinguishing certain morphologically similar groups of species such as C. vulvaria/ C. sosnowskyi, C. strictum/ C. novopokrovskyanum or C. strictum/ C. album. Unfortunately, the two recognized subspecies of C. album (subsp. album and iranicum) in Flora Iranica appear to be indistinguishable by genome size. Moreover, we have not found any hybrid plant between two species with different ploidy levels or between two species of the same ploidy level but different genome sizes and thus confirmed the view that Chenopodium species do not hybridize freely across ploidy levels.

Keywords: Chenopodium album agg., flow cytometry, genome size, ploidy level, Iran

Tulips of South-East European Russia

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In the last reviews of the genus Tulipa from 2013, Tulipa biebersteiniana Schult. et Schult. fil. is considered as T. sylvestris L. subsp. australis (Link) Pamp. However, a much more detailed taxonomy of this group exists in Russian literature. In the South-East of European Russia Tulipa biebersteiniana has two ecological forms which are sometimes consider either as two species (T. biebersteiniana s.str. and T. scythica Klokov et Zot.), or as two ecological forms. The mesophytic form grows in broad-leaved forests and meadows along large rivers such as Volga and Don, while the xerophytic one is confined to steppe habitats. These forms differ by their reproduction strategy and their morphology. The mesophytic form reproduces mostly vegetative by long lateral stolons, while the xerophytic form reproduces by seeds. In Stavropol Krai we found a single population belonging to T. biebersteiniana s.l. group, but morphologically distinctly different from the mesophytic and xerophytic form. We described the taxon as a new species: T. narcissicum N.Yu. Stepanova. Chromosome numbers were counted on metaphase plates for 32 specimens from 8 populations of mesophytic and xerophytic forms of T. biebersteiniana and T. narcissicum. DNA was extracted by CTAB method from dry leaves from 6 specimens of T. narcissicum; 15 specimens from 6 populations of the xerophytic form of T. biebersteiniana; 23 specimens from 9 populations of the mesophytic form; two specimens of T. riparia Knjaz., Kulikov et Philippov; and 1 specimens of T. sylvestris s.str. from Belarus. Ten ISSR primers were selected for fingerprinting. The results were analyzed using Structure 2.2 and PAST 3.0 software packages. We revealed that the xerophytic form of T. biebersteiniana is a diploid 2n=24, and the mesophytic form is a triploid 2n=36, rarely diploid. Tulipa narcissicum morphologically resembles the xerophytic form of T. biebersteiniana, but is a triploid and reproduces by long lateral stolons as the mesophytic form. The cluster analysis based on 125 polymorphic ISSR bands revealed two major groups. The first group comprised exclusively of T. narcissicum individuals. The second group assembled all the other specimens, and is further divided into two subclusters. The first subcluster unites the specimens of the xerophytic form, the second one includes all the specimens of the mesophytic form, as well as the samples of T. riparia and T. sylvestris s.str. from Belarus.

Keywords: Tulipa, chromosome numbers, ISSR
What drives cytotype coexistence in *Pilosella echioides*?

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A unique system of sympatric persistence of up to 5 sexually reproducing cytotypes (2x, 3x, 4x, 5x and 6x) was found in some populations of *Pilosella echioides* (Asteraceae). A surprising feature of several populations from S Moravia, Czech Republic and N Austria was the dominance (up to 70%) of triploids. Consequently performed intra- and inter-cytotype experimental crosses revealed high ploidal diversity of the progeny, but only limited prospects for triploid dominance. Hybridization data used in model of the system behaviour showed impossibility of such cytotype coexistence without existence of differential performance of particular cytotypes. As the best candidate explaining triploid’s numerical superiority was supposed asexual reproduction that favour triploids. But the asexual reproduction via seeds was rejected based on crossing and emasculating experiments. Only extensive research of clonal spread based on molecular markers (SSRs) brought us necessary clue to solve the mystery. Our data corroborated the hypothesis of differential clonal growth of cytotypes that favour triploids (index of clonal diversity R=0.11) before tetraploids (R=0.19) and diploids (R=0.89). Those findings fit the theoretical model were differential clonal growth was parametrized and was able to explain triploid dominance. It seems that clonal growth is the main force that enable cytotype coexistence of *Pilosella echioides* even in unexpected pattern. Finally, the only question remains to be solved - is the differential clonal growth the way to survive in sympatry or the means that allow unlikely intermingling of cytotypes via seed reproduction?

Keywords: *Pilosella echioides*, cytotype co-existence, clonality, triploid dominance

Genome size evolution in the *Minuartia verna agg.*

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The *Minuartia verna* agg. (Caryophyllaceae) is an evolutionarily young polyploid complex (3-15 species depending on taxonomic concept) with extremely interesting distribution pattern. It includes species with large geographic ranges (arctic areas, central and south European mountains) as well as species with small areas, often confined to refugial sites and can serve as a model example in studies aimed at the role and location of both Glacial and Holocene refuges and postglacial migration routes. We performed molecular and morphological analyses and ploidy level and genome size estimations (combined with chromosome counts) in plants from 120 European populations. All populations from northern Europe and mosts parts of central Europe (except for the Pannonian Basin) are diploid. A more complex pattern with often sympatric or parapatric di- and tetraploids was detected in southern Europe. Central European lowland populations surviving in suboptimal conditions, often on toxic substrates, have lower genome size in comparison with (high) mountain populations. Molecular analysis (cpDNA) revealed two major groups of haplotypes. The first one embrances diploid populations from Scandinavia, the majority of populations from central Europe (both in mountains and refugial lowland sites, often on heavy metal rich soils) and high mountain populations from the Romanian Carpathians and northern part of Balkan Peninsula. The second group, consisting of both diploid and polyploid populations, is more or less confined to southern Europe, with an overlap to the Pannonian Basin and south-eastern foothills of the Alps. Morphological studies confirmed a high diversity and geographical pattern of seed surface, not fully congruent with formerly published species concepts. The study will be completed by RAD sequencing (in progress) and, finally, a new taxonomic concept of the group will be proposed.

Keywords: genome size, *Minuartia*, refuges
Common coexistence of diploids and tetraploids triggers formation of cytogenetic novelty in populations of the annual weed *Tripleurospermum inodorum* (Asteraceae)

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Mixed-ploidy populations, consisting of individuals of two or more cytotypes, have been widely used as ‘natural laboratories’, mainly for the study of the conditions enabling origin and establishment of new polyploids. However, even a secondary coexistence of diploids with already established polyploids may significantly shape the further evolution of both lineages, e.g. through inter-ploidy gene flow. To get new insight into the topic, we undertook a detailed investigation of *Tripleurospermum inodorum* (L.) Sch Bip., a common and widespread annual weed of man-disturbed sites. Over 1,200 central European populations of *T. inodorum* were screened for cytotype composition during our extensive flow-cytometric ploidy screening. Strikingly common coexistence of diploids and tetraploids was observed at all the studied spatial scales, with the overall incidence of mixed-ploidy populations reaching up to 48% in some regions. Marked genetic differentiation of the cytotypes along with differences in their monoploid genome sizes suggests secondary nature of the cytotype contact. Apart from regular occurrence of triploid hybrids in mixed-ploidy populations, aneuploid individuals spanning almost continuously 3x and 5x cytotypes were occasionally present. Manipulated pollinations involving both euploid (2x, 3x, 4x, 6x) and aneuploid pollen donors/recipients were conducted to study reproductive interactions of cytotypes in a greater detail. Interestingly, most of the pollination treatments resulted at least occasionally in production of viable progeny. That was also the case of crosses involving triploid or aneuploid parents, which displayed profound variation in chromosome numbers among their progeny. Our data thus show that triploids and aneuploids have an important role in generating cytogenetic novelty within *T. inodorum* populations. The overall intensity of inter-ploidy gene flow at a landscape level was assessed using microsatellite markers on a numerous set of diploid and tetraploid individuals sampled from both uniform-ploidy and mixed-ploidy populations of the contact zone. Moreover, to test for a possible involvement of minority cytotypes in mediating the diploid-tetraploid gene flow, a subset of progeny from the pollination experiment was screened for both ploidy level and molecular genetic markers.

Keywords: aneuploidy, contact zone, gene flow, ploidy mixtures

Diploids and teraploids under metal stress: Local adaption in *Alyssum montanum*

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Heavy metal rich soils are a challenge for plant life. On the one hand it forces species to evolve tolerance mechanisms but on the other hand it offers weak competitors the possibility to establish populations in nearly unriviled habitats. In central Europe (central Germany and southern Poland, Olkusz region) diploids and tetraploids form distinct populations on normal non polluted soil and populations on copper shale spoil heaps, where the thin soil layer is heavily metal loaded. This cluster of primary and secondary habitats offers the chance to investigate the possible adaption to the high metal concentration as a powerful selection factor with a very steep gradient on a small spatial scale. Also there is the possibility to differentiate between diploids and tetraploids in their ability to form metal tolerant ecotypes. In the project the adaptation of *Alyssum montanum* L. (Brassicaceae) to high metal content of soil was investigated in the context of micro-evolution and differentiation of ecotypes. For this purpose we performed a pot experiment on soil mixtures with a gradient in metal content. 1,150 plants from 23 populations from different edaphic and geographic provenance were included. Fitness proxies were measured, population genetics with AFLP fingerprinting and DNA methylation patterns with metAFLPs were performed to reveal differences in population’s ability in forming stable populations on metal loaded soil and possible correlations with the genetic differentiation between the populations and cytotypes. First results show a strong differentiation in the fitness proxies of populations of different edaphic origin. They also indicate independent evolution of metal tolerance in populations originating from different metal soils. Interestingly there is no significant difference in metal tolerance between cytotypes. Tolerance differences in spatial closely situated populations are reflected in the population genetic structure.

Keywords: metal tolerance, epigenetic differentiation, population genetics, metAFLP
The polyploid fescues of the Festuca valesiaca group and F. laevigata in the Alps

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In the Alps occur a couple of polyploid fescue taxa showing leaf anatomy and morphology similar either to F. laevigata or to widely distributed diploid F. valesiaca. Many populations of octoploids and decaploids have been overlooked in the past and were probably allocated to F. stricta subsp. trachyphylla. Morphologically the western alpine F. laevigata seems to descend into the octo- and decaploid plants of the Central and Southern Alps, which are expected to belong to the F. valesiaca affinity until now. From this line-up, we infer two central questions. Do the octoploids from west to southeast represent the same taxon? Is there any genetic impact from F. valesiaca like plants in the central and southeast populations on high polyploids? The investigation area covered almost all large dry valleys of Central, Southern and Western Alps. More than 500 individuals from about 40 populations were sampled for AFLP analysis. All accessions were cultivated in the Botanic Gardens Jena, thus ploidy level and endophyte infection of each plant was determined prior to molecular analysis. Furthermore, individual morphological and anatomical characters were analyzed for proper species determination. First results let assume the multiple origin of octoploids and decaploids within the Alps and the existence of 3 to 4 different genetic groups. Remarkable is the genetically isolation of F. stricta subsp. sulcata, a taxon which traditionally seemed to be the closest relative to F. valesiaca and therefore a potential hybrid partner. Most of the octoploid populations cluster together with F. stricta subsp. trachyphylla. The decaploids split in different groups and cluster on the one hand with F. valesiaca and on the other hand with the various octoploids. The disjunct distribution area indicates the decaploids as a young evolutionary group with multiple origins.

Keywords: Festuca valesiaca, F. laevigata, AFLP, Alps

Xenobiotic tolerance and allopolyploidy in Spartina: Integrative approaches to unravel the impact of hybrid genome duplication under stress conditions.

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Plant tolerance to xenobiotics represents an important research area. Indeed, PAHs (Polycyclic Aromatic Hydrocarbons) constitute an alarming group of organic pollutants, largely studied because of their harmful impact on natural ecosystems and human health. In plants, abilities to absorb, metabolize and/or degrade PAHs were investigated, but signaling and detoxification processes are still poorly understood. During these last decades, polyploidization has been described as a key mechanism in the emergence of new adaptive traits that could affect species tolerance to environmental constraints. In this context, species from the genus Spartina (Poaceae, Chloridoideae) were selected to examine the impact of genome duplication in the establishment of tolerance mechanisms to PAHs. Spartina species are characterized by numerous hybridization and genome duplication events. Recent allopolyploids represent excellent models for studying the short-term impact of interspecific hybridization and polyploidization. Here, three species were considered: the hexaploid parental species S. maritima and S. alterniflora and their allododecaploid derivative S. anglica, which resulted from genome doubling of the homoploid hybrid (S. x townsendii) during the end of the 19th century. This work focused on physiological analyses in these Spartina species under phenanthrene (model PAH) induced stress. Impact on physiological functions, or content and cellular compartmentalization of xenobiotics in leaf tissues were investigated, revealing higher tolerance to phenanthrene in S. anglica compared to its parental species. These preliminary results support the hypothesis whereby genome duplication increases tolerance to organic pollutants. On the other hand candidate genes involved in xenobiotic tolerance and metabolism (referenced as the xenome) have been identified in Spartina following an in-silico investigation based on A. thaliana transcriptome analysis under PAH induced stress. A set of GST (Glutathione-S-Transferases) coding genes were selected; GSTs constitute key enzymes involved in several xenobiotic detoxification processes. Expression of selected GSTs was quantified by RT-qPCR under phenanthrene induced stress. Impact of genome duplications on genes involved in organic xenobiotic tolerance is discussed.

Keywords: Spartina, abiotic stress, PAHs, GST, phenanthrene
Effect of nitrogen and phosphorus limitation on the genome size, ploidy and photosynthesis efficiency

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Angiosperm genome sizes (GS, the amount of DNA in the unreplicated gametic nucleus) range c. 2400-fold. Nitrogen (N) and phosphorus (P) macronutrients are essential for plants and are needed for building nucleic acids and for photosynthesis. Plant growth requires N and P for production of proteins and nucleic acids. Thus cell division is N and P demanding. Photosynthesis is also demanding in N-usage, with the most demanding molecule being the protein Rubisco, which incorporates CO₂ into sugars in the dark reaction of photosynthesis. The second most N-demaning molecules are the light harvesting pigment-proteins. However, photosynthesis is also demanding in P-usage for energy light transduction into chemical energy in the form of ATP and NADPH. Here we test the hypothesis that N and P limitation influence the interaction between N and P, plant GS, ploidy level and photosynthesis efficiency. To test this hypothesis we analysed the photosynthesis efficiency under four different nutrient treatments on two types of Arabidopsis thaliana (diploid and tetraploid), Nicotiana tabacum and Nicotiana sylvestris.

Keywords: genome size, photosynthesis, nutrient limitation

Habitat segregation in an allopolyplploid and its parent species in Cardamine

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Polyploids are generally expected to have intermediate and broader environmental distributions than diploids. However, empirical data on the environment of polyploids and its diploid parents in field are limited. We quantified habitat environment of diploids Cardamine hirsuta and Cardamine amara and the allopolyploid Cardamine flexuosa originated from C. hirsuta and C. amara in their native area in Switzerland in order to examine whether habitat environment differs among species. The diploids C. hirsuta and C. amara segregated in that C. hirsuta occurred in dry, bright, and nutrient-rich habitat in contrast to C. amara, while the allopolyploid C. flexuosa occurred in environments in combination of and wider range than those of the parent species. These findings provided evidence that the parent species occurred in extreme environments, while the allopolyploid occurs in broader environments overlapping with those of the parents. We discuss the potential of C. amara, C. hirsuta, and C. flexuosa as a model system to study the molecular basis of adaptive significance of allopolyploidy in wild plants, with particular focus on phenotypes and gene expressions.

Keywords: Cardamine, habitat environment, field observation
Using RAD-seq as a genome-wide approach to understand allopolyploid evolution in Dactylorhiza (Orchidaceae)

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Genomic inferences based on RAD-seq (i.e. restriction-site associated DNA sequencing) data have recently started to be widely applied to answer evolutionary questions in diploid organisms. However, difficulties in distinguishing between homoeologs and paralogs in duplicated non-model genomes have made it challenging to use RAD-seq to answer questions related to polyploid evolution. We describe here a reliable RAD-seq bioinformatics pipeline that integrates information obtained from parental diploid genomes in order to analyse polyploid genomic data. We further apply it to a dataset containing 184 representatives of several allopolyploid Dactylorhiza species that have originated independently from the same parents and overlap in distribution, but have different ecological preferences and morphology. We first assemble an artificial genomic reference based on non-overlapping RAD loci from the diploid parental species, which are expected to present a disomic inheritance in the allopolyploid genomes. We further implement and compare two pipelines (i.e., STACKS vs. GATK) for aligning the polyploid reads to this reference and calling/filtering SNPs. Based on the 5,000+ RAD loci obtained, we finally investigate the patterns of genetic divergence and gene flow dynamics, and formulate hypotheses regarding the polyploid origins across 35 populations sampled in Europe, over the distribution range of the D. majalis complex. Our results show a phylogeographic signal, but only a minimal genetic differentiation between the allopolyploids, giving evidence for frequent and extensive gene flow between the sympatric sibling allopolyploids in Central Europe. The data further supports two possible origins of D. braunsteineri: one in Great Britain and one in Continental Europe, whereas D. majalis and D. purpurella seem most likely to be of a single origin. We conclude that, in the face of gene flow, the observed phenotypic divergence between the sibling Dactylorhiza polyploids is maintained by a strong divergent selection, potentially related to their ecological specialization.

Keywords: genomics, gene flow, homoeologs, population structure, RAD-seq

Genetic variability in the 2x-4x Lotus corniculatus complex in Northern Eurasia

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The aims of the study are: 1) to characterize genetic (SSRs and cpDNA sequences) variation in Northern Eurasian populations of Lotus corniculatus complex, 2) to study gene flow between the cytotypes and 3) to obtain the first insights into the evolutionary history of L. corniculatus complex. Markers: SSR analysis (8 loci), tml-F region of cpDNA. Methods: AMOVA, Structure, TCS, Material: SSR analysis: 47 populations sampled across a wide area in E. Europe, Caucasus, Siberia and Central Asia (425 individuals in total). Tml-F sequences: 174 Lotus specimens, representing Lotus corniculatus complex (150 ind.) and outgroups. European populations of 2x L. tenuis and L. stepposus and 4x L. ucrainicus and L. corniculatus are generally characterized by higher genetic diversity and lower genetic isolation than populations of Euro-Asian (2x L. frondosus) and Asian (2x L. kiyovii) species. Decrease of genetic variability in combination with increasing genetic isolation may be related to autogamy, which presumably plays significant role in the breeding system of the latter two species. Minimal genetic diversity was observed in L. kiyovii populations from Tyva and Mongolia. Our data support rare introgression between two ploidy levels. Phylogeographical analysis of the section Lotus by tml-F cpDNA marker revealed, that within the material studied, a haplotype of L. alpinus from Spain (the Iberian System) was the closest one to a hypothetical ancestral haplotype of the L. corniculatus complex. Derived haplotypes were spreading eastward, and two areas of diversification: in Central and Eastern Europe (of a higher diversity level) and in the Asian part of Northern Eurasia (of a lower diversity level) are known. Tetraploid species L. ucrainicus and L. corniculatus share haplotypes with European but not Asian diploid species.

Keywords: Lotus, SSRs, cpDNA
Genetic identification and population analyses of mussel *Mytilus galloprovincialis* along the eastern Adriatic coast: heterozygote/hybrid characterization

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Mussels *Mytilus* spp. belong to a group of key species in marine, coastal ecosystems. This work demonstrated the dominant presence of Mediterranean mussel *M. galloprovincialis* along eastern Adriatic coast (22 sites), from Limski kanal to Dubrovnik. Morphological variations of shell measurements and shape of sampled mussel specimens were inside normal values for *M. galloprovincialis*. However, molecular analysis gave some new insight into the genetic diversity of Adriatic mussels. Nuclear marker Me 15/16 of the adhesive byssus protein is suitable to distinguish mussels of *Mytilus edulis* complex: *M. edulis* is characterized with allele E, *M. trossulus* with allele T and *M. galloprovincialis* with allele G. Among the 110 analysed mussels of the Croatian Adriatic Sea populations 2 specimens showed a heterozygote GE and GT genotypes, while all others were identified as homozygotes GG. The AFLP analyses of GT, GE and GG mussels also showed differences at two other loci, mac-1 and EFbis. The phylogenetic analysis based on D and D 5S rDNA clones showed grouping of Adriatic *M. galloprovincialis* in three clusters; GG genotypes form one group with referent *M. galloprovincialis*, GE genotypes grouped with *M. edulis* and GT genotypes with *M. trossulus*. The presence of the E alleles of all three loci at low frequency in *M. galloprovincialis* mussels could be explained by introgression since *M. edulis* and *M. galloprovincialis* are known to hybridise and exchange genes in the European contact zone. The presence of the T alleles is very surprising. This could be explained by introducing of T alleles as a result of accidental entrance of *M. trossulus* with ballast waters. However, we could not exclude the presence of relict *M. gallorovincialis* population carrying T allele. Further detailed morphometric and genetic analyses on larger numbers of specimens are needed to clarify the processes underlying origin and evolution of Mediterranean mussels *M. galloprovincialis*.

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