

ICPHB 2009

International Conference on  
**Polyploidy, Hybridization  
and Biodiversity**

**PROGRAM and ABSTRACTS**

**May 17 – 20, 2009**  
Palais du Grand Large  
**Saint Malo – FRANCE**

[www.icphb2009.univ-rennes1.fr/](http://www.icphb2009.univ-rennes1.fr/)



The **International Conference on Polyploidy, Hybridization and Biodiversity** aims at promoting knowledge exchanges and discussions on the latest developments concerning these major drivers of genome shaping and speciation. A wide range of topics will be covered such as the *consequences of polyploidy on biodiversity, hybrid and polyploid speciation, meiosis and fertility in polyploid species, genome evolution and structure, transposable elements and DNA methylation, epigenetics and gene regulation, heterosis, phenotypic variation* ... The conference will focus sessions on all these areas and therefore illuminate mechanistic and evolutionary insights into many fundamental phenomena in biology. This understanding is critical for management and conservation of Biodiversity as well as for breeding programs as most important crop species are relatively recent polyploids.

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## SCIENTIFIC PROGRAM

### Sunday 17 may 2009

16h00: Registration

19h00-22h00: Welcome cocktail at the Palais du Grand Large

### Monday 18 may 2009

8h00: Opening of the Palais / Registration

8h30: Opening speech

## CONTRIBUTION OF POLYPLOIDY AND HYBRIDIZATION TO EVOLUTION & ADAPTATION

**Chairwoman: P. SOLTIS**

**8h45-9h10:** Y. Van de Peer (Univ. of Ghent, Belgium) Did plants with double genomes have a better chance to survive the Cretaceous–Tertiary extinction event?

**9h10-9h35:** M. Barker (Univ. British Columbia, Canada): Paleopolyploidy and the diversification of the Angiosperms

**9h35-10h00:** O. Jaillon (Centre National de Séquençage, France) Deciphering polyploidization events that occurred during evolution of eukaryotes, through complete genome sequencing

**10h00-10h15:** M.E. Schranz (Univ. of Amsterdam, The Netherlands) Multiple rounds of paleopolyploidy in the Cleomaceae and Brassicaceae: an older shared event after their divergence from the Papaya lineage followed by younger lineage-specific events

**10h15-10h30:** A. Salmon (Iowa State Univ., USA) Assessment of gene conversion events in the *Gossypium hirsutum* polyploid genome

**10h30-10h45:** J. Jacquemin (Univ Perpignan, France) Genome evolution in a recent duplication in the rice (*Oryza*) genus

**10h45-11h10:** Coffee break (in the poster room)

**Chairwoman: M. AINOUCHE**

**11h10-11h35:** J.F. Wendel (Iowa State Univ., USA) *Gossypium* as a model for exploring the origin of novel phenotypes in polyploids

**11h35-12h00:** J. Doyle (Cornell Univ., USA) The impact of polyploidy on photosynthesis in Glycine species

**12h00-12h15:** A. Chenoui (Univ. Aix-Marseille, France) Does hybridization (not polyploidy) increase evolutionary rates?

**12h15-12h30:** P. Bures (Masaryk Univ., Czech Republic) Natural hybridization and genome size

**12h30-13h30:** Lunch

**13h30-14h30:** POSTER SESSION

## MECHANISMS FOR GENE EXPRESSION IN POLYPLOIDS

**Chairman: J.F WENDEL**

**14h30-14h55:** L. Comai (Univ. of California, USA) The making of a polyploid genome: regulation and selection

**14h55-15h20:** J. Chen (Univ.of Texas, USA) A molecular mechanism for gene expression changes and growth vigor in hybrids and allopolyploid

**15h20-15h40:** K. Alix (UMR Génétique Végétale Le Moulon, France) A combined proteomic and transcriptomic approach to study differential regulation of gene expression in newly synthesized *Brassica napus*

**15h40-15h55:** M. Hegarty (Aberystwyth Univ.) Genomic consequences of hybridisation and polyploidy in *Senecio*

**15h55-16h10:** K. Adams (Univ. British Columbia, Canada) Extensive divergence in alternative splicing patterns after gene and genome duplication

**16h10-16h40:** Coffee break (in the poster room)

**Chairman: V.COLOT**

**16h40-17h05:** B. Chalhouh (INRA Evry, France) Mechanisms and relationships between genetic, functional and epigenetic changes in polyploid wheat species (*Triticum* and *Aegilops*)

**17h05-17h30:** A. Levy (Weizmann Institute of Sciences, Israel) Mechanisms of gene expression rewiring in hybrids and polyploids

**17h30-17h45:** S. Anssour (Max Planck Institute for Chemical Ecology, Germany) Changes in uni-parental gene expression in synthetic *Nicotiana* allopolyploids: consequences for anti-herbivore defense responses

## **HETEROSIS, GENE DOSAGE**

**Chairman: J. CHEN**

**17h45-18h10:** J. Birchler (University of Missouri, Columbia USA) Studies at the intersection of polyploidy and heterosis

**18h10-18h35:** R. Veitia (University of Paris VII, France) Dosage and dominant negative effects in polyploids: a theoretical point of view

**18h35-19h00:** Q. Sun (Agricultural University, Beijing, China) Molecular basis of heterosis in hexaploid wheat: From nonadditive gene expression to gene regulatory network

**Tuesday 19 may 2009**

**8h30:** Opening of the Palais

## **ECOLOGICAL CONSEQUENCES OF HYBRIDISATION AND POLYPLOIDY**

**Chairman: J. DOYLE**

**8h45-9h10:** P. Soltis (Univ. Florida, USA) Comparison of natural and synthetic polyploids in *Tragopogon*: Does evolution repeat itself?

**9h10-9h35:** M. Ainouche (Univ. of Rennes, France) Hybridisation, polyploidy and evolution of invasive species

**9h35-9h50:** O. Paun (Kew Garden, UK) Genetic and epigenetic responses driving adaptation after allopolyploidization in *Dactylorhiza* (Orchidaceae)

**9h50-10h05:** B. Mable (Univ. of Glasgow, UK) Polyploids in extreme environments: is there potential for climate change to increase polyploid speciation rates?

**10h05-10h20:** K.W. Hilu (Virginia Tech, USA) Polyploidy and biodiversity: evaluating underlying associations

**10h20-10h50:** Coffee break (in the poster room)

**Chairman: C. PIRES**

**10h50-11h15:** R. Abbott (Univ. of St. Andrews, UK) Hybrid speciation and the molecular genetics of introgressed traits

**11h15-11h30:** C. Parisod (Univ. of Oslo, Norway) Evolutionary advantages of autopolyploidy in natural populations of plants

**11h30-11h45:** P. van Tienderen (Univ. of Amsterdam, The Netherlands) Potential for exchange of adaptive traits between two tetraploid species adapted to flooding

**11h45-12h00:** P. Mráz (Univ. of Fribourg, Switzerland) Polyploidy and invasion success: lessons from the *Centaurea stoebe* complex

**12h00-13h00:** Lunch

**13h00-14h00:** POSTER SESSION

## **POLYPLOIDY: EFFECTS ON GENOME ORGANISATION AND STRUCTURE**

**Chairman: L. COMAI**

**14h00-14h25:** A. Leitch (Queen Mary Univ. of London, UK) *Nicotiana* homoploid hybrids and allopolyploids and considerations on the evolution of disomic inheritance

**14h25-14h45:** M-A Grandbastien (INRA Versailles, France) Retrotransposons and polyploidy in *Nicotiana* species

**14h45-15h10:** A. Kovarik (Academy of Science, Czech Republic) What ribosomal RNA biology can tell us about evolution of polyploid plants

**15h10-15h30:** A. d'Hont (CIRAD Montpellier, France) Evolutionary dynamics of hom(oe)ologous haplotypes (BACs) within the highly polyploid sugarcane genome

**15h30-15h45:** C. Rustenholz (INRA Clermont Ferrand, France) Gene space organisation along the bread wheat chromosome 3B

**15h45-16h00:** A. Madlung (Univ. of Puget Sound, USA) The role of mitotic instability and aneuploidy in the evolution of allopolyploids in the genus *Arabidopsis*

**16h00-16h25:** Coffee break (in the poster room)

**Chairman: B. CHALHOUB**

**16h25-16h50:** C. Pires (University of Missouri, Columbia USA) Homoeologous chromosome pairing and rearrangements identified in allopolyploid *Brassica napus* by an integrated BAC-FISH karyotype of diploid *Brassica*

**16h50-17h10:** A-M Chèvre (INRA Le Rheu, France): Genetic context of allopolyploid formation and consequences on genome evolution: case studies from *Brassica napus*

**17h10-17h25:** G. King (Rothamsted Research, Harpenden, UK) Functional significance of segmental and whole genome polyploidy in *Brassica*

**17h25-17h40:** M. Feldman (Weizmann Institute of Science, Israel) Genome downsizing in polyploids provides the physical basis for their cytological diploidization

## **MEIOSIS AND REPRODUCTION IN POLYPLOIDS**

**Chairwoman: A.M. CHEVRE**

**17h40-18h00:** J. Jahier (INRA France) Progenitor dependent chromosomal stability of hexaploid synthetic wheats

**18h00-18h20:** C. Köhler (Swiss Federal Institute of Technology, Switzerland) Mechanisms of dosage sensing in *Arabidopsis* seeds

**18h20-18h40:** T. Naranjo (Univ Complutense Madrid, Spain) Homologous interactions in early meiosis in wheat-rye additions when the chromosome structure changes

**18h40-18h55:** M. Neiman (Univ. of Iowa, USA) Are polyploid *Potamogeton ampliparus* truly obligate asexuals?

**Wednesday 20 may 2009**

**8h30:** Opening of the Palais

### **MEIOSIS AND REPRODUCTION IN POLYPLOIDS**

**8h45-9h00:** E. Jenczewski (INRA France) Genetic regulation of meiosis in polyploid species: new insights into an old question

**9h00-9h15:** S. Armstrong (Univ Birmingham, UK) Using fluorescence in situ hybridisation (FISH) and genomic in situ hybridisation (GISH) as tools to investigate meiotic recombination in *Brassica napus*

### **RETICULATE EVOLUTION, PHYLOGENY**

**Chairman: A. LEITCH**

**9h15-9h30:** O. Panaud (Univ. Perpignan, France) New phylogenomic approach to investigate species history and speciation events: example of the genus *Oryza* L.

**9h30-9h45:** F. Depaulis (ENS Paris, France) Discrimination between ancestral polymorphism and recent hybridization as causes of shared polymorphic sites between species

**9h45-10h00:** V. Mahelka (Institute of Botany, Czech Republic) Gene capture from across the grass family in the allohexaploid *Elymus repens* (Poaceae, Triticeae) as evidenced by ITS, GBSSI, and molecular cytogenetics

**10h00-10h15:** W. Albertin (Univ. Bordeaux, France) Autotetraploidy and speciation in yeast

**10h15-10h30:** J. Fehrer (Institute of Botany, Czech Republic) Extensive ancient hybridization in sexual and apomictic hawkweeds (*Hieracium*, Asteraceae) and evidence for extinct diversity

**10h30-11h00:** Coffee break (in the poster room)

### **POLYPLOIDY AND EPIGENETICS**

**Chairwoman: M.A. GRANDBASTIEN**

**11h00-11h25:** O. Mittelsten Scheid (Gregor Mendel Institute, Austria) Polyploidy-associated gene silencing in *Arabidopsis*

**11h25-11h50:** R. Doerge (Purdue University, USA) The design and analysis of gene expression in a maize polyploid series

**11h50-13h00:** Lunch

### **POLYPLOIDY AND EPIGENETICS**

**Chairman: A. KOVARIC**

**13h00-13h15:** P. McKeow (University College, Ireland) Parental genome dosage in isogenic *Arabidopsis* polyploids: effects on nuclear organisation, gene expression and plant phenotype

**13h15-13h30:** A. Banaei (Leibniz Institute of Plant Genetics and Crop Plant Research, Germany) Analysis of the relationship between intra-species hybridization and the dynamics of DNA and histone methylation in *Arabidopsis thaliana*

**13h30-13h45:** A. Kilian (Diversity Arrays Technology Pty Ltd, Australia) Genome profiling of polyploids: lessons from diversity arrays technology (DArT)

**13h45-14h10:** V. Colot (ENS Paris, France) Assessing the impact of transgenerational epigenetic variation on complex traits

**14h10-14h35:** R. Martienssen (Cold Spring Harbor laboratory, SA) Heterochromatin, small RNA and post-fertilization dysgenesis in allopolyploid hybrids of *Arabidopsis*.

**14h35-15h15** – Closure

## **SESSION 1**

### **Contribution of Polyploidy and hybridisation to evolution and adaptation**

## **DID PLANTS WITH DOUBLE GENOMES HAVE A BETTER CHANCE TO SURVIVE THE CRETACEOUS–TERTIARY EXTINCTION EVENT?**

Van de Peer Y.

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9052 Gent, Belgium

Most flowering plants have been shown to be ancient polyploids that have undergone one or more whole genome duplications early in their evolution. Furthermore, many different plant lineages seem to have experienced an additional, more recent genome duplication. Here, starting from paralogous genes lying in duplicated segments or identified in large EST collections, we have dated these youngest duplication events through penalized likelihood phylogenetic tree inference and show that a majority of these independent genome duplications are clustered in time, and overlap with the Cretaceous-Tertiary (KT) boundary. The KT extinction event is the most recent large-scale mass extinction caused by one or more catastrophic events such as a massive asteroid impact and increased volcanic activity. These events are believed to have generated global wildfires and dust clouds that cut off sunlight during long periods of time resulting in approximately 60% of plant species going extinct, as well as a majority of animals, including all dinosaurs. From recent studies demonstrating that genome doubling or merging can lead to increased fitness, we propose that polyploidization may have greatly contributed to the survival and propagation of many plant lineages during or following the KT extinction event. Due to advantages such as altered gene expression leading to hybrid vigor and an increased set of genes and alleles available for selection, polyploid plants might have been better able to adapt to the drastically changed environment, 65 million years ago.

Keywords: KT boundary – polyploidy – dicots – monocots - angiosperms

## PALEOPOLYPLOIDY AND THE EVOLUTION OF THE ANGIOSPERMS

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(2) Department of Biology,  
Indiana University, Bloomington, USA

Our understanding of green plant evolution has been revolutionized by the availability and analyses of plant genome data. Perhaps the largest discovery from these analyses is the pervasive role of duplication in plant genome evolution. In particular, recent analyses have demonstrated that numerous flowering plants experienced ancient genome duplication events, or paleopolyploidy, and that all angiosperms likely have a polyploid ancestry. However, most plant genome analyses to date have focused on model plant species, in particular *Arabidopsis*, because they have the most available data. To expand our knowledge of paleopolyploidy beyond these well studied organisms, we analyzed the growing EST and whole genome resources available for the green plants. Using a bioinformatic pipeline, we examined over 12 million EST sequences representing nearly 200 species of green plants for evidence of ancient genome duplications. Our analyses reveal more than 50 independent ancient duplication events distributed across the phylogeny of angiosperms, gymnosperms, monilophytes, and bryophytes. Surprisingly, we observe a genome duplication prior to the radiation of extant seed plants. Controlling for rate variation among lineages, our results indicate that 97.6% of angiosperm genera have experienced a whole genome duplication within  $K_s < 2$ . Consistent with the perceived high frequency of ancient genome duplications in seed plants, we find that seed plant genera have experienced an average of 0.92 genome duplications/ $K_s$ . Analyses of paleopolyploidy and diversification within this data set suggest that whole genome duplications have likely played key roles in the diversification of seed plants and other lineages of green plants.

Keywords: paleopolyploidy, diversification, evolutionary genomics, genome duplication

**DECIPHERING POLYPLOIDIZATION EVENTS THAT OCCURED DURING EVOLUTION OF EUKARYOTES, THROUGH COMPLETE GENOME SEQUENCING**

Jaillon O.

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Species are usually defined by reproductive isolation and are characterized by their gene repertoire. These two aspects are consequences of events fixed during evolution, including whole genome duplications and other polyploidizations. Thanks to the recent progress in genome sequencing, new light has been shed on these events. Evolutionary traces of such events have been evidenced in various lineages in plants, animals, fungi and protozoa. We present comparative analysis of synteny as a powerful approach to unveil evolutionary footprints of these events. According to expectations, these events would facilitate speciation since some of them are thought to be at the base of major radiations such as teleostei or eudicotyledons. After an initial amplification, the gene repertoire would be shaped by constraints such as expression level and functional interactions that would tend to maintain only a tiny fraction of the duplicates over the long term. Functional innovation from duplication may be a secondary effect, enabled by these duplicate retention mechanisms.

Keywords: Ancient Whole Genome Duplications. Ancient Polyploidizations. Evolution. Comparative Genomics

**MULTIPLE ROUNDS OF PALEOPOLYPLOIDY IN THE CLEOMACEAE AND BRASSICACEAE: AN OLDER SHARED EVENT AFTER THEIR DIVERGENCE FROM THE PAPAYA LINEAGE FOLLOWED BY YOUNGER LINEAGE-SPECIFIC EVENTS**

Schranz E.(1), Barker M.S (2), and Vogel H.(3)

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The analysis of the *Arabidopsis* genome revealed evidence of three ancient polyploidy events in the evolution of the Brassicaceae. The most recent event, with the most sets of maintained paleologs, is called the At-? or 3R event, the next oldest is referred to as the At-? or 2R event and the oldest, and with the fewest maintained paleologs, is the At-? or 3R event. It is now established that the At-? is shared with other Rosids, including Papaya and Grape. Whereas data to date suggest that the At-? and At-? are Brassicaceae specific. To address when precisely these two events occurred and which plant lineages share these paleopolyploidizations we have analyzed EST sequences from the Cleomaceae, the sister-family to the Brassicaceae. We have sequenced over 4000 normalized ESTs from the 5' end. The analysis of these Cleome sequences and homologous sequences from other Rosid genomes (*Arabidopsis*, papaya, cotton, *Populus* and grape) yielded three major findings: (1) Confirmation of a Cleome specific paleopolyploidization (named Cs-?) that is independent of the Brassicaceae At-? paleopolyploidization; (2) Cleome shares the At-? duplication with *Arabidopsis*, which is lacking from papaya within the Brassicales; and (3) Analysis of rates of molecular evolution of the aforementioned Rosid taxa shows that the herbaceous taxa *Arabidopsis* and Cleome have a faster rate of molecular evolution than the other predominantly woody lineages. These findings contribute to our understanding of the dynamics of genome evolution due to polyploidy within one of the most comprehensively surveyed plant genomic systems, the Rosids, and particularly of the complex genomic history of the ?, ?, and ? duplications of *Arabidopsis*.

Keywords: paleopolyploidy; Brassicaceae; Cleomaceae; ESTs; molecular evolution;

## ASSESSMENT OF GENE CONVERSION EVENTS IN THE *GOSSYPIMUM HIRSUTUM* POLYPLOID GENOME

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Whole genome doubling (polyploidisation) and segmental duplication (e.g tandem repeats) lead to redundant genomic information whose fate is of particular interest in genome evolution. Due to the release of functional and selective pressure, one of the duplicates can evolve independently by either neofunctionalization, pseudogenisation, or loss (divergent evolution) or undergo gene conversion via non-reciprocal transfer of genic material with its homo(eo)logous partner (concerted evolution). In polyploid cotton (*Gossypium hirsutum*), both mechanisms have already been demonstrated. Genic regions have been shown to evolve independently (using 16 loci), while on the contrary, tandemly repeated rDNAs arrays derive from only one parental genome, indicating concerted evolution.

The genomic resources developed recently in cotton make it possible to detect homeolog-specific SNPs using the ESTs sequences from *G. arboreum* (A genome), *G. raimondii* (D genome), and *G. hirsutum* (AD genome). Using a previously described homeoSNP database and new ESTs sequences obtained from next-gen sequencing methods (454 sequencing), our goal is to estimate the proportion of gene conversion events that have affected the polyploid genome of cotton. The first step of this project was to detect putative gene conversion regions, corresponding to *G. hirsutum* ESTs that present both A-genome and D-genome-specific homeoSNPs. To validate these putative gene conversion events, and to ensure that they are not due to either sequencing errors or PCR recombination, these sequences were reamplified, cloned and sequenced (10-12 clones each for 30 targeted regions). Furthermore, to determine if these putative gene conversion events were not due to post-transcriptional modifications such as co-splicing of exons from A- or D- mRNA copies, a subset of primers was designed to amplify both exonic and intronic regions.

These results will be compared to previous results from the literature, and the nature of the sequences undergoing such gene conversion process will be discussed.

Keywords: gene conversion, ESTs assembly, homeoSNPs, gene duplication

## GENOME EVOLUTION IN A RECENT DUPLICATION IN THE RICE (*ORYZA*) GENUS

Jacquemin J.(1), Goicoechea JL.(2), Wing R.(3), Cooke RM.(1)

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(2) Genoscope-Centre National de Séquençage, 2 rue Gaston Crémieux CP5706 91057 Evry-Cedex France.

(3) Arizona Genomics institute, The University of Arizona, Tucson, AZ, USA.

The fate of duplicated genes has been the object of numerous empirical and theoretical studies since Ohno (1970) suggested the importance of gene duplication in supplying new genetic material to biological evolution. The current view is that duplicate genes will suffer one of the following fates; pseudogenization, neofunctionalization, subfunctionalization or concerted evolution.

A high-quality finished genome sequence is available for the cultivated rice *Oryza sativa* ssp *japonica* c. v. *Nipponbare*. In addition, the well-studied evolutionary history of the genus, spanning the last ~25million years make it of particular interest to study the dynamics of duplicated gene evolution. Our focus is on the most recent large segmental duplication involving chromosomes 11 and 12 in rice.

We aim to answer the following questions: When did this duplication appear? Is it really specific to the genus? What are the evolutive dynamics of paralogous pairs within and between the wild rice species presenting the duplication? Can we observe changes at the structural and/or functional level within the region? Finally, we will consider its contribution to species diversification in the *Oryza* genus. Using a phylogenetic approach with a small data set of paralogous genes, we have shown heterogeneity in evolutive dynamics, some of genes displaying strong sequence conservation, suggesting the existence of a mechanism of gene conversion. We present here first results of a large-scale sequencing project of the duplicated region in representative wild species of the genus

Keywords: duplication, rice, genome evolution, gene conversion.

## **GOSSYPIMUM AS A MODEL FOR EXPLORING THE ORIGIN OF NOVEL PHENOTYPES IN POLYPLOIDS**

Wendel J.F.

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Increasingly powerful technologies are being applied to polyploids in many plant groups, resulting in dramatic discoveries of novel genomic interactions. *Gossypium* includes classic allopolyploids arising from a biological reunion 1-2 MYA of divergent diploids from different hemispheres. This serendipitous merger generated a spectrum of responses, including disruption and reconciliation of ancestral gene expression patterns. Using several microarray platforms and other technologies, we are studying global transcriptional changes in synthetic and natural *Gossypium* allopolyploids and reconstructed F1 and polyploid hybrids, using differing tissues and genetic backgrounds. Allopolyploid formation induces massive alteration in gene expression and complex transcriptomic responses, including genome-wide genomic dominance and novel (transgressive) expression patterns. Using a chip that simultaneously distinguishes transcript levels for each homoeolog of thousands of genes, we show that allopolyploidization entails significant homoeolog expression modulation that is 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. Expression in some tissues may be biased such that there is an overall unequal contribution of two genomes to the transcriptome. Homoeolog expression ratios change during fiber development, showing that duplicate gene expression modulation even characterizes the development of a single cell. We are exploring the functional consequences of gene duplication in cotton and the possibility of novel gene recruitment following genome doubling.

Keywords: Cotton, *Gossypium*, Transcriptome, Gene expression, Subfunctionalization

## THE IMPACT OF POLYPLOIDY ON PHOTOSYNTHESIS IN GLYCINE SPECIES

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It has been known for some time that various aspects of photosynthesis can differ between polyploids and their diploid progenitors. It is possible that differences in photosynthesis could contribute to the greater “success” of polyploids as inferred, for example, from range expansion. We are investigating the effect of polyploidy on photosynthesis in the legume genus, *Glycine*, which includes the cultivated soybean (*G. max*) and whose members have experienced up to three polyploid events. An informatic study of the soybean genome has shown that homoeologous gene retention after polyploidy is greater for gene families of photosystem II than for the Calvin cycle or the light harvesting complex; this same pattern exists in *Medicago* and *Arabidopsis*, but intriguingly, different gene families are involved within each functional class. Photosystem gene families appear to be evolving to maintain balance among proteins of the complexes, whereas Calvin cycle family members show more evidence of positive selection and divergence in expression patterns. Studies of photosynthesis in recently formed polyploids in the perennial subgenus show that polyploids can differ significantly from their progenitors in net photosynthesis and photoprotection. Based on Solexa/Illumina assays of the same plants used in the physiology experiments, candidate genes for photoprotection show only subtle differences in expression between the tetraploid and its progenitors. Very different suites of genes are up- or down-regulated under light stress conditions in the three different species. Preferential homoeologue expression is seen at many loci. Although the tetraploid transcriptome appears to be more like that of its paternal donor overall, more chloroplast-targeted genes appear to be expressing exclusively the maternal homoeologue.

Key words: *Glycine*, soybean, allopolyploidy, transcriptome, photosynthesis

## **DOES HYBRIDIZATION (NOT POLYPLOIDY) INCREASE EVOLUTIONARY RATES ?**

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In contrast to polyploidy, whose consequences in evolutionary rates have been and are still thoroughly studied, hybridization, per se, has never been presented as possibly impacting evolutionary rates.

We obtained and analysed data of the 28S ribosomal DNA D8 domain from 31 species of echinoderms (primary and secondary structures), and compared the rates of evolution with 16S mitochondrial DNA sequences. Striking variation in D8 evolutionary rates was evidenced among sea urchins, by comparison with both 16S mitochondrial DNA and paleontological data. In two very distant genera, the increase in D8 evolutionary rate is extreme. Their highly stable D8 secondary structures rule out the possibility of pseudogenes. These taxa are the only ones in which interspecific hybridization was reported. We discuss how evolutionary rates may be affected in nuclear relative to mitochondrial genes after hybridization, by selective processes (increase in proportion of positively selected mutations) or mutational processes (gene silencing, concerted evolution). The selective hypothesis applies to any functional nuclear DNA, whereas the mutational hypotheses only apply to multigenic families. Data from different taxa known to hybridize (probably known by researchers present at this meeting) may allow to test whether this observation is general, and, in case it is, to select among the possible explanations.

Keywords: rRNA secondary structure - Evolutionary rate - Interspecific hybridization - Concerted evolution - Polyploidy - Nucleolar dominance/selective silencing - Positive selection - Effective size

## NATURAL HYBRIDIZATION AND GENOME SIZE

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The frequency of spontaneous hybrids is non-randomly distributed among plant taxa: while some genera or families contain large numbers of hybrids, the majority contain none (Ellstrand et al. in Proc. Nat. Acad. Sci. U.S.A. 93: 5090–5093, 1996). Our model genus *Cirsium* is a typical example of a genus with a high affinity for natural interspecific hybridization. Marked differences in the frequencies of particular thistle hybrid combinations were repeatedly observed under natural conditions in Central Europe. Considerable interspecific differences in nuclear DNA amount among various diploid ( $2n = 34$ ) *Cirsium* taxa were detected: 2C-values varied from 2.14 pg in *C. heterophyllum* to 3.60 pg in *C. eriophorum* (1.68-fold difference). Relationship was found between hybrid promiscuity and genome size in *Cirsium*: species with smaller genomes hybridize frequently, while those with larger genomes produce few or no hybrids. Moreover, negative correlation between the frequency of natural hybridization and the genome size difference between parental taxa was documented in the most commonly hybridizing *Cirsium* species pairs. A similar relation between genome size and the frequency of natural hybridization was found in *Epilobium*. We tested the general model "the higher frequency of hybridization, the smaller genomes" on complete datasets about vascular floras of Great Britain, Germany, and the Czech Republic, using the data from the Plant DNA C-value Database (Kew Royal Bot. Gardens; Bennett & Leitch, <http://data.kew.org/cvalues/homepage.html>, 2005). The correlation between the average genome size and hybrid promiscuity (counted as number of hybrids / number of species within a particular genus or family) was confirmed both on generic and family level within the British, German, and Czech vascular floras. The research was supported by grants no. GA CR206/07/0859 and LC06073.

Keywords: *Cirsium*, DNA-content, *Epilobium*, interspecific hybrids

## **SESSION 2:**

### **Mechanisms for gene expression in polyploids**

**GENETICS AND MOLECULAR MECHANISMS AFFECTING POLYPLOIDY-DEPENDENT SPECIATION**

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Genome-wide duplication events are thought to have contributed to diversification of plants. We study incipient and recent polyploidy in *Arabidopsis* to understand the molecular mechanisms leading to successful hybridization and establishment of polyploids. I will describe experiments aimed at elucidating non-additive regulation deriving from hybridization. The considerable regulatory disruption observed in recent interspecific hybrids of *Arabidopsis* must be reconciled with the success and apparent stability of the natural allopolyploid *A. suecica*. To characterize the molecular events associated with adaptation and evolution of allopolyploids we have sequenced the genome of *A. arenosa*, the presumed ancestral paternal parent of *A. suecica*. By de novo assembly of Illumina reads we have constructed a framework of contigs that covers much of this genome. We compared *A. suecica* sequence generated by Illumina to the *A. arenosa* contigs and to the known genome of *A. thaliana*, the presumed maternal parent of *A. suecica*. We will present information emerging from this ongoing analysis and discuss possible mechanisms involved in the evolution and fractionation of polyploid genomes

## A MOLECULAR MECHANISM FOR GENE EXPRESSION CHANGES AND GROWTH VIGOR IN HYBRIDS AND ALLOPOLYPLOIDS

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Hybrids produced within and between species often grow bigger and more vigorously than the parents. Some crops including corn and rice are grown mainly as hybrids, and many other crops such as wheat, cotton, and canola are grown as allopolyploids. An allotetraploid is a “doubled interspecific hybrid”. We use the model plant *Arabidopsis* to study the mechanisms for gene expression changes in hybrids and allotetraploids. *Arabidopsis suecica* is a natural allotetraploid, and new allotetraploids are readily resynthesized by hybridizing the related species *Arabidopsis thaliana* and *Arabidopsis arenosa*. We found genome-wide nonadditive expression of homoeologous genes in the allotetraploids and expression and phenotypic dominance of *A. arenosa* over *A. thaliana*. The nonadditively expressed genes include microRNA targets and the genes encoding transcription factors. One set of transcription factors controls circadian clock regulation in *Arabidopsis*. Circadian clocks mediate metabolic pathways and increase fitness in plants and animals. We showed that epigenetic modifications of the circadian clock genes CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) and their reciprocal regulators TIMING OF CAB EXPRESSION 1 (TOC1) and GIGANTEA (GI) mediate expression changes in downstream genes and pathways. During the day, epigenetic repression of CCA1 and LHY induced expression of TOC1, GI and downstream genes that contain CCA1 binding site (CBS) in chlorophyll and starch metabolic pathways in allotetraploids and F1 hybrids, which produced more chlorophyll and starch than the parents in the same environment. Daily repression of *cca1* in TOC1:*cca1*-RNAi transgenic plants increased expression of downstream genes and chlorophyll and starch content, whereas constitutively expressing CCA1 or ectopically expressing TOC1:CCA1 had the opposite effects. The causal effects of CCA1 on output traits suggest that hybrids and allopolyploids gain advantages from the control of circadian-mediated physiological and metabolic pathways, leading to growth vigor and increased biomass.

Keywords: Hybrids, Polyploidy, Epigenetics, Circadian clock, Heterosis

**A COMBINED PROTEOMIC AND TRANSCRIPTOMIC APPROACH TO STUDY DIFFERENTIAL REGULATION OF GENE EXPRESSION IN NEWLY SYNTHESIZED BRASSICA NAPUS**

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Polyploidy has played a major role in the evolution of plant genomes. Success of polyploidy can be explained by the fact that a polyploid genome is not only the strict addition of the progenitor genomes, but that polyploidisation induces structural and functional modifications which represent important sources of novelty. Our project aims to evaluate the modifications of proteomic profiles which occur during the early steps of formation of an allopolyploid genome; the second purpose is to identify the molecular mechanisms responsible for the differential regulation observed. Our plant model is oilseed rape (*Brassica napus*, AACC) naturally originating from interspecific hybridization between *B. rapa* (AA) and *B. oleracea* (CC); as experimental material, we dispose of newly synthesized allotetraploids resulting from independent AA × CC crosses.

Comparative proteomics performed on early generations (F1-S0-S1) of newly synthesized *B. napus* lines led to the observation of numerous non-additive patterns (compared to the mid-parent values): while only a few (<1%) qualitative modifications were observed, a high number (25–38%) of proteins displayed quantitative non-additive patterns [1]. We revealed that interspecific hybridization triggered the majority (89%) of the deviations observed, whereas very few variations were associated with genome doubling (3%) or selfing (9%). Interestingly, further characterization of the non-additive proteins in the newly synthesized *B. napus* did not reveal any relation to protein functions or subcellular localizations [2]. A transcriptomic approach was then undertaken to evaluate the prevalence of transcriptional regulation for the origin of the non-additivity of proteins. A subset of 100 genes coding for non-additive proteins in the synthetic *B. napus* lines was analysed by RT-PCR and Q-PCR: less than one third of the genes was regulated at the transcriptional level, 70% of them displaying transcriptional additive patterns. The mechanisms responsible for the non-additivity of proteomes have still to be identified: work is in progress to estimate the role of post-translational modifications of proteins in the non-additivity observed.

[1] Albertin et al. 2006. *Genetics* 173: 1101-1113.

[2] Albertin et al. 2007. *BMC Genomics* 8:56.

Keywords: *Brassica napus*, comparative proteomics, gene regulation, polyploidy

## GENOMIC CONSEQUENCES OF HYBRIDISATION AND POLYPLOIDY IN *SENECIO*

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Interspecific hybridisation is a major force in plant evolution and speciation. The genus *Senecio* (ragworts) presents examples of both allopolyploid and homoploid hybridisation (hybridisation with and without a change in chromosome number, respectively). The homoploid hybrid *Senecio squalidus* arose from hybridisation of the diploid species *S. aethnensis* and *S. chrysanthemifolius* on Mt. Etna (Sicily). This species was transplanted to the UK in the 1700s and has subsequently spread across much of the country. During this period, *S. squalidus* has hybridised on at least two occasions with the native groundsel *S. vulgaris* (tetraploid). This results in a sterile triploid hybrid, *S. x baxteri*, which can undergo spontaneous chromosome doubling to produce a fertile allohexaploid, *S. cambrensis*. These *Senecio* taxa therefore provide interesting examples of recent hybrid speciation events and are valuable models for studying changes to gene expression resulting from both hybridisation and polyploidy. Using a transcriptomics-based approach, we performed microarray assays to identify altered patterns of gene expression in both wild and resynthesised *Senecio* hybrids, relative to their parental taxa. These assays showed large-scale changes to gene expression as a consequence of both hybridisation and polyploidy, and that these changes occur rapidly following hybrid formation.

Keywords: polyploidy hybridisation *Senecio* transcriptomics

## **EXTENSIVE DIVERGENCE IN ALTERNATIVE SPLICING PATTERNS AFTER GENE AND GENOME DUPLICATION**

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Gene duplication and alternative splicing (AS) are two processes that increase proteome diversity. To study conservation and divergence in AS after whole genome duplication we examined AS events in gene pairs from *Arabidopsis thaliana* that were retained from an ancient polyploidy event in its lineage. We analyzed 2584 duplicated gene pairs using cDNA data and found a low level of conservation of AS events between the duplicates. To more extensively and precisely characterize AS divergence between the duplicated genes we used RT-PCR with six organ types and three abiotic stress treatments to detect organ and stress-specific divergence in AS in 52 gene pairs. Differences in splicing patterns in one or more organs or under stress conditions were found between the genes in a large majority of duplicated pairs, and some of those differences affected conservation of AS events between the duplicated genes. For example a few gene pairs showed conservation of an AS event only under an abiotic stress treatment. We also examined AS events in 21 tandem duplicate pairs using RT-PCR in six organs and under two abiotic stress treatments and found patterns of AS divergence comparable to the genes duplicated by polyploidy. Our results indicate that AS events diverge considerably after gene and genome duplication. AS divergence between duplicated genes contributes to functional and regulatory evolution and may lead to preservation of duplicated genes.

Keywords: whole genome duplication, alternative splicing, paleopolyploidy, abiotic stress, *Arabidopsis*

**MECHANISMS AND RELATIONSHIPS BETWEEN GENETIC, FUNCTIONAL AND EPIGENETIC CHANGES IN POLYPLOID WHEAT SPECIES ( TRITICUM AND AEGILOPS )**

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Polyploidy induces structural, functional and epigenetic changes, which importance varies among taxa and genomic combinations. In an attempt to precise relationships and mechanisms of such changes in the wheat polyploid model, we have characterized more than 35 newly-synthesized allotetra- and allohexa-ploids, using genetic, cytology, transcriptome, DNA methylation, transposable elements (TEs) and small RNA analysis.

Wheat allopolyploids with important structural changes, such as chromosome number variations (aneuploidy), deletions, translocations, and homoeologous recombination were evidenced. On the contrary other wheat allopolyploids (mainly allohexaploids) show no apparent structural changes. This offers us the possibility to characterize functional and epigenetic changes in wheat allopolyploids that show either important or no apparent structural changes.

We have characterized reprogramming of gene expression occurring at the whole genome level of the allopolyploids, as compared to progenitors and the mid-parent values, using the Affymetrix - GeneChip® Wheat Genome Array).

In “genetically stable” wheat allohexaploids, the level of non-additively expressed transcripts varies between 2 and 8% of the ca. 28000 total expressed transcripts. On the other hand, majority of the ca. 5000 transcripts, which expression was different between progenitors ( $P > 0.05$ ), were additively expressed in allopolyploids, suggesting an advantage in gene expression. The comparative transcriptome analysis allowed us to define sets of “polyploidy-regulated” transcripts, which expression is activated or repressed in a similar manner in the different allohexaploid combinations and/or across the S0, S1 and S2 generations as well as in the natural wheat allohexaploids, representing thus potential candidate genes involved in polyploids stabilization. None of the ca. 150 silenced genes was shown to be deleted from these wheat allohexaploids, confirming their genetic stability and indicating a more likely epigenetic regulation of gene expression. Different levels of cytosine (C) methylation were revealed for both coding and TE sequences, depending on the allopolyploid combinations. Analysis of TE transcription activity and small RNAs suggest important role in the regulation of gene expression.

In comparison, when aneuploid plants (lacking one chromosome) were analyzed, functional and epigenetic changes were much higher.

More advanced results; relationships between the genetic, functional and epigenetic changes and their role in the overall stabilization of the wheat polyploids will be discussed.

Keywords: wheat, genetics, epigenetics, transcriptome, allopolyploid-stabilization

## MECHANISMS OF GENE EXPRESSION REWIRING IN HYBRIDS AND POLYPLOIDS

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Genome merging, in interspecific hybrids and allopolyploids, is associated with novel patterns of gene expression. We have analyzed the genetic and epigenetic basis for this rewiring in two model systems, namely a yeast hybrid between *Saccharomyces cerevisiae* and *S. paradoxus*, and a synthetic wheat hybrid and allopolyploid analogous to bread wheat. In yeast, we have analyzed how hybrid-specific gene expression patterns are generated from the divergence in regulatory components between the parental species. Between the species, we have distinguished changes in regulatory sequences of the gene itself (cis) from changes in upstream factors (trans). Expression divergence was mostly due to changes in cis. Changes in trans were condition-specific and reflected mostly differences in environmental sensing. In the hybrid, over-dominance in gene expression occurred through novel cis-trans interactions or, more often, through modified trans regulation associated with environmental sensing. We will discuss the phenotypic impact of hybrid-specific expression patterns. In wheat we have previously shown rapid genetic and epigenetic alterations in genes or transposons at the onset of hybridization and/or in nascent allopolyploids. As small RNAs are candidates for affecting these events, we have analyzed the changes in small RNAs (Micro and siRNAs) populations in hybrids and allopolyploids and their connection with gene and transposon expression. We show that small RNA populations are altered in hybrids and polyploids with the strongest changes occurring upon polyploidization. Overall, in the first generation of the polyploid, there was a massive suppression of siRNAs that corresponds to repeats and transposons. This is consistent with the observed transcriptional activation of transposons upon polyploidization and supports the role of siRNAs in heterochromatinization and repression of transposons. These works emphasize how different levels of regulation, namely genetic, epigenetic and environmental, can bring about hybrid-specific expression patterns in lower and higher eukaryotes.

Keywords: Hybrids, polyploidy, gene expression regulation

**CHANGES IN UNI-PARENTAL GENE EXPRESSION IN SYNTHETIC NICOTIANA ALLOPOLYPLAIDS:  
CONSEQUENCES FOR ANTI-HERBIVORE DEFENSE RESPONSES.**

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We examined the expression of *N. attenuata* (Na) and *N. obtusifolia* (No) herbivore-induced genes in synthetic auto-polyploids (NaT and NoT) and 5 independent allopolyploid *N. × obtusifolia* (N×o) lines, to understand how the expression of genes regulating complex polygenic traits is altered in the early stage of allopolyploid hybridization. In Na, applying oral secretions (OS) from *Manduca sexta* larvae to wounds rapidly increases the expression of wound-induced protein kinase (WIPK), lipoxygenase 3 (LOX3), non-expressor of PR1 (NPR1), and jasmonate-resistant4 (JAR4); this triggers JA and JA-Ile production, which then stimulates the expression of trypsin protease inhibitors (TPIs), a potent anti-herbivore defense. In No, OS elicitation attenuates NPR1 transcript accumulation. NPR1 negatively regulates SA accumulation and the elevated SA levels in turn antagonize JA and JA-mediated defenses. The responses of NaT and NoT do not differ from those of their diploid counterparts, but were delayed in their JA bursts and showed dosage effects on the accumulation of WIPK and JAR4 transcripts, as well as in JA levels in NaT. The synthetic allopolyploid N×o lines responded to wounding and OS elicitation by enhancing the accumulation of Na-WIPK, No-LOX3, No-JAR4 and Na-TPI transcripts. Although both Na- and No-NPR1 transcripts were expressed in N×o lines, their low levels resulted in a phytohormone accumulation pattern similar to that of No, in which elevated SA appear to antagonize JA accumulation. TPI transcript levels correlated with TPI activity among the N×o lines; TPI activity in N×o lines 1 and 2 was similar to that in Na, whereas in lines 3, 4 and 5, TPI activity was comparable to that in No. These results suggest that synthetic neo-allopolyploids rapidly readjust the expression of their parental defensive genes to create highly variable anti-herbivore responses. Changes in the expression pattern of key genes and post-transcriptional events might facilitate adaptive radiations during allopolyploid speciation events.

Keywords: *Nicotiana*, polyploidy, Plant–herbivore interaction, direct defenses, polygenic traits, adaptive radiations, allopolyploidy speciation, Solanaceae

## **SESSION 3**

### **Heterosis, gene dosage**

## STUDIES AT THE INTERSECTION OF POLYPLOIDY AND HETEROSIS

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A ploidy series of inbred maize was produced at the following levels, 1x, 2x, 3x, 4x and 6x. The monoploid is less vigorous than the diploid. With increasing ploidy above the diploid level for inbred derivatives, the plant vigor and stature decline. However, duplex hybrids produced by crossing tetraploid inbred derivatives are vigorous. A further increase in vigor is achieved by producing double cross quadruplex hybrids that have the potential for four different alleles at any one locus, a phenomenon referred to as progressive heterosis. Thus, the greatest vigor is achieved at the tetraploid level when allelic diversity is maximized and the least vigor is observed with maximum homozygosity. Vigor decline occurs at similar rates with the inbreeding of matched diploid and tetraploid hybrids in contrast to theoretical predictions. Triploids derived from inbred lines have reduced vigor compared to the diploid progenitors. Reciprocal triploid hybrids that carry different numbers of alleles from the two parents exhibit consistently different magnitudes of heterosis indicating an impact of allelic dosage on hybrid vigor. Global patterns of gene expression in a 1-4x inbred ploidy series vary for many genes compared to the diploid but the differences are not of great magnitude. Nonadditive expression for many genes is observed in duplex tetraploid hybrids compared to the parents. A greater number of genes show nonadditive gene expression in the quadruplex hybrids and to a greater magnitude, but there is little overlap of nonadditive genes common to all hybrid states.

Keywords: maize, polyploidy, heterosis, gene expression

## PARALOGS IN POLYPOIDS: A THEORETICAL PERSPECTIVE

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Plants are remarkable with respect to their capability to go beyond diploidy and remain in seemingly stable polyploid states. More than 50% of flowering plants are estimated to be polyploids. Theory predicts that most gene duplicates tend to degenerate and disappear, so the high level of paralog retention in plants is puzzling. The extent of paralog survival is so important that protein diversity in plants is thought to be generated primarily through gene duplication rather than by alternative splicing. The fate of gene duplicates has been explored on several occasions. This question will be reassessed from the perspective of dosage effects. In addition, there is mounting evidence of altered gene expression in allopolyploids and hybrids (non additivity). Indeed, expression of the paralogs can vary among tissues, organs or can be developmentally regulated. In some cases new tissue specificity appears. Interestingly, the expression patterns in synthetic polyploids may recapitulate those found in the naturally-occurring plants. This suggests that some patterns of expression appear just after polyploidization and persist in an evolutionary scale. Classical epigenetic mechanisms such as altered DNA methylation or chromatin structure have been proposed to explain this phenomenon. Inheritable changes such as deletions have also been documented. Alternatively, sudden changes in gene expression can be due to cis/trans mismatches arising between the transcriptional effectors and their target genes contributed by the merging genomes. This perspective will be developed.

**MOLECULAR BASIS OF HETEROSIS IN HEXAPLOID WHEAT: FROM NONADDITIVE GENE EXPRESSION TO GENE REGULATORY NETWORK**

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Whole genome expression analysis in hybrid and its parental inbreds provides a platform to identify nonadditively expressed genes in hybrids, which have given some insights into the understanding of mechanisms of heterosis. In this study, two wheat (*Triticum aestivum* L.) hybrid F<sub>1</sub> derived from same female parent but displaying contrasting heterosis in primary root are used for expression analysis by using wheat genome array. The expression polymorphism analysis between the parental inbreds indicates that up to 4% genes display expression difference, but more than 3 times more present-absent genes between the two parental inbreds are detected in highly heterotic Hybrid A than in nonheterotic Hybrid B. Differential expression (DE) analysis in hybrids and their parental inbreds identify 1019 (4.94%) and 698 (3.23%) DE genes in Hybrid A and B, respectively. It is interesting to note that heterotic Hybrid A tends to have more DE genes of dominance and partial dominance expression modes than nonheterotic Hybrid B which, however, tends to have more DE genes of negative partial dominance expression mode. By adopting the “Wooden Barrel Principle”, we propose that accumulation of dominance and partial dominance expression in wheat hybrid could be a major determinant of root heterosis. We also find that a substantial number of stress-related genes as well as retrotransposon-like genes are also included in the DE genes. We propose that as compared to the interspecific hybridization which can be a source of genomic shock as described by Barbara McClintock, hybrids derived from less distantly-related two inbreds can be a source of “mild genomic shock” or “intrinsic stress” in the hybrid genome, which, in turn, could cause expression changes of genes, especially stress-related genes and retrotransposon. Higher GAs contents were found to be correlated with the heterosis in plant height. By using the uppermost internode tissues of wheat, we examined expression patterns of genes participating in both GA biosynthesis and GA response pathways between a hybrid and its parental inbreds. Our results indicated that genes encoding enzymes that promote synthesis of bioactive GAs, and genes that act as positive components in the GA response pathways were up-regulated in hybrid, whereas genes encoding enzymes that deactivate bioactive GAs, and genes that act as negative components of GA response pathways were down-regulated in hybrid. Moreover, the putative wheat GA receptor gene *TaGID1*, and two GA responsive genes participating in internode elongation, *GIP* and *XET*, were also up-regulated in hybrid. A model for GA and heterosis in wheat plant height was proposed.

Keyword: gene

## **SESSION 4**

### **Ecological consequences of hybridization and polyploidy**

## COMPARISON OF NATURAL AND SYNTHETIC POLYPLOIDS IN *TRAGOPOGON*: DOES EVOLUTION REPEAT ITSELF?

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Gene and genome duplication are central to current evolutionary models, providing new materials for adaptive and stochastic change. However, the processes and pace of such changes are unknown. The genomes of flowering plants, often bearing the signatures of recurrent cycles of polyploidization, offer excellent experimental systems in which to address questions about the fates of duplicate genes. *Tragopogon mirus* and *T. miscellus* (Asteraceae) are allotetraploid species that arose within the past 80 years and therefore provide opportunities to investigate the tempo of genomic change. Furthermore, each of these species has formed multiple times independently from the same diploid progenitors, thereby offering natural evolutionary replicates. We examined the retention and expression of genes duplicated by whole-genome duplication (homoeologs) in natural populations of these allotetraploid species and in synthetically produced allotetraploids that correspond to each species. In a gene-by-gene approach using cleaved amplified polymorphic sequence (CAPS) analysis of 20+ genes in the natural polyploids, we found that many homoeolog pairs were not maintained in duplicate; instead, we detected homoeolog loss and homoeolog silencing, including several cases of tissue-specific homoeolog silencing. Many of these patterns were repeated across populations of independent formation. In contrast, neither F1 hybrids nor first-generation synthetic allopolyploids exhibited these changes. We are now using a genomics approach to identify a larger pool of homoeologs for further analysis. Preliminary analysis has identified >45,000 SNPs in 10,428 contigs between the parental species of *T. miscellus*, *T. dubius* and *T. pratensis*, and >28,000 of these sites provide potential allele-specific markers for analysis of differential gene expression in *T. miscellus*.

## **HYBRIDISATION, POLYPLOIDY AND EVOLUTION OF INVASIVE SPECIES**

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Natural hybridisation and allopolyploidy resulting from contacts between introduced and related native species are frequently associated with rapid population expansion of newly formed genotypes and successful species. The reunion of divergent genomes in the same nucleus and their subsequent molecular interactions at the genetic, epigenetic, and expression levels has important evolutionary consequences that have received considerable attention from the scientific community in the recent years. We will examine the consequences of such interactions in genus *Spartina* (Poaceae, Chloridoideae) where ancient and recent hybridisation and allopolyploidy have resulted in the origin of invasive species (*S. alterniflora*, *S. densiflora* and *S. anglica*) that have important ecological impact on salt marsh ecosystems in different continents.

Keywords: Genome evolution, hybridisation, allopolyploidy, Biological Invasion, *Spartina*

**GENETIC AND EPIGENETIC RESPONSES DRIVING ADAPTATION AFTER ALLOPOLYPLOIDIZATION IN DACTYLORHIZA (ORCHIDACEAE)**

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Hybridization and polyploidy are potent forces that have regularly stimulated plant evolution and speciation. Independently formed, ecologically divergent allopolyploids, *Dactylorhiza majalis*, *D. traunsteineri* and *D. ebudensis* are the result of recurrent hybridization between diploids *D. fuchsii* and *D. incarnata* and provide a useful system to uncover genomic responses to allopolyploidization, together with revealing the genetic/epigenetic basis of adaptation to divergent environments. A cDNA-AFLP approach shows a significantly increased range of gene expression in allopolyploids, demonstrating a higher potential for phenotypic plasticity as compared to either parent. Moreover, allopolyploid individuals express significantly more gene variants (including a few novel ones) than the parents which provides a strong evidence for an increase in biological complexity (neo- or sub-functionalization). Alternatively, both copies of duplicated genes may remain active and retain their original function over a long evolutionary time in polyploids if the genes involved have allele-dosage effects, providing selective advantages. Multiple origins of each tetraploid taxon contribute to differential patterns of gene expression with a geographic structure. However, many patterns are conserved within each allopolyploid taxon but are variable between taxa, indicating that habitat preference shapes similar expression patterns in independently formed allopolyploids. Several cDNA patterns are correlated with environmental parameters (especially water availability) and/or encode for proteins involved in specific functions of ecological relevance. The structural changes after allopolyploidization are often accompanied by extensive epigenetic changes. A MSAP (methylation sensitive AFLP) approach evaluated the degree of methylation alteration in the allopolyploids. As expected, there are significantly less non-additive methylation patterns in the older *D. majalis* as compared to the younger *D. traunsteineri*. Corresponding to the expressed patterns, some epimutations are specific to each allopolyploid taxon, despite their multiple origins. There is a remarkable extended methylation variation within each of the two allotetraploids as compared to the diploid parental species: allopolyploid taxa show significantly more within-species epigenetic variation that may result in modulation of phenotypes and selection. Additional to stabilizing the allopolyploid genome, the genetic and epigenetic alterations are key determinants of the adaptive success of the new allopolyploid species potentially resulting in reproductive isolation.

Keywords: adaptation, ecology, transcriptomics, MSAP, cDNA-AFLP

**POLYPLOIDS IN EXTREME ENVIRONMENTS: IS THERE POTENTIAL FOR CLIMATE CHANGE TO INCREASE POLYPLOID SPECIATION RATES ?**

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A long-standing observation is that polyploidy occurs more frequently at higher latitudes and higher altitudes, possibly because polyploids are genetically or physically more robust than their diploid counterparts. However, in plants and some animals that show this distributional bias, polyploidy has also been associated with a shift in mating system towards autogamous reproduction (either through self-fertilization or parthenogenesis), which could enhance dispersal abilities into novel environments. Whether polyploidy or the ability to reproduce without finding appropriate mating partners allows invasion of these potentially harsh environments is thus difficult to disentangle. Another possibility is that unpredictable environments mechanistically favour the production of polyploids, through disruption of meiosis. Additionally, during periods of climatic change, not only the abiotic environment will be altered, but the biotic environment as well (e.g. changes in species distributions that could promote hybridization and lead to polyploidy; changes in pathogen pressures that could selectively favour polyploidy). The purpose of this talk will be to review current geographic distributions and inferred origins of polyploid plants and animals (particularly those that remain sexual and outcrossing) to assess whether rates of polyploid formation appear to be correlated with periods of climatic change and/or currently unstable or extreme environments.

Keywords: polyploidy, plants, animals, hybridization, environmental change, distribution

## **POLYPLOIDY AND BIODIVERSITY: EVALUATING UNDERLYING ASSOCIATIONS**

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Ployploidy and hybridization played important roles in the evolution and biodiversity of flowering plant. However, the intimate correlation between these biological phenomena and diversification is not well explored. Examined here are potential underlying factors that enhance biodiversity under the influence of ployploidy and hybridization using the grass family (Poaceae) as a case study. Ployploidy is the hallmark of the Poaceae as an estimated 80% of the species are believed to be of ployploidy origin. Basic chromosome numbers in grasses ranges from  $x = 2-18$ , and somatic numbers from  $2n= 4-265$ , with a  $2C$  DNA content that varies from 0.7 to 27.6. Further, ployploidy and hybridization have been in part attributed to the success of this fourth largest flowering plant family and ecologically most dominant. Several factors pertaining to promotion and success of ployploidy in this family are examined such as length of life span, historic depth in the family tree of life, extent of geographic distribution, and prominent eco-physiological adaptations. The results of the analyses of 651 of the 785 grass genera demonstrate that life span is a crucial factor in promoting ployploidy and species diversification. The trends in the Poaceae are compared with those in other plant families as well as with some animal lineages to explore potential parallelism in ployploidy evolution and biodiversity.

Keywords: Ployploidy, Biodiversity, Life Span, Phylogeny, Grasses

## HYBRID SPECIATION AND THE MOLECULAR GENETICS OF INTROGRESSED TRAITS

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Hybridization between a native species, Groundsel (*Senecio vulgaris*,  $2n=40$ ), and an introduced species, Oxford ragwort (*S. squalidus*,  $2n=20$ ), has led to the origin of two new hybrid species and a stabilized introgressant form of *S. vulgaris* in the UK within the past 200 years. The fact that *S. squalidus* is also recognised as a recently originated diploid hybrid species, makes this group of species an excellent one for studying hybrid speciation and introgression in plants. In this talk I will briefly review recent hybrid speciation events in this group of *Senecio* and then focus on recent work that has led to the isolation of regulatory genes controlling a key floral trait (ray floret expression) transferred from *S. squalidus* to *S. vulgaris* via introgressive hybridization.

Keywords: Hybridization, Speciation, Introgression, Floral evolution, *Senecio*

## EVOLUTIONARY ADVANTAGES OF AUTOPOLYPLOIDY IN NATURAL POPULATIONS OF PLANTS

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Autopolyploid plants are more common than traditionally assumed. Autopolyploidy has been neglected by the recent progress in studying polyploidy, receiving less attention than allopolyploidy. Accordingly, the advantage(s) of genome doubling per se as contrasted with gene doubling after hybridization remain unclear. In order to shed light on the characteristics and particularities of autopolyploids, we review here for the first time a broad range of studies focused on autopolyploid species encompassing levels of biological organization from genes to evolutionary lineages. Autopolyploid taxa are characterized by polysomic inheritance, with extensive genomic structural and functional redundancies, and increased effective population size. To date, evidence accumulates suggesting that autopolyploid species neither experience substantial genomic restructuring, nor wide repatterning of gene expression in the first generations following genome doubling. Biogeographic and ecological surveys stress the prevalent origin, establishment and expansion of autopolyploid lineages under changing climates. Reasons underlying the success of autopolyploid species largely remain to be addressed. However, a few promising model species recently came to light and will be discussed here. A tentative hypothesis is that polysomic inheritance alleviates the impact of genetic load in autopolyploid populations, which may represent a short-term advantage over their disomic relatives under climate-induced range shift. Genome doubling also releases some parts of the genome from selective constraints, which may provide the genetic material for long-term adaptation and colonization.

Keywords: Autopolyploidy; ecology; genome organization; history;

## POTENTIAL FOR EXCHANGE OF ADAPTIVE TRAITS BETWEEN TWO TETRAPLOID SPECIES ADAPTED TO FLOODING

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Within the Brassicaceae, hybridisation is commonly associated with polyploidisation. If inheritance is disomic, hybrids are genome-wide fixed heterozygotes for the parental genes, and backcrosses should fail due to genomic imbalances. This would result in a distinct hybrid phenotype and hybrids may further evolve to become a distinct species. However if recombination between the parental genomes leads to polysomic inheritance, backcrosses and hybrid x hybrid crosses can give rise to a range of novel genotypes in a hybrid swarm. Hence, selection could result in gene and trait exchange among the parental lineages rather than in a distinct hybrid lineage or species.

We explore the potential for such exchange between naturally hybridising tetraploid *Rorippa amphibia* and *R. sylvestris*. As these species occur in microhabitats with different flooding regimes, we are particularly interested in potential exchange of traits associated with flooding. First, we compare the parental species in their responses to three flooding treatments representative of their natural habitats. Second, we test how these traits are expressed in F1 hybrids. Third, we used Arabidopsis GeneChip arrays to test for differences in gene expression between parents and hybrids in response to flooding. Finally, we test the mode of inheritance in F1 hybrids. Our results show that the two parents have different ways to cope with flooding. *R. amphibia* shows an avoidance strategy and tries to reach the water surface, while *R. sylvestris* has a tolerance strategy with reduced growth and activity. The F1 hybrids were mainly intermediate in phenotype and gene expression. Hybrids show inheritance intermediate between disomic and polysomic. This means there is high potential for intergenomic recombination in later generations, and hence the exchange of flooding associated traits between the species. We argue that hybridisation and introgression is likely to be an important mechanism for *R. amphibia* and *R. sylvestris* to acquire new adaptations within the dynamic habitat of river floodplains.

Keywords: plasticity, flooding, inheritance, *rorippa*, gene expression

## POLYPLOIDY AND INVASION SUCCESS: LESSONS FROM THE CENTAUREA STOEBE COMPLEX

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Spotted knapweed *Centaurea stoebe* s.l. has been introduced from Europe (EU) into North America (NA) during the late 19th century, where it has become a prominent rangeland weed. Recently we analyzed the ploidy level of c. 2700 plants from 120 native and 48 invasive populations and found a pronounced intercontinental shift in the cytotype-ratio. In Europe c. 60% of the populations were diploid (2x) and 40% tetraploid (4x), while 4x is the only cytotype confirmed in North America. Though there is no clear-cut spatial cytotype pattern in the native range, tetraploids tend to be more common in southern latitudes (southeastern Europe). Detailed analysis of rare mixed-cytotype populations revealed spatial separation, with tetraploids occurring mostly in disturbed sites. Molecular marker analyses suggest a secondary contact zone of the cytotypes rather than in situ origin of tetraploids. The extremely rare occurrence of triploid plants in natural populations, as well as the difficulty in obtaining hybrid progeny in artificial 2x - 4x crosses, indicate a strong reproductive barrier between both cytotypes. Robust cross-continental comparisons between EU2x and EU4x, and between EU4x and NA4x showed a clear niche shift towards drier and warmer conditions for tetraploids in both cases. Lower specific leaf area, higher leaf dry matter content and thicker leaves found in tetraploids in comparison with diploids indicate pre-adaptation of the 4x plants to a drier climate. Field observations and common garden experiments further showed that 2x populations are predominantly monocarpic (dying after flowering) and 4x populations polycarpic with earlier flowering time. In order to identify source regions of introduced *C. stoebe* populations and to study effects of founder events and subsequent spread on genetic variation, we sequenced two cpDNA loci in 700 plants. Our results show a strong reduction in haplotype diversity in the introduced range compared to the native range and multiple introductions to North America. Preliminary analyses suggest also that the most common haplotype probably underwent fast and successful range expansion not only in the invaded range, but surprisingly also in the native European range. In conclusion, we suggest that the invasive success of *C. stoebe* is partly due to pre-adaptation of the tetraploid cytotype in Europe to drier climate and possibly further adaptation to these conditions in the introduced range. The potential for earlier and longer seed production associated with the polycarpic life cycle constitutes an additional factor that may have favored the spread of 4x plants in the introduced as well as in native range.

Keywords: invasion, life history traits, phylogeography, reproductive barrier, spatial distribution

## **SESSION 5**

### **Polyploidy effects on genome organisation and structure**

## NICOTIANA HOMOPLOID HYBRIDS AND ALLOPOLYPLAIDS AND CONSIDERATIONS ON THE EVOLUTION OF DISOMIC INHERITANCE

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Nicotiana contains approximately 76 naturally occurring species and about half of these are allotetraploids. Detailed phylogenetic, morphometric and cytogenetic analyses have revealed the likely diploid relatives of the parental genome donors, and age estimates based on molecular clocks and geology have revealed that the polyploids have formed over widely different time frames (from <200,000 years ago to >10 million years ago). Now we have found further complexity in that homoploid hybrid speciation has also played an important role in generating species diversity, at both the diploid and tetraploid level. Several lines of evidence point to hybrid origins including gene-tree incongruence and evidence for inter-allelic recombination between likely parental alleles.

In attempts to understand why allopolyploidy is so astonishingly successful in angiosperm divergence, synthetic polyploids have been made to resemble natural species. In Nicotiana we have reported the occurrence of translocations, dysploidy, supernumerary segments, rDNA amplification and homogenisation and mobility of retroelements, but there is much variation between plants, a feature also observed in some young natural allopolyploid populations. From that variation, stable allopolyploid species evolve, a process intimately linked with regular chromosome pairing and emergence of stable chromosome structure.

Of particular importance for polyploid speciation is the transition from polysomic to disomic inheritance. Using computer simulations, we predict that the evolution of pairing genes is not essential for that regular chromosome pairing, since genetic drift, coupled with a stringency threshold for stable homologue pairing is sufficient. We also predict that stable chromosome pairing is enhanced by reduced and/or focussed chiasma frequency and by the evolution of neofunctionalisation and subfunctionalisation. The latter prediction is particularly important since subfunctionalisation might explain why it emerges so rapidly and becomes so widespread in polyploid evolution. Furthermore the data suggest that the maintenance of synteny over tens of millions of years of evolution has its origin in the evolution of subfunctionalisation, which is favoured by selection because of the emergent property of stabilising disomic inheritance in young polyploid species.

Keywords: Polyploidy, disomic inheritance, Nicotiana, homoploid speciation, subfunctionalisation

## RETROTRANSPOSONS AND POLYPLOIDY IN NICOTIANA SPECIES

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Allopolyploidization is associated with a wide range of structural, epigenetic and functional changes in the hybrid genome. Transposable elements (TEs) may play a key role in these genetic modifications. We are currently studying the genus *Nicotiana* as a model system to investigate TE-associated genomic changes induced by allopolyploidy. The genus *Nicotiana* has undergone several allopolyploidization events, one of most recent ones (ca 20,000 years ago) having led to the creation of *Nicotiana tabacum* (tobacco), generated by allopolyploidy involving two distantly related diploid *Nicotiana* parents, *N. sylvestris* (maternal genome donor) and *N. tomentosiformis* (paternal genome donor). Using Transposon Display, we analyzed insertion profiles of different endogenous retrotransposon populations in natural and synthetic tobaccos as well as in the diploid parents. In natural tobacco, each retrotransposon population has evolved differently in term of loss and amplification of insertion sites. In the S4 generation of a tobacco synthetic hybrid (Th37), a subpopulation of the Tnt1 retrotransposon has amplified significantly, and newly transposed copies originate preferentially from the activation of copies inherited from the maternal parent *N. sylvestris*. We observed that a high proportion of *N. tomentosiformis* (paternal) insertion sites are modified or not transmitted to the hybrid. Modifications include indels or the loss of Tnt1/flanking sequence junctions. Complete parental additivity was observed in the F1 and S0 generations of other synthetic tobaccos, suggesting that TE-associated genome restructuring is not generated by the initial combination of parental genomes, but by further events, such as possibly meiosis. We have commenced similar work on other young *Nicotiana* allotetraploids, and we are studying the long-term evolution of the TE content of ancient *Nicotiana* allopolyploid sections.

# This work is dedicated to the memory of our dear friend Yoong Lim

Keywords: *Nicotiana*, tobacco, allopolyploidy, retrotransposon, transposable element, stress activation

## WHAT RIBOSOMAL RNA BIOLOGY CAN TELL US ABOUT EVOLUTION OF POLYPLOID PLANTS?

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In plants nuclear ribosomal DNA (rDNA) occurs in tandem arrays of units at one or several loci. Each 35S rDNA unit contains the 18S, 5.8S and 26S rRNA genes, the internally transcribed spacers (ITS) and the intergenic spacers (IGS).

Evolution of rDNA in hybrids and polyploids has stimulated much research, e.g.: (i) expression patterns of rRNA genes can be studied at interphase by observing the behavior of nucleoli; (ii) data from the ITS and ETS are regularly used to construct phylogenetic trees. (iii) epigenetic silencing can lead to inactivation of entire rDNA arrays (nucleolar dominance); and (iv) sequences can undergo concerted evolution involving sequence homogenization.

Here we studied patterns of evolution in rDNA in natural and synthetic populations of *Tragopogon* and *Nicotiana* to determine the consequences of allopolyploidy on rDNA divergence. We frequently found cases of non-Mendelian inheritance of rDNA between populations of natural and synthetic allopolyploids. Changes in parental rDNA ratios were observed even between the S1 progeny originating from the same parents. In recently formed allopolyploids (< 80 years old) rDNA homogenization occurs mainly via repeat elimination and locus loss mechanisms. In more advanced allopolyploids (>10 000 years old) we observe locus loss, intergenomic homogenization and amplification of novel units. At the epigenetic level, gene silencing (nucleolar dominance) may occur as early as in F1 hybrids with the direction similar to that observed in natural populations.

In conclusion, rDNA locus seems to be a highly dynamic structure undergoing rapid genetic and epigenetic evolution in allopolyploid genomes.

Keywords: Allopolyploidy, ribosomal RNA genes, concerted evolution,

## EVOLUTIONARY DYNAMICS OF HOM(OE)OLOGOUS HAPLOTYPES (BACS) WITHIN THE HIGHLY POLYPLOID SUGARCANE GENOME

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Sugarcane (*Saccharum* spp.) has been recognized as one of the world's most efficient crops in converting solar energy into chemical energy and having the most favorable input/output ratio. Beside its importance for sugar production it is thus also a primary energy crop. Sugarcane also presents one the most complex crop genomes studied to date. Modern sugarcane cultivars derive from the combination of two polyploid species: *S. officinarum*, the domesticated sugar-producing species with  $x=10$  and  $2n=8x=80$ , and *S. spontaneum*, a vigorous wild species with  $x=8$  and  $2n=5x=40$  to  $16x=128$  and many aneuploid forms. Both species are thought to have an autopolyploid origin. Modern sugarcane are highly polyploid (more than decaploid) and aneuploid, with around 120 chromosomes and a genome size of around 10 Gb. They typically display 70 to 80% of chromosomes entirely derived from *S. officinarum*, 10 to 20% from *S. spontaneum* and a few chromosomes derived from interspecific recombination. Their meiosis mainly involves bivalent pairing and chromosome assortment results from a combination of polysomy and preferential pairing.

We investigated genome dynamics in this highly polyploid context by analyzing the sequence of hom(oe)ologous haplotypes (BAC clones) from the sugarcane cultivar R570. We first analyzed two homoeologous haplotypes from a gene-rich region bearing the *Adh1* gene (Jannoo et al 2007), knowing that this region has been thoroughly studied within the Poaceae family. We then analyzed seven hom(oe)ologous haplotypes from a second gene-rich region. Our results indicated that the two *Saccharum* species diverged 1.5-2 mya from one another and 8-9 mya from sorghum. The sugarcane hom(oe)ologous haplotypes showed a very high colinearity as well as very high gene structure and sequence conservation. A high homology was also observed along the non-transcribed regions to the exception of transposable elements (TEs). Conversely, TEs that represent in average 33% of the BAC clones studied, were not conserved between hom(oe)ologous haplotypes. Compared to sorghum, the sugarcane haplotypes displayed a high colinearity and a remarkable homology in most of the non-coding parts of the genome. On this basis, the high ploidy of sugarcane does not seem to have induced a major reshaping of its genome (at least at the gene level). In addition, the coexistence of potentially in average 12 hom(oe)ologous alleles at each locus does not seem to induce a decrease of conservative selection at the gene sequence level.

Keywords: Sugarcane, BAC sequence, hom(oe)ologous haplotypes, evolutionary dynamics

## GENE SPACE ORGANISATION ALONG THE BREAD WHEAT CHROMOSOME 3B

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Because of its size (17Gb), allohexaploid nature and high repeat content (>80%), the wheat genome has always been perceived as too complex for efficient molecular studies. As a consequence, our knowledge of the wheat genome structure is still very limited. In particular, gene space organisation along the wheat chromosome is poorly understood and remains a matter of controversy. Indeed, during the last 10 years, it has been subjected to several studies reaching contradictory conclusions. However, none of them used a systematic approach. Following a chromosome-specific approach, we recently constructed the first physical map of a wheat chromosome: the chromosome 3B. This map allowed for the development of unique genomic resources that can be used to decipher the structure, evolution and function of the wheat genome. Based on these novel resources, we applied complementary transcriptomics and sequencing approaches to get new insights into gene space organisation along wheat chromosomes.

First, macroarrays carrying BAC clones of the chromosome 3B minimal tiling path were hybridized with mRNA extracted from five wheat organs at three developmental stages each to identify gene-containing BACs. Then, barley Agilent 15K expression microarrays were hybridized with the minimal tiling path three-dimension pools of the chromosome 3B to locate precisely genes on the physical map. Finally, gene distribution was studied at the sequence level through the sequencing and annotation of 12 megabase-sized contigs (cumulative length of 15 Mb) representing different regions of the chromosome 3B.

These experiments resulted in complementary conclusions shedding light on the chromosome 3B gene space organisation. First, they showed that genes are distributed throughout the chromosome and not limited to distal regions. Moreover, no megabase-sized “geneless” region was identified suggesting that no large genomic regions are completely devoid of genes. Finally, deletion bin-mapping of genes revealed an uneven distribution of genes along the chromosome 3B, with a possible gradient of gene density from centromere to telomeres.

Gene space organisation will be further investigated using the wheat Agilent 44K expression microarray that will also enable us to establish fine transcription profiles of the chromosome 3B genes. These data will be used to construct a transcriptional map of this chromosome and to decipher the relationships between the hexaploid wheat genome structure and the function and regulation of genes.

Keywords: hexaploid wheat, structural genomics, gene space, chromosome 3B

## THE ROLE OF MITOTIC INSTABILITY AND ANEUPLOIDY IN THE EVOLUTION OF ALLOPOLYPLOIDS IN THE GENUS ARABIDOPSIS

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Since allopolyploid hybridization can lead to speciation, allopolyploidy plays an important role in evolution. Early generation allopolyploids display meiotic instabilities contributing to high lethality, but less is known about mitotic fidelity in allopolyploids. We study mitotic stability in somatic cells of resynthesized *Arabidopsis suecica*-like neoallopolyploids, and established, natural lines of *A. suecica* ( $2n=4x=26$ ). We use fluorescent in situ hybridization to distinguish the chromosomal contribution of each progenitor, *A. thaliana* ( $2n=2x=10$ ) and *A. arenosa* ( $2n=4x=32$ ). Surprisingly, cells of the paternal parent *A. arenosa* display substantial aneuploidy, while cells of the maternal parent *A. thaliana* are more stable. Both natural and resynthesized allopolyploids display intermediate levels of aneuploidy. Our data suggest that polyploidy in *Arabidopsis* is correlated with aneuploidy, but varies greatly in frequency by species. The chromosome composition in aneuploid cells within individuals is variable, suggesting somatic mosaicisms of cell lineages, rather than the formation of distinct, stable cytotypes. Our results suggest that somatic aneuploidy can be tolerated in *Arabidopsis* polyploids and may present a mechanism for rapid evolution or species radiation in allopolyploids.

Keywords: evolution, mitosis, speciation, allopolyploidy, *Arabidopsis suecica*

**HOMOEOLOGOUS CHROMOSOME PAIRING AND REARRANGEMENTS IDENTIFIED IN  
ALLOPOLYPLOID BRASSICA NAPUS BY AN INTEGRATED BAC-FISH KARYOTYPE OF DIPLOID  
BRASSICA**

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Study of the chromosomes of *Brassica rapa* ( $2n = 20$ , A genome), *Brassica oleracea* ( $2n = 18$ , C genome), and *Brassica napus* ( $2n = 38$ , AC genome) has been difficult due to small chromosome size and lack of distinct karyological features. To identify all 10 pairs of *B. rapa* chromosomes and integrate the karyotype with genetic and physical maps, a multicolor fluorescence in situ hybridization (FISH) procedure was developed. We used 16 bacterial artificial chromosomes (BACs) that have been genetically and physically mapped and sequenced as part of the Multinational Brassica Genome Project (MBGP). Given the close phylogenetic relationship and synteny of the A and C genomes, we were able to perform reciprocal FISH experiments: *B. rapa* and *B. oleracea* BAC clones were both co-hybridized to *B. rapa* chromosomes, and then to *B. oleracea* chromosomes. When this same BAC-FISH karyotype cocktail is applied to allopolyploid *B. napus* followed by a second sequential genomic in situ hybridization (GISH), we are able to identify all the chromosomes of *B. napus*. Our procedure was then simplified by developing a set of repetitive DNA probes that collectively identify all of the Brassica chromosomes. To our knowledge, this is the first homoeologous chromosome painting toolkit developed for allopolyploid plants. This molecular cytogenetic approach can be used to precisely identify pairing and chromosomal rearrangements that until now have only been inferred by molecular markers. More generally, our robust karyotype toolkit should facilitate the study of the chromosomal relationships among diploid and allopolyploid species in the genus Brassica.

Keywords : allopolyploidy, Brassica, cytogenetics, polyploidy

**GENETIC CONTEXT OF ALLOPOLYPLOID FORMATION AND CONSEQUENCES ON GENOME EVOLUTION: CASE STUDIES FROM BRASSICA NAPUS**

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The impact of recombination in stabilization of allopolyploid species has still to be established. It is dependent on homology shared by the constitutive diploid genomes and on genetic control preventing homeologous pairing. Synthetic allopolyploid forms provide a relevant material to analyze the dynamic of structural modifications involved in the regulation of genetic redundancy, as the exact diploid progenitor genotypes are known and as the synthetic forms can be produced in different ways (i.e. reduced vs unreduced gametes). We used as our model oilseed rape (*Brassica napus*, AACC,  $2n=38$ ), an allopolyploid species with a polyphyletic origin, produced from natural hybridization between the two closely related species *B. rapa* (AA,  $2n=20$ ) and *B. oleracea* (CC,  $2n=18$ ). Two *B. rapa* and two *B. oleracea* belonging to different cultigroups were chosen as parent lines and seven different types of F1 hybrids (AC,  $n=19$ ) were produced. Their meiotic behavior indicated a high level of chromosome pairing. Additionally, BAC FISH analyses and comparisons with the meiotic behavior of the haploid forms produced from the same diploid progenitors revealed that autosyndetic pairing is the same or superior in F1 hybrids as in haploids whereas molecular markers had an additive profiles. S0 amphidiploid plants (AACC) were produced using either colchicine or female unreduced gametes. Homeologous pairing took place during the first meiosis, as revealed by establishment of meiotic behaviour, BAC FISH observations, molecular analyses of large progeny sets produced from crosses between S0 and a natural oilseed rape line, studies of 5 to 10 S1 plants per initial S0 plants. Two additional selfing generations were also analysed with single seed descent from each S1 origin. These had unstable meiotic behaviour and a higher frequency of disappearance of molecular markers belonging to the C genome than the A genome. All this data revealed that the stability of the synthetic forms is affected (1) by the genetic background of the diploid parents, with A and C genomes being differentially retained depending on genotype, (2) by the choice of maternal parent and (3) by the use of reduced or unreduced gametes, as unreduced gametes generate more structural modifications. All of this data gave new insights into the impact of the different levels of regulation and on factors affecting the stabilisation of synthetic forms. They will be discussed with respect to data acquired on natural oilseed rape.

Keywords: Brassica

## FUNCTIONAL SIGNIFICANCE OF SEGMENTAL AND WHOLE GENOME POLYPLOIDY IN BRASSICA

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Many crop genomes are relatively large, with complex organisation arising from segmental or whole chromosome polyploidy, with consequent gene duplication and divergence. This has limited progress in identifying and characterising genes underlying many agronomic traits. Availability of the emerging genome sequence for genera such as Brassica, which contribute to major oil and vegetable crops, is making it increasingly feasible to identify locus-specific copies of relevant genes. Seed growth in the Brassicaceae is controlled and co-ordinated by endosperm, integument and embryo. The embryo is the primary storage organ, with development and ultimate size determined by early development of endosperm and integuments. Although *Arabidopsis* seeds have ephemeral endosperms, increasing endosperm proliferation early in seed development increases the final size and weight of the seed. Crosses which generate 'paternal excess' in the seed (e.g. diploid 2x seed parent X tetraploid 4x pollen parent) increase endosperm size by increasing the rate and duration of mitosis. Extending the growing period of the endosperm produces a larger endosperm resulting in a larger embryo. We are transferring these insights from *Arabidopsis* to the taxonomically related Brassica crop species, that have seeds of ~200x greater mass. We have recently verified that early endosperm proliferation due to paternal excess also occurs in Brassica, by carrying out reciprocal interploidy crosses (2x X 4x). We are characterising this endosperm-led modulation of seed size in Brassica spp, including using a set of nine monosomic addition lines that provide a full set of *B. rapa* (AA) chromosomes and one each of the haploid *B. oleracea* (C) chromosomes. This has led us recently to identify a line that produces significantly larger seed. The use of addition lines allows us to target the changes in parental contribution to 'excess' by testing different sections of the genome. We are extending such studies to characterise the epigenetic regulation of genes affecting seed development at the level of interaction between segmental and polyploid gene duplications. To this end we are developing a range of approaches including adding value to the Brassica genome sequence by establishing distribution maps of epigenetic marks at key stages of development, and also exploring the effects of stochastic and targeted demethylation of genomic regions associated with known QTL.

Keywords: Brassica, segmental duplication, epigenetics

## GENOME DOWNSIZING IN POLYPLOIDS PROVIDES THE PHYSICAL BASIS FOR THEIR CYTOLOGICAL DIPLOIDIZATION

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Previous studies in the genera *Aegilops* and *Triticum* showed that allopolyploidization led to a rapid and meaningful genome downsizing. In allopolyploids of this group nuclear DNA amount was significantly less than the sum of DNA amounts of the parental species. Newly synthesized allopolyploids exhibited similar decrease in nuclear DNA amount already in earlier generations following their formation, indicating that genome downsizing occurs during or/and immediately after the formation of the allopolyploids. The equivalent amount of DNA in natural and in synthetic allopolyploids having the same genomic combination, and the lack of intra-specific variation in the natural allopolyploids, indicate that there are no further changes in genome size during the life of the allopolyploids following the initial downsizing.

While no significant changes in DNA amount was found in typical autopolyploids (autopolyploids having multivalent pairing) of the genus *Hordeum*, i.e., they had the expected additive amount of their diploid parents, cytologically diploidized autopolyploids (autopolyploids having exclusive bivalent pairing) of the genera *Elymus* and *Hordeum* had considerably less DNA (10-20% less) than expected. A newly synthesized autotetraploid line of *E. elongatus* showed similar reduction in DNA as its natural counterpart, indicating that the reduction in genome size in the natural cytotype of this autopolyploid occurred during its formation. It is suggested that the diploid-like meiotic behavior of these cytologically diploidized autotetraploids, as well as of the allopolyploids of the *Aegilops-Triticum* group, is caused by the instantaneous elimination of a large number of DNA sequences - different sequences from different homologous or homoeologous pairs - leading to differentiation in the case of autopolyploids and further differentiation in the case of allopolyploids of the constituent genomes. The eliminated sequences are likely to include those that participate in homologous recognition and initiation of meiotic pairing. A gene system determining exclusive bivalent pairing by utilizing the differentiation between the two groups of homologues or homoeologues is presumably superimposed on the DNA reduction process.

Keywords: Allopolyploids, autopolyploids, cytological diploidization, DNA sequences elimination, genome changes

## **SESSION 6:**

### **Meiosis and reproduction in polyploids**

## PROGENITOR DEPENDENT CHROMOSOMAL STABILITY OF HEXAPLOID SYNTHETIC WHEATS

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Regularity of meiosis is one of the key of success of a new allopolyploid species. Our concern is to understand the evolution of chromosome pairing in allohexaploid wheat, which natural form originates in the fertile Crescent from a hybridization between *T. turgidum* (2n=28, AABB) and the goatgrass *Ae. tauschii* (2n=14, DD). For that we developed series of synthetic wheat allohexaploids that combines two different accessions of the tetraploid progenitor *T. turgidum* as progenitors of the AB genome from one side and from the other side different accessions of *Ae. tauschii* as progenitors of the D genome. The different resulting synthetic allohexaploids were assessed for chromosome pairing and stability at metaphase I of meiosis in the generations S0, S1 and S2. There was no evidence of evolution of chromosome pairing across the three generations. From the other side, there were important diploid and tetraploid progenitor effects and chromosome pairing and stability were significantly variable depending on progenitor combinations. A few combinations of synthetic allohexaploids were comparable for regularity of chromosome pairing and chromosome stability to natural wheat allohexaploids whereas those of others were significantly lower. We also showed that frequencies of aneuploid plants in the different synthetics is positively correlated to the number of chromosome associations and to the mean number of univalents in the pollen-mother cells.

Identification of univalents (unpaired chromosomes) was made using GISH in two synthetics wheat allohexaploids. In both of them, the frequencies of univalents was higher for chromosomes from A followed by the B and lastly the D genome. It was surprising that A and B genomes, coresident in tetraploid progenitor *T. turgidum* since 0.5 MYA, showed different univalent frequencies in synthetic allohexaploids. More investigations are needed to characterize whether the lack of pairing is chromosome-specific.

The overall comparison of the synthetic wheat allohexaploids indicate that, from its creation 10000 years ago, evolution of the natural allohexaploid wheat genome has been “stabilized” for better regular meiotic pairing and chromosome stability by different mechanisms, including natural selection and domestication (including selection). The prospect of our study is presently to identify genes of *T. turgidum* and/or *Ae. tauschii* that control stability of meiotic behavior and the subsequent aneuploid frequency. The different wheat allohexaploids are being now characterized for genetic and epigenetic regulation.

Keywords: evolution, genome, chromosome pairing, GISH, wheat

## MECHANISMS OF DOSAGE SENSING IN ARABIDOPSIS SEEDS

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Polyploidy, the presence of more than two sets of chromosomes in an organism, in plants most commonly arises from unreduced gametes that have not had their ploidy level reduced via meiosis. Polyploidization is an evolutionary mechanism that serves for plant adaptation. Evolutionary potentials of polyploids are due to flexibility in genomic organization and function. Although there is much interest in the formation of polyploid plants, the molecular mechanisms underlying the production of unreduced ( $2n$ ) gametes are not well understood, and the first gene implicated in the production of a high frequency of unreduced male gametes in *Arabidopsis* was only recently described. Our laboratory has identified a further mutant, *jason* (*jas*), that is defective in male meiosis, resulting in the production of unreduced male gametes and the formation of triploid seeds. We identified the *JASON* gene and found it to encode for an unknown plant-specific protein that is conserved within the plant kingdom. Triploid seeds suffer from developmental aberrations and frequently abort, a phenomenon called triploid block. One explanation for the failure of triploid seeds is imbalanced expression of imprinted genes. We used the *jason* mutant to elucidate the underlying cause for triploid seed failure and will present a model and the supporting data explaining this phenomenon.

Keywords: *Arabidopsis thaliana*, triploid block, male meiosis, imprinted genes

## **HOMOLOGOUS INTERACTIONS IN EARLY MEIOSIS IN WHEAT-RYE ADDITIONS WHEN THE CHROMOSOME STRUCTURE CHANGES**

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In cultivated wheats and other important crops such as barley or rye, as well as in wild related species, chiasmata are formed mainly in distal chromosome regions. Long inversions and deletions may change the position of proximal chromosome regions relative to the chromosome ends. We have studied the effect that such chromosome rearrangements produce in the pattern of synapsis and quiasma distribution in rye chromosomes added to wheat. The inversion of the long arm of chromosome 1R changes the location of chiasmata from distal to proximal. In the inverted chromosome, an apparent failure of synapsis is observed in the distal region while homologous proximal segment interactions are not affected, especially in the telocentric chromosome conformation whose centromere migrates to the telomere pole. Deletion of about 70 % of the long arm of chromosome 5R has no apparent effect on the level of synapsis in this arm, but facilitates completion of synapsis in the short arm. The frequency of chiasmata is strongly reduced in the deleted arm while increases in the short arm of the chromosome carrying the deletion. These results suggest a link between homologous recognition and crossing over and that chiasma location is sequence-dependent.

Keywords: meiosis, homologous recognition, chiasmata, deletion, inversion

## ARE POLYPLOID POTAMOPYRGUS ANTIPODARUM TRULY OBLIGATE ASEXUALS?

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Asexual animals are usually polyploid, and polyploid animals are very often asexual. However, recent studies have demonstrated that ploidy level in asexuals is often much more variable and complex than initially assumed, and hinted that covert or occasional sex may be the underlying cause for this complexity. These data suggest that the link between polyploidy and asexuality in animals may in fact be quite tenuous, which could help to explain the “paradoxical” existence of ancient asexual lineages. Here, we consider the relationship between asexuality and polyploidy in *Potamopyrgus antipodarum*, a New Zealand freshwater snail that is the subject of an extensive body of research into the maintenance of sexual reproduction in natural populations. While asexual *P. antipodarum* are presumed to be all-female and triploid, in contrast to their dioecious diploid sexual counterparts, we present several lines of evidence consistent with a more complicated relationship between sex and ploidy in this system. These data include documentation of the existence of polyploid phenotypic males, ploidy levels ranging up to  $6n$ , and genome size variation amongst “triploid” asexual lineages. Molecular genetic data demonstrate that multiple independently-derived nuclear genotypes found in asexual polyploid individuals from many different lake populations are coupled with the same ancient mitochondrial haplotype. Taken together, these data suggest that the association between polyploidy and asexuality in *P. antipodarum* is weaker than originally supposed, perhaps because polyploid *P. antipodarum* are not invariably obligate asexuals.

Keywords: asexual, sexual, triploid, sex, parthenogenesis

## GENETIC REGULATION OF MEIOSIS IN ALLOPOLYPLOID SPECIES : NEW INSIGHTS INTO AN OLD QUESTION

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The genetic regulation of recombination in allopolyploid species is a pivotal issue in evolution and agronomy but has been largely under-explored in recent years, except in wheat. Crossover (CO) suppression between homeologous chromosomes is required to ensure proper chromosome segregation and fertility and therefore this mechanism is a determining factor in polyploid speciation; otherwise complex meiotic configurations would lead to unbalanced gametes, aneuploid progenies and hence impaired fertility. Regardless their chromosome composition, allopolyploid species behave like diploids during meiosis, with strict crossover formation between pairs of homologous chromosomes. This occurs despite the fact that homeologous chromosomes are usually sufficiently similar that CO can form between them under certain conditions. In many species including wheat or oilseed rape, a major locus segregating in a background of polygenic variation was shown to be responsible for the suppression of crossovers between homeologous chromosomes. Recent years have witnessed many advances in deciphering the genetic architecture of the control of CO formation in a polyploid species, including the characterization of wheat Ph1 locus at the molecular level, the delineation of wheat Ph2 locus, the cloning and expression analysis of several other wheat meiotic genes or the raise of *Brassica napus* as a second model to compare and contrast with wheat Ph genes. This talk gives an updated summary of these findings and points to several unanswered questions that should be addressed (in particular from an evolutionary standpoint).

Keywords: Meiosis, homeologous recombination, Ph1, PrBn

**USING FLUORESCENCE IN SITU HYBRIDISATION (FISH) AND GENOMIC IN SITU HYBRIDISATION (GISH) AS TOOLS TO INVESTIGATE MEIOTIC RECOMBINATION IN BRASSICA NAPUS.**

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Brassica species play role in global agriculture as well as being closely related to the model plant *Arabidopsis*. *B. napus* (AACC  $2n=38$ ) is important to world and UK agriculture as an oilseed crop. It is an allopolyploid species containing both the genomes of *B. rapa* (A genome) and *B. oleracea* (C genome). Research into the genome constitution of allopolyploids, the pairing of homeologous chromosomes, and the identification of translocations between chromosomes of different genomes has been aided by advances in molecular cytogenetics for example fluorescent in situ hybridisation (FISH) and a related technique, genomic in situ hybridisation (GISH). We present a strategy to distinguish between the A and C genomes in *B. napus* and to identify individual chromosomes within the genome. Natural *B. napus* has evolved the ability to confine most pairing to between strict A or C homologues. The frequency of homeologous recombination is much higher in resynthesised *B. napus* compared to the natural *B. napus* and it may be possible to exploit this situation to determine whether different loci or different alleles of the PrBn ( Pairing Regulator in *B. napus*) locus (Jenczewski et al., 2003) are involved in the control of homeologous recombination in this species.

Keywords: *Brassica napus*, meiosis, chromosome pairing, GISH, homeologues

## **SESSION 7**

### **Reticulate evolution, phylogeny**

**NEW PHYLOGENOMIC APPROACH TO INVESTIGATE SPECIES HISTORY AND SPECIATION  
EVENTS: EXAMPLE OF THE GENUS *ORYZA* L.**

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The increasing genomic data available on many organisms provides new opportunities to investigate the history of species. Phylogenomic methods combine phylogenetic analyses of a great amount of genes, genomic sequences or full genome sequences. These methods differ by the way they deal with the potential incongruences between datasets and trees. It is now well established that species history may differ to the history of the molecular marker used due to phenomena such as concerted evolution, introgression or horizontal transfers.

The genus *Oryza* contains 24 diploid and tetraploid species. The phylogenetic relationships within the genus are almost all resolved. However the origin of some polyploidy species (i.e. the time of the polyploidization event) such as *Oryza minuta* still needs to be ascertained. The *Oryza* Map Alignment Project provides Bacterial Artificial Chromosomes (BACs) covering the genomes of 12 species. The extremities of these BACs have been sequenced providing "BAC-ends" sequences of around 500bp covering 10% of the different genomes. We developed a new phylogenomic approach based on simple BLASTs and Neighbor Joining analyses to infer a phylogeny of the genus. By reciprocal BLASTs of all the BAC-ends of one species on the BAC-ends of another, we identified homologous sequences. The use of stringency filters and a screening of the obtained sequences allowed us to isolate couples of potential orthologous coding sequences. We computed the percentage of sequence identity between these sequences and displayed their repartition for each couple of species using R software. The repartitions appeared to be Gaussian. The maximum of each repartition was retained and used to build a distance matrix. A Neighbor Joining analysis of this matrix gave a tree displaying the expected relationships within genus *Oryza*. This method allowed us to avoid the problem caused by incongruences between datasets: the percentage retained is given by thousands of sequence couples. Indeed among these couples, paralogous or homeologous sequences can be found but they bring only minor contribution to the final result. Then, a molecular clock allowed us to date the different speciation and allopolyploidisation events in the genus.

Keywords: phylogenomics, rice, transposable elements, polyploidy, speciation

**DISCRIMINATION BETWEEN ANCESTRAL POLYMORPHISM AND RECENT HYBRIDIZATION AS CAUSES OF SHARED POLYMORPHIC SITES BETWEEN SPECIES**

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The study of the genetic pool of two species can reveal the amount of genetic similarities and dissimilarities between them. In this study we propose studying recombining DNA sequences as a way to distinguish isolation from hybridization by the patterns of linkage disequilibrium. Ancestral polymorphism shared by two species is expected to have had more opportunities to recombine than shared polymorphism generated by more recent gene introgression. Therefore, linkage disequilibrium among shared sites might indicate introgression. For this purpose, we analyze the sequence of shared polymorphic sites and fixed differences to detect clustering of shared polymorphism. Significant clustering of shared polymorphisms along the sequence will be considered as a footprint of recent introgression. We evaluated several statistics based on number of runs, length of runs and local clustering patterns with datasets generated by coalescent simulations with recombination under scenarios of isolation or hybridization. Some of the tests show some power to detect introgression. Application of these tests might be useful to detect “speciation genes”, i.e. genes resistant to introgression.

Keywords: isolation, coalescence, hybridization, molecular evolution

**GENE CAPTURE FROM ACROSS THE GRASS FAMILY IN THE ALLOHEXAPLOID ELYMUS REPENS (POACEAE, TRITICEAE) AS EVIDENCED BY ITS, GBSSI, AND MOLECULAR CYTOGENETICS**

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Four accessions of hexaploid *Elymus repens* from its native Central European distribution area were analysed using sequencing of multi-copy (internal transcribed spacer, ITS) and single-copy (granule-bound starch synthase I, GBSSI) DNA in concert with genomic and fluorescent in situ hybridization (GISH and FISH) to disentangle its allopolyploid origin. Despite extensive ITS homogenization, nrDNA in *E. repens* allowed to identify at least four distinct lineages. Apart from *Pseudoroegneria* and *Hordeum* representing the major genome constituents, the presence of further unexpected alien genetic material originating from species outside the Triticeae and close to *Panicum* (Paniceae) and *Bromus* (Bromeae) was revealed. GBSSI sequences provided complementary information to the ITS: Apart from *Pseudoroegneria* and *Hordeum*, two additional gene variants from within the Triticeae were discovered: one was *Taeniatherum*-like, but the other did not cluster with any of the diploids sampled. GISH results were largely congruent with the sequence-based markers. It clearly confirmed *Pseudoroegneria* and *Hordeum* as major genome constituents, and it further showed the presence of a small chromosome segment originating from *Panicum*. It resided in the *Hordeum* subgenome and probably represents an old acquisition of the *Hordeum* progenitor. Spotty hybridization signals across all chromosomes after GISH with *Taeniatherum* and *Bromus* probes suggested that gene acquisition from these species is more likely due to common ancestry of the grasses or early introgression than to recent hybridization or allopolyploid origin of *E. repens*. Physical mapping of rDNA loci using FISH revealed that all rDNA loci but one were located on *Pseudoroegneria*-derived chromosomes which suggests the loss of all but one *Hordeum*-derived loci. Since homogenization mechanisms seem to operate effectively among *Pseudoroegneria*-like copies in this species, incomplete ITS homogenization in our samples is probably due to a disadvantageous position of an individual minor rDNA locus located within the *Hordeum*-derived subgenome.

Keywords: *Elymus repens*; internal transcribed spacer; GBSSI; in situ hybridization, allopolyploidy

## AUTOTETRAPLOIDY AND SPECIATION IN YEAST

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About 10 000 years ago, human history was marked by the “Neolithic revolution” whereby hunter-gatherer human populations developed agriculture and domesticated several animal, plant and microbe species. The unicellular budding fungi *Saccharomyces cerevisiae* has been exploited by humans for millenniums to ferment beverages (beer, wine) and food (bread), but the evolution of those domesticated forms remains poorly studied. We have investigated the variation of ploidy in a panel of domesticated strains for oenology, brewery, bakery and distillery coming from different geographical origins. Segregation studies and crosses between the strains and tester strains with different ploidy levels showed that part of the strains were well-balanced autotetraploids displaying tetrasomic inheritance. The presence of up to four different alleles for various loci is consistent with a polyploidization mechanism relying on the fusion of two non-reduced meiospores coming from two *S. cerevisiae* strains. Based on microsatellite polymorphism, the strains clustered on the basis on their use. Interestingly, most bakery strains were tetraploid, suggesting a link between ploidy level and human use. The null or drastically reduced fertility of the hybrids between tetraploid and diploid strains indicated that domesticated *S. cerevisiae* strains are composed of two groups isolated by post-zygotic reproductive barriers.

Keywords: yeast, microsatellite, autopolyploid, tetrasomic inheritance, reproductive isolation

**EXTENSIVE ANCIENT HYBRIDIZATION IN SEXUAL AND APOMICTIC HAWKWEEDS (HIERACIUM, ASTERACEAE) AND EVIDENCE FOR EXTINCT DIVERSITY**

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Hawkweeds are one of the largest (500-8000 spp) and most notorious plant genera comprising few diploids sexuals and many polyploid apomicts. Different ploidy levels can occur in the same species. Extensive reticulation in the past and apomixis have resulted in a continuity of forms affecting species delimitation. We present the first molecular phylogeny of *Hieracium* based on nearly complete sampling of presumed major evolutionary units: species that show a unique set of characters, lack morphological evidence of hybrid origin, and are rather easy to identify. The nrETS region and the trnT-L spacer of cpDNA were used to resolve relationships. Among 60 accessions of 46 taxa, 29 had unexpected hybrid origin, with 17 different parental combinations. Diploids and polyploids were either 'pure' species or had hybrid origin. Some species had multiple origin, and some accessions had more than two parents. Hybrids were identified by character additivity at intra-individual polymorphic sites in ETS and excluded from phylogenetic analysis. A deep split of the genus into two major lineages was found. They corresponded to species with mainly Eastern or Western European distribution and also showed significant genome size differences at the same ploidy levels, but these two groups had never been suggested in any taxonomic treatment. 20 hybrid accessions were composed of members of both major clades. Detailed visual analysis of shared intra-individual polymorphisms that were not additive and cloning of selected accessions revealed the existence of three additional ribotype lineages that occurred only in hybrids; two of them were geographically widespread. Several hybrid cpDNA haplotypes were unique and rather derived; candidate maternal parents were missing. Also, a much larger number of diploids would be necessary to generate the *Hieracium* species diversity even if the most conservative estimate of species numbers is applied. However, only 10 (of 18) diploid species did not show signs of hybrid origin, and many diploids have relict character or occur only in known glacial refuge areas. We assume that the major species groups survived in different glacial refugia and hybridized after secondary contact. Hybrid origin even of supposed major evolutionary units, multiple origin of some species, and the presence of widespread or Central European taxa in both major clades had largely obscured species relationships. In addition, missing ancestral variation (more likely extinct than unsampled) preserved only in hybrid genomes may have contributed to the taxonomic confusion. Obviously, hybrid origin cannot be inferred from morphology if at least one parent is extinct.

Keywords: reticulation, apomixis, extinct diversity, phylogeny, *Hieracium*

**SESSION 8:**  
**Polyploidy and epigenetics**

## POLYPLOIDY-ASSOCIATED TRANSCRIPTIONAL GENE SILENCING IN ARABIDOPSIS

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A small genome and low genetic redundancy were major arguments for the choice of *Arabidopsis thaliana* as a model organism for molecular biology of higher plants. Nevertheless, genome analysis has revealed remnants from probably three ancient polyploidization events. Fertile polyploid *Arabidopsis* is easily generated also from recent diploid accessions. Furthermore, even in diploid plants, a substantial portion of cells undergo endoreplication, reaching high levels of ploidy. Therefore, the plentiful resources of genetic and genomic *Arabidopsis* information have been helpful to study the consequences of auto- and allopolyploidization and expected to yield information generally relevant for polyploid species.

Polyploidization in *Arabidopsis* is associated with changes of the sequence and/or the chromatin configuration of nuclear DNA. Multiplications of chromosome numbers can thereby contribute to heritable, genetic and epigenetic diversity. We will report on the formation and stability of epialleles at transgenic and endogenous sequences and the role of chromatin-modifying factors, based on analysis with molecular, genetic and cytological approaches. While many epigenetic mutants do not alleviate polyploidy-associated transcriptional gene silencing, those that have an effect indicate that the targets are under a double-acting control of different chromatin features.

The work in the lab is supported by grants from the Austrian Science Fund (FWF), the EU Network of Excellence “Epigenome” and the GEN-AU program of the Austrian Ministry for Science and Research.

Keywords: *Arabidopsis* epigenetic epiallele chromatin mutant

## THE DESIGN AND ANALYSIS OF GENE EXPRESSION EXPERIMENTS IN MAIZE POLYPLOIDS

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Although it is widely known that polyploidy is a frequent evolutionary event, it is not fully understood why polyploids have continued to be successful. Relying on microarray technology we explore the non-additive behavior of gene expression in maize polyploids. Data that include monoploid, diploid, triploid and tetraploid maize originating from Mo17 and B73 provide both the motivation and materials for these experiments. The experimental design consists of a loop design that allows clustering of results from serial differential expression across polyploidy of B73, as well as statistical assessments of the non-additive behavior of gene expression in hybrids at different ploidy levels relative to the respective parental inbred. The statistical designs, analyses, and challenges of these experiments will be discussed for the purpose of revealing information gain from a seemingly incomplete loop.

\*\*\*This work is in collaboration with Drs. Jim Birchler (University of Missouri, Columbia); Nicole Riddle (Washington University); and Lingling An (University of Arizona).

Keywords: statistics, polyploid series, non-additive gene behavior

**PARENTAL GENOME DOSAGE IN ISOGENIC ARABIDOPSIS POLYPLOIDS: EFFECTS ON NUCLEAR ORGANISATION, GENE EXPRESSION AND PLANT PHENOTYPE**

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Maternal and paternal genomes do not make equal contributions to epigenetic and maternal effects. In plants, these differences create difficulties in crop breeding programmes and also affect the outcome of hybridisation events during naturally-occurring speciation. We have studied these parent-of-origin effects using genetically identical dosage series of the model plant, *Arabidopsis thaliana* L., and comparing triploid plants with increased dosages of maternally- or paternally-derived genomes. We find that many aspects of plant phenotype differ between paternal and maternal-excess plants, including germination rate, growth habit, and even cell size regulation. Tiling microarray analysis suggests that these changes are accompanied by significant alterations to triploid gene expression. We demonstrate that maternal and paternal excess lines have altered nuclear organisation, suggesting a mechanistic basis for these epigenetic effects at the level of plant chromatin. Our results shed light upon the roles of genome dosage in determining the outcomes of plant crosses and hence crop breeding programmes and hybridisation events during plant speciation.

Keywords: polyploidy, triploid, hybridisation, chromatin, maternal affect, *Arabidopsis*

**ANALYSIS OF THE RELATIONSHIP BETWEEN INTRA-SPECIES HYBRIDIZATION AND THE DYNAMICS OF DNA AND HISTONE METHYLATION IN ARABIDOPSIS THALIANA**

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Heterosis or hybrid vigor refers to a genetic phenomenon in which hybrid offspring often show superior phenotypes compared with their inbred parents for such adaptive traits as fertility, biomass, growth rate and flowering time. Despite the great benefit of hybrid crops to agriculture, the principles underlying heterosis are still not well understood at the molecular level. Expression of genes that deviated from mid-parent expression levels, including examples of over-dominance, has been found in hybrid offspring in several studies, although the role of these genes is controversial. Chromatin modifications newly arisen in offspring as a consequence of intra-species hybridization might be considered as one possible explanation for deviations in gene expression and observed heterosis.

The aim of our study was to monitor changes in the level and distribution of different chromatin modifications, i.e. histone H3K4me<sub>2</sub>, H3K9me<sub>2</sub>, H3K27me<sub>3</sub> and DNA methylation in hybrid offspring compared with their inbred parents. Three *Arabidopsis thaliana* inbred accessions (Col-0, Cvi and C24) and their reciprocal hybrids (Col-0xCvi, CviXCol-0, Col-0xC24, and C24XCol-0) were used as models. No major changes in the distribution of methylated DNA or methylated histone H3 (K4, K9 and K27) were found between parents and offspring by indirect immunostaining. To identify minor changes in the distribution of histone H3K4me<sub>2</sub> and H3K27me<sub>3</sub> caused by intra-species hybridization, ChIP-on-chip experiments were performed. To resolve the effects of chromatin modifications from possible effects of sequence differences between the parental genomes, comparative genome hybridization analysis (CGH) was done to identify sequence differences between Col-0, Cvi and C24. Independent from genomic sequence differences, each accession was characterized by a unique distribution of histone H3 methylation marks. ChIP-on-chip experiments revealed some modifications in the distribution of histone H3K27me<sub>3</sub> in hybrid offspring compared to their inbred parents. In contrast, histone H3K4me<sub>2</sub> seemed to be additively inherited in hybrid offspring. Methylation Sensitive Amplified Polymorphism (MSAP) experiments demonstrated that DNA methylation is a rather stable chromatin modification. Hence, the chromatin modifications studied in intra-species hybrids of *Arabidopsis* inbred accessions are mostly stable and additively inherited.

Keywords: heterosis, intra-species hybridisation, histone methylation, DNA methylation

## **GENOME PROFILING OF POLYPLIIDS LESSONS FROM DIVERSITY ARRAYS TECHNOLOGY (DART)**

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Diversity Arrays Technology (DArT) was developed a decade ago to enable cost-effective genome profiling in order to increase efficiency of crop breeding and management of natural diversity. The technology offers affordable scan of any genome with thousands of markers and has found numerous genetic and breeding applications in a variety of crops. In addition to detecting SNP and Indel variation, DArT profiles report also on cytosine methylation variation, when the method of complexity reduction is using at least one of the methylation sensitive restriction enzymes (e.g. PstI). The use of methyl-filtered representations enables some insights into epigenetics of materials under study.

At the moment DArT has been developed in over 50 crops with a large number of polyploid species. With all these species the technology performed well irrespective of genome complexity or ploidy level, including arguably most complicated among the crops – sugarcane. We will present a few examples of applications in genetic, QTL and association mapping in various polyploids and contrast DArT's good performance with other genome profiling technologies. We will also present new trends in utilisation of DArT profiles in breeding (e.g. in genomic selection) and in genomic studies (e.g. physical mapping). A novel method of linking genome profiles with phenotypic information will be presented. The results of benchmarking this method (based on statistical machine learning) against current methods of QTL detection and association analysis, will be presented.

DArT technology has been developed in an “open access” format with broad participation of technology users and has been delivered by DArT PL to may hundreds of customers in over 30 countries on all continents. Some lessons from technology development through public/private consortia and from delivery of service will be presented.

Keywords: DArT, genotyping, marker-trait association, genetic mapping, physical mapping

## ASSESSING THE IMPACT OF TRANSGENERATIONAL EPIGENETIC VARIATION ON COMPLEX TRAITS

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From a classical perspective, the heritable basis of complex traits rests solely on the transmission from parents to offspring of multiple DNA sequence variants that are stable and causative. Accumulating evidence suggests that this view may be too restrictive, insofar as chromatin variation (such as differential DNA methylation) can also be propagated across generations with phenotypic consequences, independent of DNA sequence changes. However, attempts to assess the extent of epigenetic variation in natural or experimental populations and to quantify its impact on complex traits have been hampered by the confounding effects of DNA sequence polymorphisms. To overcome this problem as much as possible, we established a unique panel of so-called epigenetic Recombinant Inbred Lines (epiRILs) in the reference plant *Arabidopsis thaliana*. The epiRILs were derived from two parents with little DNA sequence differences but contrasting DNA methylation profiles. Analysis of the epiRILs revealed a remarkably high heritability for flowering time and plant height (~30%), as well as stable inheritance of multiple parental DNA methylation variants (epialleles) for at least eight generations. These findings provide a first rationale to determine the contribution of epigenetic variation to the inheritance of complex traits using linkage or association studies. More generally, the demonstration that numerous epialleles across the genome can be stable over many generations in the absence of selection or extensive DNA sequence variation prompts a need to integrate epigenetic information into population genetics studies.

**HETEROCHROMATIN, SMALL RNA AND POST-FERTILIZATION DYSGENESIS IN ALLOPOLYPLOID HYBRIDS OF ARABIDOPSIS.**

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Naturally occurring allopolyploid hybrids between *Arabidopsis thaliana* and *A. arenosa* are phenotypically vigorous and fertile. In contrast, neo-synthesized allopolyploids have highly variable phenotypes including poor seed viability, reduced growth, and late- and non-flowering habit. They also undergo heterochromatic silencing in the form of nucleolar dominance. As these polyploids are genotypically very similar epigenetic changes must underlie this phenotypic variability.

Heterochromatin is composed of transposable elements (TE) and related repeats which silence genes located nearby, and play a major role in epigenetic regulation of the genome. We have been using microarrays and next generation sequencing technologies to investigate the roles of DNA and histone modification, as well as small RNA, in heterochromatic silencing and transposon control.

Far from being inert, heterochromatin is transcribed and small interfering RNA corresponding to heterochromatic sequences is abundant in various tissues. 24nt small interfering RNA (siRNA) corresponding to some classes of TE depends on the DNA methyltransferase MET1 and the SWI/SNF ATPase, DDM1, which are required for silencing transposons in the absence of siRNA. We have found that down regulation of DDM1 and MET1 in the pollen vegetative nucleus results in transient activation of TEs and accumulation of novel 21nt siRNA. These 21nt siRNA move into sperm cells to silence active TEs. Similar changes in heterochromatic small RNA can be detected in allopolyploid hybrids. We are exploring the possibility that differences in heterochromatic silencing between parental genomes may account for dysgenic phenotypes in hybrids, using next generation sequencing of RNA, genomic DNA and chromatin immunoprecipitations.