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**S1-1**

**COMPARATIVE QUANTITATIVE TRAIT LOCUS (QTL) SUGGESTS POLYPLOIDIZATION IN FRAGARIA LED TO DIFFERENT GENETIC KEYS FOR RECURRENT FLOWERING IN FRAGARIA**

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Polyploidisation (full genome duplication) is a prominent event in higher plants. This event is usually followed by a process of diploidization whereby gene redundancy is reduced via gene silencing, sequence elimination and rearrangement, demethylation of retroelements and relaxation of imprinting. The corresponding evolution of genome rearrangements and associated agronomical traits can be studied through comparative quantitative trait locus (QTL) mapping between polyploid species and their diploid relatives.
In this work, we analysed if the genetic control of an agronomical trait, such as recurrent flowering present in diploid and octoploid Fragaria, is conserved or not along the processus of polyploidization, knowing that high level of macrosynteny and colinearity between the octoploid and the related diploid genomes have been observed.
In Fragaria, two flowering modes affecting flowering duration exist. Flowering can occur only once a year in spring ('seasonal flowering' genotypes) or all along growing period ('recurrent flowering' genotypes). Between these two extremes, all intermediate flowering modes can exist. Breeders are mostly interested by the 'recurrent flowering' genotypes, which presents an extended production.
Genetic dissection of 'recurrent flowering' has been analyzed in diploid and octoploid segregating populations issued from crosses between genotypes with contrasting flowering modes. For the diploid and octoploid genetic mapping, a F2 population of 75 individuals and a pseudo test cross population of 213 individuals were respectively used. In the diploid population, the SFL locus conferring the seasonal flowering was evaluated previously by the flowers presence in the end of July, and located on the linkage group VI. In the octoploid population, a QTL approach was carried out. In order to evaluate the seasonal vs recurrent flowering, the number of inflorescences was measured at the end of July for seven years. The QTL analysis was performed using QTL cartographer.
Results shown that one major QTL linked to the 'recurrent flowering' in the octoploid Fragaria is located on the linkage group IV-b. Using comparative linkage map, we observed that this major QTL is not orthologous to the SFL locus in the diploid Fragaria genome (LG VI).
This result suggested different genetic keys according to the ploidy level for the same trait despite the genome organization is conserved between the octoploid and the related diploid Fragaria. These different genetic keys could be due to genetic modification throughout the polyploidization events in Fragaria genus.

Keywords: polyploidy, comparative genomics, flowering, Fragaria

**S1-2**

**AUTOTETRAPLOID CITRUS LIMONIA ROOTSTOCKS ARE MORE TOLERANT TO WATER DEFICIT THAN PARENTAL DIPLOIDS**

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Water shortage of soils is one of the main abiotic constraints affecting growth and yield in citrus. When grown in the field, tetraploid seedlings and varieties grafted on tetraploid rootstocks have been shown to be slow growing plants. In this work, we investigated the anatomy and morphology of root and leaf diploid and autotetraploid Rangpur lime (Citrus limonia) seedlings grown in control condition. The autotetraploid line arose from chromosome doubling in nucellar cells of diploid Rangpur lime and has strictly the same allelic composition than the diploid one. In autotetraploids, roots and leaves were thicker and cell size was bigger than in diploids. Leaf stomatal conductance of autotetraploids was lower than for diploids. Using 20 K cDNA microarrays, leaf gene expression was investigated in both genotypes. A very limited number of genes were significantly differentially expressed in both genotypes (? 0.5%) suggesting that gene dosage per cell or post-transcriptional events may explain the phenotypic differentiation between diploids and autotetraploids. We also investigated the tolerance to water stress of diploid and autotetraploid seedlings and also their behaviour as rootstocks of Valencia Delta orange (Citrus sinensis) and citron (Citrus medica) varieties. At the beginning of the stress, leaf stomatal conductances of autotetraploid seedlings and varieties grafted on autotetraploid rootstocks were respectively lower than those of diploid seedlings and varieties grafted on diploid rootstocks. At the end of the experiment, autotetraploid seedlings and varieties grafted on autotetraploids showed the highest tolerances. This work suggests that greater tolerance is linked to a more efficient regulation of gas exchanges in autotetraploid seedlings and varieties grown on autotetraploids genotypes. Investigations of ABA root content in diploids and autotetraploids suggest that constitutive biosynthesis of this hormone is higher in autotetraploids. Analyses of candidate gene expression were performed at the root level. The results showed that NCED1, which is involved in the last step of ABA biosynthesis was over expressed in roots of autotetraploids. To have a better understanding of the impact of diploid and tetraploid rootstocks on scion, we have also investigated gene expression using microarrays and qRT-PCR in Valencia Delta leaves grafted on diploid and autotetraploid rootstocks in control and water deficit conditions.

Keywords: Citrus, tetraploidy, salt stress, water deficit

**S1-3**

## USE OF UNREDUCED (2N) GAMETES FOR HOMOEOLOGOUS RECOMBINATION AND CYTOGENETIC MAPPING IN POLYPLOIDY LILIES (LILIUM)

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Longiflorum (L), Asiatic (A) and Oriental (O) lilies belong to section Leucolirion, Sinomartagon and Archelirion of genus Lilium respectively. These interspecific hybrids (LA and OA) are promising in lily breeding for various agronomical traits. Lilium species have the largest genomes among the flowering plants and offer unique opportunities, as well as challenges, for cytogenetic investigations. There is a view that because of their enormously high DNA values, polyploidy in Lilium might be of little consequence for influencing the phenotypes. By using 2n gametes from interspecific hybrids, we have induced a large number of sexual polyploids and obtained backcross progenies of F1 hybrids of Longiflorum × Asiatic (LA) and Oriental × Asiatic (OA) groups of lilies with Asiatic parents. Genomic in situ hybridization (GISH) technique was applied to assess the intergenomic recombination in the BC progenies of LA and OA hybrids. BC1 allotriploid LA plants were originated through the functioning of either 2n eggs or 2n pollen. Similarly the BC1 OA hybrids comprised of triploid plants which originated through functional 2n pollen from a F1 OA hybrid. In both type of crosses, a majority of the progenies originated through First Division Restitution (FDR) mechanism of functional 2n gametes either with or without a cross over with few exceptions where Indeterminate Meiotic Restitution (IMR) was the mechanism of 2n gamete formation. GISH could accurately count the number of crossover points in homoeologous chromosomes, called ‘recombination sites’ of these interspecific hybrids. Based on these recombination sites, we have constructed cytological maps of three genomes of Lilium. These data indicate that intergenomic recombination is highly asymmetrically distributed among the individual chromosomes of the three different genomes. It was found that the amount of recombination is highly variable among genotypes and sexual polyploidization can be of great significance for the origin of genetic variation and evolution of polyploids.

Keywords: 2n gametes, GISH, mapping, hybridization, Lilium

**S1-4**

**CREATING HETEROZYGOUS ALLOHEXAPLOID PLANTS FROM THE ALLOTETRAPLOID BRASSICA SPECIES**

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The Brassica "U's Triangle" consists of species with either one or two diploid copies of the A, B and C Brassica genomes, i.e. diploids and allotetraploids, but there is no naturally occurring allohexaploid species containing all three genomes. Such a species with three Brassica genomes may have a significant heterotic advantage compared to the diploid and allotetraploid species. Attempts have previously been made to create allohexaploid plants by crossing a diploid species with an allotetraploid species, e.g. AA x BBCC, and treating the resultant ABC hybrid plant with somatic chromosome doubling agents. However, this approach results in loss of heterozygosity, which is undesirable for breeding purposes. Furthermore, using a diploid parent that has not developed a system of homoeologous chromosome pairing control may explain why previously generated Brassica allohexaploids have been meiotically unstable. We have trialled a new methodology aimed at creating allohexaploid plants which are extremely heterozygous and meiotically stable. Two generations of crossing between the allotetraploid species B. napus, B. carinata and B. juncea has been undertaken. The first generation of crossing will produce hybrids with an unbalanced genome complement of AABC, BBAC or CCAB. We expect that first generation hybrids will produce relatively high frequencies of unreduced gametes with the somatic chromosome number. We hypothesised that these unreduced gametes would then combine with normal, reduced gametes from the complementary allotetraploids, e.g. AnAjBjCn gametes would combine with BcCc gametes to give a heterozygous AnAjBcBjCnCc allohexaploid. We generated 112 plants using this crossing strategy. Using flow cytometry, one plant was found to have the nuclear DNA content expected for a hexaploid (6x) and another was found to have nuclear DNA content intermediate between tetraploid and hexaploid (approximately 5x). Molecular marker characterisation is underway to determine if the three complete Brassica genomes are present in the 6x hybrid as predicted, and if unreduced gametes were involved in its formation.

Keywords: 2n gametes, hexaploid Brassica, amphidiploids

**S1-5**

## PRODUCTION, MORPHOLOGICAL, CYTOLOGICAL, AND MOLECULAR CHARACTERIZATION OF INTERGENERIC HYBRIDS BETWEEN BRASSICA RAPA AND SINAPIS ARVENSIS

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Mustard ( Brassica juncea (L.) Czern. AABB, 2n=36 ) is an important crop in both ancient and modern world and is used primarily as oilseed but as vegetables, condiment and medicines also. Its superior tolerance to high temperature arid, and infertile, suggesting its importance in future agriculture. The genetic diversity of mustard is exceptionally rich in China, however, views on its origin in China have been conflicting, a key point is the absence of wild Brassica nigra (L.) Koch. (BB, 2n=16) in China, which have been widely accepted as one of the two ancestors of Brassica juncea. However, wild type of Sinapis arvensis L. ( SarSar, 2n= 18 ) were found widely distributed in Xinjiang Uygur Autonomous Region of northwest China, which was suggested as the ancestor of Brassica nigra. So it is very interesting to clarify whether Chinese mustards were actually derived from the intergeneric hybrids between wild Brassica rapa L. (AA, 2n=20 ) and wild Sinapis arvensis L.. To clarify this hypothesis and development novel germpalsm, intergeneric hybrids were produced between Brassica rapa L. and Sinapis arvensis through sexual crossing and followed embryo rescuing, after colchicine treatment, doubled fertile hybrids were successful obtained. Morphological, Cytological, and Molecular studies were conducted to establish its hybridity. The hybrids were intermediate between parents, interestingly, its morphology at vegetative stage was similar to the landraces of B. juncea in Tibet geographically adjacent to Xinjiang, but similar to Brassica carinata Braun at flowing stage. Reciprocal crosses between the hybrids and natural Brassica juncea differed in crossability, with natural Brassica juncea as pollen parent, the compatible index was 5.7, in the reciprocal cross, the compatible index sharply decreased to 0.32. Analysis hybrids and its parents with 2 chloroplast SSR (microsatellite) and one mitochondrion SSR primer pairs proved the cytoplasm belong to the female parent Brassica rapa L. Results obtained from DNA fingerprinting with 8 nuclear SSR and 5 SRAP primer pairs indicated the hybrids were intermediate between parents. Genomic in situ hybridization(GISH) analysis of genome components of hybrids indicated the chromatins were from two parents. The result suggested it is possible that Chinese mustard were derived from y derived from the intergeneric hybrids between wild Brassica rapa L. (AA, 2n=20 ) and wild Sinapis arvensis.L..

Keywords: the intergeneric hybrids; Brassica juncea ; SSR ; GISH

**S1-6**

**GENOME SIZES AND PLOIDY LEVELS IN INTERSPECIFIC HYBRIDS OF ARABIDOPSIS THALIANA AND A. LYRATA**

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Arabidopsis thaliana (2n=2x=10) and A. lyrata subsp. petrea (2n=2x=16) were used for interspecific hybridization. These species are closely related but important differences exist. A. lyrata is an obligate long-day plant, while A. thaliana is a facultative long-day plant. In contrast to A. thaliana, A. lyrata is a self-incompatible perennial, with air-rosettes besides basal rosettes. Whereas A. thaliana develops from seed to seed in 5 to 6 weeks, it takes A. lyrata 6 to 12 months.
By hand pollination and embryo rescue seedlings were obtained. A. thaliana was used as mother, the reciprocal cross was less fertile. In F1 and F2 plants the hybrid character was proven by phenotyping and genome size analysis. The F1 progeny inflorescence types were intermediate to the parents. Leaf growth and shape were more resembling to A. lyrata. As in A. lyrata flowering was preceded by a vegetative phase. Some F1 plants showed an altered phenotype in some branches by raised fertility and bigger siliques.
For genome size analysis a flow cytometer (argon laser) and propidium iodide staining were used. The internal standard L. esculentum 'Stupice' (0.98 pg/1C) was co-chopped with the samples. Genome sizes were calculated relatively from the peak positions in the histograms. A. thaliana had a genome size of 0.15 pg/1C. In A. lyrata the genome size was larger, 0.22 pg/1C. The F1 hyrbrids had an intermediate genome size. Compared to the genome size in normal branches of the F1 plants, it was double in the altered branches. Probably somatic polyploidization occurred in these branches.
Backcrosses with the F1 plants resulted in different genomic combinations for A. thaliana (TT) and A. lyrata (LL). The F1 plants were male sterile, except for the tetraploid branches. Female fertility was very low when backcrosses were made with A. thaliana or A. lyrata. Flowers of the tetraploid branches were more fertile and resulted in more offspring. Triploids (TLL) resulted from crosses between tetraploid branches of F1 plants (TTLL) and diploid A. lyrata (LL). The tetraploid F1 branches (TTLL) were also crossed with mitotic polyploidized A. thaliana (TTTT) resulting in F2 plants (TTTL). These F2 plants showed more phenotypical variation than the F1 and resembled more to A. thaliana.
The hybrid character and genome composition were determined by genome sizes of the F2 plants and comparison to the theoretical assumed values. However, small differences in genome sizes are difficult to examine by flow cytometry. Additional methods like molecular markers or Genomic In Situ Hybridization (GISH) to confirm the hybrid nature and to visualize recombination in the F2 plants are necessary.

Keywords: fertility, flow cytometry, interspecific hybridization, somatic polyploidy

**S1-7**

**INTERPLOIDY CROSSES AND OCCURENCE OF UNREDUCED GAMETES IN BUDDLEJA**

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To enlarge genetic variability within Buddleja an interspecific breeding program using B. davidii (2n=4x=76), B. lindleyana (2n=2x=38), B. globosa (2n=2x=38) and B. weyeriana was set up. B. weyeriana originated from a cross between B. davidii and B. globosa. Chromosome counting and genome size measurement for B. weyeriana revealed however a higher chromosome number (76) and genome size than could be expected, suggesting the occurrence of 2n-gametes formation during meiosis of B. globosa.

Interspecific crosses B. davidii x B. weyeriana, B. weyeriana x B. davidii, B. davidii x B. lindleyana and B. davidii x B. globosa were made. The success rate of the crosses was about 9%. Resulting fruits aborted in an early stage of their development. Therefore, in vitro embryo rescue was used 10 weeks after pollination. Germination of the embryos occurred within 2 weeks and about 86% of the generated seedlings could be acclimatised. Hybrid nature of all seedlings was confirmed by AFLP. B. weyeriana x B. davidii seedlings had intermediate genome sizes compared to the parents, indicating the hybrid nature of the seedlings. The hybrids of the reciprocal cross B. davidii x B. weyeriana had 76 chromosomes but a lower genome size than expected, suggesting the occurrence of chromosome rearrangements in the genome of the hybrids. With B. davidii having 76 chromosomes and B. lindleyana and B. globosa having 38 chromosomes, it was presumed that the F1 hybrids B. davidii x B. lindleyana and B. davidii x B. globosa would be triploid (57 chromosomes). However chromosome counting revealed 76 chromosomes for all F1 seedlings. Moreover, genome size measurements showed that the F1 B. davidii x B. lindleyana and B. davidii x B. globosa seedlings had higher genome sizes than expected. These results of chromosome counting and genome size measurement indicated that 2n gametes are produced during meiosis of both B. lindleyana and B. globosa.

More evidence for the occurrence of 2n gametes in B. globosa came from genomic in situ hybridisation (GISH) experiments on B. x weyeriana and hybrids of B. davidii x B. weyeriana. The parental genomes could be clearly distinguished and recombinant chromosomes were observed. It was expected to observe maximum 19 B. globosa chromosomes in B. weyeriana. However, in B. weyeriana 28 chromosomes belonging to B. globosa and 12 recombinant chromosomes were distinguished, proving again the production of unreduced gametes in B. globosa.

So far, in woody plants, little efforts have been made to use 2n gametes in breeding. Our results open new perspectives to use 2n gametes in further breeding programs of Buddleja and of woody ornamentals in general.

Keywords: Interspecific hybridisation, mitotic polyploidisation, flow cytometry, genome sizes, GISH

**S1-8**

**WHY ARE THERE SO MANY BEGONIAS? THE ROLE OF HYBRIDISATION IN THE SPECIATION OF A TROPICAL HERB**

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Tropical biomes are the most species diverse in the world, but the processes that have contributed to this diversity are poorly understood. Emerging consensus supports hybridisation as a significant factor in driving speciation, with estimates suggesting over 10% of species being formed by ancient hybridisation. Reduction in fertility in hybrids and constant competition from parental species suggests that natural hybrids are unlikely to be stable and persist in the wild. Yet, plant hybrids are commonly observed in the field, and finding a mechanism to explain the success of natural plant hybrids is of great significance.

Heterosis, the phenomena of a hybrid being superior to the parental species for phenotypic traits, may play an important role in the success of natural hybrids, and provide a mechanism for explaining why F1 hybrids succeed in the presence of the parents. If vigorous interspecific hybrids are fertile then backcrossing to parental species may occur, resulting in the introgression of genes across species boundaries. An alternative outcome of interspecific hybridisation is hybrid dysgenesis, where plants show trait values below those observed in the parental species. These are unlikely to be successful in the wild or contribute to later generations.

Using the speciose tropical genus Begonia(1500+ species), we are looking at understanding the factors involved in speciation and reproductive isolation. Central Mexican species (Begonia section Gireoudia) have dramatic diversity in leaf form, and hybrids between species are known to occur in the field. Chromosomes counts (2n=28) suggest no change in the level of ploidy within the group. Few reproductive barriers exist between these deceit-pollinated species, yet species usually maintain their distinct identities. Whilst there are examples of hybrid dysgenesis within the group, most F1 plant are vegetatively vigorous, but with low fertility.

We have quantified vegetative vigour and reproductive success in F1s, including several hybrids that are known to occur in the wild, to determine their competitive abilities. Vigour and fertility are compared to a framework phylogeny and geographic distributions to shed light on the causes of reproductive isolation. We are also using a QTL analysis to study the genetic architecture of interspecific fertility barriers. We hope to determine whether hybridisation could have promoted or discouraged speciation in this very speciose group.

Keywords: Begonia hybridisation speciation heterosis dysgenesis

**S1-9**

**IMPACT OF POLYPOIDY ON SELECTED TRAITS IN ENERGETIC GRASSES FROM MISCANTHUS GENUS**

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Grasses from the Miscanthus genus are becoming a one key renewable raw material for energy production. The presented paper studies the influence of ploidy level of Miscanthus grasses on morphological, histological traits as well as on selected gas exchange parameters. The experiments were conducted on plants of different ploidy levels derived by polyploidization in culture in vitro. In experiments two species: diploid Miscanthus sinensisand triploid hybrid Miscanthus x giganteus were used. To induct the polyploidization explant, callus and single shoots were treated with colchicine. The ploidy level of regenerates was determined by the fluorescence intensities proportional to nuclear DNA content by flow cytometry. On established in soil plants stomatal size (length and wide), pollen diameter and length of spikelet were examined. In the field conditions photosynthesis rate (Pn), transpiration rate (E), stomatal conductance (GS), and internal CO2 concentration (Ci) were measured. The gas exchange parameters were measured using a gas exchange system (CIRAS-2). Results showed significantly higher value of stomatal size, pollen diameter and length of spikelet for plants on higher ploidy level in comparison with control plants. Dendrites of the shortest connections for analysed traits, determined by the Mahlanobis' distances, showed stronger similarity for plants with respect to the ploidy level rather than for plant genotypes. Higher mean values of gas exchange parameters were noticed for plants with doubled chromosome number rather than for control plants. Theoretically, higher photosynthesis rate for polyploids could give higher biomass yields.

Keywords: polyploidization, energetic grasses, Miscanthus, gas exchange parameters

**S1-10**

**ANALYSIS OF THE MITOTIC AND MEIOTIC BEHAVIOR OF THE PENTAPLOID BRASSICA HYBRIDS (AABCC) BY GENOMIC IN SITU HYBRIDIZATION (GISH)**

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The pentaploid Brassica (AABCC) with 46 chromosome numbers were obtained through the hybridization between B. napus (AACC) and the trigenomic diploids (AABBCC), which were colchicine-treated triploids hybrid (ABC) between B. rapa(AA)cultivars and B. carinata(BBCC). Using B.nigra genomic DNA as probes, we analyze the B chromosomes behavior in different stage of the mitosis and meiosis. GISH results showed that chromosome pairing configurations were composing of univalents, bivalents, trivalents, and quadrivalents in a cell. The occurrence of trivalents and quadrivalents suggested that BAA or BCC homologous pairing and exchange might happen. The variable number of lagging chromosomes belonging mainly to the B genome were observed at anaphase, which suggested that the B genome could be eliminated in the gametes of pentaploid hybrids.

Keywords: Brassica, chromosome behavior, genomic in situ hybridization (GISH), meiosis, mitosis, pentaploid

**S1-11**
**ENSCONET - EUROPEAN NATIVE SEED CONSERVATION NETWORK**

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ENSCONET, the European Native Seed Conservation Network, walked its first steps in November 2004 after a long preparation process. The network, headed by the Millennium Seed Bank (Royal Botanic Gardens, Kew), is composed of 24 institutes from 17 European countries and two associate members, covering 9 of 10 major bio-geographical regions of Europe (Alpine, Arctic, Atlantic, Black Sea, Boreal, Continental, Macaronesian, Mediterranean and Pannonian). The network is especially important for the Mediterranean as 9 partners are located here. It is a Co-ordination Action funded under the European Union's 6th Framework Programme. The project links to other relevant national and European conservation networks and comprises a wealth of seed banking experience. Its purpose is the improved quality, coordination and integration of European seed conservation practice, policy and research for native plant species, and to assist EU conservation policy and its obligations to the Convention on Biological Diversity and its Global Strategy for Plant Conservation. ENSCONET co-ordinates and enhances activities of several European seed banks, botanical gardens or institutes interested in seed conservation, in order to reduce duplicated efforts in establishing and improving technologies for seed collecting, curation and data management. That is being achieved through creating common high standard protocols for collection and curation, compiling data on species held in European seed banks. A virtual seed bank was created for European native plants. The 24 partners established priority species lists for new seed collection in each of the 9 bio-geographical regions. ENSCONET's perspective is that it is essential to preserve seed diversity in order to avoid the extinction of native species from European regions. ENSCONET work is organised in four main activities: collection, curation, data management and dissemination. The seed banking activities are also highly relevant for biodiversity and evolutionary studies, including research on polyploids, since the seed collections are an important source of potential research material. Importantly, the seed bank collections contain also a considerable amount of local endemics, which are difficult to obtain from the field.

Keywords: Conservation, Seeds, Vascular Plants, Biodiversity, Evolution

**S1-12**

**AUTO- AND ALLOPOLYPLOID SPECIATION WITHIN THE PILOSELLA ALPICOLA GROUP WITH CONSEQUENCES FOR BREEDING SYSTEM AND GENETIC VARIATION**

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The Pilosella alpicola group comprises several allopatric alpine taxa with a very polydisjunctive range across the high mountains of Central and Southern Europe. Morphological study using multivariate morphometric analyses revealed the existence of four morphologically distinguishable species: P. alpicola s.str. (Alps), P. rhodopea (Balkan Peninsula and Southern Carpathians), P. serbica (Serbia) and P. ullepitschii (Carpathians). Karyological and flow cytometric analyses indicate geographic and taxon specific patterns in cytotype distribution. Pilosella ullepitschii and P. serbica are exclusively diploid and strictly self-incompatible taxa. A complex cytotype pattern, including the mixed ploidy populations, was found in P. rhodopea including four different cytotypes, varying from diploids to pentaploids. These cytotypes, reproducing by strict allogamy, are not morphologically differentiated and this fact alongside with nuclear ITS data suggests their autopolyploid origin. Allozyme genetic diversity gradually decreases from diploids to tetraploids. In contrast, isolation, castration and flow cytometric seed screen analyses revealed that tetraploids and pentaploids of P. alpicola s.str. produce seeds apomictically. Apomictic reproduction of this taxon is reflected in significantly reduced genetic diversity when compared to P. rhodopea polyploids. Interestingly, ITS sequences proved that P. alpicola s.str. is in fact an allopolyploid taxon originating from hybridisation with another alpine species Pilosella glacialis. Allopatric distribution of cytotypes (tetraploids in the Walliser Alps and pentaploids in the Dolomites), different multilocus allozyme pattern, slightly different ITS additive polymorphism and some morphological differences are evidence for polytopic origin of P. alpicola s.str. We hypothesise that during suitable climatic conditions, polyploid cytotypes of Balkan P. rhodopea underwent range expansion in a north-westerly direction, where they subsequently hybridized, at least twice, with P. glacialis giving rise to hybridogeneous P. alpicola s.str. Interestingly, apomixis has not contributed to the further range expansion, and P. alpicola s.str. has currently very restricted distribution. Surprisingly, some level of introgression was detected also in diploid Pilosella ullepitschii, either from Balkan P. rhodopea or other closely related species. Our data show that different speciation processes, like an auto- and allopolyploidisation within the closely related taxa might result in different reproduction pathways resulting in consequences for genetic variation.

Keyword : agamospermy, flow cytometric seed screen (FCSS), Hieracium, hybridisation, ITS

**S1-13**

**VARIATION IN PILOSELLA OFFICINARUM F. W. SCHULTZ ET SCH. BIP. IN CENTRAL EUROPE: PLOIDY LEVELS, BREEDING SYSTEMS AND THEIR CORRELATION WITH MORPHLOGY**

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Pilosella officinarum F. W. Schultz et Sch. Bip. (syn. Hieracium pilosella L.; Asteraceae) belongs to a complex group of partly apomictic plants (i. e., its seeds are of clonal origin). Their enormous variability is caused especially by: polyploidization, combination of the sexual and apomictic breeding mode, widespread hybridization and vegetative reproduction (Krahulcová et al. 2000).
DNA ploidy level (Flow Cytometry used) or chromosome counts were determined for 768 plants of Pilosella officinarum from 216 localities from all over the area. Three ploidy levels were recorded within the area of Central Europe. The most widespread cytotype was the tetraploid one (2n=36, 65%), the second most common was the pentaploid (2n=45, 18%) while the least common was the hexaploid level (2n=54, 17%). Breeding systems of most of the plants were determinated and showed, that tetraploids were sexual and pentaploids were apomictic but hexaploid plants separated into two distinct groups (apomicts and sexuals) which are also devided geographically.
Morphometric analyses were carried out for individual plants of particular cytotypes (4x, 5x, 6x). Principal Components Analysis, Discriminant Analysis and Nonparametrical Classification Analysis detected that the tetraploid and the hexaploid plants are distinctly morphologically separated, while the pentaploids share morphological features of both. Nevertheless, the pentaploid cytotype keeps special characters that slightly distinguish the pentaploid plants from the others. Even the sexual and apomictic hexaploids can be distinguished on the basis of morphological features. Such results indicate that both groups of the hexaploids could be unrelated. The sexual hexaploids are considered to be of a relict origin while the apomictic hexaploids appear to be connected to a possible hexaploid cytotype distribution centre in the Carpathian Mts. as well as majority of pentaploid cytotype. Such results possibly reflect reticulate evolution of the group and they may be considered to be alternative to traditional complicated taxonomic view.

Keywords: Pilosella, polyploid, hybridization, apomixis

**S1-14**

**INDUCED TRIPLOID AND TETRAPLOID BLUE MUSSELS MYTILUS EDULIS**

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Polyploidy can be induced in bivalve molluscs by manipulation of the early embryogenic stages through chemical or physical shock. Triploid shellfish are of economic interest because of higher growth. Tetraploids are considered valuable because they can serve as genitors to produce triploids without induction treatment and pave the way to genetic improvement in these little domesticated species. Both ploidy groups are of interest for genetics studies. In the blue mussel Mytilus edulis heat shock treatments involving an 8-15°C shift in temperature successfully produced triploid and tetraploid larvae.
The timing of the change in temperature relative to the events in embryo development is crucial for the effect on the ploidy levels in the progeny. A first experiment illustrates how the timing of heat shock treatments following gamete mixing alters the yield of different ploidy classes in batches of a common progeny. We discuss the implications of these treatments for the probable mode of induction and constituent genomes of the resulting polyploids. Early treatments, a few minutes after fertilization gave a higher yield of tetraploids compared with treatments at 20 minutes, which produced a majority of triploids, probably through the known method of inhibition of reduction division leading to retention of the first polar body.
Occurrence of tetraploids in heat shock trials are discussed in comparison with results from chemical shock treatments (with cytochalasin B) with the same timing that successfully produced viable tetraploid juveniles in a parallel experiment. The differing nature of the heat and chemical treatments would suggest a differing mechanism. Indeed, when a common progeny was divided and treated on one hand with cytochalasin B and on the other with a heat shock, the former gave a high percentage tetraploidy and the later mostly triploids.
The influences of individual parentage in a cross, gamete ratio and concentration on the mechanism are discussed and a model of genetic contribution presented.

Keywords: Mollusc; Tetraploid; Induction; Cytochalasin B; Mussel

**S1-15**

**ORIGIN OF DIFFERENT PLOIDY LEVELS OF PROGENY FROM DIPLOID X TETRAPLOID SOMATIC HYBRID CROSSES IN CITRUS**

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World production of Citrus fruit is on a continuous growth, representing the first fruit crop in international trade. The main evolution during the last decades was the growth of request on small Citrus fruits (clementines and mandarins). Many breeding programs get started all over the world due to the evolution of consumer and market preferences.
One of the consumer turn-off is the excessive seed number. One of the ways chosen, to resolve this problem is the creation of sterile triploids cultivars, which have a great commercial potential because of their seedlessness.
A way for triploid creation is sexual cross between diploids and tetraploids. However the scarcity of natural tetraploid gene pool was a restriction for using this method. Citrus somatic hybridisation via protoplast fusion allowed the creation of tetraploid somatic hybrids that can be used as parents to generate triploids cultivars. Several crosses using diploids (female) and tetraploid somatic hybrids (male) were realised by CIRAD:
· Fortune mandarin (C. reticulata Blanco) x (Willow leaf mandarin SRA 133 (C. deliciosa Ten.) (WLM) + Star ruby Pomelo (C. paradisi) Tetraploid somatic hybrid),
· Eureka lemon SRA 4 (Citrus limon (L) Burm) x (Pumelo Star Ruby (C. paradisi Macfad) and Corsican citron (C. medica L.) tetraploid somatic hybrid)
· Eureka lemon SRA 4 (Citrus limon (L) Burm) x (Mexican lime (C. aurantifolia) + Shamouti orange (C. sinensis L.) tetraploid somatic hybrid).
After germination, 117 plantlets were analysed using flow cytometry for ploidy levels determination. Major part of progenies was triploid in the 3 crosses (61-76%). However diploid and tetraploid plantlets were also found for the 3 crosses.
Molecular analysis with SSR markers revealed that:
· Tetraploids were issued from a diploid male gamete and an unreduced female gamete.
· Triploids were the result of a haploid ovule and diploid male gamete.
· Diploids origin is a haploid ovule and a viable haploid male gamete in the cross with Fortune mandarin.
· Diploids origin result of Eureka lemon apomixis in the crosses with lemon.
This study reveals that these progeny ploidy variations were owed to meiotic dysfunction during meiosis of the female diploid parents leading to tetraploids. Diploids were issued from apomixes or from viable male haplogamete coming from the somatic hybrids.

Keywords: Citrus polyploid hybridization SSR marker

**S1-16**

**DOES POLYPLOIDY INFLUENCE FLORAL MORPHOLOGY ? A CASE STUDY IN NICOTIANA**

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The evolution of the genus Nicotiana (Solanaceae) has been marked by interspecific hybridization involving both allopolyploidy, where hybridization is accompanied by a multiplication of chromosome number, and homoploid hybridization, where there is no change in ploidy. The prevalence of hybridization has led to a pattern of reticulate, rather than bifurcating, evolution. Hybrids may be successful due to the presence of transgressive characters which fall outside the range of the parental characteristics and allow them to occupy niches unavailable to either parent.

Previous work has shown that nuclear genes, including the floral development gene NICOTIANA FLO/LFY (NFL), have been maintained in all polyploids examined, including those that formed 10 million years ago. These findings raise a number of questions: Why are both copies retained? Does retaining multiple copies allow for transgressive characters to become established in polyploids? Does morphological plasticity offer polyploids an advantage over their diploid progenitors?

In an attempt to answer these questions, we have examined floral morphology using geometric morphometrics to quantify shape. Here, we present evidence to answer the question: Do polyploids have intermediate floral morphology between that of their diploid progenitors? We hypothesize that younger polyploids will display floral forms more intermediate to the progenitors' whereas older polyploids will tend to resemble one progenitor or exhibit more clearly transgressive shapes. Preliminary data suggest that this is in fact the case. The morphometric data have also been used for phylogenetic reconstruction. In comparing the resulting phylogeny to known molecular trees, we can examine the different patterns present in the molecular and morphological evolution of Nicotiana.

Keywords: polyploidy, floral morphology, Nicotiana, morphometrics, transgressive characters

**S-17**

**HYBRIDIZATION OF CERASTIUM ALSINIFOLIUM: CARYOLOGICAL AND MOLECULAR EVALUATION OF SERPENTINE ENDEMIC SPECIES**

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Cerastium alsinifolium Tausch (Caryophyllaceae) is a severely threatened endemic plant species occurring on serpentine outcrops of the Slavkovský les protected area in Western Bohemia. C. alsinifolium frequently hybridizes with the common C. arvense L. The resulting
hybrids seem to be more vigorous: plants are markedly bigger with denser tufts, and their seeds are viable. C. alsinifolium, C. arvense and plants of hybrid origin are octoploids with 2C=72 chromosomes. The negative impact of hybridization on the abundance of the endemic species and the relevance of management actions are hypothesized and tested.
Plants were sampled at 5 localities (3 forest + 2 non-forest [serpentine outcrops] habitats). Nuclear DNA content determined by flow cytometry was used to classify serpentine Cerastium plants into three distinct groups (i. e., C. arvense, C. alsinifolium and hybrids). A total of more than 1000 plants were included in the study.
Genome size differs between C. alsinifolium and C. arvense, and also its hybrid. The overall difference in genome size (C. alsinifolium - C.arvense) is more than 45%. The spatial distribution of each species does not agree with previous assumptions: in serpentine forest habitats, C. alsinifolium is present predominantly, and hybrids are more or less rare. On the contrary, on serpentine outcrops the hybrids predominate (C. alsinifolium x C. arvense), and pure C. alsinifolium is even rarer. On serpentine outcrops C. alsinifolium occurs in rocky places or rocky rifts with a very poor substrate coverage, while the hybrids and C. arvense occur in grassland habitats. A few plants with characters intermediate between C. alsinifolium and C. holosteoides were observed. Hybridisation of C. alsinifolium with the participation
of unreduced gamete of C. arvense seems to be among the most probable mechanisms. Preliminary results of molecular analysis (AFLP, sequences of cpDNA) are discussed in context of flow cytometry results.
Serpentine habitats with the occurrence of C. alsinifolium are protected by Czech law (114/1992 Sb.) and by the European project Natura 2000 (Habitat Directive 92/43/EEC, Annexes II of the Directive). But management programs are mostly focused on the maintenance of forest-free areas on serpentine outcrops (e. g., by grazing and removal of self-seeding woody species). Forest habitats are thus out of the spotlight although they cover the majority of C. alsinifolium occurrences, and could even perhaps represent the real ecological optimum of the species.

Keywords: Cerastium, hybridzation, flow cytometry, serpentine

**S1-18**

**PATTERNS AND DYNAMICS OF GENOME SIZE VARIATION IN TARAXACUM STENOCEPHALUM (ASTERACEAE)**

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Taraxacum stenocephalum Boiss. et Kotschy (Asteraceae, sect. Piesis) is a unique dandelion species with the centre of distribution in the Caucasus. While most of dandelions are either sexual diploids or agamospermous polyploids, T. stenocephalum represents one of the few sexually reproducing tetraploids (2n = 4x = 32). Using propidium iodide and DAPI flow cytometry, we detected remarkable intraspecific variation in nuclear DNA amount (up to 1.23-fold) among plants collected in Georgia (both between and within populations). These findings stimulated our further research aimed at elucidating the causes and consequences of genome size variation. Reciprocal experimental crosses were made between individuals classified into five genome size categories in order to understand how the variation translates into the following generation(s). Interestingly, genome size of F1 progeny seems to be highly variable and not necessarily related to the genome size of their parents. We hypothesise that the random segregation of homeologous chromosomes of different sizes (under the absence of direct selection mechanisms) is the main cause for the fixation and persistence of genome size variation in this species. Future work will focus on the study of the adaptive value of genome size, relationships between genome size and various phenotypic and reproductive traits and on exploring molecular mechanisms behind this variation.

Keyword : genome size; intraspecific variation; Taraxacum stenocephalum; flow cytometry; crossing experiments

**S1-19**

**MOLECULAR EVIDENCE OF ALLOPOLYPLOID ORIGIN OF CARTHAMUS LANATUS**

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Carthamus lanatus (2n=44) is a polyploid of unknown origin. It is a noxious weed of wide Mediterranean distribution that has colonized other Mediterranean climatic regions in North and South America (Argentina and Chile), Australia and South Africa, and it has been necessary to design local policies to control its spread. The origin of tetraploid C. lanatus is obscure. It could be either an allopolyploid resulting from hybridization between a x = 10 and a x = 12 ancestor, or an autopolyploid originated from a x = 11 ancestor, e.g. C. divaricatus, the only species in the genus with x = 11.
In this study, we included 23 populations of all the extant species (11) of Carthamus sect. Atractylis with the exception of the hexaploids C. creticus and C. turkestanicus, under a combined molecular approach involving (1) sequencing of three non-coding chloroplast DNA regions, the intergenic spacers trnH–psbA and rpl32-trnL, and the intron trnK, and (2) sequencing of an intron of nuclear low-copy gene in the RNA Polymerase (RNAP) family (RPD2) to infer the origin of this polyploidy.
Our preliminary results support the allopolyploid origin of C. lanatus. Hybridization would most likely have involved one x = 10 and one x = 12 progenitor lineage. Accordingly, the x=12 progenitor of C. lanatus is most likely an extinct species which acted as the maternal progenitor.

Keywords: Allopolyploidy; Carthamus; Invasive plants; cpDNA; RNA Polymerase genes

**S1-20**

**APPLICATION OF HOMOEOLOGOUS RECOMBINATION TO GENE DOSAGE IN BREAD WHEAT**

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In an attempt to improve the breadmaking quality of wheat by elaborating novel gene combinations useful in wheat breeding programs, homoeologous recombination between wheat homoeologues has been induced to increase copy number of interest genes. Such approach has been applied to duplicate Glu-1 locus coding for the high molecular weight glutenin subunits (HMW-GS). These endosperm proteins play an important role in the end-use quality of wheat flour which is a complex character influenced by both genetic factors and plant growth conditions. Duplication of the Glu-A1 and Glu-D1 loci have been obtained in the hexaploid wheat cv. Courtot through balanced translocations between the long arm of chromosomes 1A and 1D. Amongst all the translocated lines produced, two lines duplicated respectively for the Dx2+Dy12 genes (Glu-D1) and Ax2* gene (Glu-A1) have been particularly characterized at the cytogenetical and molecular levels.

Keywords: hexaploid wheat, gene dosage, glutenin

**S1-21**

**INTER-CYTOTYPE INTERACTION IN POPULATIONS OF PLANTS WITH PLOIDY HETEROGENEITY: PILOSELLA ECHIOIDES (ASTERACEAE) AS A MODEL SYSTEM**

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Genome duplication (polyploidy), which is widely recognized as one of the major forces in plant evolution, is considered rare in the Cape flora. However, recent investigations have shown that some exceptions may occur. Perhaps the most blatant example of large ploidy diversification in the Cape floristic region identified so far is the genus Oxalis. Using DNA flow cytometry we estimated relative and/or absolute genome sizes in more than 320 Oxalis samples representing ~ 75 species (i.e., about one third of the overall South African diversity). Within the genus, DNA ploidy levels inferred from fluorescence values ranged from 2x to ~ 14x. In 32 species for which more accessions were available, 20 (> 60%) exhibited ploidy heterogeneity, and eight species harboured three or more different cytotypes. Of particular interest with respect to intraspecific ploidy differentiation are O. flava (encompassing 6 cytotypes), O. obtusa (5 cytotypes) and Oxalis pocockiae (5 cytotypes). Genome sizes (2C-values) ranged from 0.40 pg in 2x O. pulchella var. leucotricha to 6.16 pg in ~14x O. pardalis, spanning about a 15-fold range. Collectively, Cape Oxalis species provide a unique model system for studying the role of genome duplication in generating and maintaining biological diversity in this botanical hot-spot. Future work will focus on covering the whole diversity of the genus in South Africa and on employing molecular markers to elucidate the evolutionary history and dynamics of this key geophytic group.

Keywords: Polyploidy, Oxalis, flow-cytometry, South Africa

**S1-22**

**ORIGIN OF 2N GAMETES IN C. RETICULATA CV FORTUNE MANDARIN**

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Citrus are most important fruit crop worldwide. Seedlessness is a key characteristic for the fresh fruit market and the development of triploid hybrids is one strategy developed by several groups over the world. Indeed, triploid hybrids are generally sterile and produce seedless fruits and do not pollinate other varieties. Triploid citrus hybrids can be obtained by several strategies, including hybridization between diploid parents.

Mechanism of 2n gamete formation and its implication on parental heterozygosity restitution is a main parameter determining the genetic and phenotypic structure of the triploid population. In the case of Citrus it has been shown that the 2n gametes are of maternal origin. It has been proposed that the origin of 2n gametes is from the second division restitution (SDR) in Clementines and from the first division restitution (FDR) in sweet oranges. No data is available for other genotypes and particularly 'Fortune', a mandarin hybrid producing very high rate of triploids in 2x x 2x crosses and massively used to create triploid progenies. The aim of this work was to analyse the mechanism of 2n gamete formation in 'Fortune' mandarin genotype.

One hundred and five triploid hybrids from the crosses between 'Fortune' as female diploid parent and 'Murcott' or 'Mandarino Común' as male diploid parents were genotyped for twenty-four codominant molecular SSRs (Simple Sequence Repeat) markers using a capillary genetic fragment analyzer. Estimation of allelic doses from relative peaks area allowed inferring the female and male gamete structures and thus the heterozygosity restitution in the 2n gametes.; this demonstrated that all triploid arise from 2n megaspores. The unimodal distribution of heterozygosity restitution in the 2n megaspores among the analyzed genotypes suggests that all these 2n gametes arise from a same mechanism. Restitution of maternal heterozygosity for the used markers makes suppose that underlying mechanism in the 2n gamete formation is SDR. Indeed there are six markers with less than 50% of maternal heterozygosity restitution, which is incompatible with FDR hypothesis. SDR hypothesis is coherent with the results published in case of the clementine, which is one of the parents of the 'Fortune' variety. Under this hypothesis, the relatively high global heterozygosity restitution level (60,95%) should indicate that a majority of the analyzed markers are far from the centromeres. This genetic structuration will soon be confronted with phenotypic variability and compared with structuration obtained with other triploid creation strategies such as 2x x 4x hybridization.

Keywords: citrus, mandarin, triploid, 2n gametes, meiosis

**S1-23**

**PHENOTYPIC AND GENETIC CHANGES IN RESYNTHESIZED BRASSICA NAPUS**

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Brassica napus is an allopolyploid formed through hybridization of Brassica rapa and Brasscia oleracea. When this hybridization event is reconstructed in the laboratory (resynthesized) considerable heterosis is observed in B. napus relative to the B. oleracea and B. rapa parents. Studies of resynthesized Brasscia napus reveal that epigenetic changes are the immediate effects of hybridization and genome doubling. In subsequent generations chromosomal rearrangements and homeologous recombination result in phenotypic divergence between independent B. napus lines. Here we examine phenotypic and genetic changes to several resynthesized B. napus lines across ten generations. The lines are phenotypically indistinguishable in the first generation despite some chromosomal changes. By the tenth generation the lines differ significantly in terms of stature and flowering time.

Keywords: brassica, napus, allopolyploid, resynthesized

**S1-24**

**PACIFIC OYSTER AND BLUE MUSSEL CYTOGENETICS: POLYPLOIDY INDUCTION (TRIPLOIDS AND TETRAPLOIDS) AND CHARACTERIZATION OF GENOME ORGANIZATION**

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Up to now, polyploidy (triploidy) induction approaches are the only genetic improvement methods which had been used at a large scale on marine bivalves, particularly on Pacific oyster Crassostrea gigas. Triploid induction in Pacific oyster is almost completely based on the use of tetraploid males to fertilize eggs from diploids to produce batches of 100% triploids. However, the key point of this approach deals with the availability of viable and fertile tetraploids. The first generation of tetraploid C. gigas was induced in the early 90s from triploid females which were able to produce mature oocytes. Starting from an idea according to which tetraploid males obtained from these fertile triploid females could afterward produce new triploid descent which could be characterized by higher gametogenetic activity, we obtained a new and alternative method of generating tetraploids in bivalve molluscs directly from diploids. This direct tetraploid induction method was successfully applied to obtain viable and fertile tetraploids on both C. gigas and Mytilus edulis. For this late species, two methods of chemical induction of triploids were also optimised and used to produce different batches of triploid mussels which are tested in particular for their level of gametogenetic activity. Finally, flow cytometry using DAPI and PI, Primed in situ hydiridization (PRINS) and fluorescent in situ hybridization (FISH) were used to provide some first elements on the genome size and organisation of these newly induced polyploids.

Keywords: Polyploidy, Shellfish, , FISH, PRINS

**S1-25**

**TETRAPLOIDISATION IS A COMMON PHENOMENON IN APOMICTIC CITRUS SEEDLINGS AFFECTED BY GENOTYPE AND ENVIRONMENTAL CONDITIONS**

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Citrus species are mainly diploid (2n=2x=18) and very few natural polyploid are encountered in the citrus gene pool. However, several reports of tetraploid seedlings occurrence from apomictic citrus have been done during the last century. Apomixis in citrus is determined by adventitious embryony from nucellar cells. The morphological homogeneity of tetraploid plants obtained from a same diploid genotype leaded to propose that tetraploid seedlings arisen from chromosome doubling of nucellar cells. The interest of citrus breeder for tetraploid citrus plants, as progenitor to produce triploid seedless cultivars, increased a lot in the last 20 years. The objective of the present work was to confirm the genetic origin of tetraploid seedlings and to analyse the impact of genetic and environmental factors on the frequency of tetraploid production. The frequency of tetraploid plants was analysed by flow cytometry in seedlings of 21 genotypes of different genera and species. The identity of SSR profiles between tetraploid seedlings and mother diploid plants and the presence in a same seed of tetraploid and diploid nucellar embryos confirmed the origin of tetraploid embryos from chromosome doubling of nucellar cells. The frequency of tetraploid plants recovery was found dependant to the genotype and the environmental conditions. From seeds harvested in the same site of Valencia (Spain), the genotype producing the higher rates of tetraploid seedlings was 'Carrizo´ citrange (18.5%) while no tetraploids were found for 'Salteñita´ and 'Simeto´ mandarins. The medium rate in the tested genotypes was 6.1%. Limited variation of tetraploid rate was observed among 'Carrizo´ seeds harvested in Valencia over several years. To maximize the environmental variability, seeds of 'Carrizo´ harvested in different Mediterranean (Valencia, Spain; Corsica, France) subtropical (California, USA; San José, Uruguay; Eastern Cape, South Africa) and tropical areas (Florida, USA; States of Bahia and Sao Paulo; Brazil) were studied at IVIA. Very important differences were found between sites (from 19.2% in Valencia to 0.9% in Bahia). Higher and lower rates were found respectively in Mediterranean and tropical areas suggesting a great impact of the environmental conditions in the rate of polyploidisation. We propose that the tropical and subtropical localisation of citrus origin and diversification areas is one of the important factors explaining the apparent inconsistency between (i) the high rate of tetraploidisation events observed in the marginal cold areas and (ii) the very limited role of polyploidy on cultivated citrus evolution and domestication.

Keywords: apomixis, tetraploid, enviromental conditions, citrus

**S1-26**

**GENOME MAPPING WITHIN THE POLYPLOID FESTUCA-LOLIUM COMPLEX**

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Interspecific hybrids between ryegrasses (Lolium) and fescues (Festuca) combine desirable agronomical characters of their parents, such as rapid establishment from seed and fodder quality of ryegrass and tolerance to abiotic and biotic stresses of fescue. Agronomic superiority of hybrids stimulated breeding programs working with Festulolium cultivars. Breeding progress is possible due to permissive pairing of homoeologous chromosomes and consequent intergeneric recombination, resulting in a range of cultivars with varying proportions of parental genomes. The genomes of both genera differ enough to be readily distinguished by the genomic in situ hybridization (GISH). GISH is an elegant tool to identify parental chromosomes and their recombinant products. However, it has a defined limit of resolution and cannot identify small intergenomic exchanges. With the aim to improve the resolution of interspecific introgressions in Festuloliums, we have developed a DArT (Diversity Array Technology) chip for five important species of the Festuca-Lolium complex: F. pratensis, F. arundinacea, F. glaucescens, L. perenne and L. multiflorum. The current version of the DArTFest array contains 7680 probes derived from methyl-filtered genomic representations. In a first marker discovery experiment performed on 40 genotypes from each species (with the exception of F. glaucescens for which only 7 genotypes were used), we identified 3884 polymorphic markers. In order to evaluate the performance of DArTFest array in analysis of hybrids, we screened five Festulolium cultivars with various proportions of parental genomes and compared the results with the analysis of genomic composition by GISH. The results obtained so far indicate that the resources developed in this project will facilitate detailed analysis of intra- and interspecific diversity, development of genetic maps in Festuca and Lolium, and simultaneous marker-assisted selection for multiple traits or specific genome regions. The DArTFest array will make it possible to follow genome changes in Festuca × Lolium hybrids and thus provide new data on the evolution of hybrid and polyploidy genomes.

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Keywords: DArT, Festuca, Lolium, intergeneric hybrids, GISH

**S1-27**

**RELATION BETWEEN THE PLOIDY LEVEL AND CHARACTERISTICS ASSOCIATED WITH ACCUMULATION OF SECONDARY METABOLITES**

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Hypericum perforatum L. is facultative apomictic herb most frequently occurring in natural habitats as tetraploid with 32 chromosomes. However, diploid and hexaploid plants have also been reported. Peculiar mode of reproduction can lead to seed progeny of different ploidy level. Diploid and polyploid plants were selected on the basis of flow-cytometric screen of DNA amount in the nucleus and chromosome counts in root tips of the progeny of 4 mother plants. The polyploids comprised sequence of ploidy levels from triploids to heptaploids.
These plants of different ploidy level were used for the study of possible relation between the number of chromosome sets and some characteristics associated with formation and accumulation of the profiling secondary compounds hypericins and hyperforins.
The ploidy level significantly influenced leaf size and number and density of both, dark and translucent glands on leaves of in vitro cultivated plants. While the number of dark glands accumulating hypericins decreased with an increased ploidy, the translucent gland number showed an increasing tendency up to tetraploid level; however, the density of both type structures decreased from diploids to tetraploids. No correlation between the ploidy level and the studied markers was proved. These morphological characteristics are considered with respect to hypericin/hyperforin content.
The molecular study aimed at expression of two genes possibly involved in biosynthesis of hypericin (hyp-1and HpPKS2) did not reveal any association between ploidy and the level of the respective transcripts. Relationship between gene expression and the content of final product is discussed.

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Keywords: Hypericum perforatum, polyploids, glands, secondary metabolites, gene expression

**S1-28**

**CYTOGENETIC AND MOLECULAR GENETIC ANALYSIS OF THE AEGILOPS VARIABILIS SV CHROMOSOMES CARRYING RESISTANCE TO NEMATODES IN WHEAT**

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The allotetraploid species Aegilops variabilis Eig (2n=28, UUSvSv) belongs to the tribe Triticeae and is closely related to wheat. One accession, Ae. variabilis n°1, was found to be resistant to the cereal cyst nematode (CCN) and the root knot nematode (RKN). As the genetic variability for resistance to those two pests is limited within wheat, this accession was crossed to bread wheat. Previous work enabled the development of two addition lines and two translocation lines carrying resistance. Here, we demonstrate using genomic in situ hybridization (GISH), that there is no U-Sv interchange in the parental accession of Ae. variabilis. However there are multiple rearrangements in the Sv chromosomes. The Ae. variabilis chromosome carrying the gene CreX for resistance to CCN combined segments with homoeology to wheat groups 1, 2, 4 and 6. The CreX gene belongs to the group 1 part and it was likely to have been introduced into chromosome 1B at a similar location as the previously found QTL QCre.srd-1B for CCN resistance. The second Ae. variabilis chromosome carrying CreY and Rkn2 combined segments with homoeology to wheat groups 2, 4 and 7 on its short arm and 3 on its long arm. It was designated as 3Sv. The two genes for resistance are carried by its long arm and have been transferred to wheat chromosome 3B through homoeologous and genetically balanced recombination. Different SSR markers present in the introgressed segments could be used in marker-assisted selection.

Keywords: nematode, FISH, GISH, SSR, translocation, introgression

**S1-29**

**CYTOEVOLUTION AND DIVERSIFICATION THROUGH DYSPLOIDY, POLYPLOIDY AND HYBRIDISATION WITHIN ASPLENIACEAE- 'LOXOSCAPHOID' ASPLENIUMS AS A CASE STUDY -**

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One of the most species-rich, ecologically diverse and widespread families of leptosporangiate ferns, the Aspleniaceae, contains two genera, Hymenasplenium and Asplenium, and about 800 species. Previous cytological research revealed the base numbers x = 39, 38 and 36 for Hymenasplenium, and x = 36 for Asplenium.
Within Asplenium, the 'loxoscaphoids' form a remarkably distinct group. Except for two preliminary chromosome counts of A. theciferum, no cytological results were known for this group. Next to the new base number x = 35, we have discovered several ploidy levels ranging from tetraploid (4x) to dodecaploid (12x) in A. rutifolium s.l. and A. theciferum s.l. Morphological, anatomical and molecular phylogenetic placement of these polyploid taxa indicates their putative autopolyploid origin.
With the discovery of x=35 in the 'loxoscaphoids', four base numbers are known for the Aspleniaceae: x = 39, 38, 36 and 35. Within Aspleniaceae, we postulate two driving evolutionary forces: a punctuated recurrent descending dysploid evolution, counteracted or enhanced by recurrent hybridisation and/or polyploidization.

**S1-30**

**PLOIDY LEVEL AND REPRODUCTIVE BEHAVIOUR IN THE FACULTATIVELY APOMICTIC HIGH-POLYPLOID HIERACIUM SUBGEN. PILOSELLA**

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The reproductive behaviour and the capacity to generate variation in ploidy level was studied in heptaploid and octoploid hybrid mother-plants of Hieracium subgen. Pilosella. This polyploid agamic complex is characterized by both diverse ploidy levels and the combination of sexual and apomictic reproduction in different biotypes. The high-polyploid mother-plants under study originated via 2n + n hybridization in three hybrid swarms; in each of them, Hieracium pilosella (pentaploid or hexaploid, sexual or apomictic) was one parent, hybridizing either with H. bauhini (pentaploid or hexaploid, apomictic) or with H. densiflorum (tetraploid, sexual). Seeds were collected both from open pollinated plants in the field and from open pollinated/emasculated plants in the experimental garden. Flow cytometric seed screening (FCSS; Matzk et al. Pl. J. 21: 97–108. 2000) and a modified method of FCSS (Krahulcova & Suda Biol. Pl. 50: 457?460. 2006) were used to detect the ploidy level and reproductive origin of embryos within particular maternal arrays; chromosome counts and flow cytometric detection of DNA-ploidy level were used to analyse the variation in ploidy level within the cultivated seedlings.

Three ways in shaping the ploidy level variation were found:

1. Mating via unreduced gametes (2n + n, n + 2n) even further increased the ploidy level in the progeny of heptaploid and octoploid mother-plants up to 9x ? 12x.
2. Mating via reduced gametes (n + n) or true apomixis (2n + 0) resulted in the cytotypes commonly occurring at the locality (4x – 6x), or it conserved the maternal ploidy level (7x, 8x), respectively.
3. Haploid parthenogenesis (n + 0) reduced the ploidy level: 3x to 4x polyhaploid progeny was found.

Comparing the variation within the seeds to that within the seedlings, the selection against progeny cytotypes with extreme ploidy levels (3x to 4x or 10x to 12x) was shown. However, the high-polyploid hybrids may further increase the total ploidy variation within a population and may produce new biotypes with favourable combinations of characters.

Keywords: polyploidy, hybridization, Hieracium subgen. Pilosella

**S1-31**

**INTERSPECIFIC AND INTERGENERIC SOMATIC HYBRIDS WITH C. DELICIOSA TEN. ENLIGHTS NON ADDITIVE INHERITANCE IN ALLOTETRAPLOID CITRUS**

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Neoregulation of parental genome expression in allopolyploid plants contributes to the expression of new phenotypes. Somatic hybrids allow combining genomes without sexual recombination and are interesting models to study the immediate effect of allopolyploidisation on the regulation of gene expression and subsequent phenotype elaboration. While most of the citrus germplasm is diploid, somatic hybridization has become an integral part of citrus variety improvement programs aiming to create new allotetraploid rootstocks or to synthesize triploid hybrids by further sexual hybridization. By protoplast fusion CIRAD obtained allotetraploid hybrids between C. deliciosa and 6 others citrus species: 4 belong to Citrus genus (C. limon, lemon; C. aurantifolia, lime; C. sinensis, sweet orange; C. paradisi, grapefruit), 2 belong to Poncirus trifoliata (trifoliate orange) and Fortunella margarita (kumquat). Molecular analysis using 100 SSR markers did not reveal any inconsistency with total addition of parental genomes. Morphological description was done for leaves and fruits as well as the sugar and acid fruit contents. According to the traits and parental combination, codominance or dominance of one parent was observed and lead to conclude for an important contribution of interaction variance in phenotypic diversity elaboration. Analyze by GC-MS of the leaf volatile compounds of the same allotetraploid hybrids revealed a systematic global dominance of the mandarin profile. It was particularly marked regarding the absence of monoterpene aldehydes and monoterpene alcohols and the very low level of sesquiterpene hydrocarbons, sesquiterpene alcohols, and sesquiterpene aldehydes in all hybrids while these compounds were found at high concentrations for the non mandarin parents. 2-D electrophoresis analysis of the leaf proteome of two allotetraploid somatic hybrids combining C. deliciosa with C. aurantifolia and Fortunella margarita displayed a closer relation between the two allotetraploid hybrids and their mandarin parent than with the other parent. Similar results have been observed at transcriptome level in a genome-wide gene expression analysis on fruit pulp of allotetraploid between C. deliciosa and C. limon, using a Citrus 20 K cDNA microarray. The gene expression of the allotetraploid suggested a global dominance of the mandarin fruit pulp transcriptome. Particularly, genes down regulated in mandarin compared to lemon were also repressed in the allotetraploid hybrid. The study is now extended to an interspecific diallelic somatic hybridization scheme to have a wider understanding of genome interaction in allotetraploid citrus.

Keywords: Citrus, somatic hybrids, proteome, transcriptome, inheritance

**S1-32**

**DIFFERENTIAL EVOLUTION OF A DISEASE RESISTANCE GENE CLUSTER IN A DIPLOID (COMMON BEAN) AND A POLYPLOID (SOYBEAN) GENOME**

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The B4 resistance (R) gene cluster, located in subtelomeric region of the short arm of chromosome 4, is one of the largest clusters known in common bean (Phaseolus vulgaris, Pv). We sequenced 650 kb spanning this locus and annotated 97 genes, 26 of which correspond to Coiled-coil-Nucleotide-Binding-Site-Leucine-Rich-Repeat (CNL), the prevalent class of disease R genes in plants. Conserved microsynteny was observed between the Pv B4 locus and corresponding regions of Medicago truncatula and Lotus japonicus, in chromosomes Mt6 and Lj2, respectively. The notable exception was the CNL sequences, which were completely absent in these regions. The origin of the Pv B4-CNL sequences was investigated through phylogenetic analysis, which reveals that, in the Pv genome, paralogous CNL genes are shared among nonhomologous chromosomes (4 and 11). Our results suggest that Pv B4-CNL derived from CNL sequences from another cluster, the Co-2 cluster, through an ectopic recombination event between subtelomeric regions of two nonhomologous chromosomes. Integration of the soybean (Glycine max, Gm) genome data enables us to date more precisely this event and also to infer that a single CNL moved from the Co-2 to the B4 cluster. Indeed, a single truncated CNL sequence is present in both soybean Homoeologue 1 (H1) and Homoeologue 2 (H2) B4 R gene cluster syntenic regions. Given that the CNL ectopic recombination event is shared by Pv and Gm, a crucial question is why the B4-CNL expanded dramatically in Pv but not in Gm. Different hypotheses explaining the differential evolution of this disease R gene cluster in soybean (polyploid) and common bean (diploid) will be presented.

Keywords: Genome organization & evolution; comparative genomics; disease resistance; legume; NBS-LRR

**S1-33**

**ON THE ROAD TO IDENTIFICATION OF THE B GENOME PROGENITOR OF THE POLYPLOID WHEAT SPECIES**

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Triticum turgidum (AABB, 2n=28) and T. aestivum (AABBDD, 2n=42) are natural and relatively stable tetraploid and hexaploid wheat species that have been formed 0.5 and 0.01 MYA. The diploid progenitor of their A genome has been clearly identified as T. urartu (AA, 2n=14) and that of the D genome of T. aestivum as Ae. tauschii (DD, 2n=14). However the diploid species progenitor of their B genome remains unidentified, the closest relative being species from the Sitopsis section. Recent molecular comparisons of gene sequences, using germplasm collections clearly show that the B genome could be related to several Ae. speltoides lines but not to other species of the Sitopsis section. In this study, we have investigated for the first time, comparison between the B genome of polyploid wheat species and the S genomes of Ae. speltoides at both coding and non-coding DNA of a genomic region spanning the Storage Protein Activator (SPA) l gene.
The SPA gene was shown to be the only DNA sequence conserved between the two genomes, and in comparison to the A and D genomes, the Ae. speltoides SPA sequence is more closely related to that of the B genome. The remaining genomic sequences (about 90 kb) outside the SPA orthologous gene correspond to non-shared transposable elements (TEs), all of which inserted after the divergence of the S and B genomes. In comparison, the A and D genomes of the hexaploid wheat show respectively averages of 60 and 80% conserved TE insertions when orthologous regions are compared to their respective progenitors T. urartu and Ae. tauschii.
On the basis of our comparative analysis, although Ae speltoides appears to be more evolutionary related to the B genome of T. aestivum than the A and D genomes, the differential insertions of TEs, none of which are conserved between the two genomes led to the conclusion that the S genome of Ae. speltoides has diverged very early from the progenitor of the B genome which remains to be identified.
We are currently analyzing other species from the Sitopsis section using TE-based markers in order to identify genotypes that may share TE insertions common to the B genome of polyploid wheat species.

Keywords: wheat, comparative genomic, SPA, B genome

**S1-34**

**SEQUENCE ORGANISATION AND CONSERVATION AT HOMEOLOGOUS REGIONS IN THE RECENT ALLOTETRAPLOID COFFEE (COFFEE ARABICA L.)**

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Coffee is one of the world's largest traded commodities produced in more than 60 countries. Coffee species belong to the Rubiaceae family and commercial coffee production relies mainly on two closely related species: Coffea arabica and C. canephora, which account respectively for 65 and 35% of the coffee production. All coffee species are diploid (2n=2x=22) and generally self-incompatible, except for C. arabica which is the only tetraploid (2n=4x=44) and self-fertile. Molecular phylogenies have indicated that C. arabica is a recent allotetraploid (CaEa genome) formed by hybridisation between two related diploid species: C. canephora (C genome) and C. eugenioides (E genome) or ecotypes related to those diploid species. In spite of the close relationship between the two constitutive sub-genomes, C. arabica displays diploid-like meiotic behaviour with bivalent formation.

In order to estimate the sub-genome divergence at fine scale, a sequence comparison was performed between orthologous regions from both Ca and Ea sub-genomes of C. arabica. In particular two homeologous BAC clones, named clones 45 and 52, and sharing around 180 Kb, were analyzed for gene composition, sequence divergence and for presence/absence of repetitive elements. The analyzed regions include 17 putatively functional genes. All predicted genes were strictly conserved in the same order and orientation on both sub-genomes. On more than 31 Kb of coding sequence, around 1% of substitution rate was observed, corresponding to 1.5% of amino acid substitutions. In intronic regions the substitution rate increased to around 2%. Some repetitive elements were shared by the two sub-genomes, indicating that their insertion predated the divergence between C. canephora and C. eugenioides. Based on the comparison of the predicted coding sequences of both clones with the publicly available C. canephora ESTs, the clones 45 and 52 were attributed to the Ca and Ea sub-genomes, respectively.

Our data point out the high sequence and structure conservation between the Ca and Ea sub-genomes of C. arabica. This result is consistent with the evolutionary history of Arabica progenitors, where diploid C- and E-genome species involved in the allopolyploidization event derived from the same ancestor approximately 150,000-350,000 yr BP. Moreover, limited changes in genome structure of the analysed regions appear to be associated with polyploidy in C. arabica. Although partial, this study provides us with the first view of Arabica genome organisation and will be very useful when defining a whole genome sequencing strategy.

Keywords: Allopolyploidy, Genome evolution, subgenome, Microcolinearity, Coffee

**S1-35**

**COMPARATIVE GENOMICS TO CHARACTERIZE EFFECTS OF POLYPLOIDIZATION EVENTS AND SUBSEQUENT DIPLOIDIZATION PROCESSES IN THE BRASSICACEAE**

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Polyploidy is widespread and has been a predominant factor in the evolution and success of Angiosperms (Leitch and Bennett, 1997; Wendel, 2000). Brassica species, for which no complete genome is yet sequenced, are polyploids, and underwent additional polyploidization events in comparison to A. thaliana, rice, grapevine and sorghum, which genomes have been completely sequenced (AGI, 2000; Goff et al., 2002; Jaillon et al., 2007; Paterson et al., 2009).

As revealed from genetic and comparative genomic analyses (Schranz et al., 2006; Parkin et al., 2005; Rana et al., 2004; Town et al., 2006, Ziolkowski et al., 2006), genomes of current diploid Brassica species, such as B. rapa (AA, 2n=20) or B. oleracea (CC, 2n=18), bear the marks of a paleohexaploidization, shortly after the Arabidopsis/Brassica split (ca. 14 Mya). More recently, several independent amphitetraploidization events occurred by hybridization between these « triplicated-genome » species, leading to important crop species such as B. napus (AACC, 2n=38) (U, 1935). As a result, the Brassica genus includes the dicot crop species having the most highly duplicated genomes: a diploid Brassica species, such as B. rapa or B. oleracea would have accumulated 36 « ancestral-genomes » (AG), and the allopolyploid B. napus, 72 AG whereas grapevine has only three AG (Jaillon et al., 2007) and A. thaliana, 12 AG (Blanc et al., 2003).

The ongoing large-scale Brassica rapa sequencing project (http://www.brassica-rapa.org/) has allowed us to shed light on A. thaliana and Brassica genome divergent evolution after each of those events. We have analyzed all available BAC clones of Brassica rapa (more than 500) and identified their homology regions in A. thaliana genome. For 55 % of them, we have detected two homology regions, in accordance with last shared duplication, common to A. thaliana and B. rapa genomes. For 37 % of them, we have found only one homology region in the A. thaliana genome; this can be explained by either a loss of the A. thaliana duplicated region or a rapid evolution of the homologous B. rapa region. We have also shown that B. rapa regions are shorter by about 30 % than their homologous A. thaliana regions, indicating a global genomic size reduction in B. rapa, which has probably followed its genome triplication. Finally, we have characterized regions and genes that have differentially evolved in the A. thaliana and B. rapa homologous regions.

This study has also laid the foundations for the building of whole-genome comparative approaches of the other Brassica crops, allowing study of polyploidization effects as well as the subsequent diploidization processes.

Keywords: Brassica; genome evolution; polyploidy; diploidization; sequence analysis

**S1-36**

**EVOLUTION OF PARENTAL SATELLITE REPEATS IN THE NICOTIANA ALLOPOLYPLOIDS**

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The intermixing of genetic material between the parental subgenomes of an allopolyploid nucleus is regularly encountered, particularly in polyploids that are thousand of years old or older. The events that accompany these processes are poorly understood. Here we tested the hypothesis that intergenomic homogenization of satellite repeats blurs the differences between homeologous chromosomes.
We characterized at the DNA (Southern blot, sequencing) and cytogenetic (FISH, GISH) levels subtelomeric satellite repeats isolated from several Nicotiana allotetraploids and diploids that resemble the parental progenitors. The allotetraploids analysed were of different ages, and included three species <0.2 myrs-old (Nicotiana tabacum, N. rustica and N. arentsii), two species ~1 myrs-old (N. quadrivalvis and N. clevelandii - section Polydicliae) and four species ~5 myrs-old (N. repanda, N. nesophila, N. nudicaulis and N. stocktonii - section Repandae). We found that the diploid progenitor species have evolved species-specific subtelomeric satellites that underwent further age-dependent evolution following genome merger. N. rustica and N. arentsii showed minimal changes as compared to their diploid parents. Among these slight shifts in copy number were the most prominent. In N. tabacum, N. quadrivalvis and N. clevelandii we observed spreading of parental satellites to partner chromosomes and copy number changes. In section Repandae there was a complete or partial (N. nudicaulis) replacement of parental satellites by allopolyploid-specific satellites. We show that basic units of newly amplified satellites occur in parental diploid species in low copies.
A novel hypothesis on intergenomic satellite homogenization is presented. The data indicate that parental chromosomes are gradually colonized by highly homologous satellites in allopolyploids. We propose that intergenomic homogenization of these megabase-sized arrays can elevate the frequency of homeologous pairing by reducing sequence divergence between parental chromosomes.

Keyword: Nicotiana, satellite, homogenization, allopolyploid

**S1-37**

**UNRAVELING THE MESOPOLYPLOID HISTORY OF AUSTRALIAN CRUCIFERS (BRASSICACEAE)**

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The lowest chromosome numbers known in Brassicaceae (n=4-6) can be found in endemic Australian cruciferous species closely related to the model species Arabidopsis thaliana. We aimed to reconstruct the modes of chromosome number reduction in the Australian taxa from a tentative Ancestral Crucifer Karyotype (ACK, n=8) by comparative chromosome painting (CCP). In three Australian cruciferous species, Stenopetalum nutans (2n=8), Arabidella eremigena (2n=10) and Ballantinia antipoda (2n=12), all 24 genomic blocks of the ACK were unexpectedly found in duplicates. These data suggest that all three species experienced a relatively recent whole-genome duplication followed by massive karyotype reshuffling and chromosome fusions. The unique associations of ancestral genomic blocks shared by the ACK and the Australian crucifers suggest that the reduced crucifer karyotypes (n=4-6) descended from the ACK (n=8) via a polyploid ancestor with yet unknown chromosome number. This is the most comprehensive cytogenetic evidence of a mesopolyploid event revealed in bona fide diploid plant genomes.

Keywords: Brassicaceae, Arabidopsis, polyploidy, karyotype evolution, Australia

**S1-38**

**PLANT POLYPLOIDIZATION THROUGH 2N POLLEN – A FORWARD GENETICS APPROACH IN ARABIDOPSIS THALIANA**

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During plant evolution genome doubling has been a driving force creating highly adaptive polyploid lines. Although there is some discussion on the origin of these ancient polyploids, sexual polyploidization through 2n gametes is now thought to be the main process involved in genome duplication. These gametes, possessing the somatic rather than the gametophytic chromosome number, are mainly the result of a meiotic disfunctionment, which leads to a mitosis-like non-reduced division. Based on the unique characteristics of these gametes, plant species producing a significant percentage of 2n pollen/eggs can have interesting applications in present-day agriculture and crop breeding (e.g. apomixis, polyploid plant breeding, dihaploid production,…).

In order to unravel the genetic determinant(s) controlling the formation of 2n pollen, a forward genetics approach was set up using the model plant Arabidopsis thaliana. Based on the close pollen size-ploidy correlation, EMS mutagenized Arabidopsis M2 individuals were initially screened for the production of giant pollen grains. Out of the 4000 plants analyzed, 110 mutated plants were selected as potential 2n pollen producers. Using cytological and flow cytometric analyses of the progeny populations, mutant lines producing significant numbers of unreduced pollen were isolated and characterized. Tetrad analyses and meiotic chromosome spreading revealed that in several lines, the 2n pollen phenotype was caused by a defect in the meiotic cell division.

Through map-based cloning, these mutant lines are now used to identify genes or loci controlling the 2n pollen trait. Moreover, in order to elucidate the underlying cellular machinery, additional cytological and genetic research is being executed.

Keywords: 2n pollen meiosis polyploidization

**S1-39**

### NUCLEAR DNA AMOUNTS OF WILD WHEAT SPECIES AND EVOLUTION DURING POLYPLOIDIZATION

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The amount of nuclear DNA (C value) is an important biodiversity character and related to morphology, biology, ecology and distribution of the plants, directly or indirectly. Wheat is one of the most studied crops in the world due to its importance as food source. Nuclear DNA amounts already reported for many Triticum and Aegilops species. However, the most of those reports are quite old and estimations in these studies have been made by using outdated methods. Therefore, nuclear DNA amounts of Triticum and Aegilops species reported by different researchers are inconsistent. The objective of this study was to determine nuclear DNA amount of all Triticum and Aegilops species stored in national gene bank of Turkey by using the most recent method, flow cytometry. Approximatelly,120 accessions representing 6 diploid, 9 tetraploid and 2 hexaploid species were used in the study. Based on the results of this study, nuclear DNA amount variation was high among diploid Aegilops and Triticum species. Therefore, significant differences were also observed among polyploids. The observed nuclear DNA content of the some polyploid species were similar to the expected DNA content of the species calculated based on summation of the DNA of their constituent genomes. However, the nuclear DNA content of some polyploid species was less than expected while the others had more nuclear DNA content then expected. The results of the study indicate that gain or loss of nuclear DNA content has occurred during the evolution of the Aegilops and Triticum species and was probably a part of speciation.

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**S1-40**

**DYNAMIC OF RIBOSOMAL GENES DURING THE STABILIZATION OF SYNTHETIC OILSEED RAPE**

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Plant genomes are known to display large variability in number and position of ribosomal RNA (rDNA) genes on chromosomes. To study influences of polyploidy on rDNA variation we synthesized several lines of *Brassica napus* allotetraploids from one diploid *B. oleracea* (mother, C-genome) and *B. rapa* (father, A-genome) progenitors. From the same F1 AC hybrid, two independent lines (AACC) were obtained either from colchicine treatment or from female unreduced gametes. Two S1 plants obtained by selfing per initial S0 amphidiploids were analysed as well as one plant in S2 and S3 generations per S1. BAC-FISH mapping and southern analyses revealed an additive profile in S0 plants but non-additive numbers of 45S rDNA and 5S rDNA loci were detected in respectively 10/14 (71%) and 6/14 (42%) of allotetraploids individuals. The number of 45S loci ranged between 12-14; the number of 5S rDNA loci varied between 7-10 per diploid cell. Locus losses were far more common than locus gains. The A-genome 45S loci were slightly more sensitive to allopolyploidy-associated rearrangements (10 cases) compared to the C-genome loci (5 cases). Major active loci NORs were excluded from genetic changes suggesting that elimination affected mostly heterochromatic transcriptionally silent loci. The locus number variation was frequently but not always accompanied by variation in parental gene copy ratios. In early generations, the locus losses in one compartment were often compensated by locus gains in partner compartment while in later generations of synthetic lines losses predominated. The compensatory rDNA genotypes in S1 suggest homeologous pairing and nonhomologous recombination as likely mechanisms of rDNA rearrangements in *B. napus*.

**S2-1**

**NON-INDEPENDENCE BETWEEN MARKERS ON HOMOEOLOGOUS CHROMOSOMES IN AN INTERSPECIFIC ALLOPOLYPLOID COTTON RILS POPULATION**

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Cotton, as the world's main natural textile fibre, is the focus of many studies for genetic improvement of fibre quality. Two allotetraploid (AtDt genome, 2n=4X=52) species dominate world production: G. hirsutum (Gh) with medium fibre quality and G. barbadense (Gb) with high fibre quality, accounting for 95% / 3% of production respectively. A RIL population originating from a Gh-Gb cross is the base material of the CIRAD-Bayer CropScience-CSIRO ANR research project Cotton_RILs, aiming at the genetic and genomic dissection of cotton fibre quality for introgression of high fibre quality genes of Gb into Gh germplasm. Until now, classical breeding did not succeed at satisfactorily combining traits from both cotton species, in spite of high apparent synteny conservation between their chromosomes.

The fixed heterozygosity of allopolyploids is supposed to result in gene expression changes, or expression subfonctionalization, a partitioning of the ancestral expression domains among duplicate genes. It is hypothesized that allopolyploids should be subject to more genetic and epigenetic regulatory changes than autopolyploids.

We report here on evidence of interactions between markers on pairs of homoeologous chromosomes in the Gh-Gb RIL population studied (140 lines genotyped with 800 markers, assembled in a saturated map of 26 linkage groups). In five of the 13 homoeologous chromosome pairs (c12-c26, c6-c25, c11-c21, c3-c17 and c13-c18), markers from homoeologs displayed unexpectedly high linkage (LOD from 9 to 14). Such linkage was not observed between chromosomes from non-homoeologous pairs. The association concerns 1-3 markers from one chromosome against stretches of 5-8 or more markers on the homoeolog. Gametic disequilibrium (GD) has been assessed pairwise between all markers. Positive GD is highly dominant between markers of homoeologous chromosomes, but negative as well as positive GD are observed between markers of non-homoeologs, with uneven distribution. In positive GD, frequencies of Gh-Gb allelic combinations are very low, meanwhile in negative GD, there is uniform low frequency of Gb-Gb allelic combinations.

Diverse genetic and epigenetic mechanisms have been characterized in polyploid plants, including unequal expression of duplicate genes, segregation distortion and restricted recombination; in cotton, lesser retention of Gb alleles is a common feature of advanced-generation backcross or RIL interspecific populations. Our results support hypotheses of intergenomic incompatibility, with selection during inbreeding that favoured elimination of Gh-Gb allelic combinations that were too conflicting regarding the expression of duplicate genes.

Keywords: Cotton; interspecific RILs; intergenomic incompatibility; gametic disequilibrium

**S2-2**

**HEAT ALLOHEXAPLOIDS DISPLAY VARIABLE MEIOTIC STABILITY AND STRUCTURAL GENOMIC ADDITIVITY**

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Recent studies suggest that polyploidization can induce deep genomic changes, including DNA sequence elimination, chromosomal rearrangements and gene silencing. Although little is known about the molecular bases of such changes, their importance may vary among taxa or genomic combination.
To investigate the impact of polyploidization on wheat genome structure, we performed molecular and cytogenetic characterization of a series of synthetic wheat allohexaploids, derived from crosses between several genotypes of T. turgidum, as donors of the AABB genome; and various genotypes of two distinct subspecies (strangulata and tauschii) of the goatgrass Aegilops tauschii, as donors of the D genome. Meiotic studies using Genomic In Situ Hybridization (GISH) showed the preferential but incomplete homologous pairing in metaphase I. A variable level of chromosome pairing was observed (27.38 to 38.79 chiasmata), depending on the studied wheat synthetic allohexaploid. This is lower than the 39.87 chiasmata observed in the natural wheat hexaploid, used as control and is explained by the formation of univalents (unpaired chromosomes). This cytogenetic study also showed a correlation between incomplete paring and the higher aneuploid frequency observed in the subsequent generation of synthetic allohexaploids. Interestingly, aneuploid frequency varies depending on the genotype of the D genome progenitor and was much higher in wheat synthetic allohexaploids, with Aegilops tauschii subspp. strangulata as a D genome donor, than with Aegilops tauschii subssp tauschii.
A large and representative set of PCR-based markers derived from coding and repetitive sequences (more than 1000 markers) was used to analyse genetic and structural changes across three successive generations (S1, S2 and S3) of euploid plants from different wheat synthetic allohexaploids. These markers showed a complete additivity, with no evidence for structural changes such as DNA elimination.
Our study suggests that structural changes in wheat synthetic allohexaploids consist mainly in aneuploidy, which frequency varies depending on the tetraploid (AB) and diploid (D) genome progenitors. No evidence for DNA elimination or other structural rearrangement were observed in euploid plants of different wheat synthetic allohexaploids, irrespective of their chromosome stability and aneuploid frequency levels.

Keywords: Wheat, aneuploidy, structural changes, meiosis

**S2-3**

**DYNAMICS AND IMPACT OF TRANSPOSABLE ELEMENTS ON THE EVOLUTION OF POLYPLOID WHEAT GENOMES**

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Transposable elements (TEs) constitute more than 80% of the wheat species genome but the impact of allopolyploidizations on their dynamics remains unexplored.
Using a wide comparative genomic sequence analysis, we have characterized dynamics and proliferation of various types of transposable elements in the A and B genomes of the natural and stable wheat allopolyploids T. turgidum (AABB) and T. aestivum (AABBDD), as compared to that of their diploid progenitors (or relatives). Analysis of TE sequence proportions, ratios of complete to truncated copies and insertion dates of class I retrotransposons showed that specific types of TEs (Athila, Copia, Gypsy and CACTA elements) have undergone waves of differential proliferation in the B and A genomes of wheat. As estimated from their insertion dates and confirmed by PCR-based tracing analysis, the majority of differential proliferation of TEs in B and A genomes of wheat (87% and 83% respectively), leading to rapid sequence divergence, occurred prior to the allotetraploidization event that brought them together in T. turgidum and T. aestivum, less than 0.5 million years ago (MYA).
We have also compared the orthologous Hardness (Ha) region of the A genome from several accessions of the diploid (T. urartu), tetraploid (T. turgidum) and hexaploid (T. aestivum) wheat species. Important variations in DNA size and composition were observed. TE insertions account for 44.7 % while DNA segment deletions account for 55.3 % of DNA size variation. Deletion of DNA segments of more than 70 kb were revealed; and 97% of the deleted DNA through unequal crossing over, illegitimate DNA recombination or other unknown mechanisms, was shown to be “TEs-mediated”. The comparative study allowed determination of molecular basis of several DNA deletion events, among which the deletion of the Ha locus from the A genome of the polyploid wheat species. TEs insertions and DNA elimination could not be inferred to a specific ploidy level, as important variation between accessions of a same ploidy level were observed. However, the overall lengths of orthologous regions were 16-45% shorter in tetraploid and hexaploid wheat than in their diploid progenitor T. urartu.
Our study reveals that the natural allotetraploidization event that brought the A and B genome together in T. turgidum and T. aestivum, appears to have neither enhanced nor repressed transpositions. A trend to genome downsizing in natural polyploid wheat species, through TE-mediated DNA elimination, is revealed. TEs proliferation as resulting from insertions, removals and/or combinations of both evolutionary forces, in relation to polyploidization events, is discussed.

Keywords: Polyploidy, wheat, transposable element, dynamic evolution

**S2-4**

**PRECISION OF GENETIC MAPPING IN AUTOTETRAPLOOID POTATO : A PRACTICAL EXPERIENCE**

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Cultivated potato (Solanum tuberosum sbsp tuberosum) is considered as a tetraploid which displays tetrasomic inheritance 2n=4x=48 (Bradshaw, 1994). Genetic mapping of potato has been greatly improved and facilitated by TetraploidMap, a disposable software created by (Hackett and Luo, 2003). Potato genetic maps have been constructed at the tetraploid level mainly using AFLP and few SSR markers (Bradshaw et al., 2004; Bryan et al., 2002; Bryan et al., 2004; Meyer et al., 1998). Here we present preliminary results concerning genetic mapping using a population where different factors of resistance to late blight are segregating.

In order to have the most precise estimation of QRL position and effect, we need to have more accurate maps. Our purpose is to know how the precision of the map would be increased if SSR were used as codominant markers instead of dominant ones.

The population comprises 280 individuals. DNA was extracted using a standard CTAB method. Different types of markers where used for genotyping including SSR. 38 markers were used leading to 129 polymorphic bands. Markers were not evenly distributed on the chromosomes but concentrated on genomic regions where QRL to late blight were located in other studies. Genotyping of SSR was performed using an ABI Prism® 3130xl sequencing system.

In a first step markers were coded in a dominant way. Partial genetic maps of the targeted regions were calculated using TetraploidMap software for each parent.

The second step consisted in constructing a more precise map using SSR coded in a codominant way. This needs to make accurate hypothesis on parental configurations and hence, help to evacuate all doubtful markers configurations in the progeny that sometimes could not have been detected.

For the most polymorphic SSR markers we postulated parental configurations given the observed frequencies in the progeny. Each parental hypothesis was tested using the frequencies of each configuration in the progeny.

Preliminary results obtained are in accordance with tetrasomic inheritance of potato. Some markers exhibited up to fourteen alleles configurations in the progenies. Segregation distorsion was observed (19/129). Hypothesis on parental configurations could be confirmed or rejected observing arrangements of alleles in the progeny and their respective frequencies. However, some unexpected arrangements were observed. Some of them can be explained by genotyping errors. Others are unexplained for the moment: double reduction, ....

A comparison of partial genetic maps established using SSR coded either as dominant or as codominant will be presented and results will be discussed in a breeding perspective.

Keywords: potato, autotetraploid, genetic mapping, SSR

**S2-5**

**RDNA IN THE CONTEXT OF POLYPLOIDY AND ASTONISHING KARYOTYPE VARIATION IN PROSPERO AUTUMNALE**

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Prospero autumnale (L.) Speta, previously known as Scilla autumnalis L., is a bulbous monocot in Asparagaceae and exhibits astounding karyotypic variation. At least five genome sub-types have been described which vary in chromosome number and morphology as well as DNA content. Diploid chromosome numbers range from 2n=10-14 and DNA content of diploids alone varies by as much as ~30%. In addition to this genome sub-types combine, resulting in chromosome numbers from 2n = 10-42, with many cytotypes at the tetraploid and hexaploid level. Furthermore, supernumerary segments and B-chromosomes have been described in some populations adding further complexity. Amazingly, such variation occurs within a near morphologically uniform species.

Using 454 pyro-sequencing we will attempt to identify repetitive sequences, which are likely to be responsible for changes in DNA content between sub-genomes. Fluorescence in situ hybridisation (FISH) will be used to track changes in repeat sequence distribution between genome sub-types, and these data will be framed in a phylogenetic context to search for convergent genetic change across independent lineages. An immediate aim is to use FISH data in conjunction with a phylogeny of individuals to examine the fate of rDNA following hybridisation and polyploidy.

Early results indicate considerable variation in the number, size and location of 5S rDNA loci, even between chromosome sets that are indistinguishable at the morphological level. Such variation occurs among individuals within the same population, raising questions as to how this affects fertility. Large 5S rDNA arrays and a second smaller 5S locus have been located on B-chromosomes, and this is concomitant with a reduction in 5S rDNA signal on chromosome 2. This variation also hints that the division of genomes into distinct sub-types may require revisions that better accommodate the wide range of further chromosomal variation being discovered. Our long-term aim is to determine if there is adaptive significance to genome restructuring.

Keywords : Polyploidy, karyotype variation, rDNA, adaptive radiation

**S2-6**

**ALLOPOLYPLOIDY HAS DIFFERENT IMPACTS ON THE EVOLUTION OF RETROTRANSPOSON AND MITE INSERTION SITES - A STRUCTURAL APPROACH ON NEWLY SYNTHESIZED BRASSICA NAPUS ALLOTETRAPLOIDS**

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Polyploidy has played a major role in the evolution of the plant genome [1]. Recent studies have demonstrated that the genome of newly synthesized polyploids is unstable, dynamic and subject to genetic and epigenetic regulations [2,3]. Transposable elements (TEs) represent a major component of the genome and may have the potential to provide the host plant genome with the plasticity necessary for its adaptative response. Our study aims at evaluating the genomic modifications related to TEs (rearrangements, transpositions) that occur during the formation of an allopolyploid genome.
Our plant model is oilseed rape (Brassica napus, AACC) originating from interspecific hybridization between B. rapa (AA) and B. oleracea (CC), and we dispose of newly synthesized B. napus allotetraploids resulting from independent AA × CC crosses. To follow the evolution of TE insertion sites during the allopolyploidisation event, we developed S-SAP (Sequence-Specific Amplification Polymorphism) markers anchored in two different families of Brassica-specific TEs, Athila retrotransposons [4] and MITEs, which are contrasting TE systems regarding their genomic organisation, location in the genome, dynamics of evolution, and mode of replication. We compared S-SAP profiles of the re-synthesized B. napus polyploids to those of their diploid progenitors; the S-SAP developed from MITEs gave rise to numerous non-additive S-SAP bands between synthetics and diploids, with a significant increase of non-additive bands in the S2 generation, while Athila S-SAP bands were mainly additive. Characterization of about 60 differentially amplified S-SAP bands by cloning and sequencing showed that only one newly amplified Athila S-SAP band may correspond to a new transposition event. Most of the non-additive S-SAP bands were thus probably due to chromosomal rearrangements or restriction site polymorphisms related to DNA methylation modifications. In view of the nature of these results, it is likely that the contrasting genomic locations of the two TEs surveyed (heterochromatin for Athila vs. gene-rich regions for MITEs) made it possible to highlight epigenetic reorganisation immediately after allopolyploidy in gene-rich regions.

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Keywords: Brassica - Transposable elements – Athila – MITE – S-SAP

**S2-7**

**THE RRNA GENES FOLLOW SIMILAR NON-ADDITIVE EVOLUTIONARY TRAJECTORIES IN BOTH SYNTHETIC AND NATURAL ALLOTETRAPLOIDS OF TRAGOPOGON**

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In a previous research we have carried out analysis of rDNA inheritance of ribosomal rRNA genes (rDNA) in several populations of T. mirus and T. miscellus. In eight of 10 natural populations we examined, there are far fewer 35S rDNA units of T. dubius than there are of the other diploid parent. Using combined fluorescent in situ hybridization and genomic in situ hybridization (FISH/GISH) analyses we now show size reductions of rDNA loci affecting both T. dubius genome homologues while the loci on partner chromosomes seem to be unaffected by deletions. To establish the timeline for these changes, we studied the inheritance and expression of rRNA genes in S1 generation of allopolyploids resynthetised from respective diploid parents. We obtained data from 7 populations of synthetic T. mirus (110 individuals) and 4 populations of synthetic T. miscellus (93 individuals). In both synthetic allopolyploids frequent deviations from Mendelian inheritance of units was observed. Typically the units were skewed away from T. dubius parent. In synthetic T. miscellus there were individuals which lost one parental array completely (5% cases). There was a considerable variation in gene copies ratios within and between the lines. At the epigenetic level we observed cases of dominance of T. dubius units or codominance (additive expression). The silencing of partner (T.porrifolius and T. pratensis) rDNA loci was more pronounced in individuals with reduced numbers of T. dubius units in both – natural and synthetic plants.

In conclusion, the rRNA genes seem to follow similar evolutionary trajectories in both synthetic and natural Tragopogon allotetraploids. There appears to be a genetic predisposition of parental loci to undergo structural and epigenetic changes in response to genomic stress induced by allopolyploidy.

Keywords: polyploidy, Tragopogon, nucleolar dominance, rDNA

**S2-8**

**A VIEW ON THE DISTRIBUTION OF RRNA GENE FAMILIES OF PENTAPLOID (ROSA SECT. CANINAE) AND TETRAPLOID DOGROSES**

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Rosa section Caninae are pentaploid dogroses (2n = 5x = 35) with a unique reproduction system, known as odd (or asymmetric) meiosis. One basic genome (x = 7) derived from the seven segregating bivalents is transmitted from the pollen, whereas four basic genomes (4x = 28, one genome is derived from the segregation of the bivalents and three sets from the segregating univalents) are transmitted by the egg cell.

Chromosomes from all five genomes carry 18-5.8-26S nuclear ribosomal DNA (rDNA). Six major rRNA gene families (?, ?, ?', ?, ?, ?) have been identified in pentaploid R.canina, R. rubiginosa, R. dumalis, R. sherardii and R. caesia based on several polymorphic sites in the internal transcribed spacers (ITSs). The ? family appears to be present among all species while the distribution of other families was more restricted. While in some species (R. canina) the ? family appears to be the dominant rDNA type, in other species, e.g. R. rubiginosa, this family was reduced in copy numbers. The ? family was overrepresented in DNA extracted from pollen grains (compared to leaf) suggesting its occurrence on bivalent forming chromosomes.

Using sequencing of ITS clones and cDNA-CAPS, we investigated expression patterns of rRNA gene families thought to be differentially distributed between bivalent or univalent chromosomes. We found that the ? family was always expressed irrespective of species. However, there was variation in expression of other gene families, and some families (?) were silenced.

The data show that the ? rDNA family, which apparently occurs preferentially on bivalent forming chromosomes, dominates rDNA expression in all dogrose species. The families originating from univalent chromosomes are being frequently (but not always) silenced.

Keywords: Rosa Caninae, rDNA, polyploidy, meiosis, ITS

**S2-9**

**NUCLEOTIDE SEQUENCE OF THE ITS1-5.8S-ITS2 REGION AND DART MARKERS SHED LIGHT ON PHYLOGENETIC RELATIONSHIPS WITHIN THE MUSACEAE**

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Bananas and plantains (Musa spp.) provide a staple food for millions of people in sub-Saharan Africa, South and Central America and much of Asia. All cultivars that are grown are seed sterile clones and are thought to have been selected as naturally occurring hybrids in Southeast Asia by the earliest of farmers. In fact, many believe that banana was one of the first crops to be domesticated by man. Almost all of the 300 or more cultivars that are known arose from two seeded, diploid species, Musa acuminata Colla (A genome) and M. balbisiana Colla (B genome); they are diploid, triploid and tetraploid hybrids among subspecies of M. acuminata, and between M. acuminata and M. balbisiana. Despite the socioeconomic importance of bananas and plantains, the exact mode of their origin and phylogenetic relationships within the family Musaceae remain subjects of debate. In this study we focused on the ITS1-5.8S-ITS2 region of the ribosomal DNA locus. We have sequenced ITS region of different diploid taxa from four sections of Musa, two species of genus Ensete, one species of Musella, and a range of cultivated banana clones. Phylogenetic tree was constructed based on the neighbour joining method. The tree, which was rooted on Zingiber spp., supports the genus Musa as monophyletic group that is separated from genus Ensete and Musella. The genus Musa is divided into two distinct clades – a clade of Callimusa and Australimusa and a clade of Eumusa and Rhodochlamys. The ITS sequence data were also found useful to resolve relationships at lower taxonomic levels within the genus Musa and to discriminate A and B genomes, whose ITS sequences remain conserved in intraspecific hybrids. The ability to determine genomic constitution is important not only in taxonomy but also in breeding new hybrid banana cultivars. Our conclusions derived from the study of ITS nucleotide sequences were supported using a different phylogenetic strategy based on DArT markers to avoid a phylogenetic reconstruction based only on one locus. This work has been supported by the Grant Agency of the Academy of Sciences of the Czech Republic (grant award no. IAA600380703) and the International Atomic Energy Agency (research agreement no. 13192).

Keywords: ITS, ribosomal DNA locus, taxonomy

**S2-10**

**GISH AND FISH ANALYSIS OF HEXAPLOID CHENOPODIUM ALBUM AND THEIR PUTATIVE ANCESTOR SPECIES.**

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Chenopodium album is a complex species and consists of highly heteromorphic wild and semi-cultivated forms. Cytologically, it comprises diploid (2n=2x=18), tetraploids (2n=4x=36) and hexaploid forms (2n=6x=54). The origin of hexaploid form is still uncertain but there is more and more data indicating that it is allopolyploid plant.
In this study several accession of C. album were analyzed. Among them the majority were hexaploids and only one diploid and one tetraploid accessions were found. Using FISH with rDNA as a probe a few accessions of C. album were analyzed to find if this species exhibit polymorphism in rDNA loci organization. GISH and FISH was used to reveal the relationships between the hexaploid and selected diploid and tetraploid Chenopodium species. After GISH with genomic DNA of diploid C. album hybridization signals were observed on 18 chromosomes of the hexaploid. Similar results were obtain when C. ficifolium genomic DNA was used as a DNA probe. After slides reprobing with rDNA sequences it was indicated that genomic DNA of diploid C. album and C. ficifolium hybridized to the same chromosome set. cGISH (comparative GISH) showed that these two species had very similar genomes and it was impossible to distinguish them with this method. GISH excluded C. murale as an ancestor of the hexaploid. Also tetraploids C. berlandieri subsp. berlandieri cannot be ancestor of hexaploid C. album. However GISH results showed that C. berlandieri subsp. berlandieri probably share one genome with the hexaploid C. album. FISH with rDNA sequences was used to compare chromosomal organization of rRNA gen loci in genomes of analyzed species.

Keywords: Chenopodium, FISH, GISH, rDNA

**S2-11**

**EFFECT OF POLYPLOIDY ON SELF-INCOMPATIBILITY IN SYNTHETIC BRASSICA NAPUS**

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Interspecific hybridization is a major factor in the rapid speciation process and plays an important role in the evolution of species. Even if self-incompatible diploid parental species are involved, the resulted allopolyploid species are often self-compatible. This is the case for B. napus (AACC, 2n = 4x = 38) which is an allotetraploid species produced after a spontaneous hybridization between B. oleracea (CC, 2n = 2x = 18) and B. rapa (AA, 2n = 2x = 20). Diploid parents have generally a self-incompatibility system (genetic system under control of a complex S locus), while B. napus is self-compatible. In this study, we investigated the consequences of polyploidy on the self-incompatibility complex system by analyzing the phenotypes and the S-products in several synthetic rapeseeds. These synthetic B. napus were produced from parents showing different phenotypes of self-incompatibility. The results showed that the effect of polyploidy on the phenotype of self-incompatibility was variable depending on the progenitors and their allelic composition and they showed variable modifications following generations. Two hybrids were self fertile at the S0 generation (they have no functional S haplotype of B. oleracea parent). Two other hybrids were self-incompatible at the S0 generation, but the self-incompatibility phenotypes vary in S3 progeny. The phenotypic variation among S3 plants was structured differently according to the origin of the hybrids. Furthermore, structural or functional changes at the S locus were observed following the polyploidization. While S0 plants inherited the S loci of both parental genomes A and C, some S3 plants lost the S locus of the A parental genome. The analysis of the expression of SLG and SRK parental genes in all hybrids (S0 and S3) showed a differential expression of these genes among plants. These results are discussed in the light of current data on the different mechanisms of alteration of self-incompatibility in polyploids leading to the establishment of stable self genotypes.

Keywords: Brassica napus, self-incompatibility, polyploidy

**S2-12**

**COMPLEXITIES OF CHROMOSOME LANDING IN A HIGHLY POLYPLOID, ANEUPLOID, INTERSPECIFIC GENOME: TOWARDS MAP-BASED CLONING OF A RESISTANCE GENE (BRU1) IN SUGARCANE (2N=CA 115)**

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The genom e of modern sugarcane cultivars is highly polyploid (~12x), aneuploid, of interspecific origin, and contains 10 Gb of DNA. Its size and complexity represent a major challenge for the isolation of agronomically important genes. We have undertaken the first attempt to isolate a gene from sugarcane by map-based cloning, targeting a durable major rust resistance gene (Bru1).To overcome constraints associated with high polyploidy, we developed stategies including diploid/polyploid syntenic shuttle mapping with model diploid species (sorghum and rice) and haplotype-specific chromosome walking. These strategies allowed us to develop a high-resolution genetic map including 17 markers in an interval of 0.42 cM comprising Bru1 and to build a physical map of the target haplotype that still includes two gaps at this stage due to the discovery of an insertion specific to this haplotype. BAC clones representing seven differents hom(oe)ologous haplotypes have been sequenced. These sequence s are being used to complete the physical map of the target haplotype.

Keywords: sugarcane, resistance gene, diploid/polyploid syntenic, map-based cloning

**S2-13**

**EXTRACTION OF THE DIPLOID A GENOME AND PRODUCTION OF MONOSOMIC ADDITION LINES FROM THE ALLOPOLYPLOID BRASSICA NAPUS ( AACC, 2N=38).**

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There are two strategies available for understanding structural and/or functional modifications which took place during the stabilization of polyploid species. The first involves production of synthetic forms in order to mimic the events occurring during genome stabilization, and the second involves the extraction of the polyploid's diploid component for comparison with the present natural diploid species. On the oilseed rape model (Brassica napus, AACC, 2n=38) which is a natural hybrid between B. rapa (AA, 2n=20) and B. oleracea (CC, 2n=18), we used two methods to extract the diploid AA genome from B. napus. Firstly, AAC F1 interspecific hybrids (produced by crosses between B. napus and B. rapa) were backcrossed three times to B. napus. AAC plants were selected at each generation but the resulting AAC hybrids were male sterile and so it was impossible to eliminate the C chromosomes by selfing. Secondly, the initial AAC F1 hybrids were crossed to B. rapa and plants with AA genomes were selected for, selfed and also backcrossed to B. napus. After one cycle of such crossing, we selected AA plants with mainly the A genome of B. napus and additionally monosomic addition lines (2n=21) carrying C1, C2, C3, C5, C7 or C8 as characterized by molecular markers. The resulting AA plants had regular meiotic behaviour but a specific morphology. Monosomic addition lines provide useful material with which to identify the impact on phenotype of specific C genome chromosomes, and also to attribute unambiguously BAC or monomorphic markers to a specific chromosome. We will perform a second cycle of crosses to get diploid plants as close as possible to the A genome of B. napus as well as plants covering a complete set of monosomic C chromosome additions. From this material, it will be possible to determine the comparative evolution of the A genome in a diploid and polyploid genetic background.

Keywords: B napus diploid component monosomic addition lines meiotic behavoiur

**S2-14**

**THE FIRST MEIOSIS OF NEWLY SYNTHESIZED BRASSICA NAPUS : A GENOME BLENDER?**

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Chromosomal reshuffling occurs during meiosis in newly created polyploid species of Brassica, contributing to divergence of the parental genomes from their natural diploid form. In newly synthesized Brassica napus, studies have shown little effect of the very first meiosis in genomic restructuring. However, the frequent and non-random fixation of translocations (HNRTs) in early generations of re-synthesized B. napus supposes a major role of the first meiosis on chromosome rearrangements. The homeologous chromosome pair A1-C1 provide an appropriate model to study homeologous pairing and genome remodelling during polyploid creation, as they are completely collinear in macrosynteny in B. napus, in its progenitors and are also the most rearranged chromosome pair in natural B. napus haploids.
Using multiple lineages of synthetic B. napus, we aimed to : (i) establish that the A1-C1 frequently pairs during the first meiosis of neo-polyploids; (ii) assess precisely the impact of this meiosis on the nature, size and frequency of rearrangements generated on A1 and C1; and (iii) determine the effect of A1-C1 rearrangements on the perturbation of regular meiotic behaviour in contrasted progeny lines.
(i) Three different S0 (colchicine doubled hybrid) lineages of resynthesized B. napus have been created from 4 different diploid parents to test the effect of genetic background as well as a reciprocal cross to test maternal cytoplasmic effect. By using a BAC-FISH (Fluorescent In Situ Hybridisation) approach at meiosis of the first generation S0, it is possible to detect the implication of A1-C1 in abnormal chromosome pairing. Evidence for A-C pairing during the first meiosis exist, and this work will determine the A1-C1 pairing ability of the diploid progenitors' genome structure.
(ii) To assess the impact of the first meiosis on genome remodelling in gametes, crosses were performed between 3 amphidiploid (S0) lines and a natural B. napus line (92 ind. per population). Genetic analysis using molecular markers mapped on A1 and C1 identified a different behaviour of the B. rapa cytoplasm population, but all had highly frequent inherited chromosome rearrangements. The nature of observed rearrangements (translocation vs. deletion) will be verified on a subset of plants using BAC FISH to differentiate homeologous regions.
(iii) By establishing the meiotic behaviour of this subset of plants, we will validate the effect of A1-C1 homogenisation on further genome instability.
These data will provide new insight into genome restructuring in polyploids after genome duplication.

Keywords: Brassica, allopolyploid, meiosis, synthetic hybrids, recombination

**S2-15**

**RECONSTITUTION OF THE TETRAPLOID COMPONENT OF A BREAD WHEAT.**

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Bread wheat, Triticum aestivum (2n=42, AABBDD) originated from a cross between T. turgidum (2n=42, AABB) and Ae. tauschii (2n=14, DD) about 10000 years ago in the Fertile Crescent. Here we present the reconstitution of the tetraploid component (AABB) of the French wheat variety cv. Courtot. Initially a cross was made between cv. Courtot and the Italian durum variety cv. Creso. The pentaploid F1 was selfed and tetraploid plants recovered in F2. The latter were crossed to Courtot (female) and again tetraploids were selected in the BC1F2. A series of 6 backcrosses were made to eliminate the genetic material contributed by Creso in the initial cross. Thus we ended up with Tetra Courtot with more than 99% of the A and B genomes of Courtot. That genotype does not ressemble commonly described varieties of present-day tetraploid species. It is much less vigorous than durum varieties. It is dwarf , even shorter than Courtot which contains Rht-B1 and Rht-D1 dwarfing genes. It is also very sterile with less than five grains per spike.
Our results confirms findings by other authors. Contrary to what is generally admitted, bread wheat is not the sum of the genomes of tetraploid wheat and Ae. tauschii. In the hexaploid background, there was an evolution of A and B genomes by different mechanisms which remain to be understood or/and bread wheat has retained certain gene combinations. This was confirmed by the fact that synthetics having Tetra Courtot as tetraploid parent are meiotically more stable than synthetics produced from tetraploid wheats (see oral communication by same authors).In short we have of a nice tool to deciphering genome evolution in hexaploid wheat.
The reconstituted genomic components of bread wheat may also be of value in plant breeding. They can be combined with accessions of goat grass to produce synthetics which would be more easily used than classical synthetics by breeders. Those synthetics can also be crossed to the bread wheat varieties from which the tetraploids have been extracted. In the progeny of that kind of cross it should be easier to detect genes/QTLs potentially interesting to wheat breeders.

Keywords: evolution, bread wheat, durum wheat

**S2-16**

**RIBOSOMAL RNA GENES EVOLUTION AND ORGANISATION IN THE FAMILY ASTERACEAE**

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The study of ribosomal RNA genes has long been an important tool in research about polyploidy and hybridisation in plants. In flowering plants and animals the most common ribosomal RNA genes organisation is that in which 35S (encoding 18S-5.8S-26S rRNA) and 5S genes are physically separated occupying different chromosomal loci. However, a recent discovery in the genus Artemisia (Asteraceae) describes a new type where both 35S and 5S have been unified to a single unit and where it seems that the 5S gene has been inserted, in inverted orientation, into the 26S-18S intergenic spacer (IGS).

This research work aims to reveal possible mechanisms leading to the linked organisation, searching for species displaying intermediate statuses with both kinds of rRNA genes arrangements. In addition, we wonder if the 5S insertion that led to the linked units took place once or repeatedly during evolution and if this fact may have systematic implications in the phylogeny of the family. The Asteraceae are the largest angiosperm family, with around 23,600 species, and other objectives of our project include describing in which lineages there is an organisation similar to that of Artemisia, evaluating its extent throughout the family and knowing if variations of this structure, or perhaps other novel rDNA types, exist. To accomplish these goals we have obtained data for ca. 200 species at different levels (PCR, Southern blot, FISH and sequencing), representative of the whole family's diversity and including their currently accepted 12 major lineages.

Our results point that the dominant linked arrangement seems to be restricted to the subfamily Asteroideae (indeed the largest group, accounting for 72% of the diversity of the family). However, sensitive PCR revealed low copy units in some different, scattered members of the Asteraceae. Within the Asteroideae, many species homogenized rRNA genes towards a linked genotype, while the remaining exhibit classical unlinked arrangement. Mapping of 5S gene insertions revealed its conserved (inverted) arrangement downstream from the 26S gene, while the intergenic spacers were highly diverged across the species. Thus selection pressures appear to act on the 5S gene but not on sequences flanking the insertion.
This research will also provide a basis on to which analyse ribosomal RNA genes changes during polyploid and hybrid formation in the family Asteraceae.

Keywords: Ribosomal RNA genes, 5S, 35S, genome organisation, Compositae

**S2-17**

**EVOLUTION OF TRANSPOSABLE ELEMENTS DURING ALLOPOLYPLOID FORMATION IN NICOTIANA**

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Interspecific hybridization associated with genome duplication (allopolyploidy) has repeatedly occurred in the evolution of Nicotiana, leading to allopolyploid species of widely different ages (ca 10MY-0.02MY). Polyploidy is known to have a significant impact on plant evolution, frequently causing structural and functional genomic modifications. We focused our investigations on the most recent Nicotiana allopolyploids (probably forming ca 20,000 years ago), i.e. Nicotiana tabacum, N. rustica and N. arentsii. We aimed to determine the extent of genomic restructuring generated by the endogenous populations of transposable elements (TEs) that has occurred with the allopolyploid species divergence by comparing the TEs profiles of natural species with synthetic (interspecific) F1 hybrids and, when possible, derived allopolyploids (S0 generations). The degree of TEs-associated restructuring is analyzed and discussed in relation to the phylogenetic distance between the parental lineage, the generation of the hybrid/allopolyploid and (when known) the biology of the TE population considered.

# This work is dedicated to Yoong

Keywords: Nicotiana, allopolyploidy, transposable element

**S2-18**

**CYTOMOLECULAR IDENTIFICATION OF INTERGENOMIC CHROMOSOME REARRANGEMENTS IN THE ALLOTETRAPLOID SPECIES AEGILOPS BIUNCIALIS AND AEGILOPS GENICULATA**

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Intergenomic chromosomal rearrangements have played an important role in allopolyploid speciation. The identification of structural chromosome polymorphism is indispensable in utilizing genetic pools of the wild Aegilops species in wheat breeding programmes. Aegilops biuncialis (2n=4x=28; UbUbMbMb) and Ae. geniculata (2n=4x=28; UgUgMgMg) are distributed in the Mediterranean and Western Asiatic regions. These species have good tolerance against biotic and abiotic stresses and are used as gene sources to improve the stress tolerance of wheat (Triticum aestivum L.).

Two-colour genomic in situ hybridization (GISH) using differentially labelled total genomic DNA from Ae. umbellulata and Ae. comosa allowed the clear discrimination of the U- and M-genome chromosomes of Ae. biuncialis and Ae. geniculata. Twenty-eight Ae. biuncialis and twenty Ae. geniculata accessions from 14 countries of Europe, Asia and Africa were scored by two-colour GISH for the presence of intergenomic translocations. Chromosomal rearrangements were detected in 6 of 28 accessions of Ae. biuncialis and 5 of 20 accessions of Ae. geniculata. According to GISH analysis most of the breakpoints were located near the centromere. Sequential fluorescence in situ hybridization (FISH) with differentially labelled repetitive DNA probes (pSc119.2, Afa family, pTa71) and GISH were used for the identification of intergenomic translocations. The FISH results indicate that homeologous group 7 chromosomes were involved most frequently in the translocations. Three of six chromosomal rearrangements detected in Ae. biuncialis were 7Ub/7Mb and considered as homeologous translocations.

The karyotypic changes detected in this study may help to understand the evolution of Ae. biuncialis and Ae. geniculata. These intergenomic changes should also be taken into account when it is planned to use these Aegilops accessions for wheat breeding programmes.

Keywords: Ae. biuncialis, Ae. geniculata, intergenomic translocation, FISH, mcGISH

**S2-19**

**REVISION OF PLOIDY LEVELS OF DIOSCOREA ALATA POLYPLOID SPECIES BY CYTOGENETIC AND MICROSATELLITE SEGREGATION ANALYSIS.**

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Dioscorea alata is a polyploid species with several ploidy levels and its basic chromosome number has been considered by most authors to be x = 10. Standard chromosome counting and flow cytometry analysis were used to determine the chromosome number of 110 D. alata accessions of the CIRAD germplasm collection. The results revealed that 76% of accessions have 2n = 40 chromosomes, 7% have 2n = 60 chromosomes and 17% have 2n = 80 chromosomes. Progenies were produced from 2n = 40 types of D. alata and the segregation patterns of six microsatellite markers in four different progenies were analysed. The Bayesian method was used to test for diploid versus tetraploid (allo- and autotetraploid) modes of inheritance. The results provided the genetic evidence to establish the diploidy of plants with 2n = 40 chromosomes and to support the hypothesis that plants with 2n = 40, 60 and 80 chromosomes are diploids, triploids and tetraploids, respectively, and that the basic chromosome number of D. alata is x = 20. The findings obtained in the present study are significant for effective breeding programs, genetic diversity analysis and elucidation of the phylogeny and the species origin of D. alata.

Keywords: Dioscorea alata, polyploidy,microsatellite segregation, basic chromosome number

**S2-20**

**GENETIC MAPPING AND SYNTENY ANALYSIS ALLOWED THE IDENTIFICATION OF GENOME REARRANGEMENTS IN THE ALLOTETRAPLOID ARACHIS HYPOGAEA.**

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Cultivated peanut (Arachis hypogaea L.) is widely used as a food and cash crop around the world. It is considered to be an allotetraploid AABB (2n = 4x = 40) originated from a recent and single hybridization event between two wild diploids. Among the sixty wild species described, A. duranensis (AA) and A. ipaensis (BB) appeared to be the best candidates for the A and B genome donors, respectively. The combining effects of polyploidisation and domestication have greatly narrowed the genetic diversity and hampered the application of molecular approaches for the genetic analysis and the improvement of the cultivated peanut. Recently, the development of synthetic amphidiploids using wild diploid species allowed overcoming the reproductive barrier between wild diploids and the cultivated tetraploid species. This material is an important resource for genetic mapping, synteny analysis between the A and B genomes and molecular breeding.
The objectives of this study were to construct a wild x cultivated tetraploid genetic map using the co-dominant SSR markers, to assess the type of inheritance and the synteny between the A and B genomes.
A synthetic amphidiploid, obtained from the cross between the most probable wild progenitors of the cultivated peanut (A. duranensis, A ipaensis), was crossed to the Fleur11 variety. A population of 88 BC1F1 individuals was produced and genotyped with 277 polymorphic SSR markers.
We mapped 299 loci in 21 linkage groups (LGs), spanning a total map distance of 1843.7 cM. We determined the sub-genomic origin of the SSR alleles by comparison with the alleles of the wild diploid parents of the amphidiploid. This enabled us to confirm the disomic inheritance of all loci and to distinguish the A from the B genome linkage groups (LG). We have not observed LGs with mosaic A/B allele composition. This indicates that the chromosome pairing happened between "homologous" genomes and confirms the high affinity between the A/B genomes of the cultivated species and the A genome of A. duranensis and the B genome of A. ipaensis, respectively. We also identified the homeologous LGs with 53 SSR markers that mapped on both the A and B genomes. We observed an overall good collinearity between each pair of homeologous LGs. However, three inversions of chromosome segment were pointed out between homeologous LGs a01/b01, a03/b03 and a09/b09, as well as a major translocation involving the LGs b07 and b08. These rearrangement events are discussed regarding the divergence of the A and B genomes.
The result of this study contributes to the comprehension of the structure of the A and B genomes and the broadening of the gene pool of the cultivated peanut.

**S3-1**

**REPROGRAMMING OF GENE EXPRESSION IN GENETICALLY STABLE WHEAT SYNTHETIC ALLOHEXAPLOIDS**

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In order to characterize reprogramming of gene expression occurring at the whole genome level and their putative role in stabilization mechanisms of polyploids, we have conducted an integrated analysis of wheat synthetic allohexaploids that we have characterized as genetically stable using molecular markers levels. Towards this purpose, we have analyzed wheat allohexaploids derived from hybridization between the tetraploid T. turgidum ssp. durum line (cv. Joyau), as donor of the AB genomes and several accessions of the two subspecies strangulata and tauschii of the diploid Ae. tauschii as donors of the D genome. The different wheat allohexaploids exibit different karyotypes and meiotic behaviour stabilities. However, our survey show that all euploid plants having the expected 42 chromosomes do not show genetic rearrangements as assessed by more than 10000 PCR-based markers.
Euploid plants of two representative synthetic wheat allohexaploids were compared for gene expression changes with their respective progenitors, between each others, S0 and S1 generations as well as with natural wheat allohexaploids. The Affymetrix GeneChip Wheat Genome array, which contains 61,127 wheat probe-sets representing 55,049 transcripts covering the 42 wheat chromosomes, was used. Genes were classified based as additively or non-additively expressed in the allopolyploids, as compared to the mid-parent value. About 5000 transcripts were statistically different ($P<0.05$) between T. turgidum durum and Ae. tauschii, progenitors of the wheat allohexayploids representing about 22% of the overall detected transcriptome on the microarrays.Majority of these transcripts were additively expressed in the newly synthesized wheat allohexaploids, representing thus an advantage in gene expression of the wheat synthetic allohexaploids as compared to their both progenitors.
The overall non-additively expressed transcripts in the synthetic allohexaploids consist in an average of 8.2% of the total ca. 28000 total expressed transcripts.
Comparisons between combinations and generations allowed us to define sets of polyploidy-regulated transcripts behaving in a similar manner in the different allohexaploid combination and/or generations, and thus to highlight potential candidate genes putatively involved in polyploid stabilization mechanisms.

Keywords: wheat, allopolyploidy, reprograming of gene expression

**S3-2**

**THE ALTERATION OF GENOMIC SEQUENCE VARIATION BETWEEN SYNTHETIC HEXAPLOID WHEAT AND ITS PARENTAL SPECIES**

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Bread wheat represented one of the best characterized examples of evolution through alloploidy, and some studies have provided much insight into the alteration of genomic rearrangements, epigenetic and gene expression changes in synthetic allohexaploid wheat. In the present study, we analyzed the sequence variation at the genomic level of the newly synthesized hexaploid wheat and its parental species with DNA-AFLP, and the rate of coding sequence variation among the 208 single-copy genes was also determined.

Genomic sequence variations in F1 hybrid (ABD) and newly synthesized hexaploid wheat (AABBDD) S1 generation between tetraploid wheat DM4 (AABBB) and diploid goat grass Y199 (DD) were also investigated by using DNA-AFLP procedure. Using 18 primer pairs, 1639 clear bands were obtained, among which 1194 were polymorphic and 445 were monomorphic between two parents. For those polymorphic bands, 782 were specific to maternal parent DM4, and 412 specific to paternal parent Y199. When comparing the synthetic hexaploid wheat S1 generation with their parents, we found that 9 (1.15%) of 782 from DM4 were disappeared in synthetic hexaploid and 71 (17.23%) of 412 from Y199 were disappeared in synthetic hexaploid. To further determine whether sequence elimination occurred in triploid F1 hybrid, we compared sequence variation between synthetic hexaploid S1 generation with the triploid F1 hybrid. It was found that the majority of the disappeared bands in hexaploid S1 generation were also absent in the triploid F1 hybrid, only one bands from Y199 which was present in triploid F1 hybrid but was absent in synthetic hexaploid S1 generation.

To determine the sequence changes in the coding regions of the genome during wheat polyploidization, 208 primer pairs developed were used to amplify the DNA from the the newly synthesized hexaploid wheat (DM4/Y199) S1 generation, F1 hybrid and their parental species for SSCP analysis. We found that among the four alleles derived from D genome of parent Y199 were also absent in the synthetic hexaploid wheat DM4/Y199 S1 generation and F1 hybrid. It was indicated that sequence variation detected by SSCP was much lower than those detected by DNA-AFLP, which suggested that much less variation in the coding regions occurred in the synthetic hexaploid wheat, and sequence variations detected by DNA-AFLP could be derived mostly from non-coding regions and repetitive sequences. It was interesting to note that 3 out of the 4 genes were mapped and clustered on the long arm of chromosome 2D, which indicated that variation in coding sequences in synthetic hexaploid wheat might not be a randomized process.

Keywords: Synthetic hexaploid wheat; Polyploidy; Genome; Sequence variation

**S3-3**

**CROSS SPECIES HYBRIDISATION MICROARRAYS REVEAL CONSISTENT TRANSCRIPTOMIC CHANGES FOLLOWING NATURAL INTERSPECIFIC HYBRIDISATION AND ALLOPOLYPLOID SPECIATION IN SPARTINA (POACEAE)**

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Microarrays are being commonly used for large-scale gene expression analysis. However, due to a lack of sequence information, this technique remains essentially restricted to model systems that benefit from genomic resources (i.e. large EST data sets or fully sequenced genomes). We have explored the utility of cross species hybridisation microarrays to detect the transcriptomic changes associated with allopolyploid speciation in genus *Spartina* (Poaceae, Chloridoideae) that contain notorious examples of recent hybridisation and allopolyploid speciation. Using rice (*Oryza sativa*) oligo-microarrays, we have compared leaf expression profiles among the parental hexaploid species (*S. alterniflora* and *S. maritima*), the F1 natural hybrid (*S. x townsendii*), and the allo-dodecaploid *S. anglica* formed following genome duplication of *S. x townsendii*. We were able to detect repeatable and significant genome expression differences between the parental species (grown in similar conditions) where 1487 genes appeared differentially expressed. These species diverged ca 1-2 MYA and exhibit 95-99% sequence identity in coding regions. Deviation from parental expression additivity in the F1 hybrid was encountered in about 13% of the examined genes. The allopolyploid *S. anglica* exhibit about 15% different transcriptomic patterns than *S. x townsendii* indicating that genome doubling also induced gene expression changes. Most genes that are differentially expressed in *S. alterniflora* and *S. maritima* appear over-expressed in the allopolyploid *S. anglica*. The functional categories of the genes affected by these changes are examined in the light of the ecological traits of these species.

Keywords: Microarray, crosss species hybridization, transcriptome, Spartina, allopolyploid speciation

**S3-4**

**ISOLATION AND CHARACTERIZATION OF THREE *TASPL* GENES FROM WHEAT (*TRITICUM ASETIVUM* L.)**

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SQUAMOSA (SQUA) promoter-binding-like (SPL) genes encode plant-specific transcription factors, some of which contain complementary sequences of miRNA156. It had been reported that SPL genes play essential roles in the developmental process of young panicles, floral induction, and vegetative phase change.

In the Arabidopsis genome, 16 putative SPL genes that contained the SBP domain were predicted based on sequence analysis; and 19 rice (Oryza sativa) SPL (OsSPL) genes were identified in the rice genome. Up to date, no gene encoding SPL has been obtained in wheat. In this study, we reported the identification of TaSPL genes, which were predicted to be regulated by wheat miR156.

BLASTN was used to search the EST and Unigene database of wheat with miRNA156 mature sequence, and 3 Unigene clusters (Ta.3711, Ta.6374, and Ta.7012) which contain complementary sequences of the miRNA156 were obtained and predicted to be the target genes of miR156. In silico cloning combined with RT-PCR were applied to obtain cDNA sequences designated as 'TaSPL1, TaSPL2 and TaSPL3. The sequence comparison in GenBank revealed that these three genes are homologous to a group of genes encoding SPL protein in other plant species.

Transcript level analysis of TaSPL1, TaSPL2, and TaSPL3 in various wheat tissues and organs including roots, leaves, shoots, spikes, flag leaves and internodes below spike were examined. Based on the semi-quantitative RT-PCR of the non-target-site regions in 3'UTR, we found the expressions of TaSPL2 and TaSPL3 were stronger in internodes below spike than other tissues investigated. And TaSPL1 had higher expression level in young spikes than in other tissues. We also detected the expression level of wheat miR156, and the results indicated that expression of miR156 was higher in roots and flag leaves, but lower in spikes and internodes below spike. Such complementary expression patterns between miR156 and target genes suggested that the target TaSPL genes might be tempo-spatially regulated by miR156.

In addition, by use of a set of wheat aneuploids and deletion stocks, three TaSPL3 homology genes were located on chromosomes 6A (TaSPL3-6A), 6B (TaSPL3-6B) and 6D (TaSPL3-6D), respectively. The sequence comparison within 3'UTR region indicated that 7-bp and 8-bp insertion polymorphism were observed in TaSPL3-6B and TaSPL3-6D compared to TaSPL3-6A. And we found that TaSPL3-A and TaSPL3-D showed high expression levels in spikes and internodes below spike, but the expression of TaSPL3-B were low in all the examined tissues except in spikes (>10.0cm). Further analysis is still under way.

Keywords: SPL genes, wheat, gene expression, gene homology

**S3-5**

**EXPRESSION AND EPIGENETIC ALTERATION AMONG THREE HOMOEOLOGOUS GENES OF *TAEXPA1* IN HEXAPLOID WHEAT**

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Common wheat (Triticum aestivum) is a hexaploid species with A, B, and D ancestral genomes. Most common wheat genes are present in the genome as triplicated homoeologous genes derived from the ancestral species. Expansins are thought to be key regulators of cell wall extension during plant growth. Recently, we isolated 18 wheat expansin genes, and analyzed their sequence characteristics, and their expression patterns (Mol. Gen. Gent., 2005, 274: 548–556). In this study, the genomic and cDNA sequences of three TaEXPA1 homoeologous genes of bread wheat and their promoters were cloned and sequenced. The three sequences showed a very high conservation of the coding region and of the exon/intron structure, which consisted of three exons. By using nullisomics-tetrasomics and deletious lines of Chinese Spring, three homoeologs were mapped to 1AL-1-0.46-0.61?1BL-6-0.32-0.47 and 1DL-2-0.18-0.41, respectively. We analyzed their transcript levels by homoeolog-specific quantitative RT-PCR. The three homoeologs were transcribed differentially, and the ratio of the individual homoeologous transcripts to total homoeologous transcripts also varied with the tissue and developmental stage. In seedling leaves, B-genome homoeolog gene was specifically silenced, which correlates with the highest level of DNA methylation in promoter region of this gene. Interestingly, the transcripts of all three homoeologs were not detected in roots of hexaploid wheat and its diploid progenitors, indicating that the genomic bias in the transcription of the TaEXPA1 genes in roots of hexaploid wheat originated in the diploid progenitors and has been retained through the polyploidization. Furthermore, TaEXPA1 expression in roots and leaves correlates negatively with H3K9 dimethylation and positively with H3K4 Trimethylation, epigenetic marks for gene silencing and activation. In addition, ectopic overexpression of TaEXPA1 in Arabidopsis leads to enlarge the size of rosette, increase plant height and earlier transition to flowering. This study provides evidence demonstrating TaEXPA1 plays significant roles in growth and development, and three homoeologs are differentially regulated by epigenetic mechanisms.

Keywords: wheat, homoeolog,TaEXPA1,gene expression,epigenetic

**S3-6**

**EVALUATION OF TAGSK1 GENE EXPRESSION IN SELECTED WHEAT GENOTYPES IN IRAN**

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In order to determine the level of TaGSK1 gene expression in 9 selected wheat genotypes an experiment was carried out in Agricultural Biotechnology institute, university of Zabol. Nine lines of wheat (Triticum aestivum L.) were obtained from Zabol Agricultural Research, Sistan and Baloochestan, Iran. For each wheat line 10 seed were placed in glassware containing 50 mL of solid MS-medium with 25 gL-1 sucrose without any growth regulators. The medium was stalinized with NaCl to make the final concentrations of 0 and 200 mol m-3. Total RNA was extracted from 0.2 grams of leaves meristem using RNAsy Total RNA Isolation Kit (QIAGEN) according to manual company. First-strand cDNA was prepared from 120 ng of total RNA, using universal Oligo(dT)15 primer and 200 units of SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA), at 42 ° C for 1 h in a 20- mL reaction volume. Each reaction was performed on 5 mL of 1 : 100 (v/v) dilution of the first-strand cDNA, synthesized as described above, in a total reaction volume of 25 mL using SYBR Green PCR Master Mix (Applied Biosystems) and 270 nM of each forward and reverse primer.
For QPCR data, Relative expression for the GOI was determined using ??Ct method ( Livak and Schmittgen 2001). The expression of the GOI was relative to a control plant sample which was no exposed to salinity stress. Kavir line was chosen and termed the calibrator.
?Ct(sample) = Ct (GOI sample) __ Ct (Reference gene sample)
??Ct= ?Ct(sample) __ ?Ct(calibrator)
Relative expression = 2-??Ct
A different pattern of TaGSK1 transcript accumulation was found between 9 selected lines that were exposed on salinity stress and non stress conditions. The expression level for three lines including ER-salt-81-14, ER-Salt-85-12 and Mahdavi lines were at least 50% higher than other six genotypes. Also, The results of real time PCR showed that the Bam genotype has maximum level expression of TaGSK1 between 9 genotypes. Minimum expression was belonged to ER-Salt-85-17 line originated from Tehran province.

Keywords: TaGSK1, Real Time PCR, Relative Gene Expression, wheat genotypes

**S3-8**

## GENETIC BASIS OF HETEROSIS FOR FRUIT YIELD IN EGGPLANT (*SOLANUM MELONGENA* L.)

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Investigations were carried out in Department of Vegetable crops, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India to examine genetic basis of heterosis from one 9 × 9 half-diallel cross population and two sets of six standard generations from two crosses in eggplant. The 9 parents were grouped in 6 different clusters in the lot of 70 entries based on multivariate analysis of 18 growth, yield components, fruit yield and fruit quality traits. Of the 36 hybrids, positive mid-parent heterosis was manifested in 29 ranging from 0.11 to 107.35 percent and negative heterosis in 7 hybrids ranged between -3.10 to -24.87 percent, overall average heterosis being 30.19 percent. Highly significant t2 value (15.39**) indicated that epistasis along with dominance played important role in the expression of this character. Both additive, (1.22*) and dominance variance, ?1 (3.50 **) was significant although, dominant component was more than additive component which was also reflected by high degree of dominance effect (?2 = 3.03 **), i.e. sum total over all loci in heterozygous state. The environmental component of variations associated with individual means ( ) was insignificant. The mean degree of dominance ?1/ )½ = 1.69 suggesting over dominance. Symmetry of distribution of dominant and recessive alleles in the parent could also be corroborated by the significance and direction (sign) of , the mean covariance of and ?1. F = 0 means balanced distribution (p=q = 0.5). > 0 (+) means dominant alleles are more frequent than recessive alleles (p>q) and vice versa. The estimate, = -0.92 but insignificant hence, did not signify the prevalence of recessive alleles in the parents. In the present investigation, ?2/4?1 = 0.19 which is ? 0.25 meaning that positive and negative dominant genes for fruit yield/plant are almost symmetrically distributed in the parents. Number of block of dominant genes for plant height estimated by ?2/?2 ratio was 1.15. The narrow sense heritability estimate (h2NS) was very low (21.29%) indicating single plant selection as ineffective breeding strategy for improving fruit yield/plant. Results emanated from two sets of 6 genetic populations indicated insignificant additive gene action (d = -0.30, 0.20), highly significant dominant gene action (h= 1.14*, 4.17**), and significance of most of the epistatic components (i= 1.24*, 2.22*; j = -0.65*, -0.42; l = 0.21, 3.62**) for fruit yield/plant.

From both the studies it emerged that heterosis for fruit yield/plant resulted from the combination of non-linear interaction of genes at different loci and interaction between alleles at the same locus.

Keywords: Gene action, Heterosis, Yield, Eggplant

**S4-1**

**NATURE AND STABILITY OF SEQUENCES UNDERGOING METHYLATION CHANGES DUE TO ALLOPOLYPLOIDISATION IN BRASSICA NAPUS NEOSYNTHESIZED POLYPLOIDS**

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New phenotypes are usually associated with polyploid speciation and can contribute as well for the evolutionary success of polyploids as for their domestication. A previous study lead in our lab focusing on the genome methylation polymorphism within the Brassica oleracea species (one of the progenitor – with Brassica rapa – of Brassica napus) showed that this species displayed an important genome methylation polymorphism between different populations and lines representing the species diversity. One of the more striking aspects of that study was that a high number of methylated loci could be sampled using the MSAP method, adding the fact that bands can be picked and the sequences undergoing methylation state changes can be identified through sequencing and sequence analysis.
Because the polyploid species and its parental species can diverge after the polyploidisation event, it becomes difficult to identify the diploid parents that have actually contributed as genome donors. Synthetic allopolyploids allow examining the immediate consequences of hybrid genome duplication in a well-characterized genetic context. We have analyzed various progenies from resynthesized Brassica napus (obtained by crossing Brassica rapa and Brassica oleracea). Different B. napus polyploids were synthesized: from repetitive crosses between the same DH parents, from reciprocal crosses, from different ways of production (by colchicine doubling after hybridization and by unreduced gametes). To test the stability of genome methylation state changes and fix changes due to allopolyploidisation, each polyploid (S0 generation) was self-crossed, and 3 to 5 plants of S3 generations were studied for each progeny. The goals of this study were to: (i) identify changes of genome methylation state due to polyploidisation depending on parental genetic background, the way of production of polyploids, and the origin of the cytoplasmic genome of neosynthesized polyploids; (ii) identify the nature of the genomic regions undergoing methylation state changes.
We used the MSAP (Methylation Sensitive Amplification Polymorphism) method in order to compare genome methylation of the homeologous genomes present in the allopolyploid, to their parents by surveying non-additive patterns. The parental genomic fragments that underwent methylation state changes in polyploids were cloned and sequenced. Most of these regions (27/50 fragments) displayed homology to coding (rather than non-coding) sequences. Some of the identified genes could have important adaptive significance (e.g. SRK gene from the S locus, and MBD9, an inhibitor of FLC).

Keywords: Neosynthesized polyploids; Cytosine methylation; MSAP; Phenotypic diversity; Polymorphic methylation patterns

**S4-2**

**ROSA GENOTYPES INVOLVED IN THE EMERGENCY MODERN ROSE THROUGH HUMAN SELECTION: CYTOGENETIC APPROACH**

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For Roses, long flowering period and perfume are prized characters. The human selection led in the 1900s to Modern Roses showing the two characters.
We describe here a cytogenetic approach of some Rosa genotypes involved in the emergency of the Modern Roses.
The aim of the work is to assess for historic strategic crosses what genomic parts were inherited in interspecific hybrids.

Keywords: Rosa, FISH, GISH, biodiversity

**S4-3**

**ALLOPOLYPLOIDY IN THE SPHAGNUM SUBSECUNDUM COMPLEX**

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In the genus Sphagnum there are several complexes of species that originated through hybridization and allopolyploidy, suggesting that these processes have played a major role in evolution and speciation in this genus. The Sphagnum subsecundum complex includes seven species in North America and Europe: three haploids and four diploids.
An analysis of genome size, chloroplast DNA sequences (trnG and trnL), and 16 microsatellite markers was carried out to clarify the evolution of polyploidy in the S. subsecundum complex in North America. Our results show that the "diploid" species contain both haploid and diploid populations that are morphologically indistinguishable. Chloroplast DNA variation suggests that haploid S.lescurii is likely the maternal parent of the diploids. Microsatellite markers also reveal that S. subsecundum was the male parent of the other North American diploids.

Data of a comparative study of the mating structure between haploid and diploid S. lescurii in a population in Wake Co., NC, USA, will be also presented. Preliminary analyses show patterns of mixed mating, that the diploids are not derived from the local haploids, that there is evidence of some interploidal hybridization and that the average number of sporophyes and diameter of sporophyes bear by a haploid and diploid females differ.

Keywords: allopolyploidy bryophytes sphagnum matting

**S4-4**

**ALTERATIONS IN SMALL RNA SPECIES FOLLOWING HYBRIDIZATION AND POLYPLOIDIZATION IN WHEAT**

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In the past few years, small RNA molecules have been assigned roles in epigenetic as well as genomic changes in several species. To investigate the role of small RNAs in the genetic and epigenetic changes that have been reported in hybrid and polyploid wheat, we performed a high-throughput screen of small RNA expression in parental tetraploid Triticum turgidum var. durum and diploid Aegilops tauschii species, and in their derived synthetic triploid hybrid and hexaploid, using the Illumina Genome Analyzer. A total of more than 18 million reads were obtained from leaves of one month-old plants. Of these we analyzed about 6 million high quality reads, which corresponded to 21,787 unique sequence tags. The small RNAs sizes ranged between 18 and 32 with the two prominent classes of 21 and 24 nt length.
We aligned the small RNAs sequences to the wheat genome, namely, to wheat ESTs and wheat repeats and transposons databases, as well as to the rice genome, to the NCBI nucleotide database and to Sanger miRBase. We then assigned the small RNAs into the following classes according to their matches: genomic sequences, genes, repeats, micro RNAs, ribosomal RNAs, transfer RNAs, and non-annotated sequences. In order to assess the changes in the abundance of small RNAs that occur in the hybrid and in the polyploid, we compared the average number of hits corresponding to a given RNA, between the two parental libraries (the mid parent value (MPV)) to the number of hits in the hybrid or the polyploid.
Micro RNAs represented 30-45% of the total hits, depending on the library. On the average there was an increase in the amount of microRNAs in the polyploid. The most abundant microRNA was miR168. It is a regulator of Argonaute 1, and is up-regulated 2.7 fold in the polyploid compared to the MPV. Conversely, small RNAs that match repeats and transposons are strongly down regulated in the polyploid compared to the mid parent value and to the hybrid. For example, small RNAs that match the retrotransposon Wis2-1A are represented by several hundred reads in the parental and in the hybrid library, whereas they are not present at all in the polyploid library. This major decrease is consistent with the previously described transcriptional activation of Wis2-1 in the first generation following polyploid formation. These data supports the role of siRNAs as mediators of epigenetic changes that occur upon polyploidization, in particular, as shown here, in the activation of heterochromatin and transposons. We are further validating the involvement of small RNAs in gene expression regulation.

Keywords: polyploid, hybrid, microRNA, siRNA, epigenetic alterations

**S4-5**

**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.

Keywords: epigenetics, complex traits, heritable variation

**S4-6**

**ORIGIN AND GENOMIC CONSEQUENCES OF LOSS OF SEXUALITY IN PLANT-PARASITIC NEMATODES OF THE MELOIDOGYNE GENUS**

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Meloidogyne incognita is a widespread and polyphagous obligate asexual endoparasite of plants that causes serious and growing problems to agriculture. In the M. genus, closely related sexual species, such as M. hapla, have a narrower host range and geographic distribution than M. incognita. Therefore, adaptation of M. incognita to various environments and host plants may be due to its peculiar asexual mode of reproduction. This mode of reproduction may confer an unexpected plasticity and the observed unusual structure to its genome. Indeed, the whole genome sequence of this nematode revealed the co-existence of two highly divergent copies within individuals for the majority of the genome. Two hypotheses are currently considered to explain the presence of these two genomic copies. The first hypothesis proposes that the two divergent genomic copies represent former haplotypes that have been evolving independently one from the other in the absence of sexual recombination (the "Meselson effect"). Under this hypothesis, the two copies derive from paternal and maternal haplotypes from an ancient sexual ancestor that lost sexuality a sufficiently long time ago, allowing considerable divergence between former "allelic" regions. This mechanism has already been demonstrated in other asexually-reproducing eukaryotes like rotifers. The second hypothesis suggests that the two copies result from a hybridization event between two distinct species that gave rise to asexual hybrids. In this case, initial divergence between each copy could have increased toward the currently observed high level also by independent accumulation of mutations. Whatever the true evolutionary scenario, we suggest that the existence of the two divergent genomic copies may have provided the required pool of genetic variability for adaptation to various different environments and wide host spectrum. Mechanisms of genome evolution and adaptation in asexually-reproducing animals are still poorly understood and M. incognita is the first such metazoan whose whole genome is available. This species is hence, an ideal starting model to analyze the relation between asexula mode of reproduction, genomic alterations and success of parasitism. In addition to the genome sequence of M. hapla, genome sequencing of two additional relatives, M. javanica and M. arenaria, also known to only reproduce asexually, has been initiated by our team. Soon, this situation will allow identification of conserved syntenies duplicated genomic regions and reconstruction of ancestral genomic architectures in this genus, offering an unparalleled opportunity to study the consequences of loss of sexuality in animals.

Keywords: Loss of sexuality, Meselson effect, Hybridization

**S4-7**

**INTERCHEMOTYPIC HYBRIDIZATION FOR ELITE GENOTYPE DESIGNING IN WITHANIA SOMNIFERA (L.) DUNAL**

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Withania somnifera (2n = 4x = 48) commonly known as ashwagandha, is a plant of immense medicinal value. Out of the total germplasm collected from different parts of India, two morpho-chemically distinct groups were identified. Floral biology, pollination behaviour, breeding system and reproductive effort of Withania somnifera (family: Solanaceae) was studied in two elite contrasting chemotypes. The species reproduces sexually through seed. It is predominantly a self pollinating and self compatible species; pollination being predominantly autogamous. Autogamy is accomplished as a consequence of synchronicity of stigma receptivity and anther dehiscence. Autogamy/open pollination results in the higher fruit set and seed out put as well as seed germination. In the present study experimental hybridization of the best performing chemotypes AGB002 (a selectant from wild populations) and AGB025 (cultivated in restricted places as a commercial crop) was performed and the hybrids developed showed positive heterosis with respect to the root biomass and withaferin A content. Hybridization studies showed distinctness between these two chemotypes as evident by low fruit and seed set (5-10 %). Nuclear and chloroplast genome assays of the two populations by AFLP, internal transcribed sequence – cleaved amplified polymorphic sequence (ITS-CAPS), chloroplast DNA-CAPS, and cloning and sequencing of ITS region of ribosomal DNA reinforced the conclusions based on crossability, morphological, pharmacognostical and chemical characterization data.
The quantitative dynamics of Withaferin A production in Indian populations and interchemotypic hybrids developed have been studied. An analysis on inheritance pattern based on presence/absence of Withaferin A in hybrid plants and their respective parents is given for future studies on the chemogenetics of this complex species in details.

Keywords: Withania somnifera, chemotypes, hybridization, AFLP and ITS-CAPS**.**

**S5-1**

**DIVERSITY AND RELATIONSHIPS IN DIPLOID AND TETRAPLOID NATURAL POPULATIONS OF THE HORDEUM MURINUM L. COMPLEX IN NORTH-AFRICA**

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The genetic diversity of wild populations of the specific complex Hordeum murinum L. *s.l.* from Algeria was analysed using phenotypic, cytogenetic and molecular approaches. Sampling of natural populations was performed in a large range of bioclimatic conditions.

Two diploid, 2n=2x=14 (ssp. glaucum) and tetraploid, 2n=4x=28 (ssp. leporinum) cytotypes were encountered. One of the striking results is the existence in Algeria of a strong correlation between the distribution of 2x/4x cytotypes and the North-South bioclimatic gradient. The frequencies of the diploids increase with aridity and their chromosome meiotic behavior was regular with mainly ring bivalent configurations. Tetraploids had also a regular meiosis, however in some mountainous populations, pairing irregularities were frequent, with relatively elevated rate of tetravalents suggesting an autopolyploid origin. Moreover, chromatin bridges at late anaphase I and telophase I suggest the presence of spontaneous structural rearrangements that would represent a significant source of diversity and adaptation in these areas.

Morphological differentiation among the three subspecies (ssp. glaucum, ssp. leporinum and ssp. murinum) is encountered. Combination of hordein and randomly amplified DNA data reveal consistent polymorphism. Hordein variability is strongly correlated with genetic diversity and bioclimatic parameters. Of particular interest is the differentiation of diploid populations (ssp. glaucum) that is associated with marginal distribution and stressful ecological conditions (drought, salinity). Results are discussed in the context of the controversial nature of polyploidy in this complex.

Cytological and molecular markers that correlate to environmental conditions is of high interest in the perspective of selecting adapted genotypes and for conservation genetics.

Key words**:** Hordeum murinum, natural populations, polyploidy, Algeria**.**

**S5-2**

## CHROMOSOMAL MEIOTIC BEHAVIOR IN NATURAL POPULATIONS OF DACTYLIS GLOMERATA L. FROM ALGERIA

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Meiotic behavior of natural diploid and tetraploid populations of Dactylis glomerata L. was investigated. Populations were sampled from forty five localities in different ecological conditions of Northern Algeria. In diploid populations (2n=2x=14), occurrence of high rate of bivalents (6.7) and chiasmas per cell (11.92), indicated that the meiosis can be regarded as stable. However, some diploids populations are characterized by unexpected rod bivalents and B chromosomes. Meiosis in tetraploid populations (2n=4x=28) exhibits also regular configurations. The constant incidence of tetravalent (2.18 per cell) can be related to the autopolyploid nature of this complex. Moreover many abnormalities were observed like univalents, lag chromosomes, chromatin bridges (at anaphase I and telophase I), asynchronous divisions and micronuclei (at anaphase II, diads and tetrads). Structural heterozygosity can be at the origin of these irregularities. The meiotic behavior is discussed in the light of the correlation with the biogeographic conditions highlighted in the natural populations.

Keywords: meiotic configurations, polyploidy, Poaceae, Dactylis glomerata

**S5-3**

## ON MEIOSIS IN BRASSICA NAPUS HAPLOIDS : STAY SINGLE OR DIVORCE ?

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Precise control of chromosome pairing and homologous recombination are essential in sexually reproducing polyploids for conferring good chromosome segregation, genome stability and plant fertility. In most polyploidy species, this control has a strong genetic basis but little is know about the genes and cytological mechanisms that are responsible for the restriction of crossover formation to homologues only.
This study examines meiosis in Brassica napus haploids (AC, 19 chromosomes) in which the level of homeologous recombination is genetically determined by a major gene, PrBn, segregating in a background of polygenic variation. We provide an accurate and comparative description of Prophase I in two haploid genotypes showing different PrBn activities; one haploid shows a lot of univalents at Metaphase I (Yudal) and the other shows few univalents at MI (Darmor-bzh). Different cytological analyses were combined to provide information on the relative degree of completion of some important meiotic steps, and notably on the extent to which synapsis (intimate association of chromosomes at pachytene) is formed in these two genotypes. We observed that both Yudal and Darmor-bzh haploids undergo synapsis but our results suggest that Yudal haploids show less synapsis than Darmor-bzh haploids. These results are important to decipher the cytological mechanisms that contribute to reduce homeologous recombination in some B. napus haploid genotypes compared to others.

Keywords: B. napus haploid, meiosis, homeologous recombination

**S5-4**

**CHROMOSOME PAIRING IN FESCUE SPECIES ( FESTUCA SPP.) AND THEIR HYBRIDS WITH RYEGRASS SPECIES (LOLIUM SSP)**

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Diploidizing chromosome pairing systems prevent meiotic irregularities and provide for normal fertility in polyploids. While the nature of these systems is known in some polyploid crops including wheat, little is known about the control of chromosome pairing in polyploid fescues (Festuca spp.). Here, we analyzed chromosome pairing in allohexaploid F. arundinacea, its progenitors F. pratensis and F. glaucescens, and intergeneric hybrids L. multiflorum (2x) × F. arundinacea (6x) and L. multiflorum (4x) × F. glaucescens (4x), F. pratensis (4x) × L. multiflorum (4x) and F. pratensis (4x) × L. perenne (4x). To evaluate pairing of individual chromosomes, we analyzed tetraploid lines of L. multiflorum with monosomic or disomic substitution from F. pratensis. Genomic in situ hybridization (GISH) permitted the analysis of homoeologous chromosome pairing and recombination of different genomes involved. Diploid-like pairing system was apparent in polyploid fescues, F. arundinacea and F. glaucescens, the latter being one of the progenitors of F. arundinacea. Such pairing control system was absent in the second progenitor – F. pratensis. Detailed analysis of intergeneric hybrids confirmed the presumed haplo-insufficiency of the fescue diploidizing system, which resulted in homoeologous pairing between all component genomes. This indicated that introgressions of any specific chromosome segments from one genome to another are possible in all combinations. In the substitution lines of L. multilforum, the choice of a MI pairing partner for any F. pratensis chromosome depended on the identity of the remaining chromosomes present in the quadruplet. In monosomic introgressions, the choice of a homologous or homoeologous pairing partner was completely random; in disomics there was a slight preference for homologous pairing. Pairing preference was similar for each chromosome, suggesting that pairing affinity of all chromosomes is essentially the same and that there are no major structural differences differentiating the homoeologues. High MI pairing in these hybrids could be due to either a very permissive chromosome pairing control system or little divergence of DNA sequences responsible for pairing partner recognition and crossing over. These results may have serious implications for the design of hybrid breeding schemes in forage grasses. This work was supported by the Ministry of Agriculture of the Czech Republic (grant award NAZV QH71267) and the Czech Science Foundation (grant award 521/07/P479).

Keywords: Festuca, meiosis, GISH, interspecific hybrids, Lolium

**S5-5**

**UNREDUCED POLLEN FORMATION AND FERTILITY ESTIMATES IN HYBRIDS BETWEEN CROP WHEAT AND WILD RELATIVES**

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One of the main constraints of allopolyploid formation is hybrid sterility caused by meiotic irregularities that lead to aneuploid unviable gametes. The most common way to restore fertility in hybrids is the production of unreduced (2n) gametes which transmit the whole chromosome complement of individuals to their offspring. Several mechanisms of nuclear restitution may be involved 2n-gamete formation but in interspecific hybrids it seems to be related to the lack of meiotic pairing between parental genomes. Both genotypic and non-genotypic factors are known to affect the production of unreduced gametes.

We have estimated the rate of 2n-pollen formation and fertility in different hybrid genotypes between T. turgidum and the wild species Aegilops geniculata, Ae. triuncialis, Ae. neglecta, Ae. ventricosa and Ae. cylindrica, and also in hybrids between T. aestivum and Ae. geniculata. Nuclear restitution promotes the formation of dyads at the end of meiosis. Then we first estimated the rate of unreduced meiosis by counting dyads at the tetrad microspore stage using aceto-carmine staining. Bigger pollen size is also an indicator of the production of unreduced gametes since the volume of unreduced pollen grains will be approximately twice the usual volume of a pollen grain. Based on this assumption we estimated 2n-pollen viability in mature anthers stained with Alexander as the rate of giant stained grains. Then, these two estimations were compared with seed set after selfing, as a representative parameter of hybrid fertility.

A wide variation in the frequency of dyads and giant viable pollen grains was occasionally found between anthers from the same hybrid plant that had been sampled simultaneously. In agreement with that, seed set of some hybrid genotypes greatly differed from one harvest to another. All that evidenced the influence of uncharacterized non-genotypic factors in unreduced gamete formation. Self-fertility was generally low, but 5-10 seeds per 100 spikelets were formed in some hybrid genotypes. The analyses of dyads at the end of meiosis and mature pollen after Alexander's staining resulted useful to predict that a hybrid was fully sterile. However, some hybrids produced no seeds despite some fertility had been expected according to the unreduced pollen estimates. In those cases, which mainly affected durum wheat hybrids, we observed a decrease in 2n-pollen production from tetrads to pollen stage.

Keywords: hybrid fertility, unreduced gametes, wheat, Aegilops ssp.

**S5-6**

## GENOME SIZE INHERITANCE IN INTRASPECIFIC CROSSES WITHIN DIPLOID AND WITHIN TETRAPLOID PLANTS OF *FESTUCA PALLENS* VARYING IN GENOME SIZE

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Intraspecific variation in genome size is one of important phenomena making possible to study early phases of genome evolution in species. Compared to genomic methods, measurement of genome size by flow cytometry is fast and cheap, and may cover hundreds of plants, as needed in any study of micro-evolutionary processes in natural populations. By the study of these processes, a special attention is paid to the heritability of a newly appearing character which determinates its survival and establishment in a population or further in a separate species.

We tested the inheritance of genome size by reciprocal crosses between diploid (10 pairs) and between tetraploid (10 pairs) plants of Festuca pallens (Poaceae) differing in genome size (not caused by the presence of B-chromosomes). We have shown that differences in genome size within species do not constitute a reproduction barrier. The progeny of some maternal plants was shown to significantly vary in genome size (up to 1.081-fold, and even if the parental genome sizes were similar), which indicates that some variation in genome size is probably produced during the gametogenesis. In reciprocal crosses of diploid plants, the genome size found in progenies ranged between the sizes of parents, and only occasionally a weak maternal effect was detected. In the progenies from crosses between tetraploid plants, significantly higher variation in genome size was observed, regularly exceeding the range of parental genome sizes.

Our data indicate a very weak inheritance and easy induction of genome size variation in Festuca pallens, which implies that the establishment of a population (or possibly also of a new species) with different genome size may be a result of a random event (e.g., bottleneck or founder effect) rather than a result of an adaptive process. In this respect, a stronger adaptivity effect may be assumed in diploids.

Keywords: grasses, genome size evolution, adaptivity, polyploidy

**S5-7**

**PHENOTYPIC AND GENETIC ANALYSIS OF THE VARIATION IN REPRODUCTIVE EFFORT OF TRIPLOID PACIFIC OYSTERS (CRASSOSTREA GIGAS).**

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Triploidy is commonly used to improve bivalve aquaculture production. In Pacific oyster, it leads to partial sterility, contributing to higher growth and increased survival through energy reallocation. It also improves meat quality during the reproductive period. However, reproductive effort in triploid oysters is variable, ranging from complete sterility to normal gametogenesis (i.e. similar to diploid). In this case, triploid oysters can produce competent gametes, although these mostly give aneuploid larvae with very low survival.
We studied reproductive effort and growth in related diploid and triploid oysters (3 groups of 96 mixed families: diploids, chemically-induced triploids and triploids from 4n x 2n crosses). Comparison of reproductive patterns between groups revealed a lower (-47%), but still significant, reproductive effort in triploid groups relative to the diploid group, showing partial blockage of germline maturation between the gonial and cyte stages. A significant relationship was observed between gender and body mass within each group, and between gender and gonadic occupation in diploids only, suggesting that the link between sex, reproductive allocation and food acquisition varies between diploids and triploids.
Microsatellite-based pedigree analyses and individual models were used to study genetic parameters of growth and gonad development in the groups and changes in family value between groups. Significant co-variation between reproductive effort and growth in diploids, together with low heritability estimates, suggests that these traits are mainly under environmental control (i.e. food availability). Ranking of half-sib families for reproductive allocation was significantly modified in triploids compared with diploids. Ranking for growth rate was conserved, although the proportion of offspring phenotypic variance explained by the female component co-varied with its contribution to the offspring genome (i.e. 1/2, 2/3, 1/3 for 2n, 3nCB and 3nDT groups, respectively).
To assess whether reproductive effort can be modified by directional selection, we also produced divergent diploid and triploid progenies, using selected diploid breeders with contrasted reproductive effort. A significant response to selection was shown in 1-year-old oysters, revealing a positive diploid-triploid covariation for allocation to reproduction, which suggests a common genetic basis for reproductive effort in these two types. Our results offer new possibilities for investigating reproductive physiology and resource allocation in triploid oysters. Outcomes will also be discussed in terms of aquaculture and the environment

Keywords: triploids, oysters, reproductive effort, genetic determinism

**S5-8**

**TETRAPLOID SEGREGATION INTERMEDIATE BETWEEN DISOMIC AND TETRASOMIC: A GENERAL LIKELIHOOD-BASED MODEL**

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Tetraploid inheritance has two extremes: disomic in allotetraploids and tetrasomic in autotetraploids. The possibility of mixed, or intermediate, inheritance models has generally been neglected. These could well apply to newly formed hybrids or to diploidizing (auto)tetraploids. We present a simple likelihood-based approach that is able to incorporate disomic, tetrasomic, and intermediate inheritance models and estimates the double-reduction rate. Our model shows that inheritance of microsatellite markers in natural tetraploids of Rorippa amphibia and R. sylvestris is tetrasomic, confirming their autotetraploid origin. However, in F1 hybrids inheritance was intermediate to disomic and tetrasomic inheritance. Apparently, in meiosis, chromosomes paired preferentially with the homolog from the same parental species, but not strictly so.

Detected double-reduction rates were low. We tested the general applicability of our model, using published segregation data. In two cases, an intermediate inheritance model gave a better fit to the data than the tetrasomic model advocated by the authors. The existence of inheritance intermediate to disomic and tetrasomic has important implications for linkage mapping and population genetics and hence breeding programs of tetraploids. Methods that have been developed for either disomic or tetrasomic tetraploids may not be generally applicable, particularly in systems where hybridization is common.

Keywords: tetraploid segregation, meiotic pairing, tetrasomic inheritance, disomic inheritance, polyploid evolution

**S5-9**

**EVIDENCE OF UNREDUCED GAMETE PRODUCTION FROM INTERSPECIFIC CROSSES BETWEEN GOSSYPIUM HIRSUTUM AND G. HERBACEUM**

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In order to analyze the gene flow between the allotetraploid cultivated cotton (Gossypium hirsutum, AADD, 2n=52) and the wild diploid (G. herbaceum, AA, 2n=26), the possibility of natural hybridization between these two cotton species has been investigated. In fact, in South Africa and particularly in the KwaZulu Natal Province, where commercialisation of transgenic Bt cotton began in 1998, a wild species (G. herbaceum) is neighbouring from the cultivated cotton fields.
From reciprocal crosses performed without emasculation between G herbaceum used as female or male and G hirsutum, 148 and 335 plants respectively, have been analyzed. Neither examination of the morphological characteristics nor the flow cytometry analysis of the 335 plants resulting from the cross G hirsutum x G herbaceum, have shown any to be hybrid plants. For the cross G herbaceum x G hirsutum, three plants have shown a hybrid phenotype. Analysis of DNA content by flow cytometry and morphological traits, have clearly shown that two of them were triploid (AAD). The third one exhibited a value in flow cytometry slightly higher than G hirsutum. In addition some morphological characteristics (plant morphology, presence and size of petal spots, leaf shape...) have led us to conclude that this plant is AAAD and was the result of a fecundation with unreduced gamete AA from the female *G herbaceum* parent. Fluorescent In Situ Hybridization (FISH), and meiotic behaviour have confirmed this hypothesis. This is, to our knowledge, the first description of the occurrence of a non-reductional meiosis in the species G. herbaceum. This plant material could provide a useful tool for the study of the expression of genes duplicated in the A and D cotton genome. The possibility of obtaining an interspecific hybrid between cultivated and diploid wild cotton through fertilization with an unreduced gamete raises the question of its evolution in natural populations.

Key words: gene flow, G hirsutum, G herbaceum, meiosis, unreduced gamete

**S7-1**

**MOLECULAR PHYLOGENY AND HYBRIDIZATION OF LINEAR-LEAVED PONDWEED SPECIES (POTAMOGETON, POTAMOGETONACEAE)**

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Potamogetonaceae are a family of aquatic plants with worldwide distribution. They play an important role in wetland ecosystems. Their taxonomy and systematics is challenging, because they are characterized by high morphological plasticity and rather few characters many of which are reduced in adaptation to the aquatic habitat. This is especially true for a group of linear-leaved species which is therefore in great need of revision. Two major ploidy levels occur in Potamogeton: (i) diploid with 2n=26 (mainly) or 2n=28. All linear-leaved species are diploid, but broad- leaved species also comprise diploid taxa; (ii) tetraploids with 2n=4x=52. They are broad-leaved and presumably of autopolyploid origin. A large part of Potamogeton speciation is thought to have occurred at the tetraploid level. Tetraploid genomes appear to be 'diploidized', and multigene sequences are homogeneous. Hybrids are usually sterile. They mostly occur as homoploid hybrids at the diploid or the tetraploid level, but triploid hybrids resulting from heteroploid crosses are also known. Hybrids can persist vegetatively for a long time and are at some sites a significant component of aquatic communities. To assist species delimitation and to understand linear-leaved species relationships, we used phylogenetic analyses of the nuclear ribosomal ITS region and the chloroplast trnT-trnL intergenic spacer. Hybrid accessions were identified according to character additivity in direct ITS sequencing, and their maternal parent was determined according to cpDNA haplotype. Phylogenetic analyses of that region showed the linear-leaved species as monophyletic, and several subclades were well-supported. Hybrid haplotypes matched one of the parents inferred from ITS sequences. In contrast, the linear-leaved species were not monophyletic according to ITS, but embedded with predominantly tetraploid broad-leaved species. This marker was probably not sufficiently variable to resolve basal relationships. It is also possible that several tetraploid lineages arose independently from diploid ancestors. ITS subclades / cpDNA haplotype groups were basically the same with both markers. With respect to species relationships, the following patterns were found: (i) most species appeared as monophyletic entities with one or both markers, (ii) some species were polyphyletic, partly coinciding with geographic area and morphological differences, (iii) some species shared the same ribotype/haplotype indicating that genetically more or less identical species could show diagnostic morphological differences. The latter two patterns are difficult to reconcile with any satisfactory taxonomic treatment.

Keywords: Potamogeton, molecular phylogeny, hybridization, ITS, trnT-L

**S7-2**

## MOLECULAR CHARACTERIZATION OF THE TRANSGENE PGIC2

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We have previously reported the instance of a horizontal gene transfer between two grass species, F. ovina and a Poa. We want to know how much genetic material was transferred and how it happened. Genome walking has been performed in order to obtain sequence information upstream of the transgene, and we can see that for the first 1000 bp both donor species and transgenic species are the same. After that in the transgenic species we find sequences who show similarities to known transposable elements.

Keywords : transgene, PgiC, grass

**S7-3**

**EVOLUTIONARY HISTORY OF THE POLYPLOID ANTHOXANTHUM ODORATUM COMPLEX (POACEAE) IN EUROPE**

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Genus Anthoxanthum L. s.s. (Poaceae: Pooideae: Aveneae) comprises 15 species distributed mainly in the temperate and arcto-alpine regions of Europe, Asia and Africa. Eight Anthoxanthum (vernal grass) species have been described in Europe, among them five (or six) perennials (belonging to the complex of Anthoxanthum odoratum) and three annuals (A. gracile Biv., A. aristatum Bois. and A. ovatum Lag.). While all annual taxa are diploids (2n = 10) restricted to the Mediterranean area, perennials are both diploids and polyploids occuring either locally or throughout Europe.

Although allotetraploid origin of Anthoxanthum odoratum was repeatedly suggested, clear evidence has not yet been given. Our data based on genome size (using flow cytometry) as well as molecular (cpDNA sequencing) analyses corroborate such theory. The most likely scenario seems to be a hybridization between diploid A. alpinum and "mediterranean diploid" followed by polyploidization. Existence of putative diploid ancestor in the Mediterranean area (so far described as "mediterranean diploid") was predicted and partially supported in previous works, but it has been confirmed for the first time in our study. This diploid taxon is relatively common in Montenegro, Croatia, Italy and France (Corse) and its occurrence elsewhere in the vicinity of the Mediterranean Sea is very likely (Greece, continental France, etc.).

Clearly separated genome sizes (Cx-values) allowed us to objectively distinguish all taxa from the A. odoratum complex and therefore estimate their distribution area, morphological differences as well as species specific molecular markers.

Chloroplast DNA sequence data (trnL-trnF) revealed existence of two major haplotype groups within tetraploid A. odoratum s. str., the first of them shared with diploid A. alpinum and the second one with "mediterranean diploid", but without any obvious geographical structure.

Keywords: allopolyploid; hybridisation; Anthoxanthum

**S7-4**

## UNRAVELLING MULTIPLE CYCLES OF HYBRIDIZATION AND POLYPLOIDIZATION IN THE EVOLUTIONARY HISTORY OF MELAMPODIUM SERIES LEUCANTHA (ASTERACEAE)

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An interesting group to study processes associated with origin and evolution of polyploid genomes is the so-called white-rayed complex of the genus Melampodium (Asteraceae), whose evolutionary history has been associated with Pleistocene climate changes involving repeated cycles of hybridization and polyploidisation. It comprises three xerophytic species (M. argophyllum, M. cinereum, M. leucanthum) distributed in northern Mexico and the southern United States. Based on x = 10, both M. cinereum and M. leucanthum comprise both diploid and tetraploid cytotypes of likely recurrent autopolyploid origin. In contrast, the exclusively hexaploid M. argophyllum has been hypothesised to be of allopolyploid origin involving M. cinereum and M. leucanthum. The conspicuous restriction of tetraploids of M. cinereum and M. leucanthum to the eastern part of the species' distribution areas to the near exclusion of diploids as well as their genetic cohesiveness suggest that they may be on the verge of speciation.
We applied several plastid and nuclear sequence markers as well as AFLPs, complemented by genome size and cytogenetic data (i) to infer the phylogenetic relationships among the three species; (2) to assess the role and extent of hybridization and gene flow as well as the mode of polyploidisation; and (3) to analyse the type and the extent of genome changes, concerning karyotype structures, genome size, as well as rDNA number and localisation, in polyploids of presumably different origination modes (auto- and allopolyploids).
AFLP data clearly show that all three species constitute well separated and distinct gene pools. Whereas currently no hybrids between any of the species are known, sequence data, in particular plastid sequences and nrITS restriction patterns, show that introgression of M. leucanthum into M. cinereum has occurred repeatedly, but obviously it did not negatively affect species integrity. Polyploids in M. cinereum and M. leucanthum are of autotetraploid origin and originated recurrently, and show no significant changes in genome size or karyotype. While the evidence for ancient hybridisation between M. cinereum and M. leucanthum as well as the observed high ploidy level agree with the hypothesis of an allopolyploid origin of M. argophyllum, its phylogenetic position in the plastid and several of the nuclear markers suggests that M. argophyllum is sister species to the other two taxa and consequently of autopolyploid origin, possibly from M. cinereum or its ancestor, with subsequent introgression from M. cinereum. In agreement with its older age, M. argophyllum shows genome downsizing.

**Keywords: allopolyploid, autopolyploid, Melampodium, recurrent origin, Asteraceae**

**S7-5**

**THE ROLE OF POLYPLOID EVOLUTION IN FLOWERING PLANTS: A CASE STUDY FROM THE ALPINE SPECIES PRIMULA MARGINATA**

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Hybridization and polyploidization are frequent phenomena in angiosperms and they have been estimated to be responsible for 2-4% of speciation events. In particular, two different kinds of polyploidization may occur: autopolyploidization and allopolyploidization. Many plant species are thought to be allopolyploids that originated through a hybridization event followed by genome duplication. The occurrence of different chromosomal races at different ploidy levels within a species has also been reported. Primula marginata Curtis (Primulaceae), an endemic species of the Western Alps, represents an ideal group to study the role of polyploidization in plant evolution, because two different cytotypes (2n=6x and 2n=12x) are known to exist, but the mode of origin of these races (autopolyploidy vs. allopolyploidy) has never been investigated. Therefore, a study was undertaken in order to assess whether the two different cytotypes form two distinct phylogenetic entities (i.e. clades). Preliminary results suggest that P. marginata with 2n=12x is an allopolyploid, probably derived from hybridization between hexaploid individuals of P. marginata and P. latifolia. However, these preliminary results remain inconclusive, and multiple lines of evidence must be brought together to assess whether P. marginata 12x is an auto- or allopolyploid. We therefore aim at elucidating the nature of the different cytotypes of P. marginata by analysing phylogenetic, cytological, morphological and ecological data.

Keywords: autopolyploidy, allopolyploidy, speciation, Primula

**S7-6**

## SEXUAL REPRODUCTION AS A SOURCE OF PLOIDY LEVEL VARIATION IN AGAMIC COMPLEX OF HIERACIUM SUBGENUS PILOSELLA (H. PILOSELLA AND H. BAUHINI AS A MODEL SYSTEM)

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Apomixis is clonal reproduction through seeds. Nevertheless, when it is combined with sexual reproduction it can lead to the formation of intricately structured agamic complexes characterized by a huge diversity in morphology, ploidy level, mode of reproduction, and degree of hybridity. Study of recent hybridization in young developing agamic complexes, e.g. Hieracium subgen. Pilosella, can improve our understanding the role of sexual reproduction in the diversification within the complex. Model population from vicinity of Valov (NW Bohemia, Czech Republic) consisted of 4x sexual H. pilosella and 6x apomictic H. bauhini (parental species), and their 5x, 7x, and 8x hybrids. Crosses between parental species were performed to quantify their potential to produce ploidy level variation. Flow cytometry revealed significant ploidy level variation in the progeny, showing that reduced as well as unreduced gametes of both parents participated in crosses. Progeny from the cross where H. pilosella served as a maternal parent (273 plants) consisted of 4x progeny from autogamy (18.3%) and of 5x hybrids (81.7%). Progeny from the cross where H. bauhini served as a maternal parent (821 plants) consisted of apomictically derived 6x progeny (93.2%), of three types of hybrids (5x - 4.8%, 7x - 0.1%, and 8x - 0.7%), and of 3x parthenogenetic progeny (1.2%). To quantify ploidy level variation really formed in the field, ploidy level was estimated in the progeny that was obtained from seeds collected from parental species at the locality. Progeny of H. pilosella (317 plants) consisted of 4x progeny from auto/allogamy (98.7%) and of 5x hybrids (1.3%). Progeny of H. bauhini (486 plants) consisted of apomictically derived 6x progeny (92.4%), of four types of hybrids (5x - 2.7%, 7x - 0.4%, 8x - 2.9%, and 10x - 0.4%), and of 3x parthenogenetic progeny (1.2%). Hybridization between H. pilosella and H. bauhini generated significant variation in ploidy level under both experimental and field conditions. Nevertheless, it appears that only a part of this potential variation can influence ongoing evolution in the population as neither 3x, nor 10x adult plants were detected in the field. Some selection disadvantage of these cytotypes can be suggested. When regarded as maternal parents, sexual H. pilosella gave rise to significantly lower ploidy level variation than apomictic H. bauhini, and the proportion of 5x hybrids produced by H. pilosella was much lower in the field than in experiment. The apomictic species thus appears to be better source of ploidy variation in population, evidently due to it's ability to take advantage of diverse mode of reproduction.

Keywords: Hieracium, H. pilosella, H. bauhini, apomixis, polyploidy

**S7-7**

**DOMESTICATION HISTORY OF A HEXAPLOID, THE SWEET POTATO (IPOMOEA BATATAS (L.) LAM.)**

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Despite the importance of sweet potato as a food crop, its evolutionary history has been poorly investigated. The geographical and botanical origins of sweet potato remain unclear. Sweet potato is in the section Batatas of the genus, which also includes 13 wild relatives, almost all endemic to the Americas. I. batatas is not known in the wild state. Morphological and genetic analyses indicate that I. trifida is sweet potato's closest wild relative, but the genomic composition of I. batatas is still debated. It is still unclear whether this hexaploid is auto-, allo- or auto-allopolyploid. The range of I. trifida extends from northern Peru to Mexico, and the assumed region of origin of I. batatas is somewhere within this vast geographical area. I. trifida forms a complex of ecotypes of differing ploidy levels (diploids to hexaploids), but the distribution and origin of populations of varying ploidy levels are not documented. Morever, no genetic studies have been conducted to determine the relationships between different wild populations of I. trifida and the cultivated I. batatas, which could allow inference of sweet potato's region(s) of origin. Finally, a major domesticated trait of I. batatas is its capacity to produce edible storage roots. Some I. trifida are known to form small tuberous roots, but these have not been studied in any depth. The purpose of our study is to investigate the origin of sweet potato and particularly the role of polyploidization in its domestication history. A set of 180 I. trifida populations and 450 sweet potato landraces, distributed from Peru to Mexico, were chosen from the collection of the International Potato Center (CIP, Lima, Peru). Morphological characterisation of these plants is in progress, as well as genetic analyses using neutral chloroplast markers. We plan to evaluate ploidy levels of these different wild and cultivated samples by flow cytometry and study the genome composition of representatives of ploidy groups by genomic in situ hybridization. These analyses should lead to advances in the reconstruction of sweet potato's evolutionary history.

Keywords: Ipomoea Batatas, domestication, origin of polyploidy

**S7-8**

**RETICULATIONS AND INTROGRESSION IN AN ARABIDOPSIS SUTURE ZONE**

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Polyploidy, i.e., the duplication of entire nuclear genomes, has shaped the evolution of major lineages of eukaryotes. Although many duplicated genes will be silenced or lost through time, more than expected from classical theory are retained in the genomes of polyploids. The evolution of several polyploid lineages in Arabidopsis makes this genus a highly interesting model system for studies on the effect of genome duplication. We aim to study the consequences of polyploidisation on genetic diversity in natural populations of A. petraea and A. arenosa, which both have diploid and tetraploid populations in Central Europe. The origin of the polyploids (auto- or allopolyploids) and the amount of introgression between species and cytotypes are currently not known. We use two low-copy nuclear markers (CHS and scADH), a cpDNA marker, and microsattelites to identify the origin of the Central European tetraploids, and to investigate potential gene flow between taxa and ploidy levels. Our preliminary results show that the diploids are gentically distinct from each other. The tetraploids on the other hand, are probably a mixture of allo- and autotetraploids, and polypoloidisation events are likely to be frequent.

Keywords: polyploidy, hybridisation, recurrent origin, Arabidopsis, suture zone

**S7-9**

**ORIGIN, DIVERSITY AND PHYLOGEOGRAPHY OF THE INVASIVE POLYPLOID SPARTINA DENSIFLORA BRONGN.**

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Spartina densiflora is a robust and morphologically variable south-American Chloridoid grass colonizing salt marshes, and that was introduced to California and South-West Europe where it rapidly spread. Our recent analyses have revealed a reticulate (allopolyploid) origin of the species that appears to be heptaploid (2n=70), deriving from a hexaploid maternal lineage and a tetraploid paternal lineage. Various populations from Argentina, Chile and California including different morphotypes and taxa (e.g. “typica”, “patagonica”, “longispica” types) were analysed for phylogeographic and diversity analyses based on AFLP, nuclear and chloroplast DNA sequences. The results indicate no genetic divergence among morphotypes but instead consistent geographic pattern, with most molecular diversity exhibited on the South-East America (Argentina) from where plants were introduced to Chile and later to California.

Keywords: Spartina, Chloridoid, polyploid

**S7-10**

**UNBALANCED INTERSPECIFIC GENE FLOW OF NUCLEAR RIBOSOMAL DNA IN JASIONE SECT. JASIONE (CAMPANULACEAE) IS DETERMINED BY PLOIDY LEVEL.**

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Western Europe taxa of Jasione sect Jasione (J. laevis s.l., J. crispa s.l., J. cavanillesii) belong to a well-defined monophyletic plastidial lineage composed by perennial orophilous taxa with small size populations – extremely variable in ploidy levels–, plus J. montana, a diploid annual species widespread at medium and low-altitudes with large populations. We surveyed the variability of ITS by cloning multiple alleles occurring in individuals across a broad geographic sample (70 populations) and analyzing their distribution among populations and species, where five ploidy levels (2x, 4x, 6x, 8x, 10x) were recognized. Our results found four main ribosomal lineages, many of them present in more than one species, revealing a complex reticulate pattern and intragenomic additivity of ribotypes. This scenario points out to a generalized occurrence of hybridization events and introgressions evidencing weak interspecific barriers. One interesting case is the ribotype transfer from J. laevis (4x) alleles towards J. crispa (4x, 6x, 8x and 10x). Although both species have sympatric populations along their distributions, the presence of J. laevis ribotypes in J. crispa populations concentrates in some ranges while is totally absent in others, which suggests that 6x and 8x levels of ploidy in J. crispa have arisen independently in different geographical zones as a result of allopolyploidization with J. laevis. Another important transfer of J. montana (2x) ribotypes towards the other taxa has been detected. Its distribution and frequency follows a precise pattern: high frequency of ribotypes of J. montana is not correlated to current overlap in range or habitat between the perennial taxa and J montana, but it is correlated with the occurrence of J. crispa or J. laevis populations with diploid individuals. Frequently J. montana ribotypes percolate those populations to such extent that they become dominant among diploid individuals despite their taxonomic ascription. When several intragenomic copies coexist the intensity of ITS concerted evolution towards one particular ribotype can be determined by how frequent has been gene introgression from other taxa. However, a high ploidy level seems to preserve the genetic integrity of nrDNA modulating lateral gene flow from other diploid species with high population sizes probably through a gene dosage effect.

Keywords: Iberian mountains; introgression, allopolyploidy; glacial refugia, flow cytometry

**S7-11**

**PHYLOGENETIC STUDIES IN INDIAN POLYPLOID CURCUMA SPECIES USING AFLP MARKER**

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Economically important genus Curcuma is taxonomically critical polyploid complex. Various ploidy levels (2x, 6x, 9x, 11x, 12x and 15x) developed various modes of reproduction and exhibit different levels of morphological variability. With no recent revision and extremely complicated nomenclatoric issues, incorrect identifications are very common. So far there is also no suitable infrageneric classification. Based on ongoing taxonomic revision of Indian Curcuma and recent extensive cytological study, the goals of present AFLP study are: (1) check the reliability of the genome size groups sensu Leong-Skornickova et al. (2007) detected by the cytological study; (2) outline phylogenetic relationships among hexaploid taxa, and between hexaploids and higher polyploids and (3) assess genetic variability within and between populations to gain information about level of genetic diversity in various taxa and different ploidy levels.

A total of 19 Curcuma species from 25 populations (total 115 plants) were analysed. From nominate subg. Curcuma we sampled 13 hexaploid (2n = 42) populations belonging to three genome size groups, seven nonaploid (2n = 63), one endecaploid (2n = 77), one dodecaploid (2n = 84) and two pentadecaploid populations (2n = 105). Subgenus Hitcheniopsis was represented by one population of diploid C. vamana (2n = 22). AFLP data were analysed using NJ and PCoA as well as neighbour network and maximum parsimony analysis.

AFLP data supported former hypothesis, that genome size groups have probably different evolutionary histories. Well supported relationships between taxa within particular genome size groups G1 and G2 were confirmed. Moreover, among taxa of G2 group, which are mainly sexually reproducing hexaploids, were detected species of probable hybrid origin, so further detailed study of this events are desirable. We also gained solid outlines of relationships between taxa with different ploidy levels, which belong to the same genome size group G1. Close relations were detected between hexaploids and nonaploids as well as between nonaploids and taxa of other higher polyploid levels. We hypothesize that majority of nonaploid cytotypes plausibly originated by a fusion of reduced and unreduced gametes of different species of hexaploids, giving rise to allopolyploids. Formation of autopolyploids however cannot be ruled out. We suspect that both nonaploid and hexaploid plants were involved in formation of another higher polyploids and the reticulate evolution in Curcuma is very likely. Exact ways of polyploid formation and origin of particular higher polyploids remains as the subject of our further studies.

Keywords: genome size, network phylogeny, polyploid origin

**S7-12**

**EVOLUTION BY HOMOPLOID HYBRIDISATION IN NICOTIANA (SOLANACEAE)**

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Allopolyploidization has played a key role in the evolution of Nicotiana; approximately half of the 76 naturally occurring species have an allopolyploid origin. However, homoploid hybridisation has also been hypothesised as having an important role in generating species diversity within this genus, at both the diploid and tetraploid level. We use data from multiple low-copy nuclear genes to test this hypothesis and provide new evidence for the importance of homoploid hybrid speciation in the evolution of Nicotiana. Putative hybrid origins for several diploid species are inferred on the basis of gene-tree incongruence, evidence for inter-allelic recombination between likely parental alleles, and support for competing splits in spectral analyses. We review evidence for homoploid hybrid origins of tetraploid species and also discuss the implications that reticulation events at the diploid level may have for reconstructing the evolutionary origins of polyploids within the genus.

Keywords: Homoploid hybrid; inter-allelic recombination; phylogenetic reconstruction; polyploid; reticulate evolution.

**S7-13**

**PHYLOGENETIC RELATIONSHIPS BASED ON TWO MITOCHONDRIAL GENES AND HYBRIDIZATION PATTERNS IN ANSERIFORMES**

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Waterfowls are prone to hybridization. The evolution hybridization patterns in Anseriformes has been investigated using a cladistic analysis (morphology), which may underestimate or overestimate the phylogenetic divergence among species, or restricted only to ducks.
We produced DNA sequence data from two mitochondrial genes (cytochrome b and the NADH dehydrogenase subunit 2) to reconstruct the phylogenetic relationships among 121 species of the Anseriformes (waterfowls including ducks, geese, swans, the Magpie Goose and screamers). Phylogenetic analyses based on maximum likelihood and Bayesian inference both converged into a congruent topology and defined several well-supported clades. We calibrated a molecular clock and reconstructed ancestral biogeographical areas using Bayesian inference supporting an austral continental (Gondwanaland) origin of the waterfowls during the Upper Cretaceous (76 Myr) while ducks, swans and geese might have diversified later reaching northern distributions in Holarctic and Afrotropical regions during the Miocene (23–5 Myr ago).
Using a phylogenetic framework, genetic-based distances and a Bayesian time calibration, our data support the hypothesis based on immunological distances of slow rate of appearance of reproductive incompatibilities in waterfowls compared to other vertebrates and the view that these birds may be like frogs in having lost their interspecific hybridization potential more slowly than mammals.

Keywords: Anseriformes, hybridisation, mitochondrial DNA, molecular clock, phylogeny

**S7-14**

**ONGOING PROJECT: BOUTELOUA CURTIPENDULA: A HIGHLY VARIABLE POLYPLOID**

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Bouteloua curtipendula is a highly polymorphic taxa belonging to the Bouteloua curtipendula complex. It is native to the American grasslands and it is one of the most important grass species for its nutritional value. It is widely distributed from Canada to Argentina. México is its center of origin and diversity. Bouteloua curtipendula displays a complicated history of hybridization, polyploidy andapomixis, withchromosome numbers rangingfrom 2n= 20 <100. It is thought that the high chromosome numbers could have evolved from hybridization between diploids and tetraploids. Three varieties have been recognized: caespitosa, curtipendula, and tenuis, however, molecular data do not support the existance of these varieties. Phylogenetic analysis based on ITS and trnT-L-F data do not support the traditional classification of B. curtipendula, instead they show that itdoes not form a monophyletic group. High levels of homoplasy have led to a taxonomic confusion so the species circunscription is questioned. Our goal in this study is to determine the genetic diversity among different populations of the group known as B. curtipendula to delimit the real circumscription of the species, as well as to assert the number of haplotypes present. We will assess this problem by using microsatellites.

Keywords: Bouteloua curtipendula, Gramineae, polyploidy

**S7-15**

## CONTRASTING PATTERNS OF CYTOTYPE DIVERSITY AND DISTRIBUTION IN EASTERN ASIAN POLYPLOID CARDAMINE (BRASSICACEAE) SPECIES

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Intraspecific ploidy level variation is an important aspect of species' genetic makeup, which may lend insight into its evolutionary history and future potential. In our study, we explore this phenomenon in a group of Eastern Asian Cardamine species, which have been shrouded in taxonomic confusion and misinterpretations. First, we sought to resolve their nomenclature and revise species circumscription, and then to explore their chromosome numbers and variation in ploidy level. We sampled 59 populations from Japan and Korea, which were used in karyological (chromosome counting) and flow cytometric analyses.
We present a new taxonomic and nomenclatural concept based on morphometric studies and consultation of nomenclatural types and type localities. While studying chromosome numbers and DNA ploidy levels within species, substantial cytotype diversity was found, with strikingly different distribution patterns between the species. Two cytotypes were found in C. torrentis (4x and 8x), which display a north-south geographic pattern in Japan. We discuss hypotheses regarding the origin and colonization history of this species throughout its distribution. In the Korean populations, usually treated as C. amaraeiformis, we found only tetraploids, and considering overall morphological similarity and the same monoploid genome size we hypothesize that this species may be conspecific with the tetraploid populations of C. torrentis. C. yezoensis was found to harbour as many as six cytotypes in Japan, ranging from hexa- to dodecaploids. Ploidy levels do not show any obvious geographic patterning; ploidy-level mixed populations, containing two to four cytotypes, are frequently observed throughout the range. C. schinziana, an endemic of Hokkaido and southern Kurils, comprises hexa- and octoploid populations. We also revise previous chromosome records, showing that they are largely based on misidentified material or misinterpreted names.
Considerable variation in DNA content was recorded within several cytotypes when multiple individuals were analyzed. The intracytotype variation can be considered reliable, because analyses based on two different fluorochromes gave consistent and highly correlated data, and simultaneous analyses of individuals of the same cytotype but with divergent DNA content values yielded histograms with a bifurcated or two separate peaks.
Sampling of multiple populations and utilization of the efficient flow cytometric approach allowed us to resolve numerous taxonomic controversies, and to detect large-scale variation in ploidy level. These data will be essential in further phylogenetic and evolutionary studies.

Keywords: Cardamine, polyploidy, Eastern Asia, chromosome numbers, flow cytometry

**S7-16**

**ANCIENT AND RECENT HYBRIDIZATION BETWEEN EURASIAN ASHES**

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In forest trees, large contact zones usually exist as a result of broad geographical ranges, long-distance pollen flow and high outcrossing rates. We have been studying natural hybridization between Fraxinus excelsior L. (common ash) and Fraxinus angustifolia Vahl (narrow-leaved ash), which are indigenous to France along fluvial basins such as Loire and Sâone valleys, (Gerard et al., 2006 ; Fernandez-Manjares et al., 2006). Despite a revised molecular phylogeny of the genus (Wallander, 2008), the speciation history and phylogeography of the species complex in Europe remained unclear.

To study the extent of genetic differentiation between both species across their ranges, a wide sample of diverse populations of European ashes taxa was collected throughout their distribution. To explore more exhaustively the phylogeography of the group, the closely related F. mandshurica (Asian ash) was also considered. Nuclear ETS and nITS sequences were used to reconstruct the phylogeny using Bayesian and Maximum Likelihood approaches. The two genomic regions analysed indicated a clear differentiation between the two species. The phylogeny for the group was partially congruent with that previously published (Wallander, 2008). The lack of grouping for some individuals in the phylogeny indicated that they likely originated from natural hybridization.

An incongruence was observed between nETS and nITS phylogenies, which was interpreted as a consequence of ancient hybridization between F. angustifolia and F. mandshurica. A phylogeny taking into account ancient reticulation was reconstructed and molecular datations of major nodes were estimated. The results show that speciation and reticulation events could be traced back to the Miocene period (24-5 Mya), with occurrence of major geological events inducing changes in climate. These results and inferences indicate that allopatric speciation has been a driving factor for the diversification of the group, and that hybridization has been a recurrent factor in the history of the group.

Keywords : Fraxinus - hybridization - phylogeny - reticulate evolution

**S7-17**

**THE POLYPLOID SERIES OF CENTAUREA TOLETANA: GLACIAL MIGRATIONS AND INTROGRESSION REVEALED BY NRDNA AND CPDNA SEQUENCE ANALYSES**

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The polyploid series of Centaurea toletana comprises diploid, tetraploid, and hexaploid cytotypes. Previous studies suggested that the tetraploid was an autopolyploid, while the hexaploid was an allopolyploid and should be considered a different species, C. argecillensis. Sequencing of the ITS and rps4-trnT-trnL, ycf3-trnS, and rpL16 regions, and extensive cloning and sequencing of the ETS region have revealed that many diploid individuals and populations show different ribotypes, likely resulting from ancient hybridization events. Ribotypes found in the diploid populations are also present in tetraploid populations. The extreme difficulties in classifying the tetraploid as auto- or allopolyploid are discussed. The hexaploid C. argecillensis also shows many different ribotypes, including a ribotype not found in the diploids and making an autopolyploid origin unlikely. The pattern of introgression and gene flow in the polyploid series implicates several species from the Iberian Peninsula and the High Atlas Mountains in Morocco as genetic donors in ancient hybridization events. This long-reaching network of hybridization may trace its origin to the climatic history of the western Mediterranean during the Neogene.

Keywords: allopolyploidy; autopolyploidyglaciation; homoploid hybridization; introgression

**S7-18**

**TAXONOMY AND NOMENCLATURE OF ANIMAL SPECIES OF HYBRID ORIGIN**

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In biology, the old "species problem" has several dimensions. A distinction must be made between "species" as an evolutionary unit, a taxonomic unit (taxon), a taxonomic category and a nomenclatural rank. Another problem is the reductionist temptation to recognize only one kind of "basic entities" of biodiversity. For all users of taxonomy, any organism must be referred to a taxon of nomenclatural rank species, designated by a Latin binomen like Mus musculus. These nomina are indispensable for all biological research, gestion and conservation of biodiversity. But this does not imply that all these taxa should be referred to a single taxonomic category, a "unified concept of species". In zoology, several kinds of entities exist in nature. They can be characterized according to their patterns of speciation and the modalities of their reproduction (modes of gametogenesis, of initiation of the development and of gene flow between individuals). The well-known "mixiological" species concept points to sets of bisexual individuals, with standard eumeiosis and fertilization, in panmictic populations that are genetically independent from each other. Speciation may result from "gradual change" within a lineage, from splitting of a species in two or from interspecific hybridization. Speciation through the first two processes is usually rather slow, and results in new independent bisexual panmictic entities. In contrast, speciation through hybridization may give rise, sometimes very rapidly, to entities of various kinds. Their gametogenesis often implies an ameiosis or a metameiosis. Some are unisexual female entities in which genetic transmission is clonal, reproduction being often through parthenogenesis or other mechanisms with similar results in genetic terms. Others are unisexual or bisexual entities which depend for their reproduction, at each generation, on a another species: their metameiosis produces particular gametes which start their development either by fertilization or gynogenesis. So-called "hybridogenetic" entities are "hemiclones" with a peculiar gametogenesis involving premeiotic exclusion of the genome of one of the parental species, followed by fertilization of their gametes. These particular cases are not rare phenomena, and not necessarily "evolutionary dead-ends". Some of these forms are advantaged under certain conditions and may persist in the long term in nature. They are also in some cases the source of allopolyploid species. Systematics must deal with these different kinds of natural entities, which requires the implementation of appropriate taxonomic and nomenclatural terminologies and rules.

Keywords: Taxonomy, speciation, hybridization, gametogenesis, hybridogenesis

**S7-19**

**LOW COPY NUCLEAR GENES REVEAL HYBRID SPECIATION IN POLYSTACHYA (ORCHIDACEAE)**

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Polystachya is a genus of c. 240 species distributed throughout the tropics. Our studies into its phylogeny, cytology and genome size have revealed several instances of allopolyploidy, more commonly away from the centre of diversity on tropical Africa. One group of tetraploids is endemic to Madagascar and and the Malagasy islands, and shows a wide range of morphological variation. Another group shows little morphological variation but has dispersed rapidly throughout the tropics and radiated in Central- and South America. We have used plastid DNA, nuclear ITS and nuclear low copy nuclear gene sequences to infer their relationships and build a picture of reticulation in the genus. Nuclear low copy genes have been duplicated in the tetraploids and each copy has retained sequence similarity with the diploid species from whose lineage the genome copy was contributed. This gives us a narrow range of candidate species for the parents of each tetraploid. Further work on GISH (genomic in situ hybridization) would be necessary to confirm the results.

Keywords : hybrid speciation; low copy nuclear genes; phylogenetics; Polystachya; reticulate evolution

**S7-20**

## GENE CAPTURE FROM ACROSS THE GRASS MAMILY 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

**S7-21**

**ORIGIN AND GENOME EVOLUTION OF POLYPLOID SPECIES OF THE GENUS MELAMPODIUM (ASTERACEAE)**

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The genus Melampodium (Asteraceae) comprises 40 species distributed mainly in Mexico and the southern United States. The genus is chromosomally very diverse with five base chromosome numbers known (x = 9, 10, 11, 12, and 14). It is divided into six sections, and the largest section, Melampodium, comprises 22 species, all with chromosome numbers based on x = 10. One of the series of section Melampodium (Sericea), comprises five exclusively polyploid species, both tetraploids and hexaploids. The current study has been undertaken to infer the origin and analyze the genome evolution of these polyploids. The allopolyploid origin of all polyploid taxa has been unambiguously confirmed using plastid (matK and psbA-trnH) and nuclear (ITS, 5S rDNA spacer, and two paralogues of low copy PgiC) DNA sequence data. Allopolyploids were shown to have originated via hybridization among members of series Melampodium and series Cupulata. Several parental taxa have been involved in the formation of more than one allopolyploid, indicating that multiple hybridizations, when combined with polyploidisation, are successful for new species formation. The genomes of the two groups of diploid putative parental species show different trajectories concerning genome size evolution and only few detectable changes in chromosome structure. Conversely, the polyploids showed only minor changes in genome size and reduction of both 5S rDNA and 35S rDNA loci number compared to the expected sum of the number of loci in the parental taxa. Two hexaploid species, M. pringlei and M. sericeum, have originated independently from the same parental taxa (tetraploid M. strigosum as paternal and diploid M. linearilobum as maternal parent), but followed different pathways of genome restructuring, concerning both 35S rDNA homogenization and locus loss.

Keywords: Melampodium, allopolyploids, chromosomes, origin, genome size

**S7-22**

**PATTERNS OF HYBRIDIZATION WITHIN VERONICA SUBGENUS PSEUDOLYSIMACHIUM**

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Veronica subgenus Pseudolysimachium is one of the systematically most complex groups of Veronica with on average 25.5 synonyms for the 29 accepted species. Their showy appearance with a long bright blue, dense and long inflorescence has attracted horticulturists and botanists alike. Despite the long history of breeding the species and investigating the natural variation, we still lack basic knowledge of relationships between the species and even exact species circumscriptions. In the Balkan Peninsula, taxonomic discussions centered on the group of Veronica spicata, V. barrelieri and V. orchidea, all growing mostly on dry grasslands and steppe vegetation. All three species vary in ploidy level with diploid and tetraploid types occurring in the Balkan Peninsula. Flow cytometry allowed to investigate the distribution of cytotypes in more detail than previously using chromosome counts. AFLP fingerprints and cpDNA markers further reveal a complex relationship of these three taxa. Whereas typical individuals of the three species are quite well seperated from each other in the study area, all taxa appear to hybridize over large areas leading to the observed large variation in morphology and genotypes. The study sets the scene for future experimental studies investigating differences between homoploid (on the diploid and tetraploid level) and polyploid hybridization in the group.

Keywords: AFLP, Balkan Peninsula, cpDNA, flow cytometry, morphology

**S8-1**

## RANGE AND NICHE EXPANSION VIA INTROGRESSION IN MARGINAL POPULATIONS OF A COASTAL SHRUB

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The coastal shrub Armeria pungens (Plumbaginaceae) has a disjunct distribution. It occurs along a 500-km coastal stripe in southwest Iberia but is also present on two continental archipelagos: in the Atlantic, on the Cies Islands (offshore Galician coast, NW Spain), and in the Mediterranean, on South Corsica and North Sardinia. Our study using AFLP data, as well as ITS, plastid DNA (trnL-F, trnS-fM and matK), and low-copy nuclear GapC sequences together with morphometric data has shown that a highly diverse ancestral lineage occurring in SW Portugal has been the source for the colonization of the remaining areas. Populations in Corsica-Sardinia are the result of long-distance dispersal while those occurring in the southern coasts of Iberia from Cape S. Vicente to Cadiz (Gulf of Cadiz lineage) underwent a genetic bottleneck and colonized the area in recent times. This scenario is consistent with the results of a bioclimatic envelope modelling study that found similarities in Corsica-Sardinian and Portuguese climatic conditions as compared to the drier Gulf of Cadiz environments. This paper focuses on the northernmost and southernmost populations of the species range located in the Cies Islands and near the Gibraltar Strait (Punta Camarinal), respectively. These two populations exhibit evidence for on-going (in Cies also ancient) introgression from sympatric congeners. This situation contrasts with the finding that no introgressed genotypes seem to occur in other parts of the range of A. pungens despite the existence of sympatric species and the propensity to hybridize in this genus. We suggest that hybrid genotypes may be somehow favoured on the margins of the species range and that novel genotypes may allow for expansion beyond the geographical and ecological boundaries of the species.

Keywords: Armeria, introgression, range expansion, long distance dispersal

**S8-2**

## SEXUAL REPRODUCTION OF THE INVASIVE POLYPLOID OXALIS PES-CAPRAE IN THE MEDITERRANEAN REGION

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Oxalis pes-caprae L. is a native plant from South Africa and a widespread invasive weed in regions with Mediterranean climate. This species is tristylous (with long-, mid- and short-styled floral morphs) presenting a self- and morph-incompatibility reproduction system and being composed by three cytotypes (2x, 4x and 5x) in its native range. Previous studies have shown that in the exotic range of distribution the pentaploid short-styled morph is dominant and its reproduction is mostly asexual, by bulbils. Recently, the tetraploid long-styled morph was discovered growing in several mixed populations in the Mediterranean basin but no fruit production was observed. In the present study we screened new unexplored areas of this species in the Mediterranean area. Thirteen populations were studied for floral morph ratio, from which six were analysed in more detail for fruit production and cytotype composition. Data on pollinator's assemblage was also gathered. We report here, for the first time in this region, the occurrence of the mid-styled floral morph growing in mixed populations along with the other morphs and also the production of fruits. Flow cytometric analyses revealed the occurrence of tetraploid and pentaploid individuals within each floral morph in variable proportions. The presence of three floral morphotypes with compatible ploidy levels opened the possibility for sexual reproduction, potentially enhancing the genetic diversity of this species. By other way, the asexual reproduction may govern the maintenance of both cytotypes. The occurrence of two reproductive strategies and the detection of localized sexual reproduction provide a model system to study the origin, maintenance and evolution of this polyploid species and may provide new insights in the dynamics of the invasion process of Oxalis pes-caprae.

Keywords: cytotype, flow cytometry, heterostyly, pentaploid, tetraploid

**S8-3**

## MOLECULAR EVIDENCE FOR AN ALLOPOLYPLOID ORIGIN OF THE INVASIVE EUROPEAN GORSES, ULEX EUROPAEUS SUBSP. EUROPAEUS (FABACEAE; GENISTEAE).

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Genus Ulex is a small euploid series (2n = 32, 64, 96) of 15-20 perennial spiny shrub species and subspecies distributed in Western Europe and North Africa. While most diploid and polyploid gorses are restricted to very localized areas in the Iberian Peninsula, their primary centre of diversification, few of them have extended their distribution northward along the western European facade: U. minor (2n = 2x), U. gallii and U. europaeus subsp. europaeus (2n = 6x). Only the latter species is notorious for being an invasive weed in different continents, following introductions during the last two centuries. ITS and ETS nrDNA sequences were used to investigate the evolutionary history of Ulex taxa and to elucidate the origin of the European gorses.

Sequence analyses revealed three main haplotypes for each of the ITS and ETS arrays. Two of them showed a differential geographic distribution within Ulex : one from each nrDNA region mostly characterizing the "Atlantic" taxa, and one haplotype from each region for the "Mediterranean" taxa. The third ITS and ETS haplotypes were intermediate between the formers, respectively, suggesting that they most likely result from sequence recombination due to interlocus concerted evolution, following reticulate evolution in Ulex. Among all samples surveyed, only the hexaploid U. europaeus subsp. europaeus exhibited ITS and ETS sequence heterogeneity. Phylogenetic analyses of separate and combined ITS and ETS sequences provided new insights to best understand relationships among the diploid taxa native from either the "Atlantic" or the "Mediterranean" regions, and allowed inference on the origins of the polyploid taxa. The data suggest: (i) a very likely autopolyploid origin of both U. breoganii (4x) and U. gallii (6x) from the "Atlantic" diploid U. minor; (ii) that most other polyploids, U. densus (4x), U. jussiaei (6x), U. australis (6x), U. europaeus subsp. latebracteatus (4x) only share variants of the «Mediterranean» haplotype, which derive from the "U. parviflorus-U. baeticus-U. argenteus" diploid complex via autopolyploidy; (iii) and that the the invasive hexaploid U. europaeus subsp. europaeus unambiguously contains both "Atlantic" and "Mediterranean" ITS and ETS haplotypes, originating from the genomes of the diploid U. minor and the tetraploid U. europaeus subsp. Latebracteatus. Thus the results strongly suggest that the only polyploid species which extended its distribution out of Europe and behaves as a worldwide invasive plant, is of a recent allopolyploid origin and combines both the "Mediterranean" and the "Atlantic" genomes.

Keywords: Ulex, Phylogeny, Allopolyploid origine, Invasive

**S8-4**

**GENOME SIZE AND DIVERSIFICATION OF CENTRAL EUROPEAN PLANT LINEAGE**

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Genome size varies strongly among species at the level of ploidy, C value and chromosome number. Thanks to recent technical progress in cytochemistry measure and better knowledge of phylogenetic relationships, it could be shown that genome size varies among evolutionary lineages. Moreover, it has been shown that variation in genome size can affect the evolutionary success of species. However, it is not known whether genome variation affects the evolutionary success of entire lineages, such as rates diversification of species and life history traits. Here we analysed a phylogeny of Angiosperms from Central Europe using phylogenetically independent contrast between clades. We, first, identify where across the phylogeny genome size varies most strongly Then, we relate variation in genome size between clades to variation in clade richness. Finally, we investigate relationships between variation of genome size and diversification of life-history traits (life style, type of reproduction). First results showed a large heterogeneity in the distribution of genome size both within and across taxa. Morever, the richness of taxa increases with the chromosome number, while no significant effect was observed for C value and ploidy level. Future results of this study will test this observation and will give us more information about the potential impact of genome size variation on diversification of plant lineages.

Keywords: C value , chromosome number, ploidy level, species richness, life-history traits

**S8-5**

## POLYPLOID EVOLUTION AND HYBRIDIZATION IN THE ANDEAN GENUS LASIOCEPHALUS (ASTERACEAE) - INSIGHTS FROM GENOME SIZE DATA

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The Andean genus Lasiocephalus Willd. ex Schlecht. (Asteraceae) comprises approx. 30 species inhabiting a range of vegetation types between the montane forest and high-altitude páramo from Venezuela to Bolivia. Two main growth forms are recognized in the genus, i.e., the broad-leaved suffrutescent climbers of the montane forest and the narrow-leaved ascending subshrubs and herbs of the páramo. We employed DNA flow cytometry to assess the variation in genome size in twelve species and used the data for getting insight into evolutionary processes that shaped the diversity of the genus (incl. genome duplication and hybridization). DNA triploids were revealed for the first time in two species, forming mixed populations with their diploid counterparts. Holoploid genome size (2C-values) in diploid accessions varied ca 1.33-fold and ranged from 13.26 to 17.57 pg DNA. Although the variation in nuclear DNA content was rather continuous, several groups of species with similar genome size were recognized. Interestingly, the páramo species possessed lower genome size values than their montane-forest counterparts; the threshold was approx. 15.3 pg DNA. A putative intergeneric hybrid between Lasiocephalus and Culcitium was detected, based on intermediate genome size. Moreover, DNA content of offspring originated from achenes of the hybrid plant suggests that backcrossing with both parental taxa can occur in the field. Further research will focus on integrating flow cytometric and molecular data and on the interpretation of the genome size values in the phylogenetic context.

Keywords: hybridization, polyploidization, Asteraceae, Andes, growth forms

**S8-6**

## HYBRID ALIEN ASH: FRAXINUS EXCELSIOR × F. ANGUSTIFOLIA AND ITS POTENTIAL FOR INTERBREEDING WITH NATIVE ASH IN IRELAND

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Fraxinus excelsior L. is the only indigenous ash in Ireland. It is a native hardwood tree with important economic value. From 1993 to 2000, ash plants were imported from the continent to Ireland because home production failed to meet full demand. The imported trees soon showed very bed stem form. It is hypothesised that poor growth may be due to the source of material, which could have been: a) hybrid trees of F. excelsior × F. angustifolia, b) pure F. angustifolia and/or c) provenances (sources) of F. excelsior, which were not well adapted for the Irish environment. Moreover, the potential for introgression of genes from non-native Fraxinus into native stocks became a cause of concern. However, little is known about the morphology, phenology and population genetics of natural hybrid populations in Europe which impedes an adequate evaluation of the risks associated with this material.

The goals of this project are to examine the significance of hybrid plantations in Ireland, by analysing the key morphological characters in parental and hybrid trees, to determine how the breeding biology of both tree species may lead to the introgression of undesired gene pools and finally, by estimating gene flow at the landscape level, to quantify the hybridization potential of the introduced hybrid trees with native Irish ash. The first part on the work presented here was on F1 hybrid material obtained via artificial crosses, to investigate the morphological relationships of leaf and bud structure among the two parental species and genetic F1 hybrids. The F1 individuals showed an intermediate morphology. However, due to the widespread variation of the parent morphology, F1 hybrids are often included in one of the parental groups. The flowering time of suspected hybrid material was monitored in two plantation sites and first results showed an overlap in flowering period of some late flowering F. angustifolia-like trees with some early flowering native F. excelsior trees. Molecular analysis of suspect hybrid plantations show indeed an important F. angustifolia component but morphology (Leaf morphology) appears somewhat different from continental populations.

Keywords: Fraxinus, hybridisation, introgression, phenology

**S8-7**

## DO CYTOGENETIC DIVERSITY AND GENOME STRUCTURE DRIVE ADAPTIVE EVOLUTION IN DIPLOID VERSUS POLYPLOID ROOT-KNOT NEMATODES?

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Root-knot nematodes (RKNs), Meloidogyne spp., are obligate endoparasites of plant roots, responsible for annual losses to world agriculture estimated to exceed 100 billion €. Depending of the species considered, their life cycle and reproductive mode are very variable, ranging from classical amphimixis to obligatory mitotic parthenogenesis, the trend towards parthenogenesis being generally correlated with larger host range and increasing importance as crop pathogens. RKNs are also highly variable with respect to their chromosomal complement. Amphimictic RKN species are exclusively diploid, while diploid, triploid and rare tetraploid forms are encountered within parthenogenetic species. Moreover, although the haploid number of the genus is assumed to be n = 18, most populations have somatic chromosome numbers ranging from 30 to 50, and numbers that are perfect multiple of 18 are not frequently observed, as a consequence of aneuploidy/polysomy as well as structural rearrangements. In addition, RKNs exhibit considerable karyotypic variation at the intraspecific level, and this is particularly true for species that have completely abandoned sexual reproduction. Such variability is partly due to the fact that RKN species harbour holocentric chromosomes with a diffuse centromere lacking localized kinetochore activity. Very recently, the complete genome sequence of two parthenogenetic RKN species was made available, i.e. the diploid meiotic species M. hapla and the triploid mitotic species M. incognita. The 54 Mbp genome of M. hapla represents the smallest metazoan genome yet completed, while the assembled sequence of M. incognita spans 86 Mbp, and mostly consists of homologous but divergent segment pairs that might represent former alleles in this species. A combination of different processes could explain this peculiar genome structure in M. incognita, including polyploidy, polysomy, aneuploidy and hybridization, all features that are frequently associated with asexual reproduction. While M. incognita is extremely polyphagous, with the ability to potentially parasitize the majority of the estimated 250,000 flowering plants, and is found from temperate to tropical regions, M. hapla has both a narrower host range and geographic distribution. In this respect, RKNs constitute a unique model system to study the links between variation in genome structure, mode of reproduction, and adaptation to environment and hosts, in relation with the so successful establishment of these parthenogenetic organisms as plant parasites.

Keywords: cytogenetic diversity, genome structure, parthenogenesis, polyploidy, root-knot nematodes

**S8-8**

## MORPHOLOGICAL AND MOLECULAR RELATIONSHIPS AMONG AUTUMNAL SQUILLS (PROSPERO, BARNARDIA, HYACINTHOIDES) FROM ALGERIA

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Morphological, caryological and molecular analyses were performed on four autumnal squills from Algeria: Hyacinthoides lingulata (Poir.) Rothm., Prospero autumnale (L.) Speta, P. obtusifolium (Poir.) Speta and Barnardia numidica Poir. Multivariate analysis based on several diagnostic descriptors, showed two distinct clusters. The first one, homogeneous and well distinct (H. lingulata), presented two chromosome numbers 2n=16 which is previously reported in the literature and 2n=8 which is new. The second cluster is polymorphic and is constituted by populations of P. autumnale (2n=2x=14, 2n=4x=28 and 2n=6x=42) and P. obtusifolium (2n=8). This later taxon exhibited a high variability in the leaf width, one of the main diagnostic characters. Samples of Barnardia are also linked to this polymorphic group and are characterized by 2n=18. Phylogenetic analysis based on the sequencing of the intergenic spacer trnL-trnF (cpDNA) underline the polyphyly of the genus Barnardia and the existence of different clades within the Prospero autumnale-obtusifolium complex. Results are discussed in relation with the endemism of these species and the possibilities of hybridization in bioclimatic transitional areas.

Keywords: Hyacinthaceae, morphology, cpDNA, endemism, polyploidy

**S8-9**

## PRIMARY AND SECONDARY CONTACT ZONES OF DI- AND TETRAPLOID KNAUTIA ARVENSIS AGG. (DIPSACACEAE)

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Knautia arvensis agg., an intricate complex of di- and tetraploid cytotypes, was comprehensively screened for ploidy variation at various spatial scales in Central Europe using flow cytometry. Largely parapatric distribution of 2x and 4x cytotypes was found with a diffuse contact zone running along the north-western margin of the Pannonian basin. In addition, several populations of diploids with distinct genome size (ca 5% difference) were found scattered in the area otherwise occupied by the tetraploid cytotype. These populations inhabit relic open pine forests on serpentine outcrops and most probably represent independent evolutionary lineage, parallel to their non-serpentine Pannonian counterparts. Moreover, two serpentine populations showed a mixture of di- and tetraploid cytotypes with similar morphology and ecological preferences. Preliminary AFLP results confirmed genetic similarities between 2x and 4x serpentine plants (they grouped together), while the Pannonian diploids were clearly separated. In summary, all pieces of evidence point to the existence of both primary and secondary zones of ploidy contact within the K. arvensis agg. Interestingly, both cytotypes were more or less spatially segregated in mixed-ploidy populations either in primary or secondary contact zones. This may indicate certain differences in biological traits between diploids and tetraploids – a pattern calling for further investigation. Collectively, Knautia arvensis agg. provides a unique model system for a comparative investigation of patterns and processes in primary vs. secondary diploid-polyploid contact zones.

Keywords: contact zone, cytogeography, flow cytometry, ploidy mixture, serpentine

**S8-10**

## INTER-CYTOTYPE INTERACTION IN POPULATIONS OF PLANTS WITH PLOIDY HETEROGENEITY: PILOSELLA ECHIOIDES (ASTERACEAE) AS A MODEL SYSTEM

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The formation and maintenance of polyploids via the development of various reproductive barriers rank among the central questions of research on polyploid evolution. However, most of the recent studies dealt with model plants with quite well established diploid-polyploid pre- or postzygotic barriers and thus low frequency of fruitful reciprocal mating interaction. On the contrary Pilosella echioides with obviously poor or no barriers among at least four contemporary known cytotypes represents a unique naturally arisen model system for among-cytotypes mating interactions studies. Detailed study of populations at heathlands in the vicinity of Znojmo (SW Moravia, Czech Republic) revealed co-occurrence of up to four cytotypes (2x, 3x, 4x and 5x), even on a fine spatial scale (several square centimetres). Cytotype proportion (DNA ploidy estimation by flow cytometry) based on a set of more than 2500 adult plants is as follows: 2x – 5.7%, 3x – 76.9%, 4x – 14.6% and 5x – 2.8%. Huge predominance of the triploid cytotype is a puzzle, especially when sexual reproduction (allogamy with small contribution of autogamy) was confirmed for all cytotypes (even triploids and pentaploids). A study of DNA-ploidy levels of embryo and endosperm of seeds (or more precisely achenes) developed on mother plants of known ploidy level within subpopulations with known spatial cytotype structure was carried out. Absolutely no mature achenes on pentaploid plants (only numerous undeveloped ones) showed a female sterility of this cytotype. On the other hand the greatest portion of variability of ploidy levels was found within embryos from achenes of tetraploid mother plants. Out of almost 200 mature achenes 40.9% of triploid, 47.0% of tetraploid, 4.5% of pentaploid and 7.6% of hexaploid embryos were discovered. Up to three ploidy levels, but huge numbers of aneuploids too, were produced by triploid mother plants – ca 150 euploid achenes contained 10% of triploid, 46.7% of tetraploid and 43.3% of pentaploid embryos. Finally, the diploid mother plants produced 78.6% of diploid and 21.4% of triploid embryos (out of 250 euploid achenes). Based on the survey of produced achenes a theoretical proportion of ploidy levels should be as follows: 4.6% of diploid, 16.5% of triploid, 41.7% of tetraploid, 35.4% of pentaploid and 1.8% of hexaploid plants. The real cytotype proportion under natural conditions showed considerable shifting towards triploids and decreasing number of tetraploids and pentaploids.

Keywords: Pilosella echioides; mixed populations; inter-cytotype crossing

**S8-11**

## WHEN LINEAGES COLLIDE IN SPACE AND TIME: THE CASE OF TWO HYBRID LINEAGES OF NARCISSUS

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An extreme outcome in hybridization is the formation of new lineages in which even low fertile segregates may form stable hybrid lineage provided they have any selective advantage. Nonetheless, occasionally the survival of one or both the progenitors involved can be threatened since they can easily be replaced by either demographic swamping or genetic assimilation during hybridization. One general feature of hybridization is the potential to occur repeatedly at different times and in different geographic locations producing genetically different but morphologically similar progenies. Narcissus xalentejanus and N. xperezlarae (Amaryllidaceae) provide an example of this situation. These two hybrids are morphologically similar and have a proposed hybrid origin between N. cavanillesii and N. serotinus, in the first taxa, or between N. cavanillesii and N. miniatus, in the second taxa. Yet, the discovery of isolated hybrid populations of N. xperezlarae in East Iberian Peninsula, where the progenitor N. cavanillesii has never been reported raised several questions concerning the origin of such allopatric populations. Previous phylogenetic work has shown that N. miniatus may have a hybrid origin between N. serotinus and N. elegans, all of them members of the same group with different levels of ploidy. Thus, to uncover the origin of the two hybrids and the possible swamping process we have undertaken an integrative study based on genetic, reproductive, chromosomal and ecological data sets. First, an extensive sampling of 1435 individuals was performed to determine which species were involved in the formation of the two similar hybrid lineages. For this search, sequencing of four organellar regions (cpDNA, mtDNA), as well as ITS region (nrDNA) from nuclear genome was performed. Second, a crossing program was undertaken to probe the strength of the reproductive barriers among the putative progenitors and the hybrid, to assess whether the possibility of assimilation exist. Third, since hybrid speciation can occur rapidly through changes in chromosome number, especially by allopolyploidy, chromosome numbers of all studied populations were examined either by direct chromosome observation or by flow cytometry. Finally, since hybrid speciation requires either displacing the parental species from its niche or the colonization of a new one, an ecological niche modelling was developed to determinate ecological differences between the taxa in study. The integration of these four approaches offers a mean to explain the evolutionary history of this two hybrid lineages as well as possible causes of speciation and extinction in Narcissus.

Keywords: Narcissus, genetic swamping, demographic assimilation, Iberian Peninsula

**S8-12**

## POLYPLOIDY AND HYBRIDIZATION WITH APOMIXIS IN CRATAEGUS (ROSACEAE)

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It has been suggested since the 1940s that polyploids in Crataegus arise repeatedly from the unreduced gametes produced by diploid-diploid hybrids, that apomixis is strongly favoured in triploids, and that a complex of apomictic and sexual tetraploids has arisen from these triploids. Our investigations using morphological measurements, flow-cytometric seed screening, and molecular data have confirmed that North American native Crataegus are the expected mix of diploid sexual species with triploid and tetraploid apomictic forms, but the North American tetraploids investigated have been strongly apomictic, except for a few with partial (approx. 40%) apomixis. The only primary diploid hybrids found so far in North America are between a naturalized European species and either of two native species.

We have confirmed the existence of both auto- and allopolyploids, but morphology suggests that allopolyploids are numerous. Our artificial pollinations between ploidy levels using distantly related diploids, triploids, and tetraploids show embryos with ploidy-levels up to hexaploid, but also some apparent barriers to intercrossing. Our data include many instances of ploidy-level transitions involving unreduced female gametes, but no clear evidence as yet that unreduced male gametes can fertilize egg cells. Diploid embryos were produced by partly apomictic tetraploids, but it is not known whether such seeds are viable and represent a route for gene flow back to diploids.

Diploid hybrids are known from Eurasia, where diploid species are relatively frequent, and we are currently investigating two European sites where multiple diploid "species", hybrids, and polyploid individuals occur. Results indicate that most hybrid individuals have ploidy levels higher than diploid, and that apomictic reproduction occurs in the descendants of matings between strongly apomictic tetraploids and diploid sexual plants. Thus, apomictic polyploids might not commonly arise de novo from diploids in Crataegus but rather from diploid-polyploid hybridization.

We have confirmed that near-obligate apomictic reproduction occurs in species that are native to much of Europe and central Asia, western North America, and Canada. Polyploids whose reproduction is not yet known occur in east Asia and in the southeastern U.S. Thus, the spread of apomixis might have occurred via hybridization from just one or a few origins, across two large continents and apparently even between taxonomic sections of the genus.

Keywords: Gametophytic apomixis, apospory, Crataegus, Rosaceae, Pyrinae

**S8-13**

## FACULTATIVE APOMIXIS IN RANUNCULUS KUEPFERI (RANUNCULACEAE) ENHANCES CYTOTYPE DIVERSITY AND GEOGRAPHICAL DISTRIBUTION

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Ranunculus kuepferi is distributed along the Alps, in Corsica and in the north of the Apennines. The diploid sexuals occur only in the Alps Maritimes on the western border and at the margin of previously glaciated areas, whereas the tetraploid apomicts (flowers, pollen and fruit irregular) are more widespread and occur mainly in previously glaciated areas. This general phenomenon, known as geographical parthenogenesis (Hörandl, 2006), is being studied on population samples out of the range of the species using flow cytometry, flow cytometry seeds screen and molecular markers. In the mixed area, the putative hybrid origin of triploids and pentaploids and amount of introgression of apomixis into sexuals will be analyzed. Flow cytometry revealed that triploids and pentaploids occur in the hybrid zone but some triploids and hexaploids are present in the area of tetraploid apomicts, suggesting facultative sexuality. Pseudogamous mode of reproduction is predominant in tetraploid but also autonomous apomixes and facultative sexuality could be ascertained (Hörandl et al. 2008). Results of AFLPs show as much genetic variation within the tetraploid populations compared to the diploids, but each population has its own gene pool. Apomicts have superior colonizing abilities and grow in a broader range of altitude than the sexuals. A Mantel test computed between FST genetic distance and the geographical distance shows a weak correlation and suggests a spread of the tetraploids in the Alps from the Alps Maritimes. Analysis of private bands suggests autopolyploid origin of tetraploids and hybridization between diploids and tetraploids. Analysis of microsatellites will inform about putative enhanced levels of heterozygosity in apomicts. We conclude that maintenance of genetic diversity, a flexible mode of reproduction and founder events explain geographical parthenogenesis.

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Keywords: Apomixis, geographical parthenogenesis, DNA fingerprinting and Flow cytometry

**S8-14**

## POLYPLOID EVOLUTION AND ECOLOGICAL SEGREGATION OF CYTOTYPES IN THE ALPINE PLANT SENECIO CARNIOLICUS (ASTERACEAE)

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Senecio carniolicus (Asteraceae) is a frequent species of acidophilic alpine meadows occurring in the Eastern Alps and the Carpathians. Although the taxon was previously believed to be uniformly hexaploid, in the Eastern Alps diploid, tetraploid and hexaploid cytotypes were encountered. Cytotype mixture is frequent, with diploids and hexaploid individuals co-occurring most frequently. I will summarise published and unpublished results and present a new project exploring origin and maintenance of intrapopulational cytotype mixture in S. carniolicus following two complementary research avenues. First, origin and evolutionary relationships among different cytotypes are investigated in both space and time using a phylogenetic and phylogeographic approach mainly based on DNA sequence data. Second, mechanisms for maintenance of the cytotype mixture are explored with respect to the potential role of several pre- and postzygotic isolation mechanisms. Investigation of an altitudinal gradient on a mountain slope where di- and hexaploids co-occurred, suggested a narrow altitudinal range of the hexaploid cytotype in the low-alpine belt and a much wider range of the diploid one, spanning both low-alpine and high alpine zones. Focussing on ecological segregation and spatial autocorrelation of di- and hexaploids within a c. 20 x 60 m plot with varied micro-topography revealed that ecological factors that are correlated with micro-topography are strongly predicting the occurrence of either cytotype. The taller-growing hexaploids were restricted to fairly dense alpine grassland vegetation with presumed long snow-cover, whereas the low-growing diploids were only occurring in open habitats on a wind-blown ridge.

Keywords : cytotype mixture, ecological segregation, alpine plant, polyploid evolution

**S8-15**

**FLOWERING OVERLAP AS A PREDICTOR OF HYBRIDIZATION FREQUENCY IN NATURE**

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When natural interspecific hybridization occurs, it is a signal that some of the reproductive isolating mechanisms between populations of parental species have been broken. In this study we are focusing on the phenology of flowering as one of the basic pre-mating reproductive isolating mechanisms, considering overlap in flowering as an essential premise for interspecific hybridization. The main question is to what extent is it possible to explain differences in the frequency of particular hybrid combinations in nature by the overlaps in the flowering of the parental species.

We used all 11 Cirsium species (Asteraceae) growing in the Czech Republic as a model group. Based on 1136 herbarium specimens of Cirsium hybrids found in the Czech herbaria, we estimated the frequencies of 30 hybrid combinations in nature, and then we compared these frequencies with experimentally estimated overlaps in the blossoming of individual Cirsium species pairs.

All 11 Cirsium species were cultivated in three experimental gardens, situated in regions with different climates. During the vegetation periods 2005–2007 we visited these plots twice (2005) or once (2006–2007) a week and recorded the numbers of flower heads classified in nine predefined stages of flowering. The complete dataset gives information considering not only the flowering times, but also the seasonal dynamics of flowering intensity.

The comparison between the overlaps in flowering times (or in flowering intensities) and the frequency of individual hybrid combinations has shown that the extreme rarity or complete absence of many of the hybrid combinations in the wild cannot be explained by different flowering times as many of these species pairs flower simultaneously. If the four species that only rarely form hybrids are omitted, the flowering overlap becomes a useful predictor for the frequency of hybrids. We tested three different formulas for counting the flowering overlaps as potential predictors of the frequency of natural hybrids: (i) the overlap in relative flowering intensity (expressed as percentage from the yearly maximum of simultaneously flowering heads), (ii) the overlap in absolute flowering intensity (expressed as number of simultaneously flowering heads per one shoot), (iii) the simple overlap in flowering time. The second formula was shown to be the best predictor.

Keywords: Cirsium, interspecific hybrids, phenology, thistle

**S8-16**

## HYBRIDISATION EVENTS BETWEEN CULTIVATED POPULUS X CANADENSIS MOENCH. AND THE WILD RELATIVE POPULUS NIGRA L.

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Beside habitat destruction and modification, overexploitation and replacement by cultivated poplar plantations, hybridization and gene flow from domesticated poplars may have substantial impact on the evolution of native poplar populations. This may particularly be the case for rare native poplar species like the European black poplar (Populus nigra L.), coming into contact with abundant domesticated poplar plantations representing a limited number of poplar clones and genotypes.

The objective of this study was to improve insight in how interspecific pollen interactions (i.e. mixtures of pollen of cultivated hybrid Populus x canadensis and native European black poplar) affect siring success of the European black poplar and the frequency of hybrid formation. Crossing relationships between European black poplar and clones of P. x canadensis were studied in greenhouse-experiments by 47 hand-pollinated controlled crosses with pollen mixtures during 2005 – 2008. Hybridization events and paternity of the seedlings were defined by using molecular microsatellite markers. Hybridization events obtained under controlled conditions in the greenhouse were compared with those under field conditions by analysing open pollinated half-sib progenies of black poplar females located in Belgium.

When pollen of the hybrid poplars where included in the pollen mixtures, the controlled pollinated female black poplars produced lower number of seeds compared to the controlled crosses with black poplar pollen only. The results indicate that hybridization of P. x canadensis can pose a threat to native black poplar populations even if gene pools do not mix. The presence of hybrid plantations of P. x canadensis near natural black poplar populations can result in a waste of reproductive effort of the native black poplar populations and therefore fasten extinction of populations, even if introgression or gene flow does not occur. These findings provide some insight into the possible consequences of any introductions of genetically modified poplars into the neighbourhood of native black poplar populations. Even if the genetically modified poplars are not compatible with their wild relatives for example due to reduced hybrid fertility, hybridization without introgression and gene flow may lead to a waste of reproductive effort and reduced fitness of the natural poplar populations.

Keywords: hybridization, introgression, conservation, Populus

**S8-17**

## THE EFFECT OF MULTIPLE ORIGINS ON ECOLOGICAL SUCCESS IN ALLOTETRAPLOID WILD WHEATS OF THE GENUS AEGILOPS (POACEAE)

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Polyploidy is ubiquitous in plant evolution and is thought to be an important engine of biodiversity that facilitates speciation, adaptation, and range expansion. Polyploid species can have a high evolutionary success, e. g. expressed in the finding that established polyploid species can occupy a wider geographical range or variety of habitats than either diploid progenitor. For allopolyploid species, this is often attributed to the existence of fixed heterozgosity resulting from entire genome duplication. The phenotypic effect of the duplicated loci may result in increased ecological amplitude of the allopolyploid relative to diploid progenitors. However, multiple origins of allopolyploid species may promote their ecological success by providing genetic variability in ecological traits underlying local adaptation and range expansion. Although many studies have found evidence for multiple origins of allopolyploid species, data are lacking on whether these multiple origins increase the ecological tolerance of the species.

In our study we show that in a group of allopolyploid species, range size and abundance are correlated with the number of potential origins. We found that allopolyploid Aegilops species contain multiple chloroplast haplotypes each shared or closely related to haplotypes of the diploid progenitor species, indicating multiple origins as a major source of variation. The number of estimated origins and of haplotypes in each allopolyploid species was positively correlated to the total area occupied and the tendency for the species to be common. The number of haplotypes in each allopolyploid species was significantly correlated to the area of overlap of both progentiors, i. e. the area in which allopolyploid hybridization can occur, not to the area of overlap between diploid and polyploid species or between polyploid species. Additionally, we found differences in ecological traits among independent origins in Ae. triuncialis, the species with the largest range.

Our results suggest that multiple origins could give rise to differentially adapted lines that could represent different ecotypes within one species. Thus, multiple origins could represent one source of genetic variation that is increasing the overall ecological amplitude in a polyploid species. In addition, independent origins could face different ecological conditions and selection regimes, assuming that it is likely that they originate in different parts of the range of the diploid progenitors. This could lead to selective divergence between different origins. Recombination among these multiple origins may facilitate further expansion of the ecological niche of allopolyploids.

Keywords : Allopolyploidy, multiple origin, ecological success, wild wheat

**S8-18**

**HYBRIDIZATION IN THE TRITICUM-AEGILOPS COMPLEX**

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The domestication of wheat (Triticum sp.), one of the major crop presently cultivated, results from allo-polyploidization events involving several Aegilops species. The reproductive isolation between species of both genera is incomplete. Aegilops species were traditionally used by wheat breeders as a source of beneficial traits for selection of new varieties. Conversely, F1 hybrids between wheat cultivars and Aegilops species were regularly reported in agroecosystems. Despite these observations, long term consequences of gene transfer of wheat in the Aegilops genetic pool have been poorly documented. This topic is however of great concern for risk assessment studies associated for instance to the potential release of transgenic wheat.

The present study investigates three European Aegilops species (Ae. geniculata Roth, Ae. neglecta Req. ex Bertol. and Ae. triuncialis L.) and surveys natural Mediterranean populations growing in Spain, France, Italy and Croatia. The experimental design compared samples collected along wheat field borders to samples originating in areas isolated from agriculture. AFLP genotyping was used to assess the presence of wheat genetic markers in Aegilops populations.

The results varied according to Aegilops species: Ae. geniculata, the most autogamous species included in our study, showed no clear evidence of gene flow from wheat, except for one Ae. geniculata x Triticum F1 hybrid discovered in a Spanish population. In contrast, an unexpectedly high amount of wheat genetic markers were observed in Ae. neglecta and Ae. triuncialis samples. Interestingly, most of the affected samples originated from populations collected near to crop cultivations. In contrast, samples collected in areas isolated from agriculture showed almost no evidences of gene flow from wheat. These results provided reasonable evidence that long-term introgression of wheat markers has occurred at least in Ae. neglecta and Ae. triuncialis. Finally, Triticum turgidum (the pasta wheat) is the most likely wheat parent of introgressed Aegilops. In contrast, T. aestivum (the bread wheat) appears only to be the parent of the Ae. geniculata x Triticum F1 hybrid. Triticum turgidum and the three Aegilops species investigated in the present study are tetraploid organisms and share the same chromosome number (2n = 4x = 28). In contrast, T. aestivum is a hexaploid plant (2n = 6x = 42) and forms pentaploid hybrids with Aegilops (2n=5x=35), which are probably less fertile than the tetraploid Aegilops x T. turgidum hybrids.

Keywords: Crop, Wild relative, Introgression, Transgene

**S8-19**

## MULTIPLE ORIGINS OF TETRAPLOID VERONICA CHAMAEDRYS ON THE BALKAN PENINSULA

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Veronica chamaedrys (Plantaginaceae) is a widespread tetraploid species in Europe occurring mainly in open forests and forest margins from sea-level to the subalpine zone. Diploid individuals are mainly known from southeastern Europe, where they fall into morphologically distinct species or subspecies (V. chamaedryoides, V. chamaedrys ssp. vindobonensis, V. chamaedrys ssp. micans and V. krumovii). The distribution of these diploid species suggests survival in Pleistocene forest refugia.

Using genome size estimations, AFLP fingerprints, cpDNA markers as well as morphological characters, taxonomical and phylogeographical questions were addressed. The analyses support three geographically separated diploid taxa and one additional diploid, possibly hybridogenic taxon, all of which correspond to previously recognized morphologically defined diploid species. Tetraploids originated multiple times within the diploid groups apparently almost exclusively as autotetraploids. Despite separate origins from morphologically distinct diploids, tetraploids are morphologically not easily distinguishable. Diploid cytotypes dominate in the south of the distribution area of Veronica chamaedrys s.l., while tetraploids are increasingly important towards the North.

Keywords: Balkan Peninsula, Veronica, Aflp, cpDNA, morphometry

**S8-20**

## GENETIC DIVERSITY AND POPULATION GENETIC STRUCTURE OF *AEGILOPS TAUSCHII* IN NORTHERN IRAN

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Were studied based on nine microsatellite loci. A high level of genetic diversity was observed from the accessions collected from six regions (provinces) in Iran. The nine microsatellites revealed a total of 141 alleles, with an average of 15.6 alleles per locus. A comparison of the genetic diversity parameters indicated that subsp. tauschii possesses the highest genetic diversity, followed by unknown accessions. It was also found that the genetic diversity of T. aestivum is obviously lower than that of Ae. tauschii accessions. It is suggested that during the course of evolution from wild wheat to cultivated wheat, many alleles were lost through natural and human selection, leading to the lower heterozygosity and genetic diversity of the cultivated wheat. Moreover, the level of genetic diversity for Gilan, Golestan and Mazanderan provinces was higher than for Ardebil, Ghazvin and Semnan provinces, suggesting that these regions may be a center of genetic diversity for Ae. tauschii in Northern part of Iran.

Keywords: Ae. tauschii, genetic diversity, T. aestivum, microsatellite

**S8-21**

**CYTOTYPE DISTRIBUTION OF TUFTED VETCH (VICIA CRACCA L., FABACEAE) IN CENTRAL EUROPE: WHAT HAS CHANGED OVER THE LAST FOUR DECADES**

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Knowledge of the occurrence of different cytotypes and their geographic distribution is a stepping stone for further research on microevolutionary processes underlying plant polyploidisation events. Most of the studies exploring geographical distribution of different cytotypes come from the last decade, and we have almost no information on changes in distribution of different cytotypes over time. We studied changes in cytotype distribution over 40 years. We used Vicia cracca L. (Fabaceae) as a model, because this species was subjected to a detailed cytotype screening in Central Europe in the late 1960s. Flow cytometry allowed us to analyse a ploidy level of more than 7,000 individuals of V. cracca collected at 295 localities in the Czech Republic, the Slovak Republic, Poland, Austria, and Germany. Three different cytotypes (2x, 3x and 4x) were detected. While tetraploids predominated in the western part of the area investigated (189 populations), the diploids showed a more easterly distribution (85 populations). A contact zone was revealed near the Czech - Slovak borders where 21 populations (~7%) consisted of a mixture of 2x and 4x cytotypes. We suppose that this is a secondary contact zone. Triploids were very rare – only seven individuals were found in two otherwise diploid populations – indicating the existence of efficient breeding barriers between diploids and tetraploids. Indeed, we experimentally proved a triploid block between diploids and tetraploids. Compared to the late 1960s, the recent cytotype distribution pattern was quite similar except for the disappearance of diploids from southern Bohemia (Czech Republic). Eight diploid populations had been previously recorded in the area occupied otherwise solely by tetraploids. Nevertheless, diploids were no more found there by our field survey. This might result from a frequency-dependent selection against inter-ploidy hybrids known as minority cytotype exclusion. Another reason for diploids' disappearance could be their lower competitive ability under worse conditions due to altered management in these localities. Between-cytotype differences in fitness will be the aim of further research. The identical mean fluorescence per monoploid genome in diploid and tetraploid plants supported previously hypothesised autopolyploid origin of the tetraploid cytotype.

Keywords: Central Europe, contact zone, cytotype distribution, flow cytometry

**S8-22**

**WHAT MAINTAINS THE CYTOTYPE COEXISTENCE IN GYMNADENIA CONOPSEA? INSIGHTS ON BREEDING BARRIERS**

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Polyploidy has played a key role in plant evolution and diversification. Despite this, it is still largely unknown how different factors act in concert to achieve reproductive isolation among cytotypes in mixed populations. The present study aims to answer critical questions regarding the origin and maintenance of contact zones of various cytotypes, using Gymnadenia conopsea complex (Orchidaceae) as a study model. Specifically, we assessed cytotype distribution and coexistence in Central Europe and investigated pre- (temporal segregation, floral traits divergence, assortative mating and gametic isolation) and post-zygotic (crossing ability and hybrid viability) barriers involved in the maintenance of mixed cytotype populations. Using flow cytometry, we determined DNA ploidy levels in 2200+ individuals from 36 populations. Five different cytotypes were detected; tetraploids (60%) and octoploids (37%) clearly prevailed while other ploidies were much rarer (< 1.3%). Ploidy mixing was quite a common phenomenon, with more than half of populations harbouring two or more cytotypes. Regarding pre-mating barriers, coexistence of cytotypes in mixed populations is partly maintained by differences in floral phenology. However, cytotypes with overlapping flowering periods can freely hybridize due to similarities in floral colour and spur length, lack of assortative behaviour of pollinators and absence of gametic incompatibility. Crossing experiments revealed that minority ploidies largely originated from crosses between tetraploid and octoploid plants, with most progeny (95%) being hexaploid. Interestingly, 4x + 8x crosses often produced high proportion of seeds with similar germinability as the homoploid crosses. On the other hand, backcrossing of hexaploids with their parents produced only very low seed quality. The co-occurrence of dominant cytotypes in mixed populations is thus maintained by both partial temporal segregation and limited reproduction of hybrids. The absence of reproductive barrier precluding pollen wasting to other cytotypes as well as the genesis of hexaploid progeny is discussed. Collectively, the Gymnadenia system represents a unique group for addressing several general questions surrounding the genesis and maintenance of diversity in wild plant populations.

Keywords: assortative matting, crossing ability, fragrant orchid, phenology

**S8-23**

**ECOLOGICAL CONSEQUENCES OF POLYPLOIDY IN *CORYDORAS* CATFISHES**

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With over 150 species, the armoured catfishes of the genus Corydoras amount to a significant proportion of the freshwater ichthyofaunal diversity of South America. They are of particular interest from an evolutionary perspective as they have extraordinary variation in genome sizes and chromosome numbers, suggesting that polyploidy has played an important role in their diversification. At many (>12 known) sites throughout South America, several Corydoras species exist in sympatry. These co-occuring species shoal together and have evolved almost identical colour patterns yet differ subtly in morphology.
Here we demonstrate using mtDNA and nuclear phylogenies that sympatric species are always from different genetic clades, ruling out the possibility of their evolution via sympatric speciation. We also demonstrate using stable isotope analysis that sympatric species with different genome sizes are ecologically differentiated, with members of a single genetic clade occupying the same trophic level at multiple sites. We suggest that this may enable the long term co-existence of sympatric Corydoras species providing the stability for the evolution of identical colour patterns through predation driven natural selection. Although no causal link has yet been established between polyploidy and niche divergence, we demonstrate consistent differences in morphology and niche between species with different genomic complements.

Keywords : Catfish, mimicry, niche divergence

**S8-24**

**ORIGIN, DISTRIBUTION AND CO-EXISTENCE OF CYTOTYPES IN THE POLYPLOID ASTER AMELLUS COMPLEX**

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Polyploidization is recognized as a major mechanism of speciation in plants, still, little is known about the performance, dynamics, and evolutionary potential of polyploids. Using Aster amellus complex as study model, the aim of the ongoing investigation is to analyse the distribution of the different cytotypes and understand the factors involved in their evolution. Aster amellus is a polymorphic perennial herb with several cytotypes (diploid, tetraploid and hexaploid individuals) occurring across Europe and western Asia. In the present study we provide new insights on cytotype distribution in Central Europe, on the maintenance of the diploid-hexaploid contact zone and preliminary data on distribution of different lineages within Europe. Screening of ploidy level revealed the occurrence of a rather diffuse secondary contact zone between diploid and hexaploid cytotypes, which extends from Poland, via the Czech Republic and Slovakia to Austria. Despite growing in close proximity in many localities and even in one mixed population, no tetraploid individuals have been detected so far. The lack of between-cytotypes hybrids might be explained by the barriers observed at the breeding system, which limit natural hybridization and may be one of the main factors involved in the maintenance of the contact zone. Our future objectives are focused on the determination of the levels of pollen flow at the secondary contact zone, on the assessment of the patterns of cytotype distribution across the remaining area and on the description of the migration patterns of the different lineages within Europe.

Mots-clés breeding barriers, chloroplast DNA, experimental crosses, cytotypes, secondary contact zone

**S8-25**

**THE EFFECT OF MULTIPLE ORIGINS ON ECOLOGICAL SUCCESS IN ALLOTETRAPLOID WILD WHEATS OF THE GENUS AEGILOPS (POACEAE)**

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Polyploidy is ubiquitous in plant evolution and is thought to be an important engine of biodiversity that facilitates speciation, adaptation, and range expansion. Polyploid species can have a high evolutionary success, e. g. expressed in the finding that established polyploid species can occupy a wider geographical range or variety of habitats than either diploid progenitor. For allopolyploid species, this is often attributed to the existence of fixed heterozgosity resulting from entire genome duplication. The phenotypic effect of the duplicated loci may result in increased ecological amplitude of the allopolyploid relative to diploid progenitors. However, multiple origins of allopolyploid species may promote their ecological success by providing genetic variability in ecological traits underlying local adaptation and range expansion. Although many studies have found evidence for multiple origins of allopolyploid species, data are lacking on whether these multiple origins increase the ecological tolerance of the species.
In our study we show that in a group of allopolyploid species, range size and abundance are correlated with the number of potential origins. We found that allopolyploid Aegilops species contain multiple chloroplast haplotypes each shared or closely related to haplotypes of the diploid progenitor species, indicating multiple origins as a major source of variation. The number of estimated origins and of haplotypes in each allopolyploid species was positively correlated to the total area occupied and the tendency for the species to be common. The number of haplotypes in each allopolyploid species was significantly correlated to the area of overlap of both progentiors, i. e. the area in which allopolyploid hybridization can occur, not to the area of overlap between diploid and polyploid species or between polyploid species. Additionally, we found differences in ecological traits among independent origins in Ae. triuncialis, the species with the largest range.
Our results suggest that multiple origins could give rise to differentially adapted lines that could represent different ecotypes within one species. Thus, multiple origins could represent one source of genetic variation that is increasing the overall ecological amplitude in a polyploid species. In addition, independent origins could face different ecological conditions and selection regimes, assuming that it is likely that they originate in different parts of the range of the diploid progenitors. This could lead to selective divergence between different origins. Recombination among these multiple origins may facilitate further expansion of the ecological niche of allopolyploids.

**Mots-clés Allopolyploidy, multiple origin, ecological success, wild wheat**

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