Charles University

Faculty of Science

Genomes of plant interspecific hybrids: their structure and evolution

HABILITATION THESIS

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Content

1. Preface

Interspecific hybridization merges genomes of two different species into a single organism. Besides its key role in plant speciation, it also provides breeders an opportunity to combine agriculturally important traits from two species or to introgress one or more traits from one species (usually a wild relative) into elite cultivars of a crop species. Cytogenetic tools, such as fluorescence *in situ* hybridization (FISH), make it possible to visualize chromosomes of parental species and track their behavior in the hybrid progeny. When I first observed „painted“ chromosomes of *Festuca x Lolium* hybrids in the laboratory of Prof. Adam J. Lukaszewski, I was impressed and immediately knew I wanted to pursue my carrier in plant cytogenetics. Since then, I focused on the structure, organization, function and evolution of genomes in plant hybrids, and have never regretted.

I had a good fortune during my college years and my research carrier to meet nice and supportive persons. I would like to thank to. Adam J. Lukaszewski, who showed me not only the beauty of cytogenetics, but also how to work and how to behave. I still consider his sentence „There is no such thing as free lunch!“ as one of the most valuable advice I received in my life. Many thanks go to Jaroslav Doležel, Head of our Research Centre, always supportive and always willing to discuss various aspects of plant genetics. I wish to thank to my past teachers and supervisors: Dr. Jitka Šedivá, Dr. Dana Šafářová and Prof. Jiří Vagera, for their enthusiasm for teaching and their supervision. It was not always an easy task. Last, but not least, I would like to express many thanks to Ing. Vladimír Černoch, the most open-minded plant breeder I have ever met.

2. Introduction

Polyploidization and interspecific hybridization are two key processes underlying evolution and speciation. Many polyploidization events have been dated back to the Cretaceous-Paleogene (K/Pg) boundary (Fawcett et al., 2009) about 66 million years ago. There is evidence that all angiosperms underwent at least one round of polyploidization during their evolutionary history (Jiao et al., 2011), and it is estimated that up to 70% of recent plant species are true polyploids (Masterson, 1994). The underlying reason may be that polyploidization provides a source of genetic, biochemical and evolutionary novelty (Soltis and Soltis, 1993). For example, polyploidy is probably responsible for key innovations such as the origination of the flower (Buzgo et al., 2004), of true vessel elements in stem tissue and developmentally sealed carpels (Soltis and Soltis, 2016), and a shift to the pentamerous groundplan in *Pentapetalae* (Chanderbali et al., 2016).

There are two types of polyploids: autopolyploids and allopolyploids. Autopolyploids generate extra sets of chromosomes by intraspecific whole genome duplications (WGD), whereas allopolyploids arise by hybridization of two distinct species, preceded or followed by WGD. It has to be mentioned that interspecific hybridization can also lead directly to speciation, but such homoploid hybrids are rare. To date, only 19 putative homoploid hybrid speciation events have been documented in flowering plants (Yakimowski and Rieseberg, 2014). Allopolyploidy can generate intergenomic heterosis providing competitive advantage over diploid progenitors (Comai, 2005), mask deleterious recessive alleles leading to increased mutational robustness (Madlung, 2013), convey increased stress tolerance through such mechanisms as delayed reproduction and longer life span, and increased defense against herbivores and pathogens (Lohaus and Van de Peer, 2016). In general, allopolyploids display broader adaptation to novel environmental niches compared to their progenitor species, hence a greater ability to colonize disturbed and harsher habitats (te Beest et al., 2012), leading to increased invasiveness (Pandit et al., 2011). All these innovations associated with allopolyploidy may have triggered their radiation during angiosperm evolution (Soltis and Soltis, 2016).

Apart from the key role that allopolyploidy and interspecific hybridization play in plant evolution and diversification, these processes lay at the origin of many major crops including wheat, banana and cotton. Moreover, interspecific hybridization is frequently used in breeding programs to incorporate novel alleles into existing germplasm to either increase global genetic diversity in the genepool of a crop, or to introgress specific genes or traits to improve agronomically beneficial characteristics such as tolerance to abiotic or biotic stresses. One such example is the interspecific hybridization of ryegrass (*Lolium* sp.) and fescue species (*Festuca* sp.), giving rise to xFestulolium. Since the first commercial success in releasing a xFestulolium cultivar, many cultivars have been produced and are successful on the grass seed markets. This is mainly due to a combination of the high yield and nutrition characteristics of

ryegrasses with the tolerance to abiotic stresses and persistence gained from fescues (Ghesquiere et al., 2010).

Similar to hybridization of forage and turf grasses, cross-hybridization of wheat with its cultivated or wild relatives is also often utilized to improve elite cultivars of wheat (Trethowan and Mujeeb-Kazi, 2008). The best known intergeneric introgression in wheat is translocation chromosome 1RS.1BL, where the short arm of wheat chromosome 1B (1BS) is replaced with its rye 1RS homeologue. Originally, the 1RS.1BL translocation offered several genes for resistance to pathogens and pests of wheat (Zeller, 1973). Later on, the resistance started breaking down, however, it was found that the translocation also had a positive effect on grain yield, at least in some genetic backgrounds or environments (Carver and Rayburn, 1994; Moreno-Sevilla et al. 1995; McKendry et al. 1996). Eventually this grain yield effect was associated with larger root biomass, especially under water-stress conditions (Ehdaie et al., 2003; Waines and Ehdaie, 2007). Since the first success with introgression breeding, many new wheat lines carrying alien introgressions were developed for either scientific and/or breeding purposes.

Figure 1, Morphology of tillers/inflorescences of *L. multiflorum* (left), xFestulolium (middle) and *F. arundinacea* (right).

3. Research Interests

Allopolyploidization and interspecific hybridization represents a unique opportunity to combine characters from two different species (or even genera). However, still little is known about the behavior of two genomes in the single organism, especially in plant hybrids. Once I started my research carrier, almost nothing was known about the structure and evolution of agriculturally important hybrids of ryegrasses and fescues. Thus, my first aim was to analyze genome composition of existing cultivars. As usual, every single finding has opened a new question to be answered and broaden my research interests. Since then, I focused on many aspects of wide hybridization and polyploidization in grass hybrids, but also in cereals. This involved changes in genome constitution over the successive generations, behavior of chromosomes in meiosis (as the key factor in fertility of hybrids and stability of hybrid genomes), gene expression changes and spatial organization of parental genomes in hybrid nuclei using various methods and techniques of molecular biology, cytogenetics, microscopy and genomics. Most of this work was done in collaboration with plant breeders and our results are applicable in breeding processes. In the following chapter, I will describe my main findings from four different, but linked areas of plant hybridization and discuss them in broader context.

3a. Structure and evolution of genomes of plant hybrids

In my work, I focused on three types of interspecific hybrids (*Festuca* x *Lolium* hybrids, alien introgressions in wheat and allopolyploid *Thinopyrum*/*Elymus*, all of which possess different characteristics, as I will discuss it in the following text. My main experimental object has been xFestulolium, various hybrids between fescues (*Festuca* L.) and ryegrasses (*Lolium* L.). Ryegrasses hybridize with fescues even in nature, and several natural hybrids have been documented in southern England and Switzerland (Jauhar, 1993). However, natural hybrids are reportedly sterile. The existence of natural hybrids was picked up by grass breeders. They came up with the idea of combining agricultural characteristics of both parental genera into a single organism. Ryegrasses are high yielding, nutritious grasses with rapid establishment, fine texture and uniform turf, but suffer under climatic stresses, such as summer drought and winter freezing. On the other hand, fescues are not as yielding and nutritious as ryegrasses, but possess tolerance to abiotic stresses (Ghesquiere et al., 2010).

There are two types of xFestulolium: amphiploids and introgression forms. Amphiploids arise by hybridization of tetraploid parents, such as synthetic autotetraploid meadow fescue (*F. pratensis* Huds.) with Italian or perennial ryegrass (*L. multiflorum* Lam. and *L. perenne* L.). The F1 hybrids are further intercrossed. On the other hand, introgression forms are developed by hybridization of the parental

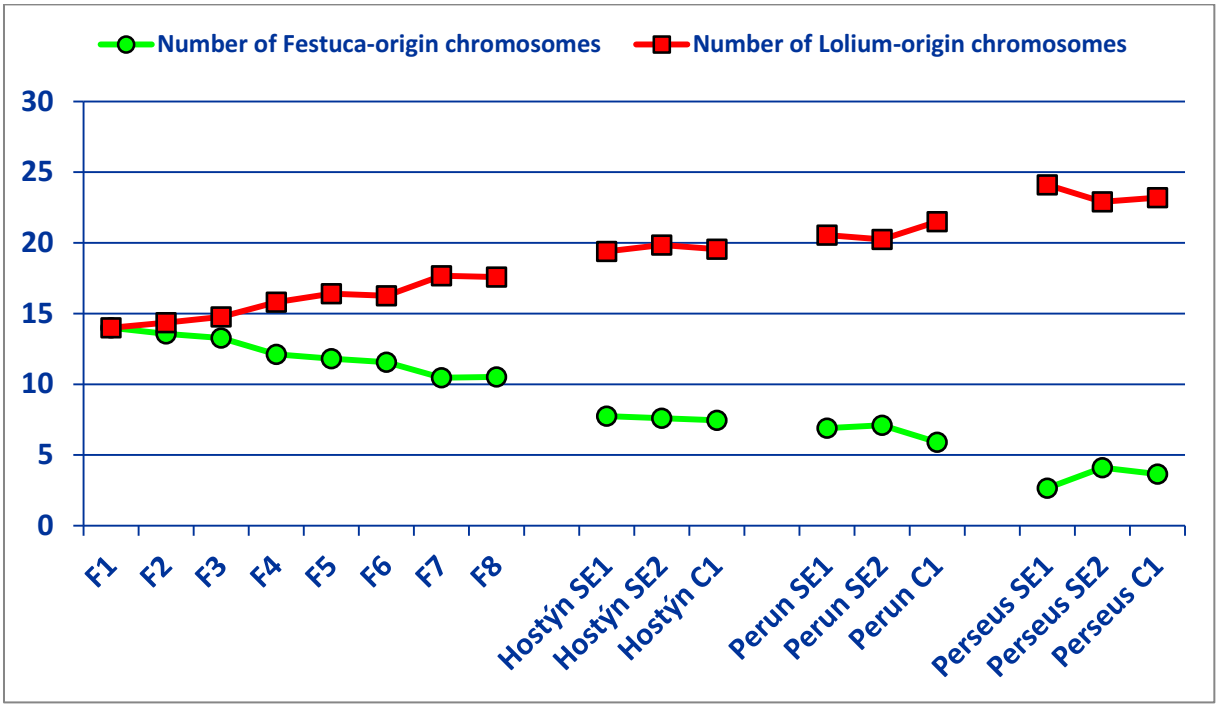
species with different ploidy levels, such as hexaploid tall fescue (*F. arundinacea* Schreb.) with diploid Italian ryegrass. Tetraploid F1 hybrids are backcrossed to one of the parental species resulting in hybrids that are morphologically and genetically closer to the parent used in the backcross with introgressed specific characteristics from the other parent (and having little chromatin from that parent). In the late 1960's, breeders from several breeding stations in Europe and USA succeeded with interspecific hybridization and in subsequent years, first cultivars of xFestulolium were released.

Despite commercial success of xFestulolium, it took a long time to uncover the genomic composition of various cultivars. Only after the introduction of molecular cytogenetic techniques, such as fluorescence and genomic *in situ* hybridization (FISH and GISH), proportions of the parental genomes in hybrids was investigated in detail. In collaboration with the plant breeding station in Hladké Životice, I karyotyped a comprehensive set of xFestulolium cultivars (Kopecký et al., 2006). Interestingly, there was a high variation within and between the cultivars, with intravarietal variation frequently exceeding the between-cultivar variation. Genomic composition ranged from almost equal proportions of the parental genomes, such as in cv. Lueur developed from a hybrid *L. multiflorum* x *F. glaucescens*, to highly introgressed forms, where only few plants in the populations carried one or very few segments of *Festuca* genome in the *Lolium* background, such as in cvs. Lofa and Bečva. Notably, there were several cultivars where no *Festuca* chromatin was detected. If there was any *Festuca* chromatin present, it was below the resolution limit of the technique. Proportions of parental chromatin frequently correspond with the breeding history of a cultivar. The cultivars where one or more steps of backcrossing into one parental species were involved, proportions of parental chromatin were highly skewed toward the backcross parent.

There are several mechanisms underlying high variation in the genomic composition within cultivars. Both ryegrasses and fescues are outcrossers (self-incompatible) and breeding is based on open pollination. Moreover, homeologous chromosomes of fescue and ryegrass pair during meiosis and recombine freely (Kopecký et al., 2006, 2008a). This is due to the absence of a chromosome pairing control system that would prevent homeologues from pairing, and close relationship (affinity) of the parental species involved in hybrids, whose DNA sequence composition is sufficiently close to initiate homeologous chromosome pairing during meiosis. This generates high gametic variability and under cross-pollination creates highly variable progeny where in essence, every single plant is genetically unique. This has two consequences. At first, it generates enormous genetic variability for selection of elite plants in the breeding process, and may lead to the development of cultivars resilient to climatic changes. On the other hand, it brings about problems in genome stabilization of individual cultivars. This variability which may affect their registration under strict distinctiveness-uniformity-stability (DUS) requirements. However, we found that despite large variability within the cultivars, proportions of

parental genomes in existing cultivars do not significantly change from generation to generation. In three successive generations of three *L. multiflorum* x *F. pratensis* cultivars we detected only minor variation between generations (Kopecký et al., 2017). This was a relief for breeders, but all three cultivars were of the amphiploid type and the stability of fescue chromatin segments in the introgression forms remains unclear. For this reason, we conducted analyses of the transmission rates of *Festuca* segment(s) in four introgression cultivars of *Lolium multiflorum* x *Festuca pratensis* (Kopecký et al., 2019a) and observed progressive elimination of *Festuca* segments in all four cultivars (about 27-32% in a single round of multiplication). At this pace, the *Festuca* chromatin would be completely eliminated within about four generations of seed multiplication. On the other hand, we have also observed that the proportion of *Festuca* chromatin in the cultivars can be increased by proper selection of mating plants (such as by using GISH). However, once selection is relaxed, the first round of the seed multiplication reverts the genome composition back to the *Lolium* type. Thus, amphiploid forms of xFestulolium with relatively stable hybrid genome constitutions appear to be a more promising material for future breeding than the introgression lines.

Figure 2, Changes in the proportion of parental genomes in successive generations (F_1-F_8) and three cultivars (three successive generations) of xFestulolium (adopted from Zwierzykowski et al., 2011 and Kopecký et al., 2017) as revealed by GISH. Note the shift towards *Lolium* in the first generations after hybridization and relative stability genomic constitution in the cultivars.



An interesting phenomenon in the genomic composition of amphiploid xFestulolium forms is the dominance of the *Lolium* genome over that of *Festuca*. All commercial amphiploid cultivars show higher proportions of the *Lolium*-origin chromosomes compared to those of the *Festuca*-origin (Kopecký et al., 2006).

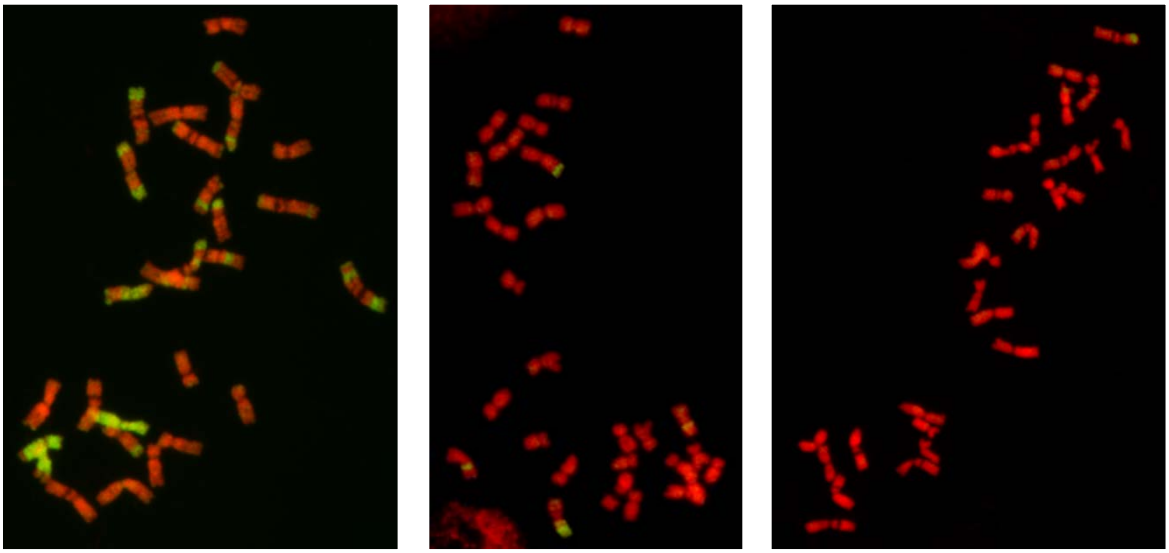
Zwierzykowski et al. (2011) studied changes in proportions of parental chromatin in eight consecutive generations of *L. perenne* x *F. pratensis* hybrids and observed a gradual shift towards the *Lolium* genome (Figure 2). However, as the hybrids were subject to selection during the breeding process, perhaps individuals with more evident ryegrass-type characteristics (such as rapid establishment from seed) were preferentially selected. For that, the team repeated the experiment with plants under random mating and observed highly similar results, with the gradual, albeit slower, replacement of *Festuca*-origin chromosome by those of *Lolium*-origin, (Zwierzykowski et al., 2012). The mechanism underlying this phenomenon is unknown. However, meiotic drive such as that observed in some hybrids, including mice (Akera et al., 2017) may play a significant role.

Genomic *in situ* hybridization is a great tool to study structure and evolution of xFestulolium hybrid genomes. However, as total genomic DNA is used as a probe, the analysis offers no information on the identity of individual chromosomes. The only known cytogenetic markers were rDNAs, with 45S rDNA located on the short arm of chromosome 3F (meadow fescue) and on three chromosomes of Italian ryegrass, while 5S rDNA is positioned on the short arms of chromosomes 2F and 2L (of *L. multiflorum*). With aim to identify cytogenetic markers specific for individual chromosomes, we developed a partial BAC library for *F. pratensis* (Kopecký et al., 2008). So far, out of 72 BAC clones tested, four turned to be chromosome-specific, or at least they provide chromosome specific hybridization patterns. Even though BAC hybridization is nowhere near as robust molecular cytogenetic markers, we were able to construct the first molecular karyotype of *F. pratensis*. The karyotype identifies all seven chromosomes and (using collinear markers from genetic maps) places them in homeologous groups of Triticeae (such as barley and wheat) with chromosome 4F to be largest, three intermediate-sized 2F, 3F and 7F, and group of three smaller chromosomes 1F, 5F and 6F.

Flow cytometry is a technique, which enable sorting individual particles based on their physical parameters. For example, chromosome 4F is different enough in size from other chromosomes and this difference creates a separate peak in the flow karyotype. Thus, we were able to successfully sort this chromosome and sequenced it using the Illumina platform (Kopecký et al., 2013). Sequence data of chromosome 4F opens new avenues in our research. We were able to assign chromosome 4F sequences to pseudomolecules of the already sequenced genome of barley using the GenomeZipper approach (Mayer et al., 2009), confirm and study in detail the main translocation (4F→4H/5H) differentiating the ancestral meadow fescue genome from modern barley and wheat genomes, analyze repetitive sequences and identify tandem repeats. These newly identified tandem repeats turned out to be a great source of robust cytogenetic markers and enabled construction of detailed molecular karyotype using FISH (Kopecký et al., 2013, Křivánková et al., 2017). Apart from the use of BAC-based probes and other molecular probes enriched with sequence repeats developed in our successive study (Majka et al., 2017), it is now possible to

use these cytogenetic markers for detailed analyses of genome constitutions of various grass hybrids.

Figure 3, Genomic composition of xFestulolium cultivars Achilles (left), Spring Green (middle) and Lofa (right) revealed by genomic *in situ* hybridization (GISH). Total genomic DNA of *L. multiflorum* was labelled with digoxigenin and detected by antiDig-FITC (green color) and total genomic DNA of *F. pratensis* was sheared and used as blocking DNA (red pseudocolor). Note the predominance of *Lolium* chromatin in all three cultivars and almost complete elimination of *Festuca* chromatin in cv. Lofa.



Despite the success and the high resolution levels of the molecular cytogenetic techniques, they still suffer from relatively low throughput. To address this bottleneck we developed a DNA chip with the aim to provide high throughput technique to analyze hundreds to thousands plants per season, hence more control in the breeding process. In collaboration with the Diversity Arrays Technology Ltd., we developed a DNA array consisting of 7680 probes (Kopecký et al., 2009a). This DArTFest array enabled us to study genomic composition of xFestulolium hybrids, to improve genetic maps of four grass species, to estimate genetic diversity of individual populations and to link genetic markers to agriculturally important traits with potential for marker-assisted selection.

We have incorporated hundreds of DArT markers into existing genetic maps of tall fescue (Dierking et al., 2015), meadow fescue, Italian ryegrass (Bartoš et al., 2011) and perennial ryegrass (Tomaszewski et al., 2012), and substantially increased their resolution. The utility of the resulting genetic linkage map for QTL analyses was demonstrated by the identification of a QTL associated with a moderate crown rust resistance (Tomaszewski et al., 2012). Similarly, 96 markers were significantly associated with freezing tolerance in xFestulolium, and five of them were genetically mapped to chromosomes 2, 4 and 7 of *F. pratensis*. Three genomic

loci associated with freezing tolerance co-localized with chromosome segments and QTLs previously identified to be associated with freezing tolerance (Bartoš et al., 2011).

To test the potential of DArT markers for analyses of genomic composition of xFestulolium, we analyzed five xFestulolium cultivars (each represented by twenty individual plants and seven bulked samples). The robustness of the technology was documented by clustering of individual plants (or bulked samples of each cultivar on the dendrogram). To estimate the genomic composition of hybrids, we identified species-specific DArT markers, based on their presence/absence in the parental species. The results highly correlated with the genomic composition analysis made by GISH (Kopecký et al., 2011).

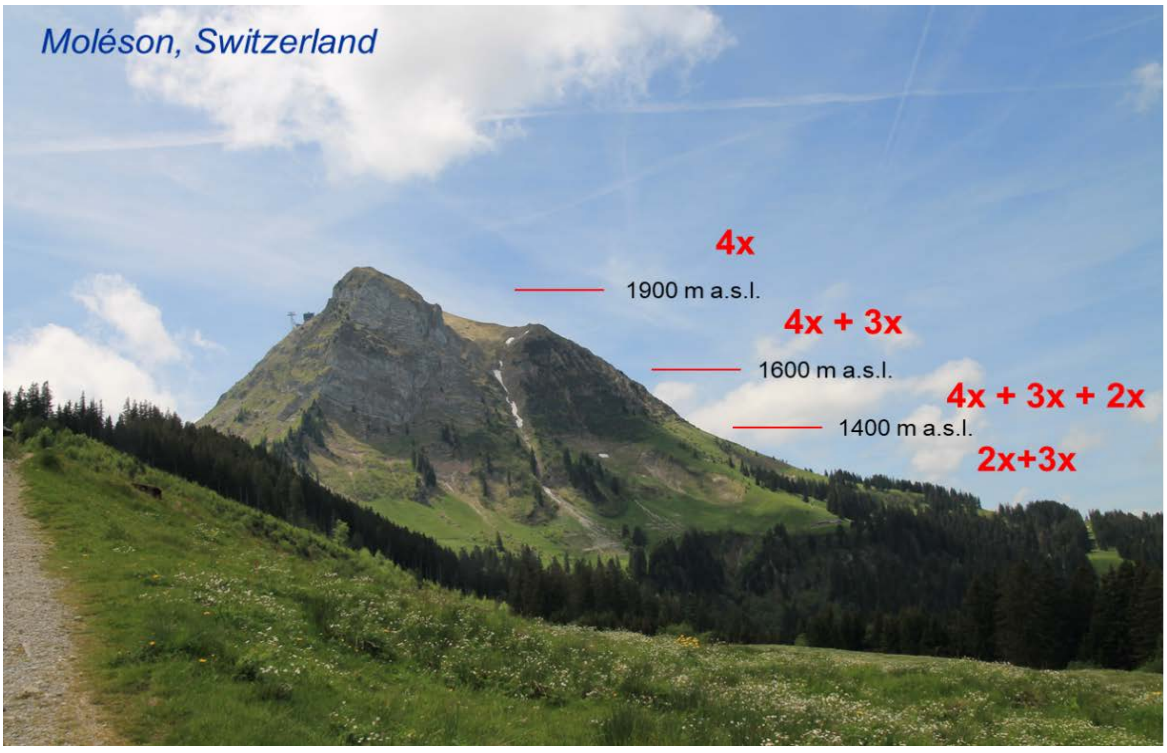
DArT markers can be employed in the protection and registration of existing and new cultivars. A study of genetic variability has been conducted with a set of turf-type cultivars of tall fescues from the USA. Interestingly, there was very little genetic diversity among over 90 entries from different breeding programs compares to the levels of diversity among forage cultivars from Europe (Baird et al., 2012). This implies massive sharing of plant material and the absence of any protection of existing cultivars in the USA. DArT array is also used for the testing of the plant material prior Distinctness, Uniformity and Stability (DUS) Testing and registration process by DLF Seeds and Science (breeding company in Hladké Životice, Czech Republic). The implementation of the DArT technology into the DUS tests would provide information on diversity and uniformity at the resolution never achieved by morphological screening.

Most of the xFestulolium cultivars originate from hybrids between meadow fescue and either Italian or perennial ryegrass. However, there is a growing interest among breeders to use wild relatives of meadow fescue. They frequently occupy sites with harsh climatic conditions, such as *F. mairei* growning in high Atlas of northern Africa or *F. apennina* at high altitudes of the Alps, Apennines and Carpathians. Most of these species, belonging to section *Schedonorus,* are polyploid with poorly understood origin. There are only two diploid species, meadow fescue as the presumable basal species involved in speciation of all or at least majority of polyploid species, and endemic *F. fontqueri* (Catalán et al., 2004). Other species are polyploid, ranging from tetraploids to decaploids. Our study on the phylogeny of the sect. *Schedonorus* using cytogenetic techniques did not offer satisfactory resolution but only indications of the possible origin of the polyploid species (Ezquerro-Lopez et al., 2017). Similarly, GISH and FISH with probes for rDNAs provided little information on the phylogeny of the fescue species from the Iberian Peninsula; more indications on the origin and relationships of the species was revealed by analyses on the genome size using flow cytometry (Loureiro et al., 2007). From a breeding point of view, the most interesting species are tetraploid *F. apennina*, *F. glaucescens* and *F. mairei*. They all possess interesting traits, such as tolerance to freezing and drought in *F. apennina* and *F. glaucescens,* and heat and drought in *F. mairei*. Moreover, they

also show diploid-like chromosome pairing indicating the presence of a system or systems preventing homeologous pairing (to be discussed in detail in the next chapter).

The origin and phylogeny of tetraploid fescues is largely unknown. There was a suggestion that *F. apennina* was either an autotetraploid of *F. pratensis* or an allotetraploid of *F. pratensis* with some unknown fescue species. In collaboration with Dr. Beat Boller (Agroscope, Switzerland) and Dr. Nicola Ardenghi (University of Pavia, Italy), I conducted expedition to collect a broad set of *F. apennina* for further study and field tests. Flow cytometric analysis of the collected vouchers revealed a presence of many triploids among the expected tetraploid *F. apennina* and diploid *F. pratensis* (morphologically difficult to distinguish in vegetative stages). Subsequent cytogenetic analysis revealed that tetraploid *F. apennina* was an allopolyploid that arose from a cross of *F. pratensis* with a species close to *F. glaucescens*. Triploids turned out to be F1 hybrids between diploid *F. pratensis* and tetraploid *F. apennina* (Kopecký et al., 2016). Interestingly, the triploid hybrids showed considerable hybrid vigor and performed well in field tests (one location in Czech Republic and three locations in Switzerland). Using molecular markers (cpDNA and DArT), we discovered that both cross directions were involved in the formation of triploids (with *F. pratensis* being maternal or paternal species) and they can propagate vegetatively by rhizomes over considerable distances (over 14 meters) (Kopecký et al., 2018).

Figure 4, Distribution of diploid *F. pratensis*, triploid hybrids *F. pratensis* x *F. apennina* and tetraploid *F. apennina* in relation to the altitude (adopted from Kopecký et al., 2016, 2018).



The taxonomy of the entire *Schedonorus* section has been debated for a long time and several nomenclatures have been proposed for various species. A recent

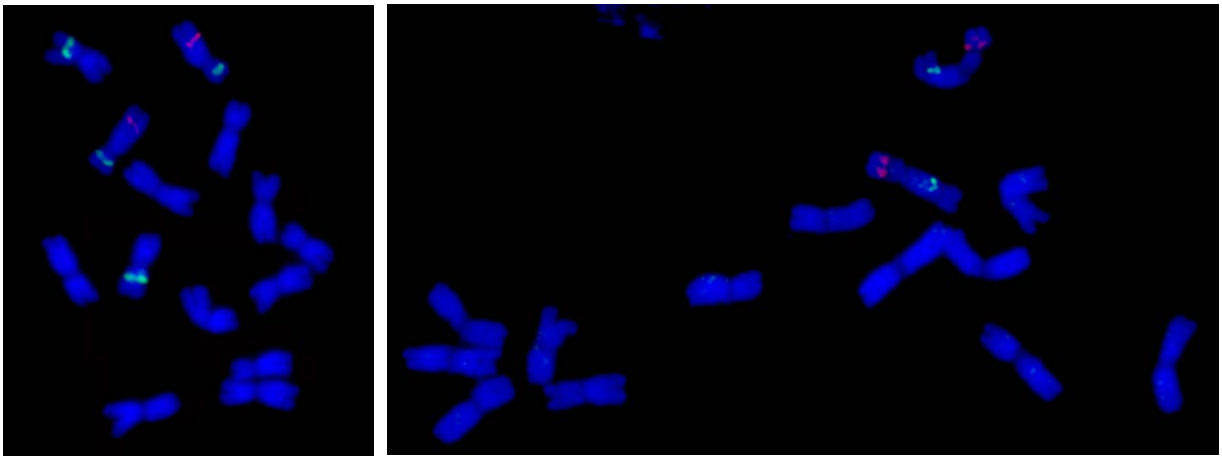
study proposed to treat all *Festuca* species belonging to this section as *Lolium* (Banfi et al., 2017). However, the terms “meadow fescue” and “tall fescue” are so well established and used by breeders, growers and researchers that it may be difficult to introduce the new nomenclature into the broad community.

Completely different example of interspecific hybridization is represented by bread wheat and its hybrids with alien relatives. It is well known that bread wheat (*Triticum aestivum*) is an allohexaploid composed of three genomes derived from wild progenitors. The species originated from a cross of of the B genome donor (unknown, but related to *Aegilops speltoides*) with the donor of the A genome (*T. urartu*). Further hybridization of the tetraploid (*T. turgidum*, 2n=4x=28, BBAA) with diploid *A. tauschii* (donor of D genome), about 8 thousands years ago, produced hexaploid bread wheat (2n=6x=42, BBAADD). Massive breeding and selection led to narrowing of genetic diversity of this species. Thus, introgression of new beneficial alleles from relatives is a desired approach in breeding, for a variety of reasons. Perhaps the most successful is the introgression (substitution) of the short arm of rye chromosome 1 (1RS) into bread wheat. This originally brought several genes for resistance to pathogens and pests of wheat (Zeller, 1973) and many recent bread wheat cultivars carry such introgression (Lukaszewski, 1990). Similarly, interspecific hybridization of wheat with *Thinopyrum ponticum* was used to develop wheat with blue aleurone. The presence of blue aleurone layer in seeds is associated with high level of anthocyanins, which are of great importance for human health due to their antioxidant, anti-inflammatory, antimicrobial and anti-cancerogenic potential. Major anthocyanidin of the blue-aleurone wheat is delphinidin-3-glucoside (Trojan et al., 2014). It is the most potent angiogenic inhibitor among anthocyanins and may be helpful in cancer prevention and treatment (Lamy et al., 2006). Delphinidin is also said to be more effective in the inhibition of tumorogenesis, by blocking activation of the mitogen-activated protein kinase (Hou et al., 2004). It was evidenced before, that blue aleurone layer is associated with the chromosome introgression of *Th. ponticum* into wheat background. However, the identity of the introgressed segment(s) was largely unknown. For this, we screened for *Th. ponticum* introgressions in various wheat lines with blue aleurone. There were six different types of introgressions, ranging from a ditelosomic addition (cv. Blue Norco) to a disomic substitution (cv. Blue Baart), substitution of complete (homologous) chromosome arms (line UC66049) and various translocations of distal parts of a chromosome arm(s). Different types of introgressions present indicates that the introgressions activate the blue aleurone trait, which is present in common wheat germplasm, but inactivated in absence of *Th. ponticum* introgression (Burešová et al., 2015).

Similar to bread wheat, many species in the tribe *Triticeace* (*Poaceae*) underwent reticulate evolution. It seems that majority of species originated from the crosses of two or more distinct species. This implies that propensity for interspecific hybridization may be higher in this tribe than elsewhere in the plant kingdom (Stebbins, 1956). In our study, we analyzed the origin of two related wheatgrass

species – *Elymus repens* and *Thinopyrum intermedium* (syn. *Elymus intermedium*), the former being a serious weed and the latter a good source of new alleles for wheat improvement. By sequencing of multicopy internal transcribed spacer (ITS) and single-copy granule-bound starch synthase I (GBSSI) DNAs in concert with genomic and fluorescent *in situ* hybridization (GISH and FISH), we have demonstrated allopolyploid origin of both species. *Elymus repens* carries two genomes of *Pseudoroegneria spicata* and one genome of *Hordeum bogdanii*. Apart from these two species as the main progenitors, we also found unexpected genetic material introgressed from *Bromus*, *Taeniatherum* and *Panicum*. Interestingly, chromatin from *Panicum bergii* was present as a small segment of rDNA, located in the interstitial part of one *Hordeum*-origin chromosome pair (Mahelka and Kopecký, 2010). We further investigated the acquisition of foreign genetic material in various *Hordeum* species (including *H. bogdanii*, a progenitor of *E. repens*) from such distant genetic sources as panicoid grass species. Using cytogenetic and genomic tools, we found that the alien DNA sequences were acquired between 1 and 5 Mya after a series of multiple events so that some current *Hordeum* sp. individuals harbor up to five different panicoid rDNA units, in addition to the native *Hordeum* rDNA copies (Mahelka et al, 2017). It appears that the alien rDNA units are not transcribed, with some showing indications of silencing via pseudogenization. Such acquisition may be a result of a horizontal gene transfer or of multiple rounds of hybridization. It is still to be determined which of the two scenarios underlies the transfer of genetic material.

Figure 5, Localization of *Panicum bergii* introgression on mitotic metaphase chromosomes in *H. pubiflorum* (left) and *H. bogdanii* (right) using GISH. Total genomic DNA of *P. bergii* was labelled with digoxigenin (green color) and 45S rDNA was labelled with biotin (red color). The chromosomes were counterstained with DAPI (blue color) (adopted from Mahelka et al., 2017).



Similar to *E. repens,* the origin and genome composition of *Thinopyrum intermedium* seems complex. We discovered contribution of distinct lineages corresponding to the following present-day genera: *Pseudoroegneria*, *Dasypyrum*, *Taeniatherum*, *Aegilops* and *Thinopyrum elongatum*. Two genomes have most likely been contributed by *Pseudoroegneria* and *Dasypyrum*, but the identity of the third genome remains obscure. Based on the results of GISH, it may be of hybrid origin, with contributions from *Thinopyrum elongatum*, *Taeniatherum caput-medusae* and *Aegilops tauschii*. Chloroplast trnL-F sequences indicated that *Pseudoroegneria* was the most likely maternal progenitor (Mahelka et al., 2011). This is in line with maternal origin in many other *Triticeae* allopolyploids where *Pseudoroegneria* was identified as the maternal parent (reviewed in Redinbaugh et al., 2000). It appears that this species is prone to receive alien pollen resulting in successful fertilization with *Pseudoroegneria* egg cell.

We further characterized both the sequence variation and genomic organization of the 45S (herein ITS1-5.8S-ITS2 region) and 5S (5S gene + nontranscribed spacer) ribosomal DNA (rDNA) families in *Thinopyrum intermedium* using sequence analysis and *in situ* hybridization (Mahelka et al., 2013). Both 45S and 5S sequences are organized within several rDNA loci within all three genomes. However, they display contrasting patterns of evolution. The 45S rDNA family has evolved in a concerted manner: homogenization toward one major ribotype via unequal crossover and/or gene conversion, and loss of certain 45S rDNA loci was found in the internal transcribed spacer (ITS) sequences residing within the arrays of two genomes. The third genome contained a minor proportion of distinct unhomogenized copies. On the other hand, 5S rDNA did undergo neither homogenization, nor loss of loci. Both 45S and 5S sequences suggest contributions from *Pseudoroegneria*, *Dasypyrum*, and *Aegilops*, the species previously identified as progenitors of *Thinopyrum intermedium*.

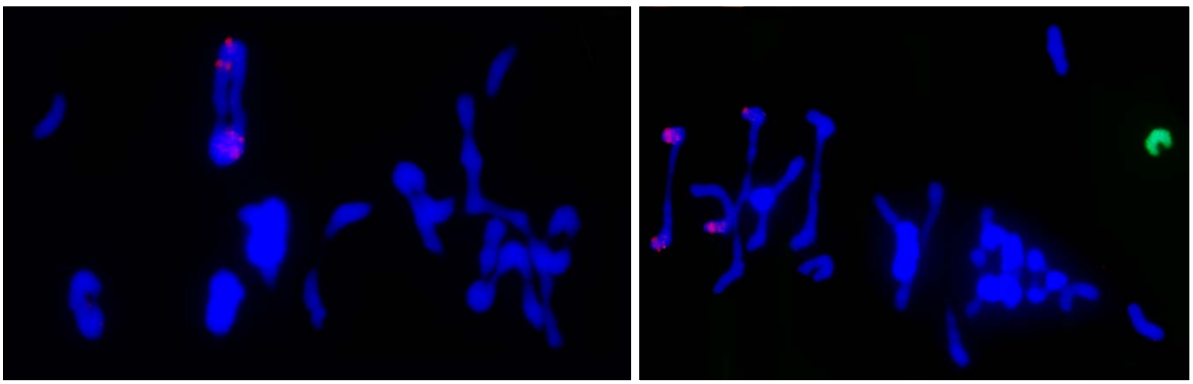
3b. Chromosome pairing and its implication for the fertility of interspecific hybrids

In newly established interspecific hybrids, homeologous chromosomes from parental species do not usually pair with each other, resulting in aneuploid gametes and, subsequently, sterility. For this reason, doubling the chromosome number of F1 homoploids (hybrids having one set of chromosomes from each parent) is a necessary step in breeding. As a consequence of doubling, homologues acquire pairing partners and chromosome segregation during first meiotic division is normal. Usually this ensures formation of euploid viable gametes and thus, fertility. Alternatively, fusion of unreduced gametes may give rise to allopolyploid F1 hybrid.

There are two possible explanations of the absence of homeologous pairing. For one, sequence dissimilarity between the two parental genomes may prevent homoeologue recognition, synapsis, and chiasma formation. Two, a genetic system may operate that prevents dissimilar chromosomes (such as homoeologues) from pairing. Such systems are known to operate in several allopolyploid species, such as wheat and Brassicas, and are suspected in many other allopolyploids. Among the most studied systems is the *Ph* (pairing homeologous) of polyploid wheat. It consists of at least two loci, *Ph1* and *Ph2*, located on chromosomes 5BL and 3DS, respectively (Riley and Chapman, 1958; Sears and Okamoto, 1958; Sears, 1977). Of these two, *Ph1* has a far stronger effect (Sears, 1984). In its absence, pairing of homeologous chromosomes takes place, and not only those from the three genomes of wheat, but also from many genomes of species related to wheat if introduced to wheat. This phenomenon has long been utilized for chromosome engineering and introgression alien chromatin into wheat (Sears, 1981).

The mode of action of *Ph1* is unknown, even though several hypotheses have been proposed ranging from a control of spatial nuclear disposition of chromosomes in all tissues of a plant (Feldman and Avivi, 1988) via the control of centromeres (Martínez-Perez et al., 2001, 2003) and the control of stringency of crossing over (Dubcovsky et al., 1995) to the effects of chromosome condensation (Mikhailova et al., 1998; Maestra et al., 2002). However, most of the above mentioned hypotheses were already disproved. To test the latter one, we compared chromatin condensation of rye chromosome arms in five wheat-rye centric translocations in the presence of the wildtype Ph1 and *ph1b* mutation (Kopecký et al., 2007). No differences in chromatin condensation was observed. That analysis was recently confirmed using three-dimensional *in situ* hybridization (Koláčková et al., in preparation).

Figure 6, The effect of presence of *Ph1* (in form of introgressed 5B chromosome) on chromosome pairing in autotetraploid rye analysed using GISH. Total genomic DNA of wheat was labelled with digoxigenin and detected by antDig-FITC (green color); 45S rDNA was labelled with biotin and detected by streptavidin-Cy3 (red color). The chromosomes were counterstained with DAPI (blue color). Note high number of multivalents in rye (left) and diploid-like pairing and bivalent formations in introgression line of autotetraploid rye having single wheat 5B chromosome (right) (adopted from Lukaszewski and Kopecký, 2010).



The *Ph1* system is well known to prevent pairing of homeologues by imposing very high stringency requirement for crossing over. As such, it affects homologues as well; in some intervarietal wheat hybrids homologues are unable to pair (Dvorak and McGuire (1981). Moreover, the *Ph1* locus extends its control to alien chromosomes introduced into wheat as well as to alien chromosomes in their native environment. Schlegel et al. (1991) found that the *Ph1* locus, when introgressed with the entire chromosome 5B into diploid rye, dramatically reduced the overall MI pairing frequency of rye chromosomes. This suggests that *Ph1* is a universal system of chromosome pairing control. To evaluate the effect of *Ph1* in alien background, we analyzed chromosome pairing in autotetraploid rye with various doses *Ph1* located on introgressed chromosome arm 5BL. The effect was striking, and appeared to be dose dependent. In genotypes with one dose of *Ph1*, the average number of quadrivalents dropped by one half and the number of univalents tripled relative to controls. With two *Ph1* copies, there was even less pairing: the average number of quadrivalents was three times lower and the number of univalents was 5 times higher than in normal rye (Lukaszewski and Kopecký, 2010). The mechanism underlying the apparent dosage effect of *Ph1* in rye is unknown. In wheat, *Ph1* behaves as a dominant gene with one copy being sufficient to prevent homeologous pairing. Perhaps the difference here is in the interaction between the *Ph1* locus and rye chromosome 5R. This chromosome is known to partially suppress *Ph1* in wheat, and the effect seems to be dose-dependent in that higher doses of 5R reduce the effect of *Ph1* more extensively (Riley et al., 1973; Lelley, 1976).

At the onset of meiosis, chromosomes are arranged into a special configuration called leptotene (or telomere) bouquet: all telomeres cluster in one pole of the nucleus opposite from the centromere pole. There is a specific reason for such arrangement. Chromosome pairing is generally initiated at telomeric regions, and it is the bouquet configuration that brings termini of all chromosomes into physical contact where recognition of homology can take place. Hence, telomeric regions of chromosome arms are the first ones to pair, and this may explain the general tendency for the first crossovers to be located distally. It had been assumed that in wheat, the prevalence of distal crossing overs was a natural consequence of terminal initiation of synapsis. This presumably favors establishment of distal chiasmata: first to synapse-first to cross over (Curtis et al. 1991; Lukaszewski and Curtis 1993) and that proximal halves of wheat chromosome arms are devoid of crossovers. Structural heterozygosity causing misalignment at the telomere drastically reduces chiasmate pairing (Moens et al. 1989) and the reduction is proportional to the degree of misalignment (Curtis et al. 1991; Lukaszewski 1997). Thus, one can expect that any region of a wheat chromosome would cross over if it was brought to the vicinity of telomere (Lukaszewski 2003). Surprisingly, Lukaszewski (2008) observed that in an inverted arm of a rye chromosome, the pattern of chiasmata was also inverted. The region of the arm which, in a normal chromosome, has the highest concentration of chiasmata (the distal region) retained the highest concentration of chiasmata when, following the inversion, it was placed in the immediate vicinity of the centromere.

Regions placed in distal positions as a consequence of inversion (proximal in a normal chromosome) did not cross over at all even in the immediate vicinity of the telomere. This implied that the proximal half of this specific chromosome arm of rye is incapable of crossing over regardless of its position on the telomere-centromere axis, and that specific segments of that arm have some assigned crossover frequencies. It was not entirely sure if these observations could be generalized to other chromosomes/species.

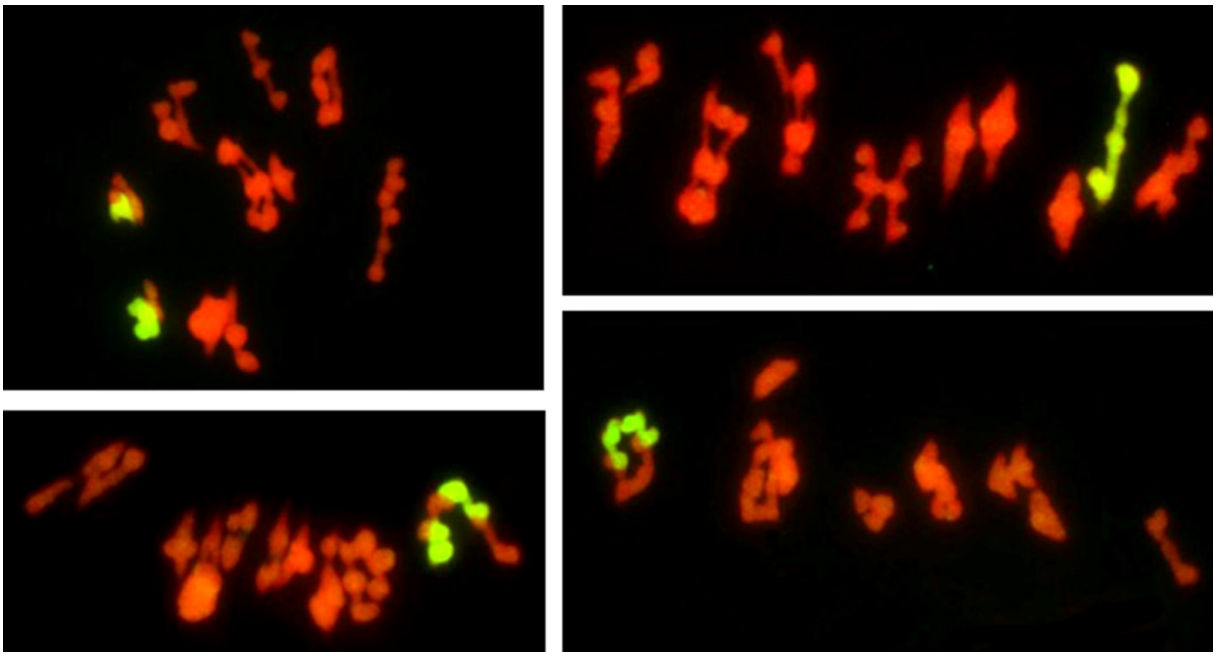
To shed more light on this phenomemnon, we investigated pairing behavior in reverse-tandem-duplications of wheat chromosomes 2BS and 4AL (Lukaszewski et al., 2012). Again, chiasmata were always located in the same regions as in structurally normal arms, and their relative frequencies remained the same. These results indicate that both in wheat and in rye, the relative crossover frequencies along chromosome arms are genetically predetermined and independent of the segment location. Segments normally not licensed to cross over do not do so even when placed in seemingly most favorable positions for it. The nature of such crossover licensing system is so far unknown and needs to be investigated in detail.

As mentioned above, chromosome pairing in xFestulolium is somewhat different from hybrids of wheat with rye or barley, or wheat by itself. Homeologous chromosomes of ryegrass and fescue pair and recombine freely. There is no *Ph*-like system in meadow fescue and in either of the two ryegrasses. High level of crossing over allows massive rearrangements of the genomes in subsequent generations, and releases unheard-of levels of genetic variability (Kopecký et al., 2006). In most cases, the level of inter-genomic recombination is assessed at the whole genome level. We decided to perform the analysis at a single chromosome level: we used monosomic and disomic single chromosome introgression lines, where individual chromosomes of Italian ryegrass are replaced by a homoeologue from meadow fescue (2n=4x=28, 27L+1F or 26L+2F), in single or double dose. In monosomic single chromosome introgression lines, the single meadow fescue chromosomes (Fp) paired with their *L. mulitflorum* (Lm) counterparts forming bivalents and quadrivalents. Disomic introgression lines offer an opportunity to observe competition for pairing partners: a pair of Fp and a pair of Lm homologues can form a quadrivalent with random or non-random positions of each chromosome; they can form two homologous or homoeologous bivalents, and various combinations of trivalent + univalent configurations (see Fig. 7). We detected statistically significant preferential pairing of homologues. However, high frequency of homeologous chromosome pairing was also present. This was different from the F1 hybrids, where homeologous pairing was observed with much lower frequencies (Kopecký et al., 2008). Overall, high rate of homeologous metaphase I (MI) pairing in xFestulolium hybrids may be due to a very permissive system of the chromosome pairing control that overlooks differences between the parental chromosomes. Given the ease of discrimination of parental genomes by GISH, the differences in repetitive DNA sequences must be substantial. On the other hand, while the DNA repeats diverged substantially during evolution, the

sequences involved in chromosome pairing remained conserved enough to facilitate regular homeologous pairing partner recognition and crossing-over.

The ability of homeologous chromosomes to pair and recombine creates ample opportunity for introgression of fescue chromosome segments into the *Lolium* background, and vice versa (direction seldom utilized in breeding). Using the complete set of single chromosome introgression lines, we conducted observations of the distribution and frequency of homeologous recombination events along individual chromosomes (Kopecký et al., 2010). Similar to the large-genome species such as wheat, rye and barley, there was an uneven distribution of recombination (in this case homeologous) along individual chromosomes, with a serious decay at centromeric/ pericentromeric regions, and increased frequencies towards distal parts of chromosomes. However, despite skewed distribution of crossing over, introgression of any chromosome segment from *Festuca* into *Lolium* background is possible. The feasibility of such approach was confirmed in our follow-up study (Barnes et al., 2014). We tested drought tolerance in xFestulolium plants and compared it with pure species (perennial ryegrass, meadow and tall fescues). The groups of diploid xFestulolium plants contained numerous introgressions of chromatin from *F. pratensis*, in the form of complete chromosomes and chromosome segments of variable length, in all possible positions in the karyotype. We noted that approximately 60% of all plants in the population which survived severe drought of Southern California carried the terminal segment of the short arm of chromosome 3 (3S from meadow fescue).

Figure 7, Chromosome pairing (metaphase I) in disomic single chromosome substitution lines of *L. multiflorum x F. pratensis*, where two (homologous) chromosomes of *L. multiflorum* are substituted by their *F. pratensis* counterparts. GISH was done with total genomic DNA of *F. pratensis* labelled with digoxigenin and detected by antiDig-FITC (green color) and used as a probe and sheared genomic DNA of *L. multiflorum* used as blocking DNA (red pseudocolor). Note various proportions of homeologous vs. homologous chromosome pairing.



The absence of a strict chromosome pairing system in xFestulolium hybrids results in a negative aspect: the problem with the stability of hybrid genomes. As discussed in the previous chapter (3a), each plant of an individual cultivar has a unique genotype and as such, cultivars are highly heterogenous. In a sense it is beneficial, as it maintains genetic diversity in grasslands (either meadows and pastures or lawns) to mitigate the negative impacts of various stresses. On the other hand, it may preclude a genetic stock to pass DUS tests and be registered as a cultivar included in national lists. Moreover, it brings the question of cultivar stability in successive generations of multiplication, and makes cultivar maintenance a serious effort. It appears that the amphiploid-type cultivars are stable enough to maintain the proportions of parental genomes. However, no apparent presence of fescue chromatin in some cultivars may indicate its potential loss in introgression lines (Kopecký et al., 2006).

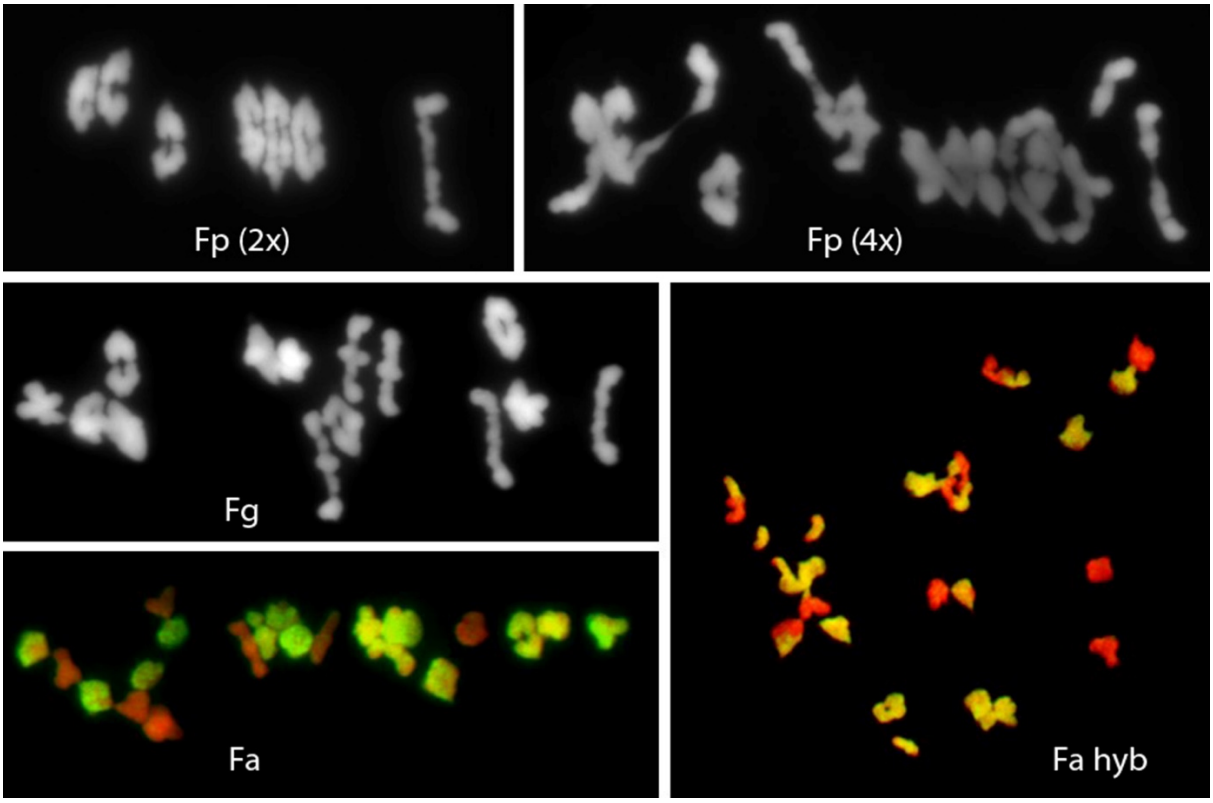
As described earlier, we observed gradual elimination of the *Festuca* segments in introgression types of Festulolium cultivars. If the pace of elimination is steady, a complete elimination of the *Festuca* chromatin may take place within about four generations of seed multiplication (Kopecký et al., 2019a). For these reasons, introduction of a diploid-like pairing control system that prevents homeologous pairing and crossing over would go a long way toward stabilization of hybrid genomes. Such a system does not exist in diploid species used for interspecific hybridization, such as meadow fescue and the two ryegrass species. However, Jauhar (1975) discovered the presence of a diploidizing chromosome pairing system in tall fescue (*F. arundinacea* Schreb.). This allohexaploid species comprises three subgenomes, two of which are close to tetraploid *F. glaucescens* and the third one to meadow fescue (2n=6x=42, FpFpFgFgFg'Fg'; Humphreys et al., 1995). The pairing control system was most likely inherited from *F. glaucescens* (2n=4x=28, FgFgFg'Fg'), as this allotetraploid shows diploid-like chromosome pairing in MI (Kopecký et al., 2009b).

Such systems also appear to be present in other polyploid fescue species from the *Schedonorus* section, such as in *F. apennina*, *F. mairei* and *F. atlantigena* (reviewed in Jauhar et al., 1994). The system in fescues, however, is different from the *Ph1* of wheat. In *Ph1*, a single copy is sufficient to prevent pairing of homeologous chromosomes, both wheat and relatives (Sears and Okamoto, 1958). The system in polyploid fescues is haplo-insufficient or hemizygous-ineffective (Jauhar et al., 1993). This means that F1 hybrids of polyploid fescues with ryegrasses (such as *L. multiflorum* x *F. arundinacea* hybrids with 2n=4x=28, LmFpFgFg') display homeologous chromosome pairing (Kopecký et al., 2009b). Similarly, pairing of homeologues was observed in tetraploid tall fescues (2n=4x=28, FpFpFgFg'). For strictly diploid pairing, two copies are necessary, which makes the approach to stabilize the hybrid genomes more difficult. The origin of the system is so far unknown. It has been described in several species, but its monophyletic or polyphyletic origin has not yet been studied. We studied the meiotic behavior in the F1 hybrids of two morphotypes (Continental and Mediterranean) of tall fescue (*F. arundinacea* Schreb.) and found frequent homeologous chromosome pairing. It is not clear if both morphotypes originated from the same progenitors (probably not), but each species shows strictly bivalent pairing (Kopecký et al., 2019b). This suggests two possibilities. Either the system evolved twice, independently in each of the morphotypes, and each is hemizygous ineffective, hence high pairing in hybrids, or it evolved only once in some (unknown) progenitor of polyploid fescues from which it was transmitted to all recent polyploid fescues and diversified enough to be incompatible in the hybrids.

Surprisingly, polyploidization of F1 hybrids does not necessarily restore the diploid-like pairing. Zwierzykowski (1980) observed frequent multivalent formation in allooctoploids obtained after colchicine treatment of tetraploid hybrids of *L. multiflorum* x *F. arundinacea,* despite the fact that all genomes were present in two copies and *F. arundinacea* pairing system must have been present in two copies, hence homozygous. Multivalents were also detected in amphiploids of *L. multiflorum* x *F. gigantea* (Morgan et al., 1988). Therefore, effectiveness of the diploidizing pairing system is not completely restored in hybrid amphiploids. At present, there is no explanation for this phenomenon. Perhaps there are interactions of this system with chromosome pairing/recognition mechanisms in diploid parents. Kleijer and Morel (1984) hypothesized that non-restoring of a diploid-like pairing was associated with the presence of the *Lolium* genome, which can suppress, to some extent, the action of the system. This correlates with our results on chromosome pairing in hybrids of *L. multiflorum* with *F. glaucescens*. If the system is hemizygous-ineffective, about 25% of F2 plants would be expected to carry it in two copies and show only bivalent pairing. However, we found only four (5.5%) out of 73 plants of tetraploid cv. Lueur (F6-F8 generation) with such pairing (Kopecký et al., unpublished). This suggests that the system consists of two complementary unliked loci. Regardless of the exact mechanism of the chromosome pairing control, the development of

genetically stable xFestulolium forms may be a more difficult task than originally believed.

Figure 8, Chromosome pairing (metaphase I) in allohexaploid tall fescue (*F. arundinacea*, Fa), its progenitors – diploid *F. pratensis* (Fp 2x) with its autotetraploid form (Fp 4x) and tetraploid *F. glaucescens* (Fg) and in inter-morphotype hybrid of tall fescue (Fa hyb) after GISH (Fa and Fa hyb). Total genomic DNA of *F. glaucescens* was labelled with digoxigenin and detected by antiDig-FITC (green color) and used as the probe; sheared genomic DNA of *F. pratensis* was used as blocking DNA (red pseudocolor). Note multivalent formation in autotetraploid *F. pratensis* (Fp 4x), while diploid-like meiosis was observed in *F. glaucescens* (Fg) and *F. arundinacea* (Fa). Inter-morphotype hybrid of tall fescue displayed frequent homeologous chromosome pairing.



3c. Gene expression

In addition to genome instability, chromosomal rearrangements including compensated and non-compensated aneuploidy, translocations, mitotic and meiotic abnormalities, large-scale changes in gene expression are commonly observed in newly formed allopolyploids. These may be due to epigenetic changes that affect cytosine methylation patterns (Song et al., 1995; Lee and Chen, 2001), gene silencing, and/or shifts in the contribution of homeologs to overall gene expression (Chen and Pikaard, 1997; Lee and Chen, 2001).

The changes in the genome constitution of xFestulolium, therefore, represent only a small piece of the puzzle underlying the *Lolium* genome dominance. For this reason, we studied gene expression in xFestulolium. We created tetraploid reciprocal F1 and F2 hybrids of *L. multiflorum* x *F. pratensis* to study the expression of parental alleles in early hybrid generations. Using the RNAseq (the Illumina platform) and assembly and annotation of the reads using the OGA approach (Orthology Guided Assembly), we identified almost 25,000 interspecific SNPs located in 5343 genes that can distinguish meadow fescue from Italian ryegrass. All identified SNPs were positioned *in silico* on the seven linkage groups (LGs) of *L. perenne* using the GenomeZipper approach (Stočes et al., 2016). Using this set of SNPs, we were able to analyze in detail genome expression in hybrids.

There are two aspects of gene expression in hybrids to be considered: the expression level dominance (ELD) and the homeologous expression bias (HEB) (Yoo et al., 2013). While ELD represents the difference in the overall gene expression between a hybrid and its parents, HEB refers to the relative contribution of homeologous alleles (from both parental species) to the hybrid transcriptome. Various studies revealed that the expression levels of genes in allopolyploids were not simply the average of the two parents (additive expression); many of the observed gene expression changes were non-additive. The frequency of non-additively expressed genes ranged from 4.5-5.8% in *B. napus* hybrids (Zhang et al., 2016) to 34% in cotton (Flagel and Wendel, 2010). Our analysis of ELD revealed a similar percentage of non-additively expressed genes. In total, only 67.3 to 68.5% of the analyzed genes showed no change in gene expression in *Festuca* × *Lolium* and *Lolium* × *Festuca* hybrids, respectively. The *Lolium* expression dominance was more frequent (15.2 and 14.6% of the analyzed genes) than the *Festuca* expression dominance (7.0 and 6.2%). Expression level dominance has been also reported in cotton (Flagel and Wendel, 2010, Yoo et al., 2013), *Spartina* (Chelaifa et al., 2010), wheat (Chagué et al., 2010) and other allopolyploids. Transgressive down-regulation was slightly more frequent than transgressive up-regulation in our *Festuca* × *Lolium* hybrids (4.8 vs. 4.4%), however, the opposite trend was observed in *Lolium* × *Festuca* hybrids (4.8 vs. 7.1%). Such differences in down- and up-regulation were also observed in the oilseed rape. Jiang et al. (2013) found that more genes were up-regulated than downregulated in the F1 generation of synthetic allopolyploid *B. napus* as compared to its parents and natural *B. napus*. However, most of the differentially expressed genes showed down-regulation in F2 - F4 generations as compared to F1 generation. More down-regulated genes than up-regulated were identified in the study of Zhang et al. (2016) in *B. napus*. Similarly, higher frequency of up-regulation as compared to down-regulation was observed in triploid F1 interspecific *Oryza* hybrids (Wu et al., 2016). Surprisingly, we identified only four and three genes displaying additivity in F1 and F2 hybrids, respectively (the overall expression level of hybrid between the expression levels of both parents, when both parents displayed different expression level).

Our homeologue expression bias (HEB) analysis revealed that the expression of homeologs in the hybrids is inherited from their parents for most of the genes (73.8% and 77.7% in *Festuca* × *Lolium* and *Lolium* × *Festuca* hybrids, respectively). This frequency agrees with the results of Wu et al. (2016) reporting 79% of such genes in triploid *Oryza* hybrids, but slightly more than 59.4-70.9% of the genes maintaining the ratio between the parental specific gene expression levels in the F1 interspecific hybrids and allopolyploids of *Gossypium* (Yoo et al., 2013). On the other hand, we found that only 1.5% and 0.9% of the genes differently expressed in parents showed equal expression of both homeologs in *Festuca* × *Lolium* and *Lolium* × *Festuca* hybrids, respectively. This is in contrast with Yoo et al. (2013), who identified 18.3-25.7% of genes expressed equally from both homeologs in diploid F1 hybrids and allopolyploids, even if these genes had significantly different expression in diploid parents. Similarly, Wu et al. (2016) identified 13% of such genes in triploid F1 *Oryza* hybrids. We further observed that over 20% of the analyzed genes showed a novel bias in hybrids. This bias was mostly caused by overexpression of a *Lolium* homeolog when the expression was at the same level in the parents (19.2% and 18.2% in *Festuca* × *Lolium* and *Lolium* × *Festuca* hybrids, respectively). Overexpression of a *Festuca* homeolog in hybrids was much less frequent (5.4% and 3.2% in *Festuca* × *Lolium* and *Lolium* × *Festuca* hybrids, respectively). Gene expression studies reported genome dominance (even though with lower frequencies) also in other polyploids, such as allotetraploid *Arabidopsis suecica* (Chang et al., 2010), *Glycine max* (Ilut et al., 2012) and in synthetic allotriploid wheat, where D-subgenome was frequently down-regulated. Interestingly, this effect was in many cases reversed by whole genome doubling of allotriploid wheat (Hao et al., 2017).

The most extreme case of HEB is a complete silencing of one of the two homeologues, but it is rarely observed. Yoo et al. (2013) found only five HEB cases out of 25317 gene pairs in synthetic allotetraploid cotton. Their results further suggest that long-term evolution and domestication does not significantly increase the frequency of one-homeolog silencing with only 0.33 and 0.38% of genes having silenced one homeolog in natural ecotype and domesticated cultivar of allopolyploid cotton. Despite the genome dominance indicated by our results and other studies, it cannot be generalized to all allopolyploids. No genome dominance has been observed in *Brassica napus* and *B. juncea*, *Nicotiana tabacum*, *Tragopogon miscellus* and other allopolyploid species (Zhang et al., 2016; Yang et al., 2016; Bombarely et al., 2012; Buggs et al., 2010; Hovav et al., 2008).

Expectedly, the regulation of gene expression is stabilized immediately after genomes merge, and subsequent changes take place during the evolution and possibly domestication of the allopolyploids. A comparison of F1 and F2 generations of *Festuca* × *Lolium* hybrids showed progressive changes in gene expression. In terms of ELD, *Lolium* expression dominance was evident more frequently in F2 generation relative to F1 (20.8% vs. 15.2% of analyzed genes). Similarly, both

transgressive down-regulation and up-regulation was more frequent in F2 generation. There were also differences in the contribution of both homeologs to the overall expression between the F1 and F2 generations. There was an increase in the number of genes with the same level of expression in the parents but different expression from both homeologs in hybrids in their successive generations. Surprisingly, genes from both classes (genes with over-expression of *Lolium* homeolog as well as genes with over-expression of *Festuca* homeolog) were increased in numbers in the F2 over F1 generations. This indicates that the hybrid genome is not well stabilized at the transcriptomic level in early hybrid generations. This is in line with the gene expression analyses in cotton and *Tragopogon*, where it is expected that the rapidly emerging genomic and transcriptomic asymmetry following allopolyploid speciation will continue to evolve under natural and/or human selection (Renny-Byfield and Wendel, 2014; Buggs et al., 2011). However, a study on hexaploid wheat indicated that the pattern of homeolog expression had been highly conserved during domestication (Li et al., 2014). It seems that there are only several genes encoding specific traits, such as increased fiber growth in cotton and increased oil content in *Brassica juncea*, which are dramatically rewired during domestication (Chaudhary et al., 2008; Yoo and Wendel, 2014; Yang et al., 2016). Similarly, a study of Yoo et al. (2013) showed that it is the genome merger via interspecific hybridization that has the greatest effect on changes in homeolog expression bias relative to the polyploidization or domestication in cotton. This phenomenon was also observed in *Senecio* (Hegarty et al., 2006), *Brassica napus* (Gaeta et al., 2009) and *Spartina* (Chelaifa et al., 2010).

Our ongoing study focuses on the gene expression changes associated with winter hardiness and aging. These two features may reverse the gene expression patterns towards *Festuca* alleles as these conditions are more favorable for meadow fescue (known to be perennial and winter hardy compared to annual/biennial and cold stress-susceptible Italian ryegrass).

3d. Spatial organization of hybrid nucleus

The structure, organization, function, and stability of hybrid genomes are closely linked and represent a complex feature. It is evident that positioning of specific sequences and chromosomes in an interphase nucleus is not random. Each chromosome occupies a so-called chromosome domain or chromosome territory (Cremer and Cremer, 2001). Each chromosome territory (CT) is a complex structure of irregular shape and appears to be fixed during the interphase of the cell cycle (Sun et al., 2000; Kozubek et al., 2002). Although CTs are spatially separated from each other by interchromosomal domains, there seem to be regions where neighboring territories intermingle (Gorkin et al., 2014).

In animals, including humans, chromosome domains seem to be arranged radially (Croft et al., 1999; Cremer et al., 2001; Habermann et al., 2001; Mayer et al., 2005). Chromosomes of plant species appear to be organized differently. There are two major configurations: Rabl and Rosette. Rabl organization in essence preserves the organization/polarity from the preceeding anaphase: centromeres clustered at one pole while the telomeres are scattered throughout the opposite pole. Chromatin is stretched over the entire volume of the nucleus (Rabl, 1885). On the other hand, in the Rosette configuration, centromeres are randomly distributed at the nuclear periphery, while telomeres congregate around the nucleolus. Centromeric heterochromatin forms distinct chromocenters while the euchromatin domains, where the majority of genes are located, create 0.2 - 2Mb loops resulting in rosette-like structures of interphase chromosomes (Fransz et al., 2002).

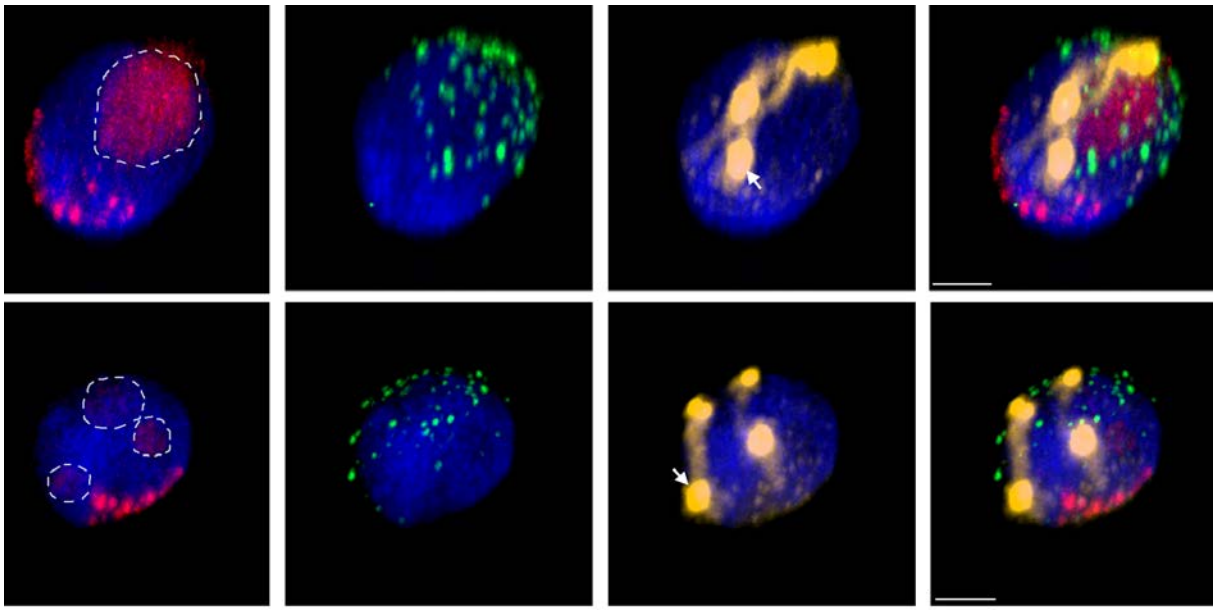
The organization of chromosome domains and parental chromatin in interspecific hybrids and allopolyploids is largely unknown. Chromosome arms of rye (*Secale cereale* L.) introgressed into bread wheat (*Triticum aestivum* L.) adopt a typical Rabl orientation of their wheat counterparts and the two homologous arms are usually spatially separated from each other (Heslop-Harrison et al., 1990; Kopecký et al., 2007). However, these findings were based on the observation of squash preparations by 2D microscopy and the third dimension was compromised. For that, we aimed to shed light on the spatial organization of parental chromatin in plant hybrids.

We used hybrids of wheat x rye with various proportions of parental genomes. Using flow cytometry to sort nuclei in a particular stage of the cell cycle, combined with molecular cytogenetics (to label DNA from both parental species), confocal microscopy (for three-dimensional analysis of the nuclear structure) and visualization in the software Imaris we analyzed positioning of chromatin from both parents. In general, chromosomes from both species were arranged in typical Rabl configuration. There were no significant differences between nuclei from different tissues (root meristem, leaf mesophyll and embryonic cells) and between nuclei at different cell cycle stages (G1, S and G2 stages). However, we found a relationship between the length of a chromosome arm and its positioning in the nucleus: long arms were located more frequently at the nuclear periphery, while short arms appeared to be located preferentially in the inner part of the nucleus. However, both short and long arms were stretched across the volume of the nucleus with telomeres and centromeres at or close to the nuclear envelope (Koláčková et al., in preparation).

Positioning of chromosome arms (including their telomeres) probably plays a key role in the stability of genomes in wide hybrids. Besides a few peculiar examples of fertile wide hybrids with homeologous chromosome pairing, such as xFestulolium (discussed in detail in chapter 3b), accurate pairing of homologues during the meiosis is necessary for proper segregation of chromosomes into the gametes and thus, for the fertility of hybrids. At times, newly formed amphiploids are unstable and in

extreme cases, one of the parental genomes may be completely eliminated. There are known octoploid triticales (amphiploids of bread wheat with rye) that revert back to bread wheat (loss of the entire rye genome) during seed multiplication (Tsunewaki, 1964). This elimination of rye chromosomes is caused by their reduced pairing, hence reduced transmission to the gametes. Reduced pairing of rye chromosomes in wheat is a common feature of wheat-rye disomic additions and substitutions as well as in tetraploid triticale (Orellana et al., 1984; Lukaszewski et al., 1987).

Figure 9, Out-positioning of the rye telomeres (arrowed) in disomic 1R introgression in wheat. GISH was done with total genomic DNA of rye labelled with TRITC (yellow color); centromeres of both wheat and rye chromosomes were visualized by FISH with oligonucleotide probe (red color), and telomeres were higlighted by FISH with a FITC-labeled probe (green color). Nuclear DNA was counterstained with DAPI (blue color). Nucleoli are indicated by white dashed lines (pseudocolor) (adopted from Perničková et al., 2019b).



Pairing of homologous chromosomes starts at the telomeric regions of chromosome arms in leptotene. Normally, chromosomes are attached, by their telomeres to the nuclear envelope via protein complexes. This ensures sliding of the telomeres on the inner surface of nuclear envelope into the telomere bouquet configuration, which makes homologue recognition possible. Naranjo (2014) hypothesized that reduced pairing of rye chromosomes in wheat appears to be a consequence of disturbed migration of rye telomeres into the leptotene bouquet. Similarly, Murphy and Bass (2012) observed that a desynaptic (dy) mutant of maize displays multiple defects in telomere-nuclear envelope interactions, homologous chromosome synapsis, recombination and chromosome segregation. In our study, we attempted to explain the odd phenomenon of a telomere of an inverted chromosome arm finding its pairing partner at the centromere pole and discovered that the rye telomere fails to be incorporated in the bouquet. Instead, with a considerable frequency it occupies various positions throught the nuclear volume, and that this failure of incorporation correlates well with the MI pairing frequency

(Perničková et al., 2019a). Moreover, rye chromosome arms were about 20 times more likely to fail to migrate into the bouquet, explaining much higher rate of pairing failure of rye chromosomes in wheat-rye amphiploids (Perničková et al., 2019a). Additionally, we found that improper positioning of rye telomeres in the nuclei of wheat-rye hybrids is actually a systemic error, not limited to meiosis. Incorrect positioning (out-of-bouquet and/or non-attached to the nuclear envelope) of rye telomeres in nuclei of somatic tissues was virtually the same as in the leptotene (Perničková et al., 2019b; Figure 9). Thus, it appears that the aberrant arrangement of telomeres in leptotene is only an extension of their erratic behavior in somatic tissues.

4. Conclusions

This habilitation thesis summarizes the results of focused effort to improve our knowledge on fundamental processes involved in allopolyploidization, one of the main mechanisms of plant speciation. Using broad spectrum of molecular biology methods, we were able to monitor the genome composition and evolution of hybrid genomes in economically and ecologically important crops. Merging two different genomes opens a question if these two genomes rather cooperate or compete. The answer is more complex than we would believe. We observed changes after intial hybridization including chromosome rearrangements, altered meiotic behavior and modifications in gene expression. Regarding hybrid genome stability, the most intriguing feature of newly established hybrids is frequent selective elimination of one parental genome in both types of hybrids: those where intermingling of parental genomes takes place via homeologous chromosome pairing in meiosis and, surprisingly, also in those where parental genomes are separated (their chromosomes do not pair in meiosis). On the other hand, high frequency of allopolyploid species in the worldwide flora demonstrates the successful cooperation of two genomes in the single organism. Similarly, wide hybridization opens a way for introgression of specific trait from one species to another and may contribute to the sustainable agriculture and food security. Results of our work provided new insights into biological processes of wide hybridization and has been shown to be utilizable in the modern grass breeding, as demonstrated from extensive cooperation with breeding stations and programs.

5. References

Akera, T., L. Chmatal, E. Trimm, K. Yang, C. Aonbangkhen, D.M. Chenoweth, et al. 2017. Spindle asymmetry drives non-Mendelian chromosome segregation. Science 358: 668-+. doi:10.1126/science.aan0092.

Baird, J.H., D. Kopecky, A.J. Lukaszewski, R.L. Green, J. Bartos and J. Dolezel. 2012. Genetic Diversity of Turf-Type Tall Fescue Using Diversity Arrays Technology. Crop Science 52: 408-412. doi:10.2135/cropsci2011.02.0106.

Banfi, E., G. Galasso, B. Foggi, D. Kopecky and N.M.G. Ardenghi. 2017. From Schedonorus and Micropyropsis to Lolium (Poaceae: Loliinae): New combinations and typifications. Taxon 66: 708-717. doi:10.12705/663.11.

Barnes, B.D., D. Kopecky, A.J. Lukaszewski and J.H. Baird. 2014. Evaluation of Turf-type Interspecific Hybrids of Meadow Fescue with Perennial Ryegrass for Improved Stress Tolerance. Crop Science 54: 355-365. doi:10.2135/cropsci2013.03.0198.

Bartos, J., S.R. Sandve, R. Koelliker, D. Kopecky, P. Christelova, S. Stoces, et al. 2011. Genetic mapping of DArT markers in the Festuca-Lolium complex and their use in freezing tolerance association analysis. Theoretical and Applied Genetics 122: 1133-1147. doi:10.1007/s00122-010-1518-z.

Begheyn, R.F., N. Roulund, K. Vangsgaard, D. Kopecky and B. Studer. 2017. Inheritance patterns of the response to in vitro doubled haploid induction in perennial ryegrass (Lolium perenne L.). Plant Cell Tissue and Organ Culture 130: 667-679. doi:10.1007/s11240-017-1255-y.

Bombarely, A., K.D. Edwards, J. Sanchez-Tamburrino and L.A. Mueller. 2012. Deciphering the complex leaf transcriptome of the allotetraploid species Nicotiana tabacum: a phylogenomic perspective. Bmc Genomics 13. doi:10.1186/1471-2164-13-406.

Buggs, R.J.A., N.M. Elliott, L.J. Zhang, J. Koh, L.F. Viccini, D.E. Soltis, et al. 2010. Tissue-specific silencing of homoeologs in natural populations of the recent allopolyploid Tragopogon mirus. New Phytologist 186: 175-183. doi:10.1111/j.1469-8137.2010.03205.x.

Buggs, R.J.A., L.J. Zhang, N. Miles, J.A. Tate, L. Gao, W. Wei, et al. 2011. Transcriptomic Shock Generates Evolutionary Novelty in a Newly Formed, Natural Allopolyploid Plant. Current Biology 21: 551-556. doi:10.1016/j.cub.2011.02.016.

Buresova, V., D. Kopecky, J. Bartos, P. Martinek, N. Watanabe, T. Vyhnanek, et al. 2015. Variation in genome composition of blue-aleurone wheat. Theoretical and Applied Genetics 128: 273-282. doi:10.1007/s00122-014-2427-3.

Buzgo, M., D.E. Soltis, P.S. Soltis and H. Ma. 2004. Towards a comprehensive integration of morphological and genetic studies of floral development. Trends in Plant Science 9: 164-173. doi:10.1016/j.tplants.2004.02.003.

Carver, B.F. and A.L. Rayburn. 1994. COMPARISON OF RELATED WHEAT STOCKS POSSESSING 1B OR 1RS.1BL CHROMOSOMES - AGRONOMIC PERFORMANCE. Crop Science 34: 1505-1510. doi:10.2135/cropsci1994.0011183X003400060017x.

Catalan, P., P. Torrecilla, J.A.L. Rodriguez and R.G. Olmstead. 2004. Phylogeny of the festucoid grasses of subtribe Loliinae and allies (Poeae, Pooideae) inferred from ITS and trnL-F sequences. Molecular Phylogenetics and Evolution 31: 517-541. doi:10.1016/j.ympev.2003.08.025.

Chague, V., J. Just, I. Mestiri, S. Balzergue, A.M. Tanguy, C. Huneau, et al. 2010. Genome-wide gene expression changes in genetically stable synthetic and natural wheat allohexaploids. New Phytologist 187: 1181-1194. doi:10.1111/j.1469-8137.2010.03339.x.

Chanderbali, A.S., B.A. Berger, D.G. Howarth, P.S. Soltis and D.E. Soltis. 2016. Evolving Ideas on the Origin and Evolution of Flowers: New Perspectives in the Genomic Era. Genetics 202: 1255-1265. doi:10.1534/genetics.115.182964.

Chang, P.L., B.P. Dilkes, M. McMahon, L. Comai and S.V. Nuzhdin. 2010. Homoeolog-specific retention and use in allotetraploid Arabidopsis suecica depends on parent of origin and network partners. Genome Biology 11. doi:10.1186/gb-2010-11-12-r125.

Chaudhary, B., R. Hovav, R. Rapp, N. Verma, J.A. Udall and J.F. Wendel. 2008. Global analysis of gene expression in cotton fibers from wild and domesticated Gossypium barbadense. Evolution & Development 10: 567-582. doi:10.1111/j.1525-142X.2008.00272.x.

Chelaifa, H., A. Monnier and M. Ainouche. 2010. Transcriptomic changes following recent natural hybridization and allopolyploidy in the salt marsh species Spartina x townsendii and Spartina anglica (Poaceae). New Phytologist 186: 161-174. doi:10.1111/j.1469-8137.2010.03179.x.

Chen, Z.J. and C.S. Pikaard. 1997. Transcriptional analysis of nucleolar dominance in polyploid plants: Biased expression/silencing of progenitor rRNA genes is developmentally regulated in Brassica. Proceedings of the National Academy of Sciences of the United States of America 94: 3442-3447. doi:10.1073/pnas.94.7.3442.

Comai, L. 2005. The advantages and disadvantages of being polyploid. Nature Reviews Genetics 6: 836-846. doi:10.1038/nrg1711.

Cremer, T. and C. Cremer. 2001. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nature Reviews Genetics 2: 292-301. doi:10.1038/35066075.

Croft, J.A., J.M. Bridger, S. Boyle, P. Perry, P. Teague and W.A. Bickmore. 1999. Differences in the localization and morphology of chromosomes in the human nucleus. Journal of Cell Biology 145: 1119-1131. doi:10.1083/jcb.145.6.1119.

Curtis, C.A., A.J. Lukaszewski and M. Chrzastek. 1991. METAPHASE-I PAIRING OF DEFICIENT CHROMOSOMES AND GENETIC-MAPPING OF DEFICIENCY BREAKPOINTS IN COMMON WHEAT. Genome 34: 553-560. doi:10.1139/g91-085.

Dierking, R., P. Azhaguvel, R. Kallenbach, M. Saha, J. Bouton, K. Chekhovskiy, et al. 2015. Linkage Maps of a Mediterranean x Continental Tall Fescue Population and their Comparative Analysis with Other Poaceae Species. Plant Genome 8. doi:10.3835/plantgenome2014.07.0032.

Dong, F.G. and J.M. Jiang. 1998. Non-Rabl patterns of centromere and telomere distribution in the interphase nuclei of plant cells. Chromosome Research 6: 551-558. doi:10.1023/a:1009280425125.

Dubcovsky, J., M.C. Luo and J. Dvorak. 1995. DIFFERENTIATION BETWEEN HOMOEOLOGOUS CHROMOSOMES 1A OF WHEAT AND 1A(M) OF TRITICUM-MONOCOCCUM AND ITS RECOGNITION BY THE WHEAT PH1 LOCUS. Proceedings of the National Academy of Sciences of the United States of America 92: 6645-6649. doi:10.1073/pnas.92.14.6645.

Dvorak, J. and P.E. McGuire. 1981. NONSTRUCTURAL CHROMOSOME DIFFERENTIATION AMONG WHEAT CULTIVARS, WITH SPECIAL REFERENCE TO DIFFERENTIATION OF CHROMOSOMES IN RELATED SPECIES. Genetics 97: 391-414.

Ehdaie, B., R.W. Whitkus and J.G. Waines. 2003. Root biomass, water-use efficiency, and performance of wheat-rye translocations of chromosomes 1 and 2 in spring bread wheat 'Pavon'. Crop Science 43: 710–717. doi:10.2135/cropsci2003.7100.

Ezquerro-Lopez, D., D. Kopecky and L.A. Inda. 2017. Cytogenetic relationshps within the Maghrebian clade of Festuca subgen. Schedonorus (Poaceae), using flow cytometry and FISH. Anales Del Jardin Botanico De Madrid 74. doi:10.3989/ajbm.2455.

Fawcett, J.A., S. Maere and Y. Van de Peer. 2009. Plants with double genomes might have had a better chance to survive the Cretaceous-Tertiary extinction event. Proceedings of the National Academy of Sciences of the United States of America 106: 5737-5742. doi:10.1073/pnas.0900906106.

Feldman, M., L. Avivi. 1988. Genetic control of bivalent pairing in common wheat: the mode of Ph1 action. In: The Third Kew Chromosome Conference. KEW CHROMOSOME C, Kew Publishing, Richmond, Surrey, UK, pp. 269-279.
Flagel, L.E. and J.F. Wendel. 2010. Evolutionary rate variation, genomic dominance and duplicate gene expression evolution during allotetraploid cotton speciation. New Phytologist 186: 184-193. doi:10.1111/j.1469-8137.2009.03107.x.
Fransz, P., J.H. de Jong, M. Lysak, M.R. Castiglione and I. Schubert. 2002. Interphase chromosomes in Arabidopsis are organized as well defined chromocenters from which euchromatin loops emanate. Proceedings of the National Academy of Sciences of the United States of America 99: 14584-14589. doi:10.1073/pnas.212325299.
Gaeta, R.T., S.Y. Yoo, J.C. Pires, R.W. Doerge, Z.J. Chen and T.C. Osborn. 2009. Analysis of Gene Expression in Resynthesized Brassica napus Allopolyploids Using Arabidopsis 70mer Oligo Microarrays. Plos One 4. doi:10.1371/journal.pone.0004760.
Ghesquiere, M., M.W. Humphreys and Z. Zwierzykowski. 2010. Festulolium. Fodder Crops and Amenity Grasses 5: 293-316. doi:10.1007/978-1-4419-0760-8_12.
Gorkin, D.U., D. Leung and B. Ren. 2014. The 3D Genome in Transcriptional Regulation and Pluripotency. Cell Stem Cell 14: 762-775. doi:10.1016/j.stem.2014.05.017.
Habermann, F.A., M. Cremer, J. Walter, G. Kreth, J. von Hase, K. Bauer, et al. 2001. Arrangements of macro- and microchromosomes in chicken cells. Chromosome Research 9: 569-584. doi:10.1023/a:1012447318535.
Hao, M., A.L. Li, T.W. Shi, J.T. Luo, L.Q. Zhang, X.C. Zhang, et al. 2017. The abundance of homoeologue transcripts is disrupted by hybridization and is partially restored by genome doubling in synthetic hexaploid wheat. Bmc Genomics 18. doi:10.1186/s12864-017-3558-0.
Hegarty, M.J., G.L. Barker, I.D. Wilson, R.J. Abbott, K.J. Edwards and S.J. Hiscock. 2006. Transcriptome shock after interspecific hybridization in Senecio is ameliorated by genome duplication. Current Biology 16: 1652-1659. doi:10.1016/j.cub.2006.06.071.
Heslopharrison, J.S., A.R. Leitch, T. Schwarzacher and K. Anamthawatjonsson. 1990. DETECTION AND CHARACTERIZATION OF 1B/1R TRANSLOCATIONS IN HEXAPLOID WHEAT. Heredity 65: 385-392. doi:10.1038/hdy.1990.108.
Hou, D.X., M. Fujii, N. Terahara and M. Yoshimoto. 2004. Molecular mechanisms behind the chemopreventive effects of anthocyanidins. Journal of Biomedicine and Biotechnology: 321-325.
Hovav, R., J.A. Udall, B. Chaudhary, R. Rapp, L. Flagel and J.F. Wendell. 2008. Partitioned expression of duplicated genes during development and evolution of a single cell in a polyploid plant. Proceedings of the National Academy of Sciences of the United States of America 105: 6191-6195. doi:10.1073/pnas.0711569105.
Ilut, D.C., J.E. Coate, A.K. Luciano, T.G. Owens, G.D. May, A. Farmer, et al. 2012. A COMPARATIVE TRANSCRIPTOMIC STUDY OF AN ALLOTETRAPLOID AND ITS DIPLOID PROGENITORS ILLUSTRATES THE UNIQUE ADVANTAGES AND CHALLENGES OF RNA-SEQ IN PLANT SPECIES. American Journal of Botany 99: 383-396. doi:10.3732/ajb.1100312.
Jauhar, P.P. 1975. GENETIC-CONTROL OF DIPLOID-LIKE MEIOSIS IN HEXAPLOID TALL FESCUE. Nature 254: 595-597. doi:10.1038/254595a0.
Jauhar, P.P. 1993. Cytogenetics of the Festuca-Lolium complex. Relevance to Breeding. Monogr. Theor. Appl. Genet. 18, p. 255.
Jiang, J.J., Y.L. Shao, K. Du, L.P. Ran, X.P. Fang and Y.P. Wang. 2013. Use of digital gene expression to discriminate gene expression differences in early generations of resynthesized Brassica napus and its diploid progenitors. Bmc Genomics 14. doi:10.1186/1471-2164-14-72.
Jiao, Y.N., N.J. Wickett, S. Ayyampalayam, A.S. Chanderbali, L. Landherr, P.E. Ralph, et al. 2011. Ancestral polyploidy in seed plants and angiosperms. Nature 473: 97-U113. doi:10.1038/nature09916.
Kleijer, G. and P. Morel. 1984. CYTOGENETIC STUDIES OF CROSSES BETWEEN LOLIUM-MULTIFLORUM LAM AND FESTUCA-ARUNDINACEA SCHREB .2. THE

AMPHIDIPLOIDS. Zeitschrift Fur Pflanzenzuchtung-Journal of Plant Breeding 93: 23-42.
Kopecky, D., J. Loureiro, Z. Zwierzykowski, M. Ghesquiere and J. Dolezel. 2006. Genome constitution and evolution in Lolium x Festuca hybrid cultivars (Festulolium). Theoretical and Applied Genetics 113: 731-742. doi:10.1007/s00122-006-0341-z.
Kopecky, D., D.C. Allen, M. Duchoslav, J. Dolezel and A.J. Lukaszewski. 2007. Condensation of rye chromatin in somatic interphase nuclei of Ph1 and ph1b wheat. Cytogenetic and Genome Research 119: 263-267. doi:10.1159/000112072.
Kopecky, D., A.J. Lukaszewski and J. Dolezel. 2008a. Cytogenetics of festulolium (Festuca X Lolium hybrids). Cytogenetic and Genome Research 120: 370-383. doi:10.1159/000121086.
Kopecky, D., A.J. Lukaszewski and J. Dolezel. 2008b. Meiotic behaviour of individual chromosomes of Festuca pratensis in tetraploid Lolium multiflorum. Chromosome Research 16: 987-998. doi:10.1007/s10577-008-1256-0.
Kopecky, D., J. Bartos, A.J. Lukaszewski, J.H. Baird, V. Cernoch, R. Koelliker, et al. 2009a. Development and mapping of DArT markers within the Festuca-Lolium complex. Bmc Genomics 10. doi:10.1186/1471-2164-10-473.
Kopecky, D., J. Bartos, Z. Zwierzykowski and J. Dolezel. 2009b. Chromosome pairing of individual genomes in tall fescue (Festuca arundinacea Schreb.), its progenitors, and hybrids with Italian ryegrass (Lolium multiflorum Lam.). Cytogenetic and Genome Research 124: 170-178. doi:10.1159/000207525.
Kopecky, D., M. Havrankova, J. Loureiro, S. Castro, A.J. Lukaszewski, J. Bartos, et al. 2010. Physical Distribution of Homoeologous Recombination in Individual Chromosomes of Festuca pratensis in Lolium multiflorum. Cytogenetic and Genome Research 129: 162-172. doi:10.1159/000313379.
Kopecky, D., J. Bartos, P. Christelova, V. Cernoch, A. Kilian and J. Dolezel. 2011. Genomic constitution of Festuca x Lolium hybrids revealed by the DArTFest array. Theoretical and Applied Genetics 122: 355-363. doi:10.1007/s00122-010-1451-1.
Kopecky, D., M. Martis, J. Cihalikova, E. Hribova, J. Vrana, J. Bartos, et al. 2013. Flow Sorting and Sequencing Meadow Fescue Chromosome 4F. Plant Physiology 163: 1323-1337. doi:10.1104/pp.113.224105.
Kopecky, D. and B. Studer. 2014. Emerging technologies advancing forage and turf grass genomics. Biotechnology Advances 32: 190-199. doi:10.1016/j.biotechadv.2013.11.010.
Kopecky, D., J. Harper, J. Bertas, D. Gasior, J. Vrana, E. Hribova, et al. 2016. An Increasing Need for Productive and Stress Resilient Festulolium Amphiploids: What Can Be Learnt from the Stable Genomic Composition of Festuca pratensis subsp. apennina (De Not.) Hegi? Frontiers in Environmental Science 4. doi:10.3389/fenvs.2016.00066.
Kopecky, D., D. Simonikova, M. Ghesquiere and J. Dolezel. 2017. Stability of Genome Composition and Recombination between Homoeologous Chromosomes in Festulolium (Festuca x Lolium) Cultivars. Cytogenetic and Genome Research 151: 106-114. doi:10.1159/000458746.
Kopecky, D., T. Felder, F.X. Schubiger, V. Mahelka, J. Bartos, J. Dolezel, et al. 2018. Frequent occurrence of triploid hybrids Festuca pratensisxF-apennina in the Swiss Alps. Alpine Botany 128: 121-132. doi:10.1007/s00035-018-0204-7.
Kopecky, D., L. Horakova, M. Duchoslav and J. Doležel. 2019a. Selective elimination of parental chromatin from introgression cultivars of xFestulolium (Festuca × Lolium). Sustainability 11: 3153. doi: 10.3390/su11113153
Kopecky, D., S.K. Talukder, J. Zwyrtkova, M. Trammell, J. Dolezel and M.C. Saha. 2019b. Inter-morphotype hybridization in tall fescue (Festuca arundinacea Schreb.): exploration of meiotic irregularities and potential for breeding. Euphytica 215. doi:10.1007/s10681-019-2419-0.
Kozubek, S., E. Lukasova, P. Jirsova, I. Koutna, M. Kozubek, A. Ganova, et al. 2002. 3D Structure of the human genome: order in randomness. Chromosoma 111: 321-331. doi:10.1007/s00412-002-0210-8.
Krivankova, A., D. Kopecky, S. Stoces, J. Dolezel and E. Hribova. 2017. Repetitive DNA: A Versatile Tool for Karyotyping in Festuca pratensis Huds. Cytogenetic and Genome

Research 151: 96-105. doi:10.1159/000462915.
Lamy, S., M. Blanchette, J. Michaud-Levesque, R. Lafleur, Y. Durocher, A. Moghrabi, et al. 2006. Delphinidin, a dietary anthocyanidin, inhibits vascular endothelial growth factor receptor-2 phosphorylation. Carcinogenesis 27: 989-996. doi:10.1093/carcin/bgi279.
Lee, H.S. and Z.J. Chen. 2001. Protein-coding genes are epigenetically regulated in Arabidopsis polyploids. Proceedings of the National Academy of Sciences of the United States of America 98: 6753-6758. doi:10.1073/pnas.121064698.
Lelley, T. 1976. INDUCTION OF HOMOELOGOUS PAIRING IN WHEAT BY GENES OF RYE SUPPRESSING CHROMOSOME 5B EFFECT. Canadian Journal of Genetics and Cytology 18: 485-489. doi:10.1139/g76-059.
Li, W., Y.M. Wei, L.L. Xu, A.J. Liu, Y.Z. Sheng, Z.E. Pu, et al. 2014. Evaluation of geographic distribution of high-molecular-weight glutenin subunits (HMW-GS) in wild and cultivated Triticum species. Genetic Resources and Crop Evolution 61: 1105-1119. doi:10.1007/s10722-014-0094-5.
Lohaus, R. and Y. Van de Peer. 2016. Of dups and dinos: evolution at the K/Pg boundary. Current Opinion in Plant Biology 30: 62-69. doi:10.1016/j.pbi.2016.01.006.
Loureiro, J., D. Kopecky, S. Castro, C. Santos and P. Silveira. 2007. Flow cytometric and cytogenetic analyses of Iberian Peninsula Festuca spp. Plant Systematics and Evolution 269: 89-105. doi:10.1007/s00606-007-0564-8.
Lukaszewski, A.J. 1990. FREQUENCY OF 1RS 1AL AND 1RS 1BL TRANSLOCATIONS IN UNITED-STATES WHEATS. Crop Science 30: 1151-1153. doi:10.2135/cropsci1990.0011183X003000050041x.
Lukaszewski, A.J. 1997. The development and meiotic behavior of asymmetrical isochromosomes in wheat. Genetics 145: 1155-1160.
Lukaszewski, A.J. 2008. Unexpected behavior of an inverted rye chromosome arm in wheat. Chromosoma 117: 569-578. doi:10.1007/s00412-008-0174-4.
Lukaszewski, A.J. and C.A. Curtis. 1993. PHYSICAL DISTRIBUTION OF RECOMBINATION IN B-GENOME CHROMOSOMES OF TETRAPLOID WHEAT. Theoretical and Applied Genetics 86: 121-127. doi:10.1007/bf00223816.
Lukaszewski, A.J. and D. Kopecky. 2010. The Ph1 Locus from Wheat Controls Meiotic Chromosome Pairing in Autotetraploid Rye (Secale cereale L.). Cytogenetic and Genome Research 129: 117-123. doi:10.1159/000314279.
Lukaszewski, A.J., B. Apolinarska, J.P. Gustafson and K.D. Krolow. 1987. CHROMOSOME-PAIRING AND ANEUPLOIDY IN TETRAPLOID TRITICALE .1. STABILIZED KARYOTYPES. Genome 29: 554-561. doi:10.1139/g87-093.
Lukaszewski, A.J., D. Kopecky and G. Linc. 2012. Inversions of chromosome arms 4AL and 2BS in wheat invert the patterns of chiasma distribution. Chromosoma 121: 201-208. doi:10.1007/s00412-011-0354-5.
Madlung, A. 2013. Polyploidy and its effect on evolutionary success: old questions revisited with new tools. Heredity 110: 99-104. doi:10.1038/hdy.2012.79.
Maestra, B., J.H. de Jong, K. Shepherd and T. Naranjo. 2002. Chromosome arrangement and behaviour of two rye homologous telosomes at the onset of meiosis in disomic wheat-5RL addition lines with and without the Ph1 locus. Chromosome Research 10: 655-667. doi:10.1023/a:1021564327226.
Mahelka, V. and D. Kopecky. 2010. Gene Capture from across the Grass Family in the Allohexaploid Elymus repens (L.) Gould (Poaceae, Triticeae) as Evidenced by ITS, GBSSI, and Molecular Cytogenetics. Molecular Biology and Evolution 27: 1370-1390. doi:10.1093/molbev/msq021.
Mahelka, V., D. Kopecky and L. Pastova. 2011. On the genome constitution and evolution of intermediate wheatgrass (Thinopyrum intermedium: Poaceae, Triticeae). Bmc Evolutionary Biology 11. doi:10.1186/1471-2148-11-127.
Mahelka, V., D. Kopecky and B.R. Baum. 2013. Contrasting Patterns of Evolution of 45S and 5S rDNA Families Uncover New Aspects in the Genome Constitution of the Agronomically Important Grass Thinopyrum intermedium (Triticeae). Molecular Biology and Evolution 30: 2065-2086. doi:10.1093/molbev/mst106.

Mahelka, V., K. Krak, D. Kopecky, J. Fehrer, J. Safar, J. Bartos, et al. 2017. Multiple horizontal transfers of nuclear ribosomal genes between phylogenetically distinct grass lineages. Proceedings of the National Academy of Sciences of the United States of America 114: 1726-1731. doi:10.1073/pnas.1613375114.

Majka, J., T. Ksiazczyk, A. Kielbowicz-Matuk, D. Kopecky and A. Kosmala. 2017. Exploiting repetitive sequences and BAC clones in Festuca pratensis karyotyping. Plos One 12. doi:10.1371/journal.pone.0179043.

Martinez-Perez, E., P. Shaw, L. Aragon-Alcaide and G. Moore. 2003. Chromosomes form into seven groups in hexaploid and tetraploid wheat as a prelude to meiosis. Plant Journal 36: 21-29. doi:10.1046/j.1365-313X.2003.01853.x.

Masterson, J. 1994. STOMATAL SIZE IN FOSSIL PLANTS - EVIDENCE FOR POLYPLOIDY IN MAJORITY OF ANGIOSPERMS. Science 264: 421-424. doi:10.1126/science.264.5157.421.

Mayer, R., A. Brero, J. von Hase, T. Schroeder, T. Cremer and S. Dietzel. 2005. Common themes and cell type specific variations of higher order chromatin arrangements in the mouse. Bmc Cell Biology 6. doi:10.1186/1471-2121-6-44.

Mayer, K.F., S. Taudien, M. Martis, H. Simkova, P. Suchankova, H. Gundlach, T. Wicker, A. Petzold, M. Felder, B. Steuernagel et al. 2009. Gene content and virtual gene order of barley chromosome 1H. Plant Physiol 151: 496–505. doi:10.1104/pp.109.142612.

McKendry, A.L., D.N. Tague and M.E. Miskin. 1996. Effect of 1BL.1RS on agronomic performance of soft red winter wheat. Crop Science 36: 844-847. doi:10.2135/cropsci1996.0011183X003600040003x.

Mikhailova, E.I., T. Naranjo, K. Shepherd, J.W. Eden, C. Heyting and J.H. de Jong. 1998. The effect of the wheat Ph1 locus on chromatin organisation and meiotic chromosome pairing analysed by genome painting. Chromosoma 107: 339-350. doi:10.1007/s004120050316.

Moens, P.B., C. Bernelotmoens and B. Spyropoulos. 1989. CHROMOSOME CORE ATTACHMENT TO THE MEIOTIC NUCLEAR-ENVELOPE REGULATES SYNAPSIS IN CHLOEALTIS (ORTHOPTERA). Genome 32: 601-610. doi:10.1139/g89-488.

Morenosevilla, B., P.S. Baenziger, D.R. Shelton, R.A. Graybosch and C.J. Peterson. 1995. AGRONOMIC PERFORMANCE AND END-USE QUALITY OF 1B VS 1BL/1RS GENOTYPES DERIVED FROM WINTER-WHEAT RAWHIDE. Crop Science 35: 1607-1612. doi:10.2135/cropsci1995.0011183X003500060015x.

Morgan, W.G., H. Thomas and E.J. Lewis. 1988. CYTOGENETIC STUDIES OF HYBRIDS BETWEEN FESTUCA-GIGANTEA VILL AND LOLIUM-MULTIFLORUM LAM. Plant Breeding 101: 335-343. doi:10.1111/j.1439-0523.1988.tb00306.x.

Murphy, S.P. and H.W. Bass. 2012. The maize (Zea mays) desynaptic (dy) mutation defines a pathway for meiotic chromosome segregation, linking nuclear morphology, telomere distribution and synapsis. Journal of Cell Science 125: 3681-3690. doi:10.1242/jcs.108290.

Naranjo, T. 2014. Dynamics of Rye Telomeres in a Wheat Background during Early Meiosis. Cytogenetic and Genome Research 143: 60-68. doi:10.1159/000363524.

Orellana, J., M.C. Cermeno and J.R. Lacadena. 1984. MEIOTIC PAIRING IN WHEAT-RYE ADDITION AND SUBSTITUTION LINES. Canadian Journal of Genetics and Cytology 26: 25-33. doi:10.1139/g84-005.

Pandit, M.K., M.J.O. Pocock and W.E. Kunin. 2011. Ploidy influences rarity and invasiveness in plants. Journal of Ecology 99: 1108-1115. doi:10.1111/j.1365-2745.2011.01838.x.

Perničková, K., G. Linc, E. Gaal, D. Kopecký, O. Šamajová and A.J. Lukaszewski. 2019a. Out-of-position telomeres in meiotic leptotene appear responsible for chiasmate pairing in an inversion heterozygote in wheat (Triticum aestivum L.). Chromosoma 128: 31-39. doi:10.1007/s00412-018-0686-5.

Perničková, K., V. Koláčková, A.J. Lukaszewski, C. Fan, J. Vrána, M. Duchoslav, G. Jenkins, D. Phillips, O. Šamajová, M. Sedlářová, J. Šamaj, J. Doležel, D. Kopecký, D. 2019b. Positioning of rye introgressions in somatic nuclei of wheat and its possible association

with meiotic instability. International Journal of Moleculer Sciences 20:1448. doi:10.3390/ijms20061448.
Rabl, C. 1885. Über Zellteilung. Morph. Jahrb. 1885, 10: 214–330.
Redinbaugh, M.G., T.A. Jones and Y.T. Zhang. 2000. Ubiquity of the St chloroplast genome in St-containing Triticeae polyploids. Genome 43: 846-852. doi:10.1139/gen-43-5-846.
Renny-Byfield, S. and J.F. Wendel. 2014. DOUBLING DOWN ON GENOMES: POLYPLOIDY AND CROP PLANTS. American Journal of Botany 101: 1711-1725. doi:10.3732/ajb.1400119.
Riley, R. and V. Chapman. 1958. GENETIC CONTROL OF THE CYTOLOGICALLY DIPLOID BEHAVIOUR OF HEXAPLOID WHEAT. Nature 182: 713-715. doi:10.1038/182713a0.
Riley, R., V. Chapman, T.E. Miller. 1973. The determination of meiotic chromosome pairing, In: Sears, E.R., Sears, L.M.S. (eds.) Proc 4 th Int Wheat Genet Symp, Agric Exp Station, University of Missouri, Columbia, pp. 731–738.
Schlegel, R., A. Boerner, V. Thiele and G. Melz. 1991. THE EFFECT OF THE PH1 GENE IN DIPLOID RYE, SECALE-CEREALE L. Genome 34: 913-917. doi:10.1139/g91-140.
Sears, E.R. 1977. INDUCED MUTANT WITH HOMOEOLOGOUS PAIRING IN COMMON WHEAT. Canadian Journal of Genetics and Cytology 19: 585-593. doi:10.1139/g77-063.
Sears, E.R., M. Okamoto. 1958. Intergenomic relationships in hexaploid wheat. In: Proc Xth Inter Congress Genet 2, pp. 258-259.
Sears, E. R. 1981. Transfer of alien genetic material to wheat. Wheat science - today and tomorrow, pp. 75-89.
Soltis, D.E. and P.S. Soltis. 1993. MOLECULAR-DATA AND THE DYNAMIC NATURE OF POLYPLOIDY. Critical Reviews in Plant Sciences 12: 243-273. doi:10.1080/713608048.
Soltis, P.S. and D.E. Soltis. 2016. Ancient WGD events as drivers of key innovations in angiosperms. Current Opinion in Plant Biology 30: 159-165. doi:10.1016/j.pbi.2016.03.015.
Song, K.M., P. Lu, K.L. Tang and T.C. Osborn. 1995. RAPID GENOME CHANGE IN SYNTHETIC POLYPLOIDS OF BRASSICA AND ITS IMPLICATIONS FOR POLYPLOID EVOLUTION. Proceedings of the National Academy of Sciences of the United States of America 92: 7719-7723. doi:10.1073/pnas.92.17.7719.
Stoces, S., T. Ruttink, J. Bartos, B. Studer, S. Yates, Z. Zwierzykowski, et al. 2016. Orthology Guided Transcriptome Assembly of Italian Ryegrass and Meadow Fescue for Single-Ncleotide Polymorphism Discovery. Plant Genome 9. doi:10.3835/plantgenome2016.02.0017.
Sun, F.L., M.H. Cuaycong, C.A. Craig, L.L. Wallrath, J. Locke and S.C.R. Eigin. 2000. The fourth chromosome of Drosophila melanogaster: Interspersed euchromatic and heterochromatic domains. Proceedings of the National Academy of Sciences of the United States of America 97: 5340-5345. doi:10.1073/pnas.090530797.
te Beest, M., J.J. Le Roux, D.M. Richardson, A.K. Brysting, J. Suda, M. Kubesova, et al. 2012. The more the better? The role of polyploidy in facilitating plant invasions. Annals of Botany 109: 19-45. doi:10.1093/aob/mcr277.
Tomaszewski, C., S.L. Byrne, A. Foito, S. Kildea, D. Kopecky, J. Dolezel, et al. 2012. Genetic linkage mapping in an F2 perennial ryegrass population using DArT markers. Plant Breeding 131: 345-349. doi:10.1111/j.1439-0523.2011.01944.x.
Trethowan, R.M. and A. Mujeeb-Kazi. 2008. Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat. Crop Science 48: 1255-1265. doi:10.2135/cropsci2007.08.0477.
Trojan, V., M. Musilova, T. Vyhnanek, B. Klejdus, P. Hanacek and L. Havel. 2014. Chalcone synthase expression and pigments deposition in wheat with purple and blue colored caryopsis. Journal of Cereal Science 59: 48-55. doi:10.1016/j.jcs.2013.10.008.
Tsunewaki, K. 1964. GENETIC STUDIES OF 6X-DERIVATIVE FROM AN 8X TRITICALE. Canadian Journal of Genetics and Cytology 6: 1-&.
Waines, J.G. and B. Ehdaie. 2007. Domestication and crop physiology: roots of Green-Revolution wheats. Annals of Botany 100:991-998. doi: 10.1093/aob/mcm180.

Wu, Y., Y. Sun, X.T. Wang, X.Y. Lin, S. Sun, K. Shen, et al. 2016. Transcriptome shock in an interspecific F1 triploid hybrid of Oryza revealed by RNA sequencing. Journal of Integrative Plant Biology 58: 150-164. doi:10.1111/jipb.12357.

Yakimowski, S.B. and L.H. Rieseberg. 2014. THE ROLE OF HOMOPLOID HYBRIDIZATION IN EVOLUTION: A CENTURY OF STUDIES SYNTHESIZING GENETICS AND ECOLOGY. American Journal of Botany 101: 1247-1258. doi:10.3732/ajb.1400201.

Yang, J.H., D.Y. Liu, X.W. Wang, C.M. Ji, F. Cheng, B.N. Liu, et al. 2016. The genome sequence of allopolyploid Brassica juncea and analysis of differential homoeolog gene expression influencing selection. Nature Genetics 48: 1225-1232. doi:10.1038/ng.3657.

Yoo, M.J., E. Szadkowski and J.F. Wendel. 2013. Homoeolog expression bias and expression level dominance in allopolyploid cotton. Heredity 110: 171-180. doi:10.1038/hdy.2012.94.

Yoo, M.J. and J.F. Wendel. 2014. Comparative Evolutionary and Developmental Dynamics of the Cotton (Gossypium hirsutum) Fiber Transcriptome. Plos Genetics 10. doi:10.1371/journal.pgen.1004073.

Zeller, F. J. 1973. 1B/1R wheat-rye chromosome substitutions and translocations. Proc. 4th Intern. Wheat Genet. Symp. 1973, Columbia, Missouri, pp. 209—221.

Zhang, D.W., Q. Pan, C. Tan, B. Zhu, X.H. Ge, Y.J. Shao, et al. 2016. Genome-Wide Gene Expressions Respond Differently to A-subgenome Origins in Brassica napus Synthetic Hybrids and Natural Allotetraploid. Frontiers in Plant Science 7. doi:10.3389/fpls.2016.01508.

Zwierzykowski, Z., E. Zwierzykowska, M. Taciak, A. Kosmala, R.N. Jones, W. Zwierzykowski, et al. 2011. Genomic structure and fertility in advanced breeding populations derived from an allotetraploid Festuca pratensis x Lolium perenne cross. Plant Breeding 130: 476-480. doi:10.1111/j.1439-0523.2010.01839.x.

Zwierzykowski, Z., T. Ksiazczyk, M. Taciak, M. et al. 2012. Genome constitution in selected and unselected plants of F2-F4 generations derived from an allotetraploid Festuca pratensis x Lolium perenne hybrid. In: Strategies for Sustainable Forage and Turf Grass Improvement II. Springer, Amsterdam, pp. 75-79.

6. List of appendices

Structure and evolution of plant hybrid genomes

1. **Kopecký, D.**, Loureiro, J., Zwierzykowski, Z., Ghesquiere, M., Doležel, J.: Genome constitution and evolution in *Lolium* × *Festuca* hybrid cultivars (Festulolium). – Theor. Appl. Genet. 113: 731-742, 2006.
2. Loureiro, J., **Kopecký, D.**, Castro, S., Santos, C., Silveira, P.: Flow cytometric and cytogenetic analyses of Iberian Peninsula fescues. – Plant Sys. Evol. 269: 89-105, 2007.
3. **Kopecký, D.**, Lukaszewski, A.J. and Doležel, J.: Cytogenetics of Festulolium (*Festuca* × *Lolium* hybrids). – Cytogenet. Genome Res. 120: 370-383, 2008a.
4. **Kopecký, D.**, Bartoš, J., Lukaszewski, A.J., Baird, J.H., Černoch, V., Kölliker, R., Rognli, O.A., Blois, H., Caig, V., Lübberstedt, T., Studer, B., Shaw, B., Doležel, J., Kilian, A.: Development and mapping of DArT markers within the *Festuca* – *Lolium* complex. – BMC Genomics 10:473, 2009a.
5. Mahelka, V., **Kopecký, D.**: Gene Capture from Across the Grass Family in the Allohexaploid *Elymus repens* (L.) Gould (*Poaceae*, *Triticeae*) as Evidenced by ITS, GBSSI, and Molecular Cytogenetics. – Mol. Biol. Evol. 27: 1370-1390, 2010.
6. **Kopecký, D.**, Bartoš, J., Christelová, P., Černoch, V., Kilian, A., Doležel, J. Genomic constitution of *Festuca* x *Lolium* hybrids revealed by the DArTFest array. – Theor. Appl. Genet. 122: 355-363, 2011.
7. Bartoš, J., Sandve, S.R., Kölliker, R., **Kopecký, D.**, Christelová, P., Stočes, Š., Ostrem, L., Larsen, A., Kilian, A., Rognli, O.A., Doležel, J.: Genetic mapping of DArT markers in the *Festuca-Lolium* complex and their use in freezing tolerance association analysis. – Theor. Appl. Genet. 122: 1133-1147, 2011.
8. Mahelka, V., **Kopecký, D**., Paštová, L.: On the genome constitution and evolution of intermediate wheatgrass (*Thinopyrum intermedium*: *Poaceae*, *Triticeae*). – BMC Evol. Biol. 11:127, 2011.
9. Tomaszewski, C., Byrne, S.L., Foito, A., Kildea, S., **Kopecký, D.**, Doležel, J., Heslop-Harrison, J.S., Stewart, D., Barth, S.: Genetic linkage mapping in an F2 perennial ryegrass population using DArT markers. – Plant Breed. 131: 345-349, 2012.
10. Baird, J.H., **Kopecký, D.**, Lukaszewski, A.J., Green, R.L., Bartoš, J. and Doležel, J.: Genetic Diversity of Turf-Type Tall Fescue Using Diversity Arrays Technology. – Crop Sci. 52: 408-412, 2012.
11. Mahelka, V., **Kopecký, D.**, Baum, B.R.: Contrasting patterns of evolution of 45S and 5S rDNA families uncover new aspects in the genome constitution

of the agronomically important grass *Thinopyrum intermedium* (*Triticeae*). – Mol. Biol. Evol. 30(9): 2065-2086, 2013.
12. **Kopecký, D.**, Martis, M., Číhalíková, J., Hřibová, E., Vrána, J., Bartoš, J., Kopecká, J., Cattonaro, F., Stočes, Š., Novák, P., Neumann, P., Macas, J., Šimková, H., Studer, B., Asp, T., Baird, J.H., Navrátil, P., Karafiátová, M., Kubaláková, M., Šafář, J., Mayer, K., Doležel, J.: Flow sorting and sequencing meadow fescue chromosome 4F. – Plant Physiol. 163: 1323-1337, 2013.
13. **Kopecký, D.**, Studer, B.: Emerging technologies advancing forage and turf grass genomics. – Biotechnol. Adv. 32: 190-199, 2014.
14. Barnes, B.D., **Kopecký, D.**, Lukaszewski, A.J., Baird, J.H.: Evaluation of turf-type interspecific hybrids of meadow fescue with perennial ryegrass for improved stress tolerance. – Crop Sci. 54: 355–365, 2014.
15. Dierking, R., Azhaguvel, P., Kallenbach, R., Saha, M., Bouton, J., Chekhovskiy, K., **Kopecký, D.**, Hopkins, A.: Linkage maps of a Mediterranean x Continental tall fescue population and their comparative analysis with other Poaceae species. – Plant Genome 8: 1-18, 2015.
16. Burešová, V., **Kopecký, D.**, Bartoš, J., Martinek, P., Watanabe, N., Vyhnánek, T., Doležel, J.: Variation in genome composition of blue-aleurone wheat. – Theor. Appl. Genet. 128: 273-282, 2015.
17. **Kopecký, D.**, Harper, J., Bartoš, J., Gasior, D., Vrána, J., Hřibová, E., Boller, B., Ardenghi, N.M.G., Šimoníková, D., Doležel, J., Humphreys, M.W.: An increasing need for productive and stress resilient Festulolium amphiploids: What can be learnt from the stable genomic composition of *Festuca pratensis* subsp. *apennina* (De Not.) Hegi? – Front. Environ. Sci. 4: 66, 2016.
18. Mahelka, V., Krak, K., **Kopecký, D.**, Fehrer, J., Šafář, J., Bartoš, J., Hobza, R., Blavet, N., Blattner, F.R.: Multiple horizontal transfers of nuclear ribosomal genes between phylogenetically distinct grass lineages. – Proc. Natl. Acad. Sci. USA 114: 1726-1731, 2017.
19. Ezquerro-López, D., **Kopecký, D.**, Inda, L.Á.: Cytogenetic relationships within the Maghrebian clade of *Festuca* subgen. Schedonorus (Poaceae), using flow cytometry and FISH. – Anal. Bot. Madrid 74: e052, 2017.
20. **Kopecký, D.**, Šimoníková, D., Ghesquière, M., Doležel, J.: Stability of genome composition and recombination between homoeologous chromosomes in Festulolium (*Festuca* × *Lolium*) cultivars. – Cytogenet. Genome Res. 151: 106-114, 2017.
21. Křivánková, A., **Kopecký, D.**, Stočes, Š., Doležel, J., Hřibová, E. Repetitive DNA: A versatile tool for karyotyping in *Festuca pratensis* Huds. – Cytogenet. Genome Res. 151: 96-105, 2017.
22. Majka, J., Książczyk, T., Kieøbowicz-Matuk, A., **Kopecký, D.**, Kosmala, A.: Exploiting repetitive sequences and BAC clones in *Festuca pratensis* karyotyping. – PLoS ONE 12: e0179043, 2017.

23. Banfi, E., Galasso, G., Foggi, B., **Kopecký, D.**, Ardenghi, N.M.G.: From *Schedonorus* and *Micropyropsis* to *Lolium* (*Poaceae*: *Loliinae*): New combinations and typifications. – Taxon 66: 708-717, 2017.
24. **Kopecký, D.**, Felder, T., Schubiger, F.X., Mahelka, V., Bartoš, J., Doležel, J., Boller, B.: Frequent occurrence of triploid hybrids *Festuca pratensis* × *F. apennina* in the Swiss Alps. – Alpine Bot. 128: 121-132, 2018.

Chromosome pairing and its implication for the fertility of plant hybrids

25. **Kopecký, D.**, Lukaszewski, A.J., Doležel, J.: Meiotic behavior of individual chromosomes of *Festuca pratensis* in tetraploid *Lolium multiflorum*. – Chromosome Res. 16: 987-998, 2008b.
26. **Kopecký, D.**, Bartoš, J., Zwierzykowski, Z., Doležel, J.: Chromosome pairing of individual genomes in tall fescue (*Festuca arundinacea* Schreb.), its progenitors, and hybrids with Italian ryegrass (*Lolium multiflorum* Lam.). Cytogenet. Genome Res. 124: 170-178, 2009b.
27. Lukaszewski, A.J., **Kopecký, D.**: The Ph1 locus from wheat controls meiotic chromosome pairing in autotetraploid rye (*Secale cereale* L.). – Cytogenetic and Genome Research 129: 117-123, 2010.
28. **Kopecký, D.**, Havránková, M., Loureiro, J., Castro, S., Lukaszewski, A.J., Bartoš, J., Kopecká, J., Doležel, J.: Physical distribution of homoeologous recombination in individual chromosomes of *Lolium multiflorum*/*Festuca pratensis* substitutions. – Cytogenet. Genome Res. 129: 162-172, 2010.
29. Lukaszewski, A.J., **Kopecký, D.**, Linc, G.: Inversions of chromosome arms 4AL and 2BS in wheat invert the patterns of chiasma distribution. – Chromosoma 121: 201-208, 2012.
30. **Kopecký, D.**, Horáková, L., Duchoslav, M., Doležel, J.: Selective elimination of parental chromatin from introgression cultivars of xFestulolium (Festuca × Lolium). – Sustainability 11: 3153, 2019a.
31. **Kopecký, D.**, Talukder, S.K., Zwyrtková, J., Trammell, M., Dolezel J., Saha, M.C.: Inter-morphotype hybridization in tall fescue (Festuca arundinacea Schreb.): exploration of meiotic irregularities and potential for breeding. – Euphytica 215: 5, 2019b.

Gene expression

32. Stočes, Š., Ruttink, T., Bartoš, J., Studer, B., Yates, S., Zwierzykowski, Z., Abrouk, M., Roldán-Ruiz, I., Książczyk, T., Rey, E., Doležel, J., **Kopecký, D.**: Orthology Guided Transcriptome Assembly of Italian ryegrass and meadow fescue for single-nucleotide polymorphism discovery. – Plant Genome 9: 1-14, 2016.

Spatial organization of hybrid nucleus

33. **Kopecký, D.**, Allen, D.C., Doležel, J., Lukaszewski, A.J.: Condensation of rye chromatin in somatic interphase nuclei of Ph1 and ph1b wheat. – Cytogenet. Genome Res. 119: 263-267, 2007.
34. Perničková, K., Linc, G., Gaal, E., **Kopecký, D.**, Šamajová, O., Lukaszewski, A.J.: Out-of-position telomeres in meiotic leptotene appear responsible for chiasmate pairing in an inversion heterozygote in wheat (*Triticum aestivum* L.). – Chromosoma 128: 31-39, 2019a.
35. Perničková, K., Koláčková, V., Lukaszewski, A.J., Fan, C., Vrána, J., Duchoslav, M., Jenkins, G., Phillips, D., Šamajová, O., Sedlářová, M., Šamaj, J., Doležel, J., **Kopecký, D.**: Positioning of rye introgressions in somatic nuclei of wheat and its possible association with meiotic instability. – Int. J. Mol. Sci. 20:1448, 2019b.

7. Appendices