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Review of the Habilitation Thesis by David Kopecký

The candidate is interested in the genome structure and its evolution/changes/mechanism of plasticity and maintenance, respectively, employing as a model system interspecific hybrids between meadow fescue (*Festuca pratensis*) and ryegrass (*Lolium sp.*), designated as xFestulolium. The work has a large impact on the basic understanding of the fundamental mechanisms of general biological importance with substantial implications in the plant breeding.

The candidate is presenting his research interest divided into three subsections: i) structure and evolution of genomes of plant hybrids, ii) chromosome pairing and its implication for the fertility of interspecific hybrids, iii) gene expression and iv) spatial organization of hybrid nucleus.

The applicant contribution to the field is complex, ranging from karyotyping of xFestulolium hybrids (Kopecky et al., 2006), generation of partial BAC library of *F. pratensis* (Kopecky et al., 2008) and isolation and sequencing of one of its (4F) chromosomes (Kopecky et al., 2013), developing new tool, the DArTFest DNA array (Kopecky et al., 2009), useful in e.g. identification of new QTL associated with crown rust resistance (Tomaszewski et al., 2012) through analysis of possible mechanism of homeologous pairing, e.g. (Kopecky et al., 2007; Lukaszewski and Kopecky, 2010). Dr. Kopecky and his team also demonstrated allopolyploid origin of couch grass (*Elymus repens*) and intermediate wheatgrass (*Thinopyrum intermedium*) (Mahelka and Kopecky, 2010) and identified the potential role of horizontal gene transfer during the evolution of one of the barley species, *Hordeum bogdanii*, a progenitor of couch grass (Mahelka et al., 2017). They also described a set of interspecific SNPs, useful in the studies on the genome interactions affecting transcription of hybrid chromosomes in the parental-specific way (Stoces et al., 2016) and studied the role of hybrid chromosome orientation in the interphase nuclei (Koláčková et al., in preparation), providing an evidence for the importance of telomeres in the proper chromosome localization both in the interphase nuclei of somatic cells and meiosis (leptotene) (Pernickova et al., 2019b; Pernickova et al., 2019a).

The outputs of the candidate results are published in solid journals with mostly mid- to high-range IF.

The data were evaluated and successfully past the peer-review process, therefore I do not feel authorized to evaluate its quality (which anyway I do not doubt about).

Based on my professional experience and field of interest, I have following questions:

1. The candidate is mentioning that in one of his early work (Kopecky et al., 2006), they found high level of intravarietal genomic variability that sometimes even exceeded the intervarietal variability. The (probably rather trivial) question could be how the individual varieties are defined (at the genome level)? The candidate is also mentioning high stability of amphiploid xFestulolium forms in comparison to introgression lines (Kopecky et al., 2019). Is there known/could the candidate speculate on the underlying molecular mechanism?
2. Applicant is also mentioning that using the much lower variability has been observed in the turf-type cultivars of tall fescues in the U.S.A. compared to the (higher?) levels of diversity among diverse forage cultivars in Europe (Baird et al., 2012). The applicant is concluding that "...This implies massive sharing of plant material and the absence of any protection of existing cultivars in the USA.". I do not understand that justification. Could the applicant provide a bit more detailed explanation to me?
3. Applicant has made a very interesting observation on various types of chromosomal rearrangements after introgression of various fragments of tall wheatgrass (*Thinopyrum ponticum*) genome into the bread wheat (*Triticum aestivum*) associated with the blue aleurone layer formation. The applicant concludes that based on a wide spectrum of the observed rearrangements, "...the introgressions activate the blue aleurone trait, which is present in common wheat germplasm, but inactivated in absence of *Th. ponticum* introgression (Buresova et al., 2015)." Could the applicant comment on that hypothesis a little bit more in detail, particularly on the possible molecular mechanism and whether he has any clue on the locus in the wheat genome that could get misregulated upon interaction with the wheatgrass genome?
4. Applicant is describing an interesting phenomenon of spatial-specific distribution of the chromosome ability to recombine (in the proximo-distal axis). Interestingly, this phenomenon is preserved even after chromosomal rearrangements, e.g. inversions. Is that known if this type of spatial information is preserved throughout several generations? Are there known any mutations affecting the aforementioned recombination distribution?

Overall, I do find the submitted work of high quality and in spite of my professional focus is different, it was a pleasure for me reading through the submitted overview of the impressive applicant work. Considering the previous publication record and its citation response, the candidate is apparently well accepted by the community and thus undoubtedly worth of the academic recognition.

Kind regards,

JAN Hejáltko

References

- Baird, J.H., Kopecky, D., Lukaszewski, A.J., Green, R.L., Bartos, J., and Dolezel, J. (2012).** Genetic Diversity of Turf-Type Tall Fescue Using Diversity Arrays Technology. *Crop Science* **52**, 408-412.
- Buresova, V., Kopecky, D., Bartos, J., Martinek, P., Watanabe, N., Vyhnanek, T., and Dolezel, J. (2015).** Variation in genome composition of blue-aleurone wheat. *Theor Appl Genet* **128**, 273-282.
- Kopecky, D., Lukaszewski, A.J., and Dolezel, J. (2008).** Cytogenetics of Festulolium (Festuca x Lolium hybrids). *Cytogenet Genome Res* **120**, 370-383.
- Kopecky, D., Horakova, L., Duchoslav, M., and Dolezel, J. (2019).** Selective Elimination of Parental Chromatin from Introgression Cultivars of xFestulolium (Festuca x Lolium). *Sustainability-Basel* **11**.
- Kopecky, D., Loureiro, J., Zwierzykowski, Z., Ghesquiere, M., and Dolezel, J. (2006).** Genome constitution and evolution in Lolium x Festuca hybrid cultivars (Festulolium). *Theor Appl Genet* **113**, 731-742.
- Kopecky, D., Allen, D.C., Duchoslav, M., Dolezel, J., and Lukaszewski, A.J. (2007).** Condensation of rye chromatin in somatic interphase nuclei of Ph1 and ph1b wheat. *Cytogenet Genome Res* **119**, 263-267.
- Kopecky, D., Bartos, J., Lukaszewski, A.J., Baird, J.H., Cernoch, V., Kolliker, R., Rognli, O.A., Blois, H., Caig, V., Lubberstedt, T., Studer, B., Shaw, P., Dolezel, J., and Kilian, A. (2009).** Development and mapping of DArT markers within the Festuca - Lolium complex. *BMC Genomics* **10**, 473.
- Kopecky, D., Martis, M., Cihalikova, J., Hribova, E., Vrana, J., Bartos, J., Kopecka, J., Cattonaro, F., Stoces, S., Novak, P., Neumann, P., Macas, J., Simkova, H., Studer, B., Asp, T., Baird, J.H., Navratil, P., Karafiatova, M., Kubalakova, M., Safar, J., Mayer, K., and Dolezel, J. (2013).** Flow sorting and sequencing meadow fescue chromosome 4F. *Plant Physiol* **163**, 1323-1337.
- Lukaszewski, A.J., and Kopecky, D. (2010).** The Ph1 locus from wheat controls meiotic chromosome pairing in autotetraploid rye (*Secale cereale* L.). *Cytogenet Genome Res* **129**, 117-123.
- Mahelka, V., and Kopecky, D. (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. *Mol Biol Evol* **27**, 1370-1390.
- Mahelka, V., Krak, K., Kopecky, D., Fehrer, J., Safar, J., Bartos, J., Hobza, R., Blavet, N., and Blattner, F.R. (2017).** Multiple horizontal transfers of nuclear ribosomal genes between phylogenetically distinct grass lineages. *Proc Natl Acad Sci U S A* **114**, 1726-1731.
- Pernickova, K., Linc, G., Gaal, E., Kopecky, D., Samajova, O., and Lukaszewski, A.J. (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.
- Pernickova, K., Kolackova, V., Lukaszewski, A.J., Fan, C., Vrana, J., Duchoslav, M., Jenkins, G., Phillips, D., Samajova, O., Sedlarova, M., Samaj, J., Dolezel, J., and Kopecky, D. (2019b).** Instability of Alien Chromosome Introgressions in Wheat Associated with Improper Positioning in the Nucleus. *Int J Mol Sci* **20**.
- Stoces, S., Ruttink, T., Bartos, J., Studer, B., Yates, S., Zwierzykowski, Z., Abrouk, M., Roldan-Ruiz, I., Ksiazczyk, T., Rey, E., Dolezel, J., and Kopecky, D. (2016).** Orthology Guided Transcriptome Assembly of Italian Ryegrass and Meadow Fescue for Single-Nucleotide Polymorphism Discovery. *Plant Genome-U S* **9**.

Tomaszewski, C., Byrne, S.L., Foito, A., Kildea, S., Kopecky, D., Dolezel, J., Heslop-Harrison, J.S., Stewart, D., and Barth, S. (2012). Genetic linkage mapping in an F2 perennial ryegrass population using DArT markers. *Plant Breeding* **131, 345-349.**