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Subject: Evaluation of the PhD thesis of Julia Dluzewska, MSc BSc

Vienna, July 17, 2023

To whom it may concern,

I was assigned with the joyful task to read and grade the doctoral thesis of Julia Dluzewska, performed in the laboratory of Prof. Piotr A. Ziolkowski (Professor and principal investigator at the Adam Mickiewicz University in Poznań, Poland, Faculty of Biology, Institute of Molecular Biology and Biotechnology, Laboratory of Genome Biology), with the title “Crossover control by mismatch detection protein MSH2 in response to the chromosome heterozygosity pattern in *Arabidopsis thaliana*”.

The thesis represents a “cumulative thesis”, compiling the already published research manuscript of Julia Dluzewska. It has a volume of 246 pages, encompassing abstracts (in English and Polish), a list of abbreviations, an introduction to meiosis, meiotic recombination and DNA mismatch repair and the specific aims and objectives. The introduction sets the stage and delineates open questions in the field. She focuses on meiotic recombination and repair to provide a solid justification of her research aims. The main part of the thesis is represented by three original research manuscripts and one review article. The impressive research output of Julia Dluzewska is as follows:

1. Nadia Kbiri, Julia Dluzewska, Ian R. Henderson, Piotr A. Ziolkowski. Quantifying meiotic crossover recombination in Arabidopsis lines expressing fluorescent reporters in seeds using *SeedScoring* pipeline for Cell Profiler. In: Lambing, C. (eds) Plant Gametogenesis. Methods in Molecular Biology, 2022, 2484:121-134. Humana, New York, NY; DOI: 10.1007/978-1-0716-2253-7_10

This manuscript represents a technical paper to quantify meiotic recombination in Arabidopsis seeds at specific locations that are bordered with seed-specific fluorescent markers. The authors describe in a detailed manner how to set up a high-throughput experiment and how to use the corresponding software. The described procedure represents a technical leap and substitutes a very cumbersome procedure with an elegant and time-effective protocol, allowing the design of sophisticated recombination experiments in Arabidopsis.

Question to the candidate:
How would you set up a test-system to score the contribution of male and female meiosis to the overall meiotic recombination rates (either genome-wide or regarding a specific locus)?

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2. Alexander R. Blackwell[1], Julia Dluzewska[1], Maja Szymanska-Lejman, Stuart Desjardins, Andrew J. Tock, Nadia Kbiri, Christophe Lambing, Emma J. Lawrence, Tomasz Bieluszewski, Beth Rowan, James D. Higgins, Piotr A. Ziolkowski, and Ian R. Henderson. MSH2 shapes the meiotic crossover landscape in relation to interhomolog polymorphism in Arabidopsis. The EMBO Journal, 2020; 39:e104858; DOI: 10.15252/embj.2020104858
[1] - joint first authors

This manuscript presents the remarkable finding that MSH2 has a pro-cross over role in pericentromeric regions of Arabidopsis hybrids, a region relatively rich in polymorphisms (including SNPs). The authors established that CO levels were positively correlated with SNPs up to certain threshold and that this correlation depends on MSH2. Importantly, the results held true for various hybrid combinations. The authors also created sophisticated recombination tester lines with a juxtaposition of homozygous and heterozygous regions to demonstrate the positive and MSH2 dependent effect of SNPs on local recombination rates.

Questions to the candidate:
The findings described above were published in 2020. Can you describe the most recent ideas how MSH2 actually stimulates class I CO formation in polymorphic regions in Arabidopsis?
Can you speculate on the mechanistic base for the differences observed in Arabidopsis and other organisms regarding the loss of MSH2?

3. Julia Dluzewska, Wojciech Dziegielewski, Maja Szymanska-Lejman, Monika Gazecka, Ian R. Henderson, James D. Higgins, Piotr A. Ziolkowski. MSH2 stimulates interfering and inhibits non-interfering crossovers in response to genetic polymorphism. BioRxiv, 2023.05.03.539183. DOI: 10.1101/2023.05.03.539183

The research presented in this manuscript further investigates the stimulatory role of MSH2 during recombination in polymorphic regions in Arabidopsis. The authors dedicated their efforts to understand the impact of polymorphisms and MSH2 with respect to interfering and non-interfering cross-over pathways. Interestingly, it was found that MSH2, while stimulating class I (interfering) COs, supresses class II (non-interfering) COs in polymorphic regions. To reach their conclusions, the authors generated a large set of double and triple mutants, in the recombination tester background….a remarkable achievement.

Question to the candidate:
Can you speculate how, mechanistically, MSH2 may suppress class II CO formation in polymorphic regions in Arabidopsis?

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4. Julia Dluzewska[1], Maja Szymanska[1], Piotr A. Ziolkowski. Where to cross over? Defining crossover sites in plants. Frontiers in Genetics, 2018; 9:609. DOI: 10.3389/fgene.2018.00609
[1] – joint first authors

The review, written in 2018, comprehensively introduced consideration of cross over patterning. It should be noted that also in this publication, Julia Dluzewska prepared all figures showing her in-depth knowledge of the subject and her ability to transform complex information into intelligible graphics.

Question to the candidate:
Can you describe an overarching framework that would explain why, in evolutionary terms, recombination stimulation at polymorphic sites could be advantageous.

All sections are appropriately referenced and Julia Dluzewska clearly states which parts of the research work presented in the published manuscripts have been generated by her. Each chapter (separate manuscripts as outlined above) concludes with a short synopsis of the research work to allow the non-specialist reader to understand the major findings and the relevance. The thesis concludes with a summary of all major findings and a reference list.

The given thesis compiles very interesting and relevant research findings. The results have already been published (or are under review in one instance), in peer-reviewed, high-impacts journals, demonstrating the appreciation of the research community. It is important to note, that Julia Dluzewska has been extremely productive and in addition to the work presented in this thesis authored further research papers, also published in high-impact, peer-reviewed international journals. The thesis work certainly fulfils all the formal requirements for a PhD dissertation and thus I recommend the thesis to be accepted and the candidate, Julia Dluzewska, to be awarded the best possible grade for her PhD degree. In congratulate her, the entire research team and her supervisor, Dr. Ziolkowski, for the achievements!

Please contact me, in case you need further information.

Sincerely,

Univ. Prof. Dr. Mag. Peter Schlögelhofer