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Review of the PhD thesis of Maja Szymanska-Lejman

entitled

"Impact of DNA interhomolog polymorphism on meiotic crossover formation at the genome-wide and recombination hotspot scale in *Arabidopsis thaliana*"

The PhD thesis of Maja Szymanska-Lejman has been prepared at the Faculty of Biology of the Adam Mickiewicz University in Poznan under the supervision of Prof. Piotr Ziolkowski. The topic of the dissertation focusses on the interplay between meiotic recombination and DNA polymorphisms. This work has been initiated by Pr. Ziolkowski in the laboratory of Pr. Ian Henderson in Cambridge, UK (Ziolkowski et al, eLife, 2015). Meiotic recombination is at the heart of Mendelian heredity, evolution and breeding. It ensures both faithful chromosome transmission and allelic shuffling over generations through the formation of crossovers (COs), *i.e.* reciprocal exchanges of DNA fragments between chromosomes, which are one of the two meiotic recombinant products. To guarantee these two basic roles, meiotic crossovers must occur between polymorphic parental chromosomes. Yet, the interplay between sequence polymorphism and meiotic recombination varies from species to species. In *S. cerevisiae*, it has been shown that the presence of polymorphisms between parental chromosomes may destabilize directly the recombination intermediates or trigger the Mismatch Repair Machinery (MMR) that will reject or correct the heteroduplex. However, outside budding

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yeast, the effect of DNA polymorphisms and the interplay with MMR on CO formation had not been studied. The PhD candidate choose to tackle this question in plants in which the topic is at the heart for plant breeding to introduce gene of interest such as disease or hydric stress resistance gene in crops.

The PhD dissertation consists in 4 publications two of which are research in high impact journals (*The EMBO Journal, Nature communications*), one is a review (*Frontiers in Genetics*), and one is describing a methodology (*Methods in Molecular Methodology*). Maja Szymanska-Lejman is first author of the *Nature communications* paper, one of the two first joint authors in the review in *Frontiers in Genetics*, the second author among three in the methodology paper in *Methods in Molecular Biology*, and the third among thirteen authors in the article in the *EMBO Journal*. Contributions of co-authors are appropriately described in the included documents.

The set of publications is preceded by a thirty pages introduction that emphasizes the position of the project as it relates to the state of the art. This chapter is very helpful to understand the biological questions that Maja Szymanska-Lejman wanted to raise and answer during her PhD. The chapter ends with a paragraph dedicated to the main theses and achievements of each article.

The publication in *Nature Communications 2023* provides an exhaustive analysis of the relationships between SNPs density and the distribution of meiotic crossovers in polymorphic hotspots. The main conclusions are that crossovers (COs) occur preferentially within polymorphic hotspots and that this effect depends on the mismatch repair gene *MSH2*. The authors developed a seed-typing method that enables massive parallel fine-mapping of crossovers by sequencing. They performed an incredible amount of work in selecting a series of new regions containing clusters of hotspots. The Chp region is of high interest because this is the first time that a cluster of hotspots has been localized and analyzed in the pericentromeric region of a plant. Pericentromeres are highly polymorphic between Arabidopsis accessions and are particularly suitable for analyzing the effect of SNPs on CO hotspot activity. The authors performed crosses between the Col accession and a series of

accessions with diverse polymorphisms in the Chp region. All hybrids, except one, had a significantly higher CO frequency in the Chp region than in the corresponding inbred region. Moreover, the loss of the mismatch repair protein MSH2 results in a decrease in COs formation in the most polymorphic hotspots. This is a very intriguing result, as inactivation of *MSH2* in the budding yeast *S. cerevisiae* gives the opposite result. To consolidate their results, the authors constructed new lines in which only the Chp region was heterozygous, to eliminate the effect of genome-wide heterozygosity. Using this unique material, the authors confirmed that local polymorphisms stimulate CO formation. The authors also showed that in the ChP region there was no competition between hotspots which again is at the opposite of which has been described in budding yeast.

This detailed analysis provides new insights into the role of mismatch repair proteins during meiotic recombination. It is of primary interest not only for people working on meiosis, but also for people working on the role of mismatch repair during recombination and DNA damage repair.

My questions to the PhD Applicant concerning this part of the thesis are as follows:

1) Could you hypothesize what could be the reasons for the differences observed in your study and in the yeast *Saccharomyces cerevisiae* when the MSH2 protein is absent?

2) Could you hypothesize on the underlying mechanisms that result in more COs in the most polymorphic region when the mismatch repair protein is active? Could you find a way to distinguish between a mismatch repair pro-crossover MSH2 protein or SNP binding stabilizing effect of the MSH2 protein without a role in mismatch repair? How general is the rule that you have observed in the pericentromeric interval?

3) Could you hypothesize why there is no hotspot competition in your study compared to the studies performed in the yeast *S. cerevisiae* ?

In the article published in *The EMBO Journal 2020,* Maja Szymanska-Lejman set up the Genotyping by sequencing (GBS) allowing the detection of the position of crossovers in a series of hybrid F2 population constructed with Arabidopsis parental lines harboring various levels

of polymorphisms. With the data obtained, a correlation was observed between SNP density and CO. Surprisingly when compared to the literature, in a *msh2* deficient background a redistribution of COs was observed from the more toward the less polymorphic regions. About this work I have a few questions:

1) Could you hypothesize what could be the reasons for the differences observed in your study and in the genome wide study conducted by Lian et al. (doi: 10.1038/s41467-022-31509-8.) in which they show that the crossover landscape is largely independent of sequence divergence?

2) Could you hypothesize on the differences of CO landscape pattern observed in male and female Arabidopsis meiosis? would it be possible that CO formation react differently in male and female meiosis to the SNPs density?

Maja Szymanska-Lejman contributed to the redaction of a review in *Frontiers in Genetics, 2018.* This review had a focus on understanding the underlying mechanisms that shaped the CO distribution in plants. This review was written in the first year of her PhD and it certainly gave to the candidate the bases necessary to handle her PhD subject.

I have one question about this review: in the light of the recently obtained results, would you change some of the conclusions of your review and if yes which ones?

In the methodology paper, Maja Szymanska-Lejman participated to the writing of the CrispR Cas9 protocol that she used to generate among other things a deletion in the ChP hotspot in Landsberg *erecta*. I have no specific questions on this part of the dissertation.

To summarize, Maja Szymanska-Lejman produced an impressive amount of work during her PhD which allowed her to participate to the redaction of four papers in excellent international scientific journals. The results obtained on the relationships between the DNA polymorphisms

and CO density have modulated the dogma on the negative role of the MSH2 protein on CO frequency. More studies are now needed to understand the underlying mechanisms.

The presented work fulfills all requirements required of a PhD dissertation. Hence, I am applying to the Council of the Discipline of Biological Sciences of the Faculty of Biology of the Adam Mickiewicz University of Poznan to award Maja Szymanska-Lejman the title of PhD in Biological Sciences. Furthermore, the high quality of the dissertation would justify to be reward by a distinction

Sincerely

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