

In this manuscript, Clairet et al. study nucleosome architecture and its relationship with genome structure and levels of gene expression in four phytopathogenic fungi with different lifestyles and genome organizations. This study supposes an opportunity to better understand the molecular mechanisms that underlie pathogenicity in these fungi from a new perspective, which is investigating directly the differences in DNA packaging.

I find the manuscript well written, the methods are appropriate and the results well presented, but I have some general comments.

- I do think, that findings should be much more discussed in light of the current state of the art. Authors make a very good job at including relevant information published elsewhere, but in general fail to make a direct connection between the findings of this manuscript and this other work.

- Also, the *Conclusion* section, would benefit from a deeper reflection on the impact of the current results towards our understanding of pathogenicity, since this is mentioned in the abstract and the experiments performed in this study on fungal growth could be used as a baseline to study patterns specific to the infection process. In line with this, I wonder whether the authors could discuss their findings in light of the different lifestyles that each pathogen has and that are highlighted in the introduction.

- In the *Methods* section authors present here a new tool to analyse MNase-seq and RNA-seq data. Being such a valuable contribution, it would be useful to detail the highlights of this new bioinformatic tool and how it differs from the ones currently available.

- Finally, since this paper will be of interest to both, fungi and nucleosome specialists, I recommend to define the concepts that are field specific (e.g. dispensable chromosomes, nucleosome occupancy (which is not defined until page 6), as opposed to positioning, etc.). Other minor aspects that could further improve the manuscript are the following.

Other comments:

Materials and Methods, Strains and culture conditions. It would be helpful to introduce this section with a sentence clarifying why the culture conditions were different. If they were performed according to previous studies or current state of the art, etc.

I would also include in the Materials and Methods sections the analyses that are done with MSTs toolkit. Define how the normalization of the nucleosome signal intensity is achieved, phasograms, etc.

Results and Discussion, Establishing nucleosome landscapes...: This is the first and only time that the Ascomycota subdivision is specified. If this has no relevance, it should be removed. Otherwise, the relevance and the subdivision should be mentioned in the Introduction.

Results and Discussion, Genome-wide nucleosome spacing:

- Regarding the definition provided of phasogram "i.e., frequency distributions of read coverage per base genome-wide for all four species". Perhaps the word "read" should be removed, and refer only to the coverage per base genome-wide?

- It is not clear to me why the nucleosome signal decays in intensity over these 1,200 bp sliding windows. Would it be possible for authors to clarify that?

- At the end of the section authors say that Lmb presenting longer NLR than Lml may be due to the "large AT-rich regions displayed in the genome". Would it be possible for the authors to clarify how the two processes are related?

Results and Discussion, Nucleosome distribution profiles: This section is of particular interest, since it revolves around genome organization in specific regions of each fungus genome. I wonder whether the impact of these findings would be more clear if the differences between read density profiles across fungi were first discussed and then the comparative phasograms for each fungus species would be moved to a new section, where authors explicitly lay out the motivation as to why they are performing these analyses. Also, for *B. cinerea*, the results are put into context relative to the state of the art, and this could be used to discuss further the results from this study. e.g. What

could the increased nucleosome occupancy in BOTY rich regions in fungus grown in axenic culture suggest?

I think results would be more clear if they were presented in the same order as in the previous section. Similarly, if in panels B and C in Figures 2-4 the phasograms for the whole genome could be represented (in grey, or shaded) for each species as a sort of reference, that would also be helpful.

I couldn't help but notice the striking nucleosome density in dispensable chromosome in Lmb. Is this the only dispensable chromosome in this species? I find it astonishing, and I think it would be worth it to discuss this further.

Results and Discussion, Nucleosome landscapes of fungal gene units: This section is of great interest to have an overall understanding on what are the broad patterns that can be observed in regards to the relationship between gene localisation and nucleosome depleted regions. Even though this is outside of my area of expertise, I do wonder whether with this dataset it would be possible to somehow perform a comparative analysis of housekeeping genes vs. genes related with pathogenicity to investigate differences in nucleosome occupancy.

I think this section would also improve if authors discussed the implications of the differences in distances between NDR and ATG sites, as well as provide more details on how they are subject to evolutionary forces.

Figure 3A seems to be cropped.