Evolution at two time-frames: ancient and singular origin of two structural variants involved in local adaptation of the European plaice (*Pleuronectes platessa*)

Le Moan et al.

Overall, I think the manuscript has improved from the previous version. The objectives of the study are more clear and the flow/organization helps understand the context of the study better.

Title – The title wording is a bit strong. From the paper it is not definitive that both SVs are associated with local adaptation, and this has not be explicitly tested with environmental data. Also I'm not sure about the use of 'singular origin', given that it has not been confirmed that these are indeed SVs, and timing of SV evolution can be difficult given different dynamics of recombination and selection. While analyses suggest that timing of SVs are similar, the use of 'singular origin' seems a bit strong. Consider revising.

Abstract Line 12 – Are these confirmed to be SVs? Or are they putative SVs? This should be clarified in the Abstract/Introduction.

Introduction – Overall, I think the introduction is more balanced now with a better context for exploring population structure/history as well as structural variants.

Line 245-247 – Clarify wording here. Does this mean FST was calculated between the three genotype groups with just the SNP data from the SVs? I would remove the word "population" here, as it is confusing. Perhaps: "Using SNPs within each SV, pairwise FST was calculated between the three haplogroups identified by DAPC for each SV separately in hierfstat…" If that is not what was intended, then please clarify.

Line 252 – It was unclear initially what this FST represented. Move sentences (Lines 255-257) above to describe groups for calculating differentiation before discussing the quantreg R package (Lines 252-254).

Lines 259-260 – Were these visualized with heatmaps (what R package? Gplots?)? Also indicate that for each SNP "mean" pairwise LD between all loci along the chromosome was calculated.

Line 364-366 – This is useful to know. Provided this, I assume that a PCA using SNPs from both SVs, would produce 9 groups (and not 3 genotype groups) if they are independent? In some cases (translocation), combining data would should the same 3 genotype clusters.

Line 370 – Figure 2 – It would be useful to fit a line to these relationships.

Line 398-401 – Can this information about diversity in the SV provide information about the potential orientation of the SV? For example, if it is an inversion, is lower diversity expected in the rearranged orientation compared to the non-rearranged orientation?

Line 407 – Figure 3 – I'm not sure, but it might help to change the span parameter for loess in ggplot for these plots? It might track the changes across the chromosome better to do smoothing at a finer scale. I would indicate in the caption or text what span was used for the plots.

Line 403-405 – On Chromosome 21, it's possible that the loci in the other LD peak may actually be physically close to the primary SV. Perhaps an assembly of the plaice genome would help clarify this in

the future. -- I see later that this is addressed in the Discussion, but wonder if it could be somehow mentioned in the Results for clarity.

Line 427 – Indicate the name of these two genes here.

Line 436-438 – What are the time for haplogroups for each SV separately?

Line 519-520 – Indicate the date of this split here.

Line 575 – should this fsv19 subscript indicate "derived" as well?

Line 577 – So these are also 'derived' alleles? Not indicated, but states, the "same allele".. Based on Figure 3,S5 – it seems this is the case for SV21, but not sure that SV19 shows that same clear pattern. Allele frequencies in the North are not that different from allele frequencies in the North Sea/Kattegat.

Line 599-602 – I don't think this study identifies strong evidence of local adaptation associated with both structural variants. For example, previous studies found an association with salinity, but in this study, for SV 21, the frequency of derived allele in the Baltic is not different from other locations. This isn't clear from this sentence. In the case of SV 19, perhaps salinity could be a driver, as the Baltic has a higher frequency of the derived allele than other locations, as discussed. But without investigating the association with environment, it is not possible to determine whether these SVs are indeed associated with local adaptation to environment. And besides environmental features, like salinity, SVs can also be associated with life history variation, which may be the case here. Some caution to the interpretation could be added here.

Line 622 – the process "where" several ancient..

Line 626 – "repeatedly" rather than "repetitively"

Line 634 – I'm not sure about the use of 'singular origin' here. Perhaps just "suggesting these SVs evolved at similar times"? They likely didn't evolve at exactly the same time, which is what the term 'singular origin' would suggest to me.

Line 638 – In conclusions/perspectives, it's worth mentioning that confirmation that these are structural variants is still needed. And what methods would be used to do this. Long-read nanopore sequencing - to confirm that they are inversions and to identify exact breakpoints?