Dear Maren Wellenreuther,

We now went through the comments of reviewer 3 and tried to provide answers to all of them. We also included a small modification to the paragraph discussing the demographic history of Iceland. In the previous version, we mentioned that "a founder event" was suggested in the previous work by Hoarau et al, (2002) to explain the reduction of diversity observed in Iceland. However, this was not accurate as they only refer to a "recent bottleneck". Thus, we slightly rephrased this paragraph (from line 530 to 548) in order to be more congruent with previous work about the European plaice.

We are really satisfied with the overall reviewing provided by the PCI in Evolutionary Biology and we are sincerely grateful for it.

Kind regards ☺
Alan

Reviewer 1:

After reading the revised manuscript and re-reading my comments, I think that this revision adequately addresses my concerns, and I'm happy to endorse the manuscript.

Thank you very much ☺

Reviewer 2:

The authors have made a good job of answering and fixing all my comments. I'm happy with this version. Congrats to the authors for a nice paper!

Thank you very much ☺

Reviewer 3:

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.

We have reworded “singular” to “common” origin but we decided to keep the local adaptation in the title. We agree that the environmental factors driving the allelic frequency of the SVs are not yet fully understood. However, our previous study linking the allele frequencies of the SVs to the salinity gradient, the high allelic differences observed here and the fact that (except for Iceland) the European populations seem to be part of the same glacial lineage are all good arguments to justify that the SVs are involved in local adaptation.

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

Done

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

Thanks ☺

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.

The Fst was calculated between populations and not between haplogroups at this stage. We changed the sentence to: “Then, we used the DAPC groups as genotype input to calculate the pairwise F_{ST} between populations at each SV using hierfstat (Goudet, 2005).”

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).

Done

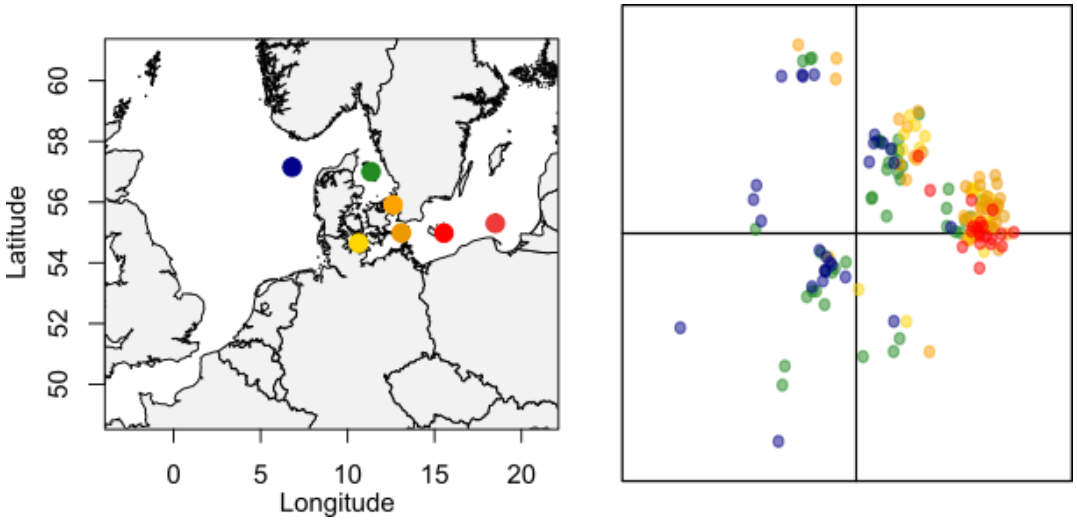
Lines 259-260 – Were these visualized with heatmaps (what R package? Gplots?)? Also indicate that for each SNP “mean” pairwise LD between all loci along the chromosome was calculated.

Thanks for pointing this out, it was missing in the Materials and Methods section. We used LDheatmap for the LD. The sentence “In addition, we used LDheatmap (Shin et al., 2006) that integrate the information about the physical position of the SNP on the chromosome to represent the heatmap of LD between loci.” was added at the end of the paragraph to clarify this.

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.

It's something that we discuss in the first manuscript, here is a copy of what we wrote and of the PCA:

"*Interestingly, these SVs were already revealed by discrete groups on the PCA (Figure 1e), which corresponded to different combinations of the two alleles at the two putative SVs. Theoretically, they should result in a maximum of nine clusters (3 genotypes x 3 genotypes) from which only seven-eight were sampled in our study.*"



In the present study, it is slightly more complex as it seems that we still have a little bit of structure in the chromosome 19 and 21 (especially for SV19), which tends to make the signal less clear on the PCA. We hope to examine this further in future studies.

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

As we tried to illustrate the higher dispersion of the pairwise Fst when calculated with the SVs in comparison to without the SVs, we feel that adding a line will make the signal less clear.

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?

Yes, it is mentioned in the beginning of the discussion line 469 "The low genetic diversity of haplogroup 1 (yellow, Figure 3c) and their long branches in the phylogenies (Figure 4) suggest that they are the derived form of the SVs."

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.

We agree that, in principle, different values of the span parameter could be used for smooting. While this may have affected the fit, we do think that the current parameter accurately captures the overall patterns in the data. Also, our data resolution is likely not sufficiently high to examine patterns at very fine scales. Consequently, we have decided to keep the current span parameter, but have included the value of the span used in the legend of the figure 3 as well as in the Materials and Methods section, as suggested.

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.

We feel that including this already in the Results section would lead to less clear structure of the manuscript and potentially confuse readers. Consequently, we prefer to keep this in the Discussion.

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

Done

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

Information has been added (line 440)

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

Done

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

Yes, thanks. Done.

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.

SV19 is much more polymorphic than SV21, which could be the reason why the signal in less clear. Yet, in our opinion, this is still the overall signal for both SVs, i.e. an increase of the derived allele at both edges. We mention the allelic surfing as one hypothesis, so we feel that the discussion is OK in its current form. We would also like to study this pattern further with additional samples in the future.

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.

We see your point. However, as the SV show clear patterns of population structure, we think that these data may indicate an association with some sort of local adaptation. This was also supported by our previous study where we found associations between SV alleles and salinity. Even if these SVs are associated with life history variation, these traits are most likely not independent from the environment and may therefore also be involved in local adaptation. However, the potential environmental factors involved in shaping the distribution of the SV alleles should be explored further; we hope to get the opportunity to do so in future work.

Line 622 – the process "where" several ancient.

Done

Line 626 – "repeatedly" rather than "repetitively"

Done

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.

We have replaced "singular" with "common"

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?

Done, we added the sentence "Moreover, longer genomic sequencing reads, as provided by PacBio or nanopore technologies, could confirm if these SVs are chromosomal inversions." Line 674-676

Thanks for all of your comment ☺