

1 **COMMENTS TO AUTHORS:**

2 I now had three reviewers provide comments about the manuscript that has been submitted to PCI  
3 Evolutionary Biology entitled 'Evolution at two-time frames shape structural variants and population  
4 structure of European plaice (*Pleuronectes platessa*)'. This manuscript explores the population  
5 structure and variation in two structural variants (SVs) in the European plaice in the North and Baltic  
6 Sea. Previous work identified these two SVs on chromosome 19 and 21 and this study further explores  
7 this variation by incorporating additional sampling locations and attempting to date the SVs. The paper  
8 also investigates whether the SVs could have been introduced through introgression from another  
9 species that is known to hybridize with plaice. Overall, I think the paper is interesting, and this was also  
10 shared by the reviewers. Given the increasing awareness about the importance of SVs to population  
11 structure, I think this paper would be of interest to many readers.

12 However the reviewers have also highlighted a number of issues that need attention and have provided  
13 detailed and constructive comments below on how the manuscript could be improved. The major areas  
14 in need of attention are:

15 1. The general framework needs to be broadened, particularly in the Introduction, to include general  
16 details about why the authors investigate the population structure of plaice. Right now, the authors  
17 mostly focus in SVs in their Introduction.

18 2. All of the reviewers found that the Methods lacked critical detail and explanations (e.g. lack of details  
19 about sampling and dataset sizes). Please go over the comments that the reviewers have made on a  
20 point by point basis and clarify this section.

21 3. Reviewer 3 added some thoughts about the dating of the SVs, and possible problems with it. Further,  
22 it would also be good for the context and interpretation if the authors could provide a bit more detail  
23 about the genes that are located in the SVs, and to qualify statements like '...many of these were  
24 involved in ion transport' (how many?).

25 4. The reviewers felt that some of the statements were too vague, and were rather descriptive and  
26 lacked quantitative support, and I agree with that. I suggest the authors go over the manuscript again  
27 and qualify some of these (many-say how many, most of the times-how often?).

28 Sincerely,

29 Maren Wellenreuther

30  
31 **We went through the extensive comments of the three reviewers and found that they raised  
32 interesting and fair points. Thus, we have now modified the manuscript with several changes,  
33 including the following major revisions:**

34 1- **We changed the first paragraph of the introduction, which was focusing too much on the  
35 structural variants, to make it broader in order to include the entire framework of the  
36 manuscript (basic population genomic and contribution of the SVs).**

37 2- **We provided more details in the method section about the geographic sampling and the  
38 number of SNPs analyzed, and included some formal tests that were missing for the discussion  
39 (e.g. correlation between pairwise  $F_{st}$  genome-wide and pairwise  $F_{st}$  for the SVs to support  
40 the fact that they are not correlated)**

41 3- **We included a more formal test to check for gene enrichment within the SVs in order to  
42 discuss the potential functional implication of the SVs. Previously, we highlighted the role of**

43 ion transport, but this new analysis allowed us to identify strong enrichment in immune  
44 related genes. We discuss it briefly, in order to avoid unnecessary speculation mentioned by  
45 reviewer 2.

46 4- We also agree with reviewer 3 that the age of the split between Iceland and the European  
47 shelf can provide interesting information about the age of the SVs. However, despite the  
48 numerous biases that can exist in using the phylogeny to age SVs, we also think it provided  
49 additional relevant information. Thus, we have decided to discuss the phylogeny and the  
50 demographic inference together, in order to support the hypothesis that these SVs are at least  
51 older than the age of the gradients in which they are currently segregating.

52 5- We reorganized the discussion and provided statistical support to clarify the vague sentences  
53 of the previous version. As suggested by reviewer 1, we removed the paragraphs on the  
54 maintenance of the SVs in the population that was too speculative. In addition, we have  
55 included a paragraph about how the plaice SVs seem to fit with the model of evolution at two  
56 time-frames at the end of our discussion, as suggested by reviewer 2.

57  
58 All the reviewers also raised some concerns about the data filtration based on Hardy-Weinberg  
59 equilibrium.

60 We understand this comment, however, as we first described the plaice SVs using the same pipeline  
61 in Le Moan et al, 2019 (<https://www.biorxiv.org/content/10.1101/662569v1>),  
62 we have decided to retain it here. Also, this filter only removed SNPs that deviate from HWE in more  
63 than 60% of the populations, which is not a stringent filtration step as it only removed 37 SNPs from  
64 the data set. These 37 SNPs were located on 17 out of the 24 chromosomes. None of the removed  
65 SNPs were found on the chromosome carrying the first SV and only 3 were located on the  
66 chromosome of the second SV. Consequently, we think that this HWE filtering step has limited  
67 consequences on the SV signals that we detected.

68 All the reviewers also commented on the relevance of using the collinear regions of chromosome 19  
69 that carries one of the SVs to infer a phylogeny representative of the genome wide divergence. We  
70 understand the issues, however, we decided to keep it in the manuscript, as it shows that the  
71 divergence is limited to what we think is a chromosomal rearrangement and that no signal is detected  
72 in the immediate vicinity of the putative SVs. This provides relevant information about the strength  
73 of such a barrier, that does not seem to affect at all the rest of the genome, even when we looked  
74 within the same chromosome. Nevertheless, we have now included a sentence in the manuscript to  
75 justify our choice as this was unanimously raised by the 3 reviewers:

76 *“Chromosome 19 was used to infer phylogenies both within the SV and to represent a genome wide*  
77 *phylogeny in order to estimate any effects that the SVs may have on collinear regions of the same*  
78 *chromosome”*

79  
80  
81 In addition to this, we went through all the specific comments raised by each of the reviewers and  
82 have responded to individual comments below.

83

84 **We feel that all of these modifications have greatly improved our manuscript. We were really**  
85 **impressed by the quality of the reviews and the relevance of the comments. Thus, we are sincerely**  
86 **grateful for this. The number of comments also partially explained why we took such a long time**  
87 **before resubmitting our manuscript. We are sincerely sorry for this delay, but we hope that you will**  
88 **understand.**  
89 **Kind regards,**  
90 **Alan**

## 91 **Reviews 1**

92 *Reviewed by anonymous reviewer, 2019-08-13 13:43*

93 I read "Evolution at two-time frames shape structural variants and population structure of European  
94 plaice (*Pleuronectes platessa*)" by Le Moan et al. I found the MS interesting and the findings novel and  
95 relevant. I think the methods used are mostly appropriate to lead to the conclusions the authors arrive  
96 (except for some minor confusing cases). On the downside, however, I found the MS difficult to read at  
97 places, some important details missing and many descriptive results that need quantitative support. I  
98 also feel the discussion can be consolidated and/or improved in some of the sections. This paper will  
99 be a nice contribution showing the relevance of Structural Variants in the evolution of divergence and  
100 demographic change

101

102 Below I include many comments. All of my comments are either minor details or are intended to add a  
103 more detailed explanation around the major issues I found in this MS:

104 1) Lack of details about sampling and dataset sizes etc.

105 2) Many different concepts are included without properly considering each of them in-depth or without  
106 consolidating a proposed mechanism to connect them. e.g,v"edge effect", "founder effect", "IBD",  
107 "allele surfing".

108 3) Some inferences are rather descriptive and lack quantitative support

109

### 110 **Comments:**

111 L 9-11 At this early point in the MS, it is a bit unclear why SV "provide evidence" for evolution at two-  
112 time frames. I think in general this sentence is a bit confusing.

### 113 **This was rephrased:**

114 ***"Changing environmental conditions can lead to population diversification through differential***  
115 ***selection on standing genetic variation. Structural variant (SV) polymorphisms provide examples of***  
116 ***ancient alleles that in time become associated with novel environmental gradients."***

117

118 L 22 - What global distribution? If the plaice is not distributed globally, or at least its global distribution  
119 is not assessed here...

120 **We have changed "global" to "northern"**

121

122 L 70-71 This argument needs to be developed a bit further and/or a reference included

123 **The sentence was: *These co-adaptations are expected to arise continuously de novo after the SV***  
124 ***associates with an environmental barrier. We could have referred to "reinforcement" here but we***

125 **decided to remove this sentence from the current manuscript as it is not directly related to our**  
126 **current framework.**

127

128 L 83 This needs a reference, a combo later used in the text would work well here (e.g. Jones et al., 2012;  
129 Morales et al., 2018)

130 **Done**

131

132 L 118 - "to examine multiple hydrographic gradients" It is not very clear which are these gradients.  
133 Would be good to mention them here.

134 **Done**

135

136 L 118-123 Given that the evolutionary history / temporal framework of SV's is the highlighted aspect of  
137 the MS, I'm surprised this is not included in the goals here

138 **We rephrased the main goals of the manuscript:**

139 ***In the present study, our overall objective was to increase our understanding of the origin of the SVs***  
140 ***and their effects in contemporary populations of the European plaice. Specifically, the main goals of***  
141 ***the study were to: i) re-assess the population structure in European plaice from northern Europe and***  
142 ***from Iceland with the use of a population genomics approach, ii) evaluate the contribution of SVs to***  
143 ***population structure, iii) test for a potential flounder origin of SVs; iv) estimate the age of the SVs in***  
144 ***plaice with a phylogenetic approach; and v) provide relevant data to understand the extent to which***  
145 ***selection is involved in maintaining the allelic clines observed at the SV. This work thus provides***  
146 ***increased insight into the relative roles of environmental gradients, demographic history,***  
147 ***hybridization and genomic structural changes in the evolution of a widespread and highly abundant***  
148 ***species.***

149

150 L 145-146 "Most of THE northern limit of the plaice distribution was covered with this sampling design"  
151 What about the baltic distribution? How much of the Baltic distribution is included in this sampling?

152 **All of the Baltic Sea distribution. From what we know, the plaice is not found after Bornholm nor in**  
153 **the north of the Baltic Sea.**

154

155 L 184 Why a multiple-testing correction was not used for Hardy-Weinberg?

156 **We did the test per population and we only removed SNPs with a significant deviation from Hardy-**  
157 **Weinberg in more than 60% of the populations. At the end, this filtration is not so stringent (we**  
158 **removed 37 SNPs out of 28000). It is a kind of correction for multiple testing since we removed only**  
159 **the SNPs that deviate from HWe in more than 4 population out of the 7.**

160

161

162

163 L 191 - 195 I suggest to add the final sample sizes for all these different dataset

164 **We have included a table with detailed sampling information to the method section. Moreover, we**  
165 **provide more details about the size of the different dataset throughout the entire manuscript.**

166

167 L 211 - 222 I'm a bit confused behind the logic of "examine the demographic histories associated with  
168 the major population breaks identified in the overall dataset" aren't the largest breaks Icel, Norws and  
169 Katte? And then the most interesting to include Bals to date the Baltic colonisation? Maybe I'm being a  
170 bit dense and cannot fully understand what the authors are trying to teste here. Also, the results of the  
171 demographic modelling seem to have very little weight in the discussion later on, so it feels a bit forced.  
172 In any case, I would like to see cartoons of the different demographic models tested in the SI

173 **By major breaks, we meant between the continental shelf and outside the continental shelf. We have**  
174 **rephrased this part to:**

175 ***"We used an approximation-of-diffusion approach, as implemented in the software from  $\delta\delta i$***   
176 ***(Gutenkunst et al., 2010), to examine the demographic histories associated with the major population***  
177 ***breaks that separate the populations sampled along continental shelf from the Icelandic population***  
178 ***(also described in Hoarau et al., 2002). Specifically, we assessed the demographic history of the***  
179 ***divergence of Iceland and its closest continental shelf population..."***

180 **A cartoon of the model can be found in the supplementary material figure S9.**

181

182 **Additionally, the demographic history of the North Sea and the Baltic Sea was already inferred in our**  
183 **other paper (Le Moan et al, 2019: Beyond parallel evolution: when several species colonize the same**  
184 **environmental gradient, available in bioRxive.) Here, it seems that the Baltic Sea has evolved in face**  
185 **of continuous gene flow from the North Sea (IM model).**

186

187 L 232 Why is this a haplotype allele frequency? Are not these markers for the entire chromosome?  
188 Thus, unlikely they represent a single haplotype?

189 **We understand this comment, and therefore we changed the word "haplotype" by the word**  
190 **"haplogroup", which is more accurate.**

191

192 L 248 I don't understand why the genomic architecture is invoked here? "The genomic architecture of  
193 differentiation was examined using SNP specific  $F_{ST}$  values"

194 This comes back in line 334. I think this is not about the genomic architecture of the SVs, given that SVs  
195 are a feature of the genomic architecture. This refers more to the genetic or spatial structure of SVs or  
196 something along those lines. I suspect the authors are misusing the definition of genomic architecture  
197 here.

198 **We agree with the reviewer and we have changed "genomic architecture of the structural variants"**  
199 **into "Genomic analyses of the structural variants"**

200 L 255 Was LD calculated pairwise across the entire chromosome? With a sliding-window? Please clarify.  
201 **Done, it was calculated pairwise**

202

203 L 277-281 How these coordinates were defined? By eye? Please explain.

204 **The answer to this question was written in lines 281-283. We have now moved it to the part about**  
205 **the gene content of the SVs (line 265 – 269) and added more details about how we defined the limits**  
206 **of the SVs.**

207

208 Also why the authors did not use the other chromosomes to represent the genome-wide estimates?  
209 How likely/unlikely it is that these regions within the same chromosome are subject to some linked  
210 effects? E.g. How well the boundaries of the SV are defined and how long is the LD decay?

211 **See general comments**

212

213

214 L 316-320 I do not fully understand what the authors are trying to say here. I find it difficult to appreciate  
215 a correlation within the blue dots with only 3 comparisons, also I cannot see the different effects  
216 between panel a and b of Fig. 2

217 **We agree with the reviewer that the blue color is not needed here, it just highlight the most distant**  
218 **pairwise comparisons. Therefore, we have removed it from the presentation. Nevertheless, the two**  
219 **graphs show that when including the SVs results in a noisier IBD pattern, which can then be improved**  
220 **when the SVs are removed ( $r^2$  improvement of 0.4, 0.56 in Figure 2a and 0.96 in 2b). This noise is**  
221 **clearly shown also in Figure 3a-b.**

222

223 Methods: there are some details that are missing in the methods when specific things are presented  
224 e.g. How many SNPs the authors started with, how many were filtered in each step and how many they  
225 end-up with. Also the final sample sizes of the different "datasets". Or how many genes were found  
226 where they say "and all genes with more than 80% mapping were recorded". Among other things.  
227 Would be good for the authors to double check the methods section and add all these small but  
228 important specific details. Particularly how many samples and markers go into each of the analyses.

229 **We agree with the reviewer that our manuscript lacked details about the different datasets.**  
230 **Therefore, we have added this information to all of the relevant parts. We have also included a**  
231 **supplementary table summarizing the number of SNPs obtained after each filtration step.**

232

233 Figures: the style for presenting panels as "(a)" or "A" and the way they are presented in the legends  
234 (e.g. before or after their corresponding explanation) varies between figures. Please consolidate in a  
235 single style.

236 **Corrected**

237

238 L 356 "the SV21 FST was elevated only in pairwise comparisons including Nors/Katte ... (Fig. 3B)" But  
239 this Figure does not show comparisons with Katte

240 **Corrected by referring to the supplementary table S3, high value of Fst are highlighted in bold**

241

242 L 359-360 "The genome(-)wide differentiation outside... " Ideally this would be backed-up qualitatively,  
243 I think mean and sd Fst of SV and collinear will suffice.

244 **Done**

245 L 360 "Several SNPs" How many? Quantitative support needed...

246 **Done**

247

248 L 326-364 The decrease  $F_{ST}$  / increase  $\pi$  inference really relies on a visual pattern that to me is not  
249 immediately obvious. This also rests support to the statement in L498-499. Some way of quantifying  
250 this pattern would add support to this statement

251 I think this section tends to be very descriptive and need to be backed-up with more quantitative data  
252 when possible

253 **We agree with the reviewer, but with the genomic coverage we have, this is all we can do. The fit on**  
254 **the 95% upper quantile clearly shows the drop of  $F_{ST}$  in the center of each structural variant.**  
255 **Moreover, loci with  $F_{ST}$  of 1 tend to be localized on the breaking point of the inversion (but there are**  
256 **only few of these loci). We can not completely rule out at this stage that this pattern is due to the**  
257 **random sampling of the ddRAD approach. However, the pattern seems quite consistent across both**  
258 **SVs. We hope to go deeper in the analysis of this pattern in future studies with increased genomic**  
259 **coverage.**

260 **We removed all the parts referring to this pattern, since it does not provide any important**  
261 **information for the purpose of the manuscript, but it will be something that we would be interested**  
262 **in investigating more carefully in future work with increasing coverage.**

263

264 Just a minor note, Figure 3D The quality of the image is not enough to appreciate the differences  
265 between red and purple in the triangular LD plots. Either include a higher quality figure in next version  
266 or maybe change the colour palette a little bit.

267 **We have the PDF of the figure, all figures will be resubmitted in high quality pdf format.**

268

269 L 408-410 I do not understand what the authors mean by "decoupled from the species' geographic  
270 distribution"

271 **The "decoupled" has been rephrased to "not correlated" and we have included a Mantel test to test**  
272 **the correlation between the  $F_{ST}$  genomewide (without the SV) and the  $F_{ST}$  for each SV to add**  
273 **statistical support to this claim.**

274

275 L 416 and L 459 "to our knowledge is one of the clearest" and "is the first example described " I  
276 personally consider this type of statements unnecessary, but if the authors feel are needed they  
277 definitely need at least some support from the literature

278 **We have toned this statement down**

279

280 L 446-460 This section is very difficult to follow because it jumps between different  
281 demographic/evolutionary models without much structure or connection. Namely, "edge effects",  
282 "founder effects" and IBD. These have obvious connections to each other and to the observed pattern  
283 of genetic diversity, but I feel the discussion here does not do a good job at making these connections.  
284 I found the edge effect particularly confusing as at some point the author seems to imply that edge  
285 effect and founder effect are the same things?

286 **We refer to "founder effect" when we speak about the Icelandic population (which is an island**  
287 **population living in isolation from the rest of the continental shelf population).**

288 **Then, we refer to the "edge effect" when we speak about the Baltic Sea and the Barents Sea which**  
289 **are, to our knowledge, are both on the edge of the plaice distribution range.**

290 **We have rephrased the paragraph about the IBD to include the connection between edge effect and**  
291 **IBD.**

292

293 L 462 "The two large SVs were polymorphic in most of the sites studied" I'm interested in entertaining  
294 the idea that the SV's are not in fact polymorphic but that individuals from different groups were  
295 combined in a single site. For example, there is not enough information in the Methods section to know  
296 how the sampling was conducted and where these individuals come from. Is it possible that individuals  
297 come from different micro-habitats or that there some cryptic variation in such a way that individuals  
298 could be grouped into some kind of ecotype with alternative SV's? i.e. the SV's are not really  
299 polymorphic.

300 This, of course, does not invalidate that SV's are polymorphic to the site level. Feel free to ignore this if  
301 it does not make sense given what is know about the ecology of the plaice

302 **At this stage, we cannot exclude that the structure we detect along the gradient between the North**  
303 **Sea and Baltic Sea also occur at more fine scale somewhere else in Europe (for example, between**  
304 **coastal and offshore locations, as found in the European anchovies or in the European Sea horse).**  
305 **However, with our current sampling design, it is difficult to evaluate this hypothesis**

306

307 L 474 - 477 This edge effect is not super obvious by only looking at the pie-charts in Fig.3. I suggest that  
308 authors to add a figure supporting this statement, e.g. correlation of frequency with latitude or  
309 something like that.

310 **We agree with the reviewer in that our sampling design is probably not ideally suited to thoroughly**  
311 **evaluate this hypothesis. However, we still mention it as one hypothesis to be further evaluated in**  
312 **future work. In addition, the latitude does not covered the position of the Baltic Sea on the edge.**

313

314 L 477-478 "the ancestral haplotypes for SV19 disappeared" This is one more example of how the lack  
315 of detail about sampling does not allow to evaluate this type of statements. E.g. I cannot see if the  
316 sample size is lower/enough in this region, potentially preventing the authors to not see the less  
317 common haplotype.

318 **The sampling sizes were detailed in the first table of the supplementary material. We have now**  
319 **moved it to the main manuscript text. We have also included a column to mention the number of**  
320 **analyzed individuals after data filtration**

321

322 L 491-494 It would be good to give an example of what the authors refer to here. A good citation would  
323 be Faria et al. 2019; Molecular Ecology; 28: 6, 1375-1393 where they show how to find inversions in  
324 non-model organisms with the type of approaches you talk about in this sentence.

325 **Ok, done.**

326

327 L 539 - 540 I do not see where the evidence for 2.5 times more net divergence is coming from? I might  
328 just have missed it, though.

329 **It is the ratio between the long and the short branch in the phylogenic tree:  $0.14/0.06=2.33$  and**  
330  **$0.12/0.05=2.4$ . This has been clarified in the discussion**



331 I think the section "The evolution and maintenance of the structural variants" could be summarised a  
332 bit further given that it contains a lot of speculation. It is a nice discussion, it just feels a little bit too  
333 long.

334 **We agree with the reviewer and therefore we have condensed and moved this interesting part of the**  
335 **paragraph to the paragraph "*Implication of structural variants for population structure of European***  
336 ***plaine*: " line 586-592**

337  
338 L 584-585 I'm not sure what the authors talk about here, the first and the second? what? SV's? This  
339 sentence is rather unclear.

340 **We have added a full paragraph entitled "*Structural variants promoting evolution at two-time***  
341 ***frames*:" that we hope will clarify our conclusions.**

342  
343 L 588 Why waiting the entire MS to propose the SV's are inversions? It is kind of irrelevant at this point  
344 and quite unexpected... I think the author should either not compromise and call them SV's all  
345 throughout, or to bring this on earlier and justify it.

346 **We now bring this up to the beginning of the discussion, after the first paragraph summarizing the**  
347 **main results**

348  
349 L 589 "decoupled from geography" does not make sense as they might have a different geographic  
350 distribution, but they cannot be "decoupled" from geography.

351 **We have changed the sentence:**  
352 ***The structural variants are likely two large chromosomal inversions with high variation in allele***  
353 ***frequencies across population that leads to population structure different from the isolation-by-***  
354 ***distance described genome-wide.***

355  
356 The "Conclusions" section is very little of conclusions and a lot of future directions.

357 **We have change the title of the section to "Conclusion and perspective"**

358

359

360 **Reviewer 2**

361 This paper presents an analysis of population structure in the European plaice, using RAD-seq data and  
362 concentrating especially on the contribution of two previously detected large structural variants. The  
363 population genetic data suggest a genomic background of isolation by distance (strong correlation  
364 between  $F_{st}$  and geographic distance), from which the structural variants deviate. They make the case  
365 that the structural variants are likely to be ancestral polymorphisms, possibly maintained by balancing  
366 selection.

367 In my opinion, the main merits of the manuscript are that it uses standard methods in a sensible way,  
368 and that it is well written, especially the Introduction and Discussion sections.

369 **Thanks !**

370

371 I don't have any major criticisms, but several minor comments and questions about framing, some  
372 details that are missing from the Methods section, and some of the Results that could be more clearly  
373 presented.

374

375 **Minor comments**

376 Title and conclusions (lines 583-590): The framing of the paper as about the "evolution at two-time  
377 frames" (by the way, shouldn't it be "at two time-frames"?) strikes me at a somewhat odd choice, since  
378 it's not that clear to me what this model entails in terms of testable predictions, and how it fits with the  
379 data. This becomes especially clear in the Conclusions section, that highlights this model to the  
380 exclusion of other results, but in very general terms. It is almost a necessity that any widespread  
381 polymorphism first is established in a population, then spreads, but what else does the model predict  
382 that fits, or doesn't fit, with the plaice data? I acknowledge that this may just be an issue of how the  
383 model is presented, or a matter of personal taste.

384 **We added a full paragraph to further develop how the plaice inversion seems to fit to the model of**  
385 **evolution at two time-frames at the end of the discussion entitle *Structural variants promoting***  
386 ***evolution at two-time frames:***

387

388 Throughout the paper: I would advice against using bespoke abbreviations. "SV" for "structural variant"  
389 might be acceptable (though my preference would be to spell that out too), but I see no reason to for  
390 example abbreviate "North Sea" to "Nors". This saves little space and impairs readability. I would also  
391 encourage spelling out isolation by distance, since the abbreviation "IBD" unfortunately has two  
392 meanings in population genetics (even if it should be obvious from context that you aren't talking about  
393 identity by descent).

394 **Ok, we have only kept SV, LD, and kya/Mya for the abbreviations**

395

396 Lines 38-39: The sentence says that because structural variants can harbour several genes, they may  
397 have functional consequences. This does not follow, so I guess the sentence is trying to say something  
398 else?

399 **Our main point here was simply to say that, in comparison to mutations associated with single base**  
400 **pairs, SV often affect several genes, which make them likely to have functional consequences.**

401

402 Lines 76-81: The idea of "evolution at two time-frames" could be fleshed out in more detail.

403 **See earlier response**

404

405 Line 98: This is the first mention of the European plaice in the introduction. I recognize that this might  
406 be a matter of personal style, but in my opinion, the study system and the question to be addressed  
407 would benefit from being introduced much earlier.

408 **We understand the comment, but we feel that it will break the flow if we mention the plaice earlier**  
409 **in the introduction.**

410

411 Line 114: The notion that structural variants explain most of the differentiation could be made more  
412 precise. How much, and what differentiation?

413 **We have incorporated more precision from the previous paper where we identified these SVs:**  
414 ***In a recent study of the genomic basis underlying the colonization of the Baltic Sea, we identified two***  
415 ***large polymorphic SVs (Le Moan et al., 2019) carrying a strong signal of selection between the North***  
416 ***Sea and the Baltic Sea plaices (66% of the top 0.1% outlier loci localized within two SVs).***

417

418 Line 160: Does "DNA extractions were standardized" mean that samples were diluted?

419 **Yes, we have changed the wording.**

420

421 Line 182: What does it mean that a SNP is "present" in 80% of individuals? That 80% of individual had  
422 genotype calls for that SNP, or something else?

423 **Yes, we have changed the word "present" to "genotyped"**

424

425 Line 184-185: Hardy-Weinberg equilibrium filtering of SNPs is problematic even in the best cases, and  
426 this threshold ( $p < 0.05$ , presumably uncorrected) is very low.

427 How many variants were removed because of this, and is this really appropriate?

428 **We used HWe filtration to remove SNPs that show a significant departure of HWe in 60% of the**  
429 **populations. At the end, we removed a very low number of SNPs (34 in total), likely mostly associated**  
430 **with potential paralogous SNPs that are polymorphic within one of the paralogous and monomorphic**  
431 **in the other, which can lead to strong departures from HWe.**

432 **The HWe filtering would be highly problematic if the plaice SVs were associated with important**  
433 **heterosis effects or unfit hybrids, but this is definitively not the case here. In addition, we identified**  
434 **the SVs with the same pipeline in our previous study, and we have preferred to keep procedures**  
435 **identical in order to facilitate comparisons of the studies.**

436

437 Lines 187-191: Could you explain in detail how and why "size selection was slightly shifted"? This  
438 sentence is not clear to me. What happened, why did it happen, and  
439 how did you detect it (could the evidence be shown in a supplementary figure)?

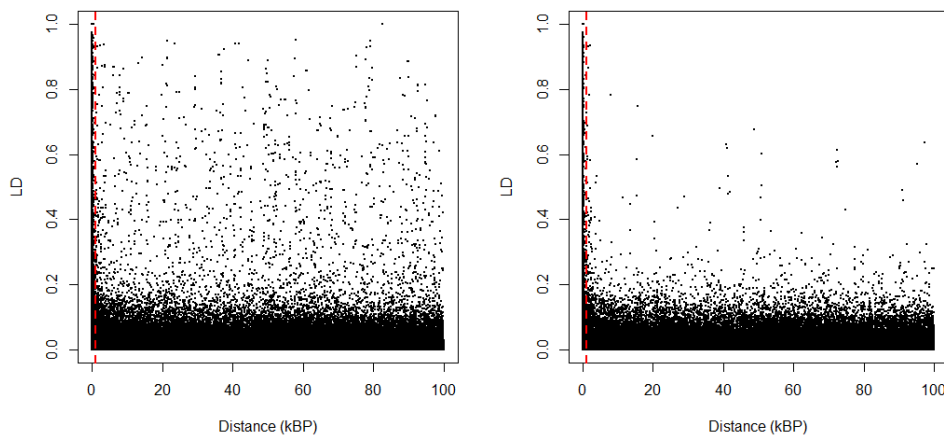
440 **This is a "library prep" effect. When we prepared the first set of libraries, an inset size between 350**  
441 **and 450 bp was targeted, but for the last library, the size selection was between 300-400bp. With**

442 **ddRAD, this is quite problematic and we therefore also discuss the implications thoroughly in the**  
443 **manuscript.**

444 Line 199: How did you choose which SNP to keep in each bin, at random? Given the strength of LD, is  
445 the 1 kbp limit enough to remove the effect of LD?

446 **Yes, we only kept one random SNP over bins of 1kb for the population structure analyses. This clearly**  
447 **not enough to remove the SV signals, which is why we repeated the analyses with and without the**  
448 **chromosomes carrying the SVs. However, it is sufficient for the rest of the genome (at least with the**  
449 **ddRAD).**

450 **Here is a plot showing the LD decay with and without the SVs on 100kb with (left) and without (right)**  
451 **the SVs. The red line shows the 1kb.**



452 **We have included this figure in the supplementary material.**

453  
454  
455  
456 Line 207: The Mantel test is never discussed again. Is this an omission?

457 **The Mantel test is not specifically mentioned but the results are shown in brackets, which comprise**  
458 **all the correlation and p values from 349 to line 363. We have included “(Mantel test:  $r = -0.56$ , p-value**  
459  **$< 0.01$ ) in the first bracket only to clarify this.**

460  
461 Lines 243-256 and elsewhere: Is "genomic architecture" really a good term for diversity and  
462 differentiation?

463 **Thanks for pointing this out. We have rephrased genomic architecture to “genomic analyses of the**  
464 **structural variants”**

465  
466 Line 258: Could you give reference genome coordinates of the extracted sequences?

467 **The coordinates are given in lines 277-281 (lines 265-269 in the revision).**

468  
469 Line 262: What does 80% mapping mean precisely?

470 **We did this part again using the genome annotation directly and not through blast. Thus, we re-wrote**  
471 **the entire method section about the gene content of the SVs.**

472

473 Lines 267-269 and 284-286: This procedure could be better explained. I also worry that the selection of  
474 random alleles might mean the creation of haplotypes that are not even present in the population. Is  
475 there a risk of getting the sequence wrong? Would statistical phasing help?

476 **We understand this concern. However, by focusing only on the homozygote individuals, this protocol**  
477 **will not really affect the estimate of divergence between the two alleles of the SV, since we work on**  
478 **individuals that are necessarily "pure" for one or the other major allele of the SV. However, the**  
479 **statistical phasing would provide valuable information regarding the potential presence of sub**  
480 **structure within each of the haplogroups of the structural variants. It will be something to dig into in**  
481 **future work (it is mentioned briefly in the conclusion/perspective paragraph).**

482

483 Lines 295-296: Is a strict molecular clock using human mutation rate really the best possible way to date  
484 this polymorphism? Are there no mutation rate estimates from fish available?

485 **We used the mutation rate used in the sea bass population genomic for similar analyses (c.f. Tine et**  
486 **al, 2014, Nature communication 5:5770 doi: 10.1038/ncomms6770). We refer to this publication in our**  
487 **manuscript.**

488

489 Lines 307-311: The observed heterozygosity may be a useful descriptive statistic, but its importance  
490 here is never really explained. Why are these results presented here?

491 **We have included some details in the paragraph about the plaice.**

492 ***However, the off-shelf population shows a higher genetic diversity than most of the population from***  
493 ***the northern distributional range of the plaice (Hoarau et al., 2002), which could suggest that this***  
494 ***population has a more ancient origin than what is currently thought.***

495

496 Lines 318-323: and Figure 2: What, exactly, is "expected under an IBD scenario", and what is it that  
497 "disappears" in panel B compared to panel A? From what I can tell, the relationship pictured in panel B  
498 is stronger than the one in A. This part of the paragraph, with multiple patterns that are lost under  
499 various removals is very hard for me to follow. It would also help to provide some context about what  
500 this is investigating. Could it be rephrased?

501 **We have rephrased this section and have added the Mantel test to provide statistical support to our**  
502 **claims.**

503

504 Line 321: The p-value can't be precisely 0, can it?

505 **It is actually 0.000001, it was rounded... We have rephrased it as p-value<0.01.**

506

507 Lines 375-377: Drawing any kind of conclusion about gene function based on 900 genes is highly  
508 questionable.

509 **We agree and this was the reason why we tried to be cautious during the discussion of gene function.**  
510 **However, this question seems to divide the reviewers as reviewer 3 ask more details about the genes**  
511 **and their potential function to discuss the selective forces acting on the SVs. We have now performed**  
512 **a formal gene enrichment analysis in order to obtain some statistical support to discuss relevant**  
513 **functions potentially affected by SVs.**

514

515

516 Lines 391-400: I'm afraid I do not understand this paragraph. Could it be rephrased?

517 **We have rephrased it, it is now in lines 434-440**

518

519 Lines 500-502: I do not understand the sentence about fission/fusion of chromosomes. Could it be  
520 clarified?

521 **The entire paragraph was re-written and shortened. We have also moved it to the beginning of the**  
522 **discussion.**

523 *The two SVs covered nearly half of chromosomes 19 and 21 of the Japanese flounder genome, where*  
524 *a strong LD was maintained over 9Mbp. These large linkage blocks are expected with chromosomal*  
525 *rearrangements such as inversions, duplications and translocations which can formally be*  
526 *distinguished by use of a linkage map or genome sequencing (e.g. Faria et al., 2019), but not with the*  
527 *reduced representation approach used in this study. However, our data filtration steps (filtering by*  
528 *heterozygosity) would have resulted in the loss of duplicated regions. Moreover, assuming a high*  
529 *degree of synteny between European plaice and Japanese flounder genomes, the size of the LD blocks*  
530 *and their central position are consistent with the presence of at least two major inversions in the*  
531 *genome (Kirkpatrick et al., 2010).*

532

533 Lines 575-581: The Discussion about what might be maintaining these polymorphisms is thoughtful and  
534 interesting, but it ends by mentioning process studied by the Kirkpatrick & Barton (2006) without going  
535 into detail. Could you the implications of this model and how it fits with the data?

536 **We have rephrased the last paragraph, but as more details are provided in the introduction, we did**  
537 **not want to expand too much on this again.**

538

539 Data availability: Will the data be made available in a standard repository? This is not mentioned in the  
540 manuscript. Depending on journal (or actually, even if the journal does not require it), a clear data  
541 availability statement would be nice.

542 **Thanks for pointing this out. We have included this information in the manuscript**

543 **Reviewer 3:**

544 **Evolution at two-time frames shape structural variants and population structure of European plaice**  
545 **(*Pleuronectes platessa*)**

546 This paper explores population structure and variation in two structural variants (SVs) in European  
547 plaice. Previous work identified these two SVs on chromosome 19 and 21 and this study further  
548 explores the variation by incorporating additional sampling locations and attempting to date the SVs by  
549 comparison with other species. The paper also investigates whether the SVs could have been  
550 introduced through introgression from another species that often hybridizes with plaice. Further, the  
551 paper also uses the new data to re-assess population structure in the species and examine demographic  
552 histories to investigate the strong divergence that has been found between Icelandic populations and  
553 other European populations.

554 The results of the population structure and demographic analyses reveal that the divergence of  
555 Icelandic population may be explained by the possibility of a different glacial refugium (different from  
556 other European populations). This is supported by their genetic divergence, demographic modelling,  
557 and by no clear reduction in genetic diversity in the Icelandic population. In addition, the study finds  
558 support for isolation by distance particularly when excluding SVs from the analysis. The authors claim  
559 this is the strongest IBD documented for a marine fish. Although population structure is generally weak.  
560 In addition, the study finds that the two SVs are old (>220,000 years) and shows that the frequency of  
561 the derived haplotype increases at the range edge along with a reduction in genetic diversity (genome-  
562 wide). The SVs were diverged from other species and the study finds no support of introgression of SVs.

563 **That is an impressive summary. Everything is mentioned.**

564

565 Overall, I think the paper is interesting. Given the importance of SVs to population structure, I think this  
566 paper would be of interest to many readers. While the study investigates an important question, I have  
567 several concerns that the authors should address. These concerns are all detailed below; however, a  
568 few major points include:

569 1- Flow and organization of the manuscript: The introduction provides extensive information on  
570 structural variation, but this is not the only thing that the authors are interested in. The authors also  
571 investigate overall population structure and diversity, as well as investigate demographic histories. This  
572 is not well established in the Introduction, and (as detailed below) it is not clear from the Introduction  
573 why the authors investigate demographic history with the Icelandic population.

574 This needs better context and requires some reorganization of the Introduction and Methods to  
575 improve flow.

576 **Thanks for pointing this out. We have rewritten the introduction to broaden it and include all aspects**  
577 **of our work.**

578 2- Methods – I think there are a few cases where parts of the methods need to be clarified. Why  
579 were loci pruned for LD if the primary point of the paper is to investigate SVs, which will have many loci  
580 in high LD? And by removing loci that are out of HWE, could this remove some loci that are of interest  
581 for complex structural variation? This is important for neutral population structure, but authors don't  
582 attempt to remove other outliers before investigating population structure. I would also suggest that  
583 authors investigate overall population structure with neutral loci (not all loci). This also relates to the

584 methods that were chosen here to investigate population structure (PCA). Structuring can be explained  
585 by a few important loci. It is not clear which loci contribute to the population structure overall, as there  
586 is no information on the loadings or significance of loci on PC axes. Was it many loci or just a few loci  
587 that are under selection that separate the populations?

588 **The loci were pruned for LD only in the analyses of the overall patterns of population structure and**  
589 **not when we analysed the genetic diversity along the SVs. We have now clarified this throughout the**  
590 **manuscript. Additionally, we have included the loading plots from the PCA in the supplementary**  
591 **material (Figure S4) to show the contribution of the SVs to the current population structure.**  
592

593 3- In addition, I am not sure about the dating of the SVs. Selection and reduced recombination  
594 on SVs can quickly lead to divergent haplotypes, I am not sure if the branch length of trees will be  
595 informative for dating the inversion. My thought would be that the earliest date that can be given to  
596 the formation of these SVs is at the timing of the split between Iceland and other populations (what is  
597 this timing, it is not clearly stated in the text?). However, if there were two refugia it is possible that the  
598 derive haplotype could have evolved only in the European refugium (southern) and then have been  
599 introduced into Iceland after the last glacial maximum (through secondary contact), so it may be even  
600 more recent than the split. I would like the authors to explain how these analyses with the SVs do not  
601 violate the assumptions. If they do, then I am not sure how informative they are. Further, the trees are  
602 compared to a collinear region of chromosome 19 to represent genome-wide average. But using this  
603 chromosome may be problematic given that the SV exists on this chromosome (only 5MBP away). I  
604 would suggest using a chromosome that does not contain SVs.

605 **These are all relevant and fair comments. Unfortunately, there is no robust procedure to take**  
606 **variation in selection and recombination into account. Our main point with the dating of the SVs was**  
607 **mainly to investigate if the SVs are potentially were older than the age of contemporary populations**  
608 **(e.g. in the Baltic Sea). All analyses seem to support this hypothesis. See the answer to reviewer 3 on**  
609 **line 959.**  
610

611 4- There is a very short section on functional annotation in the SV regions. But not much detail is  
612 provided and I could not find access to Supplemental File 1 (List of genes). I would like to see a bit more  
613 discussion on the genes in the SVs. Have they been found in other SVs in other species (particularly  
614 marine fish)? Did the authors perform an enrichment analysis? With over 1800 genes in the SVs, all that  
615 the authors say is that many are associated with ion transport and other functions like sexual  
616 recognition. There are >1800 genes, so how many are associated with ion transport? This section needs  
617 more details and these details may provide some hints as to the function of the SVs. And as mentioned  
618 below, it may be most interesting to examine which genes are found within the SNPs where  $F_{ST}$  and  $H_o$   
619 are 1.

620 **Thanks for pointing this out. We have tried to improve this part by adding functional enrichment**  
621 **analyses and expanding the discussion, but we have also kept it relatively short in order to avoid**  
622 **unnecessary speculation, as mentioned by reviewer 2. In addition to functional enrichment analyses,**  
623 **we also found a few heat shock protein genes on the first SV, which are already discussed as**  
624 **candidate genes for local adaptation in the context of the Baltic Sea in other marine fishes (sprat,**  
625 **flounder and cod). We were also able to find a relatively convincing signal of immune related gene**



626 **enrichment, which could be a reason why these SVs seem to be associated with various**  
627 **environmental gradients.**

628

629 More detailed comments are provided here below. Authors should also be careful to correct  
630 grammatical errors throughout the text. In addition, there are many areas throughout the text where  
631 authors should be more specific, instead of saying “many” or “some” or “several”, please indicate how  
632 many there actually are (see comments below for examples).

633

634 **Abstract** – The abstract requires more details as many parts of the study are not presented in the  
635 Abstract. The findings of the Icelandic population representing a potential different glacial refugium  
636 seem important in the text, but I don’t see it mentioned in the Abstract? Additionally, I understand that  
637 the range of dates for the SVs is 550-220 kya, but the Abstract only indicates that they evolved around  
638 220 kya. Also, there is no mention of the tests for introgression.

639 **We have modified the abstract to make it clearer. We decided not to highlight the specific timing**  
640 **here because of all the potential bias that can be introduced when aging SVs. We also decided not to**  
641 **highlight the introgression in the abstract, as we only rule out the flounder as a source of the origin**  
642 **of the SVs, but introgression may still have occurred from another species.**

643

644 Line 13-15 – Indicate that this is known from previous work.

645 **Done**

646

647 Line 14 – and “shows” strong genetic differentiation...

648 **Done**

649

650 **Introduction** – There is a lot of information on SVs here, which provides a nice summary of a lot of the  
651 literature. However, the Introduction misses an important part of the study. The introduction focuses  
652 solely on SVs, which is not the only thing that the paper investigates. There is no mention of the  
653 Icelandic population here and analyses of demographic history. The inclusion of analyses on population  
654 structure and demographic history need to be better outlined in the Introduction. It is not clear from  
655 the Introduction why the dadi analysis (demographic history) was done with Northern Europe and  
656 Iceland. This needs more background and better context in the Introduction. In the study, the authors  
657 suggest a previously unknown refugium may have existed in Iceland, but it is not clear why this question  
658 was even investigated. There should be more discussion about other aspects such as population  
659 structure and not just a focus on SVs.

660 **We have included a broader paragraph at the beginning of the introduction and a more specific**  
661 **statement about Iceland in the last paragraph about the plaice.**

662

663 Line 34-35 – Is this always true? If an inversion is introduced through secondary contact/hybridization,  
664 couldn’t both homozygotes be present initially? Maybe indicate “*de novo*” or “initially”, or reword for  
665 clarity.

666 **They must have occurred at some point at an initial frequency of  $1/2N_e$ , even in a different refuge**

667 Line 40 – “SVs are likely to evolve incompatible alleles”. Why? Explain.

668 **The lack of recombination and therefore the independent co-evolution.**  
669 Line 43-44 – I’m not sure if I understand what is meant by “become trapped in environmental  
670 gradients”. Also, physical barriers to gene flow would imply that populations are NOT fully connected?  
671 Please clarify this section.  
672 **“Trapped” is a common word used in the literature (by Barton for example).**  
673 **Nevertheless, these 3 sentences were removed from the new version of the manuscript, as they**  
674 **were not essential.**  
675  
676 Line 49 – Is “evolving” necessary here? Consider rewording this part.  
677 **We have changed “Structural variants are also important for evolving and maintaining locally**  
678 **adapted populations in the face of gene flow” to “Structural variants are also important features**  
679 **promoting the evolution and the maintenance locally adapted populations in the face of gene flow”**  
680 **that is further developed in the rest of the paragraph.**  
681  
682 Line 54 – Consider changing to “maladapted” instead of “unadapted”  
683 **Done**  
684  
685 Line 56 – Fix wording here.  
686 **We have removed this sentence with the reorganization of the introduction**  
687  
688 Lines 61-63 – Not sure why allele frequency clines are relevant in this sentence? Why  
689 does having mutations in SVs make clines more likely? Not clear.  
690 **Adaptive mutations in a SV are more likely to resist swamping. We have rewritten this paragraph.**  
691  
692 Line 70-71 – But this can only occur if genes with functional relevance are found within  
693 that region of the chromosome/SV?  
694 **Yes, we agree with reviewer 3. However, we have decided to remove this sentence (“these co-**  
695 **adaptation are expected to arrive continuously de-novo after the SV associates with an**  
696 **environmental barrier”) since it is not essential in the context of our study.**  
697  
698 Line 106-108 –Provide references for these biological characteristics.  
699 **Done**  
700  
701 Line 108 – What type of markers/how many? Microsatellites?  
702 **Done, it is 6 microsatellite loci**  
703  
704 Line 115 – in European plaice specifically? Or in all four species? This was not clear. I think more  
705 information on this study in European plaice is needed here (Lines 112-118).  
706 **In the plaice only. We wrote more information about our previous study to make this paragraph**  
707 **clearer.**  
708

709 For the “larger geographical scale (line 118)” indicate where these new samples are from to provide  
710 better context.

711 **This information is more detailed in the next paragraph in the methods section. We did not want to**  
712 **repeat ourselves because we think that the paper is long enough in the present state.**

713

714 Line 119 – There is no mention of the Icelandic population here and the examination of demographic  
715 histories.

716 **We have included more details about Iceland in the last paragraphs:**

717 ***However, the off-shelf population shows a higher genetic diversity than most of the population from***  
718 ***the northern distributional range of the plaice (Hoarau et al., 2002), which could suggest that this***  
719 ***population has a more ancient origin than what is currently thought.***

720

## 721 **Methods**

722 Line 138 – Samples were collected during spawning. What do we know about the distribution of these  
723 fish outside of the spawning season? I am wondering if they remain close to these spawning grounds  
724 or are they highly migratory?

725 **It’s difficult to answer this question for marine species like the plaice which are not studied a lot.**

726 **The few existing tagging data suggest some site fidelity for spawning grounds but movement to**  
727 **feeding grounds up to 250 km have already been recorded. See**

728 **<https://royalsocietypublishing.org/doi/abs/10.1098/rspb.2003.2473> for more details.**

729

730 Line 182- What does present mean here? Does it mean that it was genotyped in >80% individuals  
731 (missing in <20%)? Or something else?

732 **Yes, we have changed “present” to “genotyped”**

733

734 Line 185 – I wonder if removing SNPs that are out of HWE could remove potentially informative SNPs  
735 associated with complex structural variation. Can the authors comment on this?

736 **See the answer in the general comment address to all three reviewers**

737 Line 196 – Population structure should also be explored for neutral markers. I suggest performing  
738 analysis with all loci, neutral loci, and SV dataset(s). Or perhaps a neutral, outlier, and SV dataset.  
739 Although there are many ways to identify outliers, it might be easiest to use a method such as PCAdapt  
740 and remove loci that are outliers on the first couple of axes that explain a lot of the variation. It is  
741 difficult to know whether the patterns observed in Figure 1b are due to potential adaptive differences  
742 among populations or due to neutral structure. In a PCA differences can be driven by a few important  
743 loci. If such is the case, it would also be nice to see which part of the genome are responsible for these  
744 differences observed here (Fig. 1b). Other types of analyses for population structure may also be  
745 helpful, such as STRUCTURE (or ADMIXTURE).

746 **We understand this comment, but we feel that including a neutral vs outlier analysis would change,**  
747 **and potentially confuse, the focus of our manuscript significantly. Thus, we have decided to keep the**  
748 **focus on the contrasts between SV and collinear regions of the genome rather than basic neutral vs**  
749 **outlier. Nevertheless, we have now included a PCA as well as the loading plot without the structural**

750 **variants in the supplementary material to illustrate the contribution of the SVs to the structure**  
751 **detected in the overall PCA in Figure 1a.**

752

753 Line 199 – If you are looking for structural variants, shouldn't you not prune for LD? This may remove  
754 loci that are linked due to SV?

755 **This was confusing in the original version. We only pruned for physical linkage for the overall**  
756 **population structure analyses and the PCA as these “individual-based” analyses are sensitive to it**  
757 **and to missing data, which can be problematic with ddRAD as missing data are not randomly**  
758 **distributed. See the answer to reviewer 1 line 448**

759

760 Line 211-222 – It was not clear from the Introduction why this analysis is being conducted. It needs to  
761 be clear how this is related to SVs (the focus of the paper).

762 **We have made the introduction broader and have included specific statements about Iceland in the**  
763 **paragraph about plaice population structure**

764

765 Line 238 – How many loci had HO and FST of 1 and for which inversions? These loci may be especially  
766 important for understanding the functional differences between genotypes. The genes that these SNPs  
767 are located within may be especially informative.

768 **This information has now been added in the results section.**

769

770 Line 253-255- It is not clear to me what was done here to calculate average/smoothed FST?

771 **We used the loess regression using ggplot on the value of the upper 5% quantile. We have clarified it**  
772 **in the text.**

773

774 Line 280 – Why did you choose a collinear region of chromosome 19 to represent genome-wide  
775 average? Why not choose a different chromosome that does not contain any SVs, as the dynamics of  
776 collinear regions on chromosome 19 could still be influenced by the presence of the SV (which is only  
777 5Mbp away). This does not seem like the best approach.

778 **We understand this comment, which was also highlighted by reviewer 1. However, as we cannot**  
779 **detect any structure even within the same chromosome, it provide interesting additional information**  
780 **about the strength of barriers to gene flow, which seems to be limited to the rearranged parts of the**  
781 **chromosome.**

782

783 Line 295 – These inversions are likely not neutral (or at least that is what this study suggests). I have  
784 often wondered how to appropriately date inversions. I don't see how these analyses can be applied to  
785 inversions without violating many assumptions. Please explain.

786 **See our response to line 953**

787

## 788 **Results**

789 Line 317 – Typo (need space between 'the' and 'Transition')

## 790 **Fixed**

791 Line 333 – Provide information about timing in the text.

792 **Done**

793 Line 334-342 – It seems confusing to go back to Figure 1 here after this population structure has already  
794 been discussed above (Lines 302-307). This is an area where better organization could improve the  
795 manuscript.

796 **The figure was moved in the result part (line 346)**

797

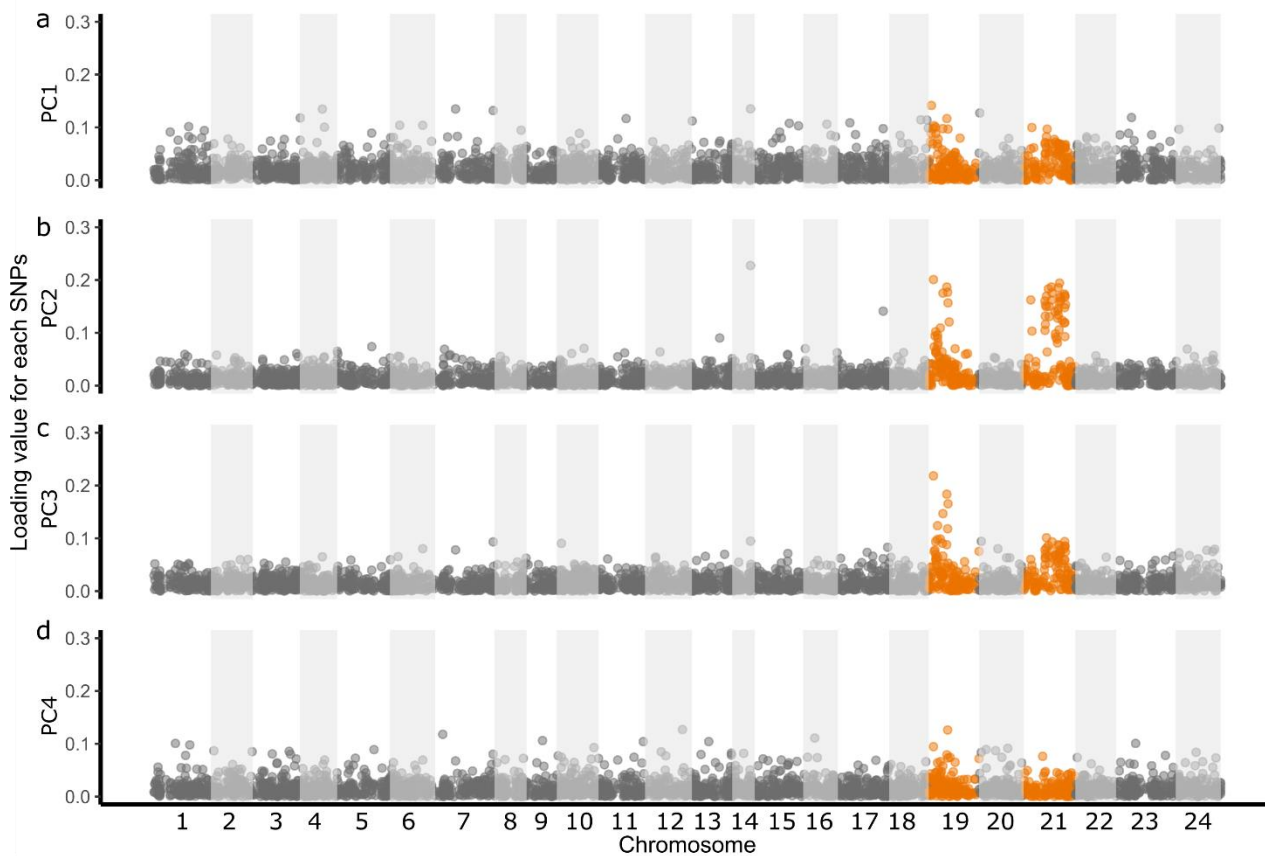
798 As mentioned, the demography history (above; Lines 327-333) seems out of place in the middle of this.  
799 This is where providing better context and describing all objectives in the Introduction would help with  
800 the flow of the manuscript.

801 **We have moved this paragraph up, after the presentation of the FST between Iceland and the  
802 continental shelf, in order to justify the removal of Iceland when analyzing the IBD.**

803

804 Line 336-337 – Manhattan plot or data to support this statement?

805 **Manhattan plot with the egeinvalue has been added in Figure S4 of the manuscript.**



806

807

808 Figure 3 (D1/2) – What does  $R^2$  indicate in this figure? The mean  $R^2$  for the locus with all other loci on  
809 the chromosome?

810 **Yes, it has been added to the text. We calculated the pairwise LD between any pair of loci along  
811 chromosomes 19 and 21.**

812

813 Line 360 – How much lower?

814 **Added:**

815 *The genome-wide differentiation outside the SVs was lower (e.g. mean  $F_{ST}$  North Sea vs Baltic Sea=*  
816 *0.004,  $sd=0.031$ ) than inside the SVs (mean  $F_{ST}$  SV19= 0.203,  $sd=0.177$  & mean  $F_{ST}$  SV21=0.167,*  
817  *$sd=0.142$ ; Figure 3b and Figure S8).*

818

819 Line 360 – Be specific in the text. “Several SNPs” -> How many?

820 **Added:**

821 *The individuals from haplogroups 1 and 3 were differentially fixed for eight and 30 SNPs within SV19*  
822 *and SV21, respectively*

823

824

825

826

827 Line 362-363 – Not clear where the reduction in FST is in the figure? Do you mean the huge gap in SV21  
828 (where there is low LD), which likely due to differences in position along the chromosome compared to  
829 the reference genome of flounder? Or another region? I also don't see a specific region of low FST for  
830 SV19? Moreover, I don't see a concordant increase in  $\pi$ . This needs to be better labeled in the figures.

831 **Most reviewers pointed to this part of the manuscript as a weak part. Here, it was referred to the**  
832 **slight drop in Fst in the middle of both SVs, with an absence of Fst of 1 between haplogroup 1 and 3**  
833 **(many with Fst of 0.98 – 0.95). However, this signal could also be due to random sampling of the**  
834 **ddRAD method. We removed this part from the manuscript, but we would definitely be interested in**  
835 **looking into this pattern in future work.**

836

837 Line 376-377 – There are over 1800 genes here, how did you determine that ion transport was  
838 important? Looked for over enrichment? Also I could not find access to Supplementary File 1?

839 **We included a gene enrichment analyses.**

840

841 Line 379-390 – Again, I am not sure that using chromosome 19 is the best option for the genome-wide  
842 estimates.

843 **See general answer to all three reviewers**

844

845 Lines 391-400 – How many SNPs were used here? It seems clear from the tree that this is not a case of  
846 ancient introgression (Line 398-400)

847

848

849 **Discussion**

850 Line 411 – derived “form”

851 **Done**

852

853 Line 412 – edge “of” the plaice distribution...

854 **Done**

855

856 Line 417 – How much greater is this IBD relative to other marine fish? What is the magnitude of  
857 difference?

858 **We have toned this part down.**

859

860 Line 417-420 – Was the geographic distribution of previous studies similar to this one? It seems  
861 surprising that if this is the strongest IBD detected for a marine fish that all types of markers should be  
862 able to resolve this pattern.

863 **Earlier work had a higher geographical coverage but only 6 microsatellite loci. The results from**  
864 **previous work is no surprise, given the high  $N_e$  of this species and the low degree of differentiation**  
865 **(<0.015). There are several examples of marine species that were thought to be genetically panmictic**  
866 **with few microsatellite, but where clear population structure has been identified thanks to the**  
867 **increased number of markers.**

868 Line 433- Are there any mitochondrial data from this species that would support a different glacial  
869 refugium? Or is such a scenario observed in other marine species? This would be useful to know. In  
870 addition, a scenario of secondary contact was most likely. How does secondary contact fit into this  
871 history? I don't see it mentioned in the Discussion here.

872 **Previous studies on the plaice mitochondria have shown quite a lot of differences between Iceland**  
873 **and the continental shelf samples:**

874 (<https://www.sciencedirect.com/science/article/abs/pii/S1385110103001321>).

875 **However, it is not clear from previous work if the Icelandic population shows private mitochondrial**  
876 **haplotypes. Consequently, we have preferred not to extend the discussion on the Icelandic sample**  
877 **in the current manuscript.**

878

879 Line 449 – References for these time frames?

880 **We have now included an entire paragraph about this**

881

882 Line 421-460 – This section seems a bit out of place. The entire Introduction focuses primarily on  
883 structural variation, so the population structure doesn't flow well with the general goals of the paper  
884 (as discussed above).

885 **We have added a paragraph to the introduction in order to be make it broader.**

886

887 Line 462 – I'm not sure that it was shown that the large SVs were responsible for the 'main population  
888 differences'. They explain individual differences, but without their inclusion you see more IBD? It seems  
889 based on  $F_{ST}$  they are important for differentiating the North Sea and Baltic, but other comparisons  
890 don't seem to show as high  $F_{ST}$  for both SVs? Based on the PCA, it is not clear what genomic regions  
891 are driving population differences. The PCA should be done without the SVs at the very least (and also  
892 with outliers removed).

893 **The PCA without the SVs has now been included in the supplementary material. Also, we have**  
894 **included estimates of  $F_{st}$  within and outside the SVs to be more clear about our statement that the**  
895 **SVs were responsible for the main population differences.**

896

897 Line 469-471 – There are no environmental data in this paper. I understand that the environmental  
898 break in the Baltic is well known but you need environmental data to back up this conclusion here. It  
899 would be possible to incorporate data from online databases. MARSPEC and Bio-ORACLE have salinity  
900 and temperature data that could be used here to better explore these associations. These can be  
901 accessed online and also using the ‘sdmpredictors’ package in R. This may be useful to the authors or  
902 even just to show on a map to those who are unfamiliar with this region. Further, since the fish were  
903 collected during spawning, do the environmental conditions at their capture site reflect the  
904 environmental conditions they would usually experience? Or are they migratory species?

905 **The analyses based on environmental data were performed in the other manuscript (Le Moan et al,**  
906 **2019, Beyond parallel evolution: when several species colonize the same environmental gradient.**  
907 **BioRxiv) together with 3 other species to study adaptation to the Baltic Sea environmental gradient.**  
908 **The allele frequencies of the structural variants were indeed correlated with the salinity gradient**  
909 **(together with 3 other small regions of the genome). We would prefer not to include more detailed**  
910 **analyses to the present manuscript as it is already relatively long.**

911  
912 Line 471-472 – Why not examine selection in your study? Test of selection could be performed on the  
913 genotype groups?

914 **Cf earlier (line 751 of the reviewer comments), but mainly because the formal test for selection were**  
915 **done in our previous study.**

916  
917 Line 498 – 499- Please provide examples of this phenomenon in other species/inversions? I was not  
918 aware of this pattern (which is not easy to see in the figures). In another recent study with a large  
919 inversion in a salmonid, they actually find an increase in FST in the middle of the inversion and a drop  
920 in nucleotide diversity (<https://www.biorxiv.org/content/10.1101/504621v1.abstract>).

921 **We have decided to remove this part for the moment but we would be very interested in coming**  
922 **back to this pattern with more data to analyse.**

923 **Thanks for sharing this paper, it is very interesting. We are not sure that the pattern they observed**  
924 **is different from the one we described in the plaice. The trout inversions seem to show higher values**  
925 **of Fst in their breaking point. This pattern is even clearer in the trout as they have two inversions and**  
926 **therefor two breaking points side by side (which look like a peak of FST in the center of the LD block).**  
927 **The substantial drop of diversity in other breaking points of their inversions is also visible. In fact, the**  
928 **trout study has a better dataset to look for these rare events of recombination between the alleles**  
929 **of an inversion.**

930  
931 Line 506 – wordin-g

932 **We have rephrased this paragraph (which is now line 484-486)**

933  
934 Line 512-514 – If this is true, would there not be drift occurring in these SVs? Should Iceland look  
935 different compared to the rest of the populations given the long divergence time?

936 **It was something that we wanted to check but the shift in the insert size during the library prep made**  
937 **this question difficult to address. At the moment, it is briefly mentioned in the perspective.**

938



939 Line 515 – “do not include effects from selection or recombination” – does this mean it is likely shorter  
940 or longer? I am not sure that you can accurately date an inversion by branch length given that inversion  
941 are much different than other parts of the genome. Selection and reduced recombination could quickly  
942 lead to very divergent haplotypes. Assuming that Hap 2 is the ancestral allele, then given that the SV19  
943 inversion is polymorphic in Iceland and that Iceland contains Hap1 (derived) for SV21, the best date you  
944 may be able to give both inversions is only as early as the time of the split using other loci. What is the  
945 split date between Iceland and other populations (this was obtained from demographic modeling?).  
946 Demographic history also suggested secondary contact after isolation, so it could be possible that  
947 variation in the inversion was introduced into Iceland after the split and was not present before. I’d be  
948 happy to learn how these methods are appropriate and also better than just using other loci to date  
949 the split.

950

951 **To answer these question in detail:**

952

953 **1- if there is an absence of recombination between haplotypes/alleles, it is good, because it means**  
954 **that aging the alleles with a basic phylogeny can work, as they behave like different species. However,**  
955 **recombination between the main haplotypes/alleles seems to occur (although rarely). Such events**  
956 **of recombination will tend to decrease the estimates of divergence because it will reduce the number**  
957 **of sites that are differentially fixed between the two alleles.**

958

959 **2- selection (positive and background) will increase the divergence in the region surrounding the**  
960 **mutation that are positively selected or counter selected due to the effect of selection at linked site.**  
961 **Nevertheless, it will not have consequences on the entire SV when they are long enough and if the**  
962 **alleles are frequent, because recombination still occurs in the homozygous individuals, and therefore,**  
963 **decreases the size of the sweeps**

964

965 **Thus, it is difficult to know how wrong we are because the two mechanism act in opposite directions,**  
966 **and the answer will depend of the balance between the two mechanisms. It should be possible to**  
967 **estimate the recombination rate between the divergent alleles with full genome sequencing, and**  
968 **then, jointly infer the divergence time and the selection forces acting on the SV under an ABC**  
969 **framework. This ABC analyses is something that we would like to do in the future with deeper**  
970 **genomic coverage. The only biases to infer selection is that the selection forces acting on the**  
971 **structural variant are likely variable over the time (see the Faria et al, 2019 cited in the manuscript),**  
972 **which can be a bit problematic to infer.**

973

974 **Nevertheless, in the paper, we highlighted the divergence of the ancestral allele (haplotype 2 in blue),**  
975 **which is likely the allele that has been the least affected by selection (i.e. it is the ancestral state that**  
976 **shows the highest diversity, which makes it less likely to diverge due to background selection and**  
977 **positive selection). This estimated time of divergence is already enough to say that it is older than**  
978 **the Baltic Sea, which was the main reason why we conducted included these analyses.**

979

980 **Then, we agree that the Icelandic population is also an interesting reference point, which we now**  
981 **used as additional data to confirm that the structural variants are old.**

982  
983

984 Line 522-529 – This is a really cool idea. Are there any other candidate species that could represent a  
985 possible source of introgression of this SV? Could you provide examples of some candidate for future  
986 studies?

987 **Maybe a species found in the western Atlantic through trans-Atlantic migration. An alternative could**  
988 **be from a Pacific species that can meet the European plaice somewhere in the North of Siberia. At**  
989 **this stage, all of these hypotheses remain extremely speculative.**

990

991 Line 55-547 – Why not test for selection in this region? Perhaps the composite likelihood ratio (CLR)  
992 method in SweeD would be appropriate here (it is more robust to variation in recombination,  
993 ascertainment, and demography than Tajima's D). You could test it within each haplotype group  
994 (homozygotes for Hap1 and Hap2).

995

996 **We are limited by the number of markers. SweeD, Tajima's D or other similar statistics are statistics**  
997 **that are calculated for sequences. It will be definitively something to do I follow-up work.**

998

999 Line 550 – I would also be interested to know if there are some life history traits that may be associated  
1000 with this inversion. It seems many inversions underlie complex phenotypes (e.g., mating and migration  
1001 strategies). Are there any traits that could be of interest, such as migration behaviour? Or age-at-  
1002 maturity? Colour morphs? I am not sure. But it would be interesting to know about any possibilities.

1003 **All these questions are questions we do also have 😊. However, we just want to avoid too much**  
1004 **speculation at this stage. Still, they certainly provides exiting perspectives for future work!**

1005

1006 Line 578 - Fix wording here.

1007 **Fixed**

1008

1009 Line 596 – There is not really much discussion on the genes in the inversions. I think this would be  
1010 interesting to explore a bit further. Were any processes over-enriched? Are any of the genes in the  
1011 inversions found within inversions in other species?

1012 **We added more analyses to answer this question. They seem enriched for immune system genes.**

1013

1014 Figure S6 – What does “mean  $F_{ST}$ ” represent? What is the comparison that the points represent? Needs  
1015 more information in caption.

1016 **Corrected**

1017

1018 Figure S7 – Include something to indicate the position – or at least which end is the startof the  
1019 chromosome.

1020 **We are not sure we understand this question as the chromosomes are separated by long stretch of**  
1021 **white.**