Institute of Science and Technology
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3400 Klosterneuburg, Austria

Klosterneuburg, 28th September, 2018

Dear Marianne Elias,

We are thankful for reconsidering our work. We have made changes in response to all referee's concerns (printed in blue and interspersed with your original email), and hope that you will now find the manuscript suitable for recommendation. The new version has been deposited on the bioRxiv website, and a 'revised tracked changes' manuscript has been uploaded on the PCIEvolBiol website.

With best regards,

Christelle Fraïsse

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On behalf of all authors.

Please, note that line numbering corresponds to the revised bioRxiv manuscript (not the tracked-changes manuscript on the PCIEvolBiol website).

Decision

by Marianne Elias, 2018-05-23 18:02
Manuscript version: https://doi.org/10.1101/239244

***Fraisse et al.*: revisions requested**

Dear Dr Fraïsse,

The same referees (Tatiana Giraud and Thomas Broquet) have evaluated the revised version of your manuscript. While they acknowledge improvements and consider the manuscript original and of general interest, there are still many concerns. I outline below the main points that need to be addressed carefully. The reviews provide more details on those, as well as additional comments, which should be addressed thoroughly.

Focus of the manuscript: this has obviously improved since the first version, but only in some places. Notably, the introduction and the discussion of the manuscript are still very tuned on adaptation, as if the main goal of the paper were to investigate adaptive introgression (several examples are highlighted by the referees, and many more can be found throughout the text). As such, the current version looks a bit 'schizophrenic'. Introduction (and, to a lesser extent, discussion) and the questions should be entirely reframed to refocus the manuscript around the fine-scale genetic structure in the Kerguelen and the effect of introgression from foreign lineages (see the suggestions of both referees in this respect). Adaptive introgression can of course be mentioned (briefly in introduction), and discussed (in light of your results and literature on the topic in the discussion), but as pointed out at the previous round of reviews and here again, your data do not enable to test for adaptive introgression. Also, the manuscript lacks a general conclusion that extends beyond mussels.
We acknowledge our previous prose put too much place to the usual interpretation of adaptation from admixture variation and this did not serve the main message of the paper. We substantially altered the Introduction and Discussion to remove any reference to *adaptive introgression*. The message of our paper is now reframed to focus on the micro-geographic genetic structure in the Kerguelen Island and the contribution of introgression from Northern lineages. Also, we better explain the two alternative hypotheses that underlie these patterns, namely (i) local adaptation and (ii) habitat-associated connectivity revealed by introgression gradients between two genetic backgrounds maintained at the scale of the island. We show in the paper why we think that the second hypothesis is the most probable. And we end the discussion by the general conclusion that patterns of genetic-environment associations can sometimes be erroneously interpreted as signal of local adaptation.

General clarity: as highlighted by both referees, the manuscript is complex, and lacks clarity in many instances. In addition to streamlining the text, improving general consistency and making sure appropriate vocabulary/phrasing is used, you should find a way to outline in a clear way 1) the hypotheses you want to test, 2) the data you have (you have different sets of samples and different sets of markers, which makes things hard to follow) and 3) the methods you implement for each hypothesis test. This could take the form of a kind of a synthetic section at the beginning of Material and Methods, and/or a figure, or something else, but it is essential that those methodological aspects are clarified.
We agree. We followed Tatiana Giraud's suggestions, and we now better introduce our questions and their related methods (and data) at the end of the Introduction.

Tatiana Giraud raised an important point during the first round of reviews, which has not been

satisfactorily addressed: please clearly explain (in the ms, not only in the reply to the referees) on what principle the methods used for that purpose disentangle ILS from introgression, so that the reader can assess how reliable the inference is.
We would like to emphasize that shared polymorphisms are preponderant in our data, as a consequence of both ongoing incomplete lineage sorting (ILS), and past and recent introgression. We also have fixed ILS in the phylogeny owing to the rapid successive splits of the four taxa (*M. trossulus*, *M. edulis*, *M. platensis* and *M. galloprovincialis*). The latter type of ILS should not be confounded with ancestral shared polymorphism (ongoing ILS), the lineages have been sorted but in a different topology than the average topology. We now make this point clearer in the text (L390 - L403 and L539 - L544). In addition, we endeavoured to better explain in the ms the principles of the three methods used to detect introgression.

Another point raised by Tatiana Giraud relates to accounting for spatial distances in the environment-genotype analyses. This is done by using spatial coordinates, while mussel dispersal around the Kerguelen is likely more constrained by coastal distances. I don't know if even a crude measure of such distance can be incorporated in the analyses, but if not, the discussion should not be so affirmative that spatial structure does not have any effect.
We now highlight this caveat in our analyse.

Although Thomas Broquet acknowledges the improvement provided by the new simulations to the link between fine-scale genetic structure and connectivity break (in the absence of local adaptation), he calls for caution for interpreting the results because of the lack of confidence intervals.
We clarify this point by providing the variance in allele frequency across loci at outlier and non-outlier markers.

Additionally, although the different analyses that enable to assess gene flow do indicate introgression from northern species (provided that these analyses are reliable), the patterns detected are somewhat different (e. g., introgression from galloprovincialis (Treemix) versus mostly edulis (Twisst) or both (dadi); see also Thomas Broquet's comment on the ancient gene flow inferred by dadi versus claims of secondary contact). Why do you think this is so?
We agree that the admixture results from the different methods are quantitatively different regarding the contribution of each Northern species to the genetic composition of the Kerguelen mussels. In fact, the different methods use distinct kind of information in the data. *TreeMix* is based on allele frequencies and may have a reduced power to detect migration events. Moreover, it infers single admixture event under the hypothesis of homogeneous introgression, while genetic barriers are often semi-permeable in *Mytilus* (Roux *et al.* 2014, Fraïsse *et al.* 2018, and see Table S5). *Twisst* is based on classification of gene genealogies, while *∂a∂i* uses the site frequency spectrum to explicitly model migration. We believe it is a strength to have these different approaches revealing different aspects of the history of mussels rather than a weakness. Overall, the divergence history of Northern and Kerguelen mussels appears to be quite complex with both ancient migration events and current gene flow. We captured this complexity by implementing three additional models with *∂a∂i* that better fit the data than the previous one-period models (see our reply to Thomas Broquet's comment #3).

A few minor comments, in addition to those mentioned in the review:
L. 53: should read 'thereby generally showing', or 'therefore they generally show'
Changed: "*therefore they generally show*" (L42 - L43).

L. 217: please briefly explain how KASPar works.
We now specify: "*We used KASPar (Kompetitive Allele Specific PCR, Smith & Maughan 2015), a fluorescence-based genotyping assay of allele-specific PCR products, to genotype*

the 58 SNPs, of them, 44 SNPs were successfully amplified." (L180 - L182)

L. 263 and below: the 'delta' of dadi is not written the same way as in the results.
Changed to *∂a∂i* throughout the manuscript.

L. 342: you never mention how many demes you model.
We now specify: "*that meet twice on a circular stepping stone model of 30 demes*" (L310 - L311).

L. 549: the % given are those of platensis, not edulis.
Thanks for spotting this error. We corrected by: "*RdA has by far the highest level of M. edulis ancestry (31% compared to <19% elsewhere)*" (L455 - L456).

L. 559: should read 'provided'.
Corrected (L466).

L. 585: 'in agreement' with…?
Replaced by "*Accordingly*" (L489).

L. 787: 'our results are very promising that…' doesn't have a correct syntax.
This sentence has been removed in the revised version of the manuscript.

Please check the text thoroughly for additional inconsistencies, typos etc that we may have missed.

Good luck with the revision,
Marianne Elias

Reviews

Reviewed by Thomas Broquet, 2018-04-16 19:31

I had two main comments on the first version of this manuscript. The first one was criticizing the interpretation of the results in terms of local adaptation. This comment was partly due to the fact that I took phrasing such as "genetic-environmental association" for indications of local adaptation, but this whole aspect of the study is now clearer in the revised version.

The second comment called for an improved presentation of the introduction and discussion sections, and I find that this can still be improved in places (as detailed below).

I find that the new analyses presented in this revised manuscript help discussing the admixture vs incomplete lineage sorting hypotheses, and the newly performed simulations also help illustrating how the fine scale patterns observed could theoretically be produced in absence of local adaptation. However, these new analyses come at a cost: they add to an already complex set of analyses, and one of the new results (allelic frequency spectrum, dadi) calls for more caution in some of the interpretations. Hence below I suggest some places where the text could be streamlined and I also comment on the results from dadi. Finally, I have two new comments on aspects that I did not notice in the first round of review but that probably call for some clarification.

Overall, all comments on this revised version call for relatively minor text improvement, and I think that the main message (impact of reticulated evolution on present fine scale genetic structuring) is of interest to a wide audience.
We thank you for your interest and for the time you spent on our manuscript.

1) It is difficult to follow what is called the "Southern lineage" (L. 659-660) given that most analyses focus on the genetic structure of Kerguelen mussels relative to Northern populations. Samples from the Southern Hemisphere were used in some of the analyses, but these samples are not described, and it is not clear why they were not included in the main analyses. The first mention of such samples (I think) is on line 259: "the Chilean mussels", but at this stage of the paper we don't know what these samples are and why they are used here and they were not used e.g. in the genetic network analysis. I don't mean that these samples should necessarily be included in these analyses, but their role in the analyses and interpretation could be clarified.
We agree that this was not clear in the previous version, as the relevant information was included in the Supp. Info only. We now introduce them in the Materials & Methods: "*We additionally included other samples from the Southern Hemisphere (Gérard et al. 2008) to assess the genetic relationships previously proposed by Borsa et al. (2012) with a handful of markers: one sample from Western Australia in Nedlands (AUS, n=12 individuals); two samples from New Zealand, Dunedin (DUN, n=8) and Wellington Harbour (WHL, n=10); two samples from Tasmania, the Simpson's Bay (SIM, n=8) and Hobart (HOB, n=9); one sample from Chile in Maullin (MAU, n=15)*".

2) The genetic break observed between sites PAF and RdA is interesting, and its interpretation and comparison with simulations suggest that the scenario proposed in the paper is plausible at least. However, I suggest to be slightly more cautious in the interpretation for the following reasons: first, there are no confidence intervals on allelic frequency estimates in Fig. 4B, and it is expected that average frequency at all loci but four (empty symbols) will be less variable that the average frequency at four loci (filled symbols). Hence the difference in variation around allelic frequencies is not a strong argument (contrary to L. 544).
Because the allele frequencies at the four outlier loci are significantly correlated (Figure S4), the variance is actually much reduced compared to that of the other loci, such that the genetic break observed between sites PAF and RdA is still completely clear. This is expected as selection tends to correlate allele frequency at the underlying selected loci, while genetic drift will ultimately fix one of the two alleles at each locus randomly, and so the variance in allele frequency across the non-outlier loci is maximized. We made this point now by adding in the Results: *"Notably, the variance in allele frequency at the four loci was weak (0.0208 in average across localities). This is in sharp contrast with the pattern observed at the other loci (open symbols) of which the average frequency remained similar across all sites (Figure 4B), and variance was stronger (0.0938 in average across localities)".*

Second, there is another possible (not discussed) break around site "BOBO". I could not find this site on the map, but it seems that this break occurs within a really small geographic distance, so small that the barrier-to-dispersal scenario may not hold there (it could be random error around the allelic frequency estimate linked with sample size). So overall, I would generally more cautious in the text.

Thanks for spotting our mistake with the sample name on the map: Bocentre was indicated instead of BOBO. While we agree that there is a change in allele frequency at site BO100am within the Henri Bossière Fjord, we do not think it is related to the genetic break observed between PAF and RdA sites. First, as you noted, the genetic change occurs at a very small geographic distance (<500m), much too small for our hypothesis of barrier to dispersal (cf simulations, and remind the average dispersal distance is mussels is around 10-100km). In addition, Figure S4 shows that the allele frequency at the four outlier loci is not so well correlated in this sample. Finally, the average proportion of *M. edulis* ancestry inferred from an ADMIXTURE analysis (Figure 4B) is similar to the surrounding localities, which is not the case for site RdA.

3) The dadi analysis suggests a scenario of ancient migration, not secondary contact. It still means that past admixture has played a role, but then the text needs to be clarified because it often refers to "secondary contact" (e.g. see simulation conditions), or "secondary admixture", "secondary genetic exchanges", etc. So you may want to clarify throughout whether your interpretation is that of a secondary contact, ancient migration, or unresolved past admixture that could be one or the other (or both), and why trust more one approach over the other.
We agree that we found evidence for both current and past admixture events with the different methods, and we should not emphasize one at the expense of the other. So we removed “secondary/secondarily” from most occurrences.
To better understand the complex evolutionary history between Northern mussels and the Kerguelen island, we performed additional *∂a∂i* analyses. We implemented three extra models that include several periods of migration during species divergence: (i) periodic ancient migration and (ii) periodic secondary contacts, which allow two rounds of ancient migration or secondary contact; and (iii) a three-period model, which includes an ancient migration period, followed by a period of isolation and then current gene flow. In agreement with our findings of a complex migration history, the best models are periodic ancient migrations between the Kerguelen mussels and *M. edulis* or *M. galloprovincialis*, and the three-period model between the Kerguelen mussels and *M. trossulus* (Table S5). Related results are described from L369 to L385.

Other specific comments:
L. 87: "local" where?
Replaced by “*population-specific*” (L71).

L. 112: are those mussels *M. platensis*?
Yes, we now specify: *”the isolated Kerguelen Islands harbor M. platensis mussels*” (L88).

L. 118: this sentence is really difficult to understand without reading your other published work (Gerard *et al.* 2015).
This sentence has been removed.

L. 139: "for many molluscs": add "including mussels" with ref. Otherwise, we wonder throughout the paper what do Macrocystis seaweeds have to do with mussels.
We followed your suggestion: “*serving as substrate and refuge for many molluscs species, including Mytilus (Adami & Gordillo, 1999), in areas exposed to wave action.*” (L587 - L589).

L. 149: point ii) has not been introduced so far, so I suppose it is going to be difficult to understand at this point of the text. This could be improved at the beginning of the intro.

We substantially modified the Introduction to better introduce these concepts. And this specific sentence has been removed.

L. 153: what handful of markers?
This sentence has been removed in the revised version.

L. 154-171: long summary of the results. Since the text is long and complex, reductions would be welcome and this is one place where this could be done.
We significantly shortened this section. We also clarified the questions and related methods as suggested by Tatiana Giraud.

L. 219: seven loci out of 44, that seems like a lot of errors between the two sequencing methods. You could add somewhere that quantitative results for the comparison of the two genotyping methods (I mean in general, not for the Mytilus case) will be welcome.
Agreed. We added in the Materials & Methods: "*More generally, these discrepancies call for studies that quantitatively compare the two genotyping methods.*" (L186 - L187).

L. 258: "we defined…" : difficult to understand why at this stage, and what are these Chilean samples. Same thing L. 341.
We now introduce the Chilean mussels in the Materials and Methods (see our reply to your comment #1). And we highlight in the text that the Chilean mussels belongs to the *M. platensis* clade, like the Kerguelen mussels: "*We defined M. edulis and the M. platensis Chilean mussels as ancestral populations from which the Kerguelen individuals derive their ancestry.*" (L223 - L225).

L. 353: what is a "bi-locus haploid genotypes at a barrier locus"?
We rephrased with a more explicit formulation: "*Selection acts in haploid individuals on a two-locus incompatibility, which is linked to a neutral marker located 1cM away and unlinked to a second neutral marker.*" (L320 - L321).

L. 375: what are those samples and why were they not used in genetic network analyses? I don't mean that they should be, but that this aspect could be clarified in the text.
Please, see our reply to your comment #1 above.

Figure 1: "internal" and "external" denominations: not very intuitive.
Replaced by "*embedded*" and "*peripheral*" throughout the text.

L. 413: "three migration edges": on Figure 1 there are four. And 50% seems like a very permissive threshold. A 50% supported edge seems not significant. Can you justify this threshold? Or at least take it into account when discussing the results?
We agree that the *TreeMix* analysis provide moderate support for migration events; and this may be due to a limited power of inferring migration based on allele frequencies when rates of incomplete lineage sorting are so strong. Moreover, *TreeMix* infer single admixture event under the assumption of homogeneous introgression across the genome, while we detected in our *∂a∂i* analysis that barriers are certainly semi-permeable (Table S5). So, we rephrased by "*They were generally weakly supported, with only four migration events that had more than 50% bootstrap support (Figure 2A and Table S8).*" (L358 - L359).

L. 453: this whole analysis says that incomplete lineage sorting is important to consider. It does not seem to bring any support to any of the scenarios tested, so it could probably be sent to entirely to sup mat (but keep the emphasis on ILS in text of course).
We think that the *Twisst* analysis is particularly useful to highlight the presence of two different types of incomplete lineage sorting (ILS): (i) ongoing sorting of shared ancestral polymorphism, which results in unresolved relationships; and (ii) ancient ILS that resolved into alternative topologies among the four species (*M. edulis*, *M. galloprovincialis*, *M. trossulus* and *M. platensis*). Moreover, the *Twisst* analysis shows clear cases of secondary introgression (see Figure 3 A2, B2 and C2) and helps understanding the variance in admixture rates along the genome, as suggested by our inferences with *∂a∂i*. So we think that both introgression and ongoing ILS contributed to the high rate of unresolved topologies in the *Twisst* analysis. We make these points clear along lines L390 - L403 and L539 - L544.

L. 527: mention here that these loci are ancestry-informative.
We now specify: "*except at the three most differentiated loci, which are ancestry-informative*" (L432 - L433).

L. 544: isn't that expected just because the number of loci is not the same?
Please, see our reply to your comment #2 above.

L. 544-546: see first comment above: where does that result come from? Where is the associated methods described (perhaps I just missed it)?
The associated method is described in the Materials & Methods as follow: "*We also performed a supervised ADMIXTURE analysis (in which the reference populations were provided) on the Kerguelen individuals with the KASPar SNPs (K=2 clusters and 50 replicates). We defined M. edulis and the M. platensis Chilean mussels as ancestral populations from which the Kerguelen individuals derive their ancestry. Individual ancestries are provided in Text S3 for the GBS analysis and Text S4 for the KASPar analysis*".

L. 566-571: already in the methods.
You're right, we have shortened this part: "*We then tested for genetic-environment associations in the Kerguelen by performing a RDA*" (L473).

L. 588-591: I could not follow this sentence.
It has been removed.

L. 592: "sharp". It looks sharp, but what is the uncertainty associated with these frequency estimates?
Please, see our reply to your comment #2 above.

L. 601: predicted by what?
We clarified this point as follow: *"but not in the direction predicted by their geographical origin*" (L501).

L. 604: what signal? Do you mean that there is an adaptation signal but that it is not visible?
We meant that the signal of genetic-environmental association is not visible. We now say: "*This imperfect association between genotypes and habitats supports the hypothesis that enhanced genetic drift and intense gene flow in the island grambled the signal of genetic-*

environment association at our markers rather than environmental differential selection." (L503 - L506).

L. 615: "surprisingly": why is it surprising? If you consider that you have two genetic backgrounds then it does not seem surprising that the allelic frequencies are roughly balanced when mixing the two types of individuals.
We acknowledge that our formulation was misleading. What was surprising to us is the observation of polymorphism at species-specific loci in the island. It is actually not surprising to get intermediate allele-frequencies under the hypothesis that we are mixing two genetic backgrounds. We removed "*surprisingly*" to avoid confusions here.

L. 617: local adaptation again seems to be the null hypothesis.
We replaced "*local adaptation*" by "*micro-geographic structure*" (L512).

L. 659: I don't find clear where this is demonstrated.
In the PCA of the Northern and Southern samples (Figure S2), the Kerguelen mussels cluster together with the Chilean mussels, in accordance with them being both named *M. platensis*. This is why we refer to the Kerguelen mussels as being a Southern lineage.

L. 665-669: I could not follow this sentence.
We removed this sentence as it provides unnecessary extra information, and we tried to shorten the manuscript.

L. 679: 51% is not "most". Plus this is 51% of 17%. That means essentially no support except for ILS. So I would be more cautious here.
We rephrased by "*In 51% of these resolved regions, we found clear evidence of admixture, i.e., the Kerguelen haplotypes were all (or part of) nested within a Northern clade*" (L544 - L545).

L. 708: this part is difficult to follow. The local genetic structure (between sites within the island) does not depend on the global population size of the island (it depends on drift within local populations more or less connected by gene flow).
We agree that the phrasing was misleading. We removed this sentence.

L. 733 what could be the effect of habitat on connectivity?
We hypothesize that the two genetic backgrounds may be partially associated with different habitats, and so the gradient of introgression correlates better with habitat than geography. We also think that when connectivity is not well correlated with distance, it is very easy to find one of many ecological variables that better correlates with the genetic structure than distance.

L. 765 to the end: a large section on local adaptation, whilst that hypothesis is not the most plausible. Yet it would be interesting to discuss the case of marker Glu-5' in the light of the results of the present study. Is it or is it not affected by local adaptation?
We substantially shortened this section, and attenuated the focus on local adaptation.

Reviewed by Tatiana Giraud, 2018-04-16 17:04

This is a revised version of a study reporting genomic analyses of mussel populations for investigating introgression and adaptation. Overall, I am sorry to say that I have the same main concerns as with the previous version: the text is unclear and hard to read in many places, with even inappropriate or ambiguous wording in some cases (see below for some examples, some may seem details, but it is tiring to read a ms where you have to interpret many sentences to try to understand what the authors really mean, and science reports should be precise and exact). Furthermore, there are still too many confusing details while it is difficult for a reader not familiar to the system to get a global understanding.

Most importantly, I am still not convinced by the interpretation that introgression is more likely than incomplete lineage sorting. No attempt has been made in the manuscript to explain how the methods used can disentangle incomplete lineage sorting and introgression. The manuscript should explain, briefly and clearly, on what principle is based these methods and how they can reliably infer introgression when there is so much incomplete lineage sorting.
We are very sorry the referee did not enjoy our work of revision. We endeavoured during this new revision to clarify and explain all the points that remained obscure. We also paid a special attention to better explain what we can and cannot conclude from the various analyses we used. The evidence of introgression comes from three different approaches: (i) the f-statistics in *TreeMix*, (ii) the study of gene genealogies with *Twisst*, and (iii) the study of the joint allele-frequency spectrum with ∂a∂i. We now endeavoured to explain these methods in the main text.
We received with sadness the critic that we could have overinterpreted the outcome of the analyses. Introgression was detected by these methods, this has nothing to do with conviction. The referee said "*when there is so much incomplete lineage sorting*" (ILS) which suggests a possible misunderstanding. In mussels we have a lot of "shared polymorphisms" that are mainly explained by introgression and to a lesser extent by ILS (i.e., shared ancestral polymorphism) according to our inferences. And we also have ancient ILS which resolved into alternative topologies, such that *M. trossulus* is not always outgroup of the *M. edulis*, *M. platensis* and *M. galloprovincialis* group, and the branching among the latter three species vary between gene genealogies. We now introduce the two types of ILS and explain their difference. We tried to better explain that they are accounted for in the methods and that introgression is nonetheless detected. The objective of this work was to understand the fine scale genetic structure and genetic-environment associations in the Kerguelen. If one denies both introgression (against the results though) and local adaptation (something we concur very easily with, despite against an abundant literature) to explain the results, which hypothesis remains? It seems less parsimonious to us to argue for false positives in a migration-drift model in a 120 km long island when the average dispersal distance is 50 km per generation and panmixia is usually observed over 1000s km. In addition, outliers are not random but ancestry-informative loci. Locus specific differentiation or associations with the environment is the basis of the genome scan approach. If our outliers were false positives, why should they be those loci that are more differentiated in the Northern Hemisphere? We endeavored to make this clearer in this new revised version of the ms.

Another important concern is that the whole text seem to assume there is local adaptation in the Kerguelen, while this can only be assessed by experiments; please state it local

adaptation has really been demonstrated (by experiments and not by just genetic differentiation at a few markers, which most likely reflects gene flow more than adaptation), otherwise be more cautious in formulations. For example, one main goal of the study is stated to be to assess whether introgression can promote adaptation, but I do not think this question can be addressed with the data at hand. The results show fine scale genetic differentiation and heterogeneous introgression both in the genome and geographically, but this could result from neutral processes including barriers to dispersion, while adaptive explanations are still too much put forward as explanations for these patterns.
We are again surprised by this critic. Our intention was to provide a balanced interpretation in the first version, and to tone down the hypothesis of adaptation in the second version following Thomas Broquet's concern. If Tatiana Giraud still thinks we are overselling adaptation it means we obviously failed. We therefore endeavoured to remove any place in the manuscript that could suggest we believe direct local adaptation explains the results. We even removed the idea that this was the interpretation made by many other articles with similar results. We hope the referee will be satisfied by the new version.

I do think this study is sound and interesting for a broad audience in evolutionary biology, and it would be a pity if the manuscript would stay hard to read and not convincing enough.
We agree and we thank you for your time devoted to help us improving the clarity of our manuscript.

More specific comments are found below, some explaining more precisely the general comments above.

-L27: this is the question that you can indeed address the data, while I don't think you can address the question L21.
We substantially rewrite the abstract to focus on the micro-genetic structure within the Kerguelen.

-L22: the sentence is not optimal: precisely because they are semi isolated species, *Mytilus* may not be a good model to assess the general importance of introgression.
We think that we can assess the importance of introgression relative to new mutations as a source of genetic variation in *Mytilus*, precisely because these species can still hybridize, and exchange parts of their genome. To note, this sentence has been removed in the revised version of the manuscript.

-L33: what panel?
We were referring to the panel of 33 SNPs genotyped on 695 mussels across 35 sites in the Kerguelen. This part has been reformulated in the revised version with: "*a panel of 33 SNPs, including SNPs that were more differentiated than the genomic average between Northern species (i.e., ancestry-informative SNPs)*" (L30 - L32).

-L38: it would be important to compare the level of fine scale differentiation to those found in Northern species.
You are right. We now make the point in the Results: "*Only Glu-5' revealed significant genetic differentiation among and within geographic regions, and between habitats, despite the very small geographical scale. This pattern is striking as intraspecific genetic variation*

rarely shows significant differentiation even over large distances in Mytilus (e.g. within the Mediterranean Sea, Fraisse et al. 2016)" (L102 - L105).

-L41: "we believe" is not scientific, evidence or rationale should be resented instead.
This sentence has been removed.

-L42: this appears at first sight hard to understand ("describe" is not the best term, see also L84), but actually after reading twice the manuscript, this is the best conclusion we can draw from the data: I would recommend to re-write the introduction in the abstract and the manuscript to further tone down adaptation and introduce better these ideas so that they will be clearer (eg L59, the idea is only introduced In a few words while adaptation is the focus of the whole following paragraph, that does not seem relevant to the study); similarly, the sentences L76-81 are unclear before having read the whole manuscript.
We extensively modified the Introduction by removing any reference to adaptive introgression, and by emphasizing on the coupling hypothesis (i.e., when contact zones are trapped by ecological boundaries).

-L58: local adaptation precisely usually leaves no footprints genome wide because of gene flow, only a few genes are differentiated among populations, so an approach like the one used in the manuscript would likely not detect it.
We agree with the referee, we face a standard paradox in "landscape genetics": we captured genetic-environment associations with a modest number of loci while simple theory of local adaptation predicts we shouldn't. Some authors have sometimes argued that >10% of some genomes could have been affected by recent selective sweeps. We concur this is not very realistic. Alternatively, high linkage disequilibrium and coupling between multifarious barrier loci makes it much easier to detect local adaptation with a few loci in secondary hybrid zones (found as soon as in the 80's with allozymes, e.g. in toads, grasshoppers or mussels). At any rate, following the recommendations, we have continued to tone down the hypothesis of local adaptation in the revised version of the manuscript.

-L66: "adaptation from hybridizing species" is an incorrect shortcut, it should be "adaptation allowed by gene flow from hybridizing species".
This sentence has been removed.

-A schema recapitulating species divergence on a map and history would be helpful.
We agree that this would be helpful for readers, so we added on Figure 1 a map with the localities of the GBS samples, and we indicated the estimate of divergence times between the Northern mussels.

-The figures should be either at the end or where they are cited, it is tiring to look for them in the text.
We have now placed all Figures at the end, after the Tables.

-L112-145: there are too many unneeded details and, more generally, too much focus is given to the marker Glu-5', just one historical marker that did not allow powerful inferences, you have much better power now, I would think Glu-5' is just not relevant anymore.
We shortened this part, but we kept the reference to Glu-5', as this marker motivated the present study by carrying a Northern heterospecific polymorphism in the Kerguelen mussels.

Moreover, Glu-5' correlates with the three outliers that we found (Figure S4), and shows genetic-environment association in the Kerguelen. So it is entirely part of our argument.

-Logical links are not appropriately used in many places (e.g., accordingly, as such, indeed… are not appropriately used), it renders the text hard to read because we have to interpret each time what you mean.
We revised as much as possible logical links throughout the manuscript.

-L131-132: I do not think it is relevant to introduce that adaptation has been suggested based on 3 allozymes….
This has been removed.

-L135-136: similarly, I find misleading to place the introduction of differentiation at four markers within a paragraph on adaptation; genetic differentiation at a few markers cannot say anything on adaptation in my opinion, even if associated with environmental variables.
We now say more clearly why we think that this genetic structure is at odd under neutrality, by referring to the fact that it happens at a very small geographical scale for mussels. We wrote: "*Only Glu-5' revealed significant genetic differentiation among and within geographic regions, and between habitats, despite the very small geographical scale. This pattern is striking as intraspecific genetic variation rarely shows significant differentiation even over large distances in Mytilus (e.g. within the Mediterranean Sea, Fraisse et al. 2016)*".
Moreover, we warn the reader in the following lines to not hastily conclude in the presence of local adaptation: *"As such, local adaptation was invoked to explain the fine-scale maintenance of polymorphism at Glu-5', although alternative interpretations involving admixture could not have been refuted."*.

-L137 and elsewhere: "correlated" is wrongly used at several places; correlation is a precise statistical test, it should not be used for describing associations with non-quantitative variables.
Throughout the manuscript, we changed "*correlated/correlation*" by "*associated/association*", where incorrectly used.

-L142: this is an example where the text seems to assume there is local adaptation while I am not convinced this is the case from what is presented. In addition, here and elsewhere, the formulation is too vague: "adaptation in Kerguelen populations" seems to mean "local adaptation within the Kerguelen" while the sentence would mean "adaptation in the Kerguelen compared to Northern populations" (see also L195).
We removed this sentence. Also, see our reply to your comment "*L135-136*" above.

- L148 and L617: examples where the text assumes there is local adaptation, while I am not convinced this is the case.
We removed this sentence.

-L148-149: I do not think these are questions you can/did address, while the questions formulated at the end of introduction are very important for the reader to understand the manuscript. As it stands, one expects some kinds of results or experiments that never come and one has a hard time to understand the results because one has to guess the questions addressed. The question 1 is just not addressable with the data, the question 2 is not well

formulated, in particular it is not that introgression “ease the investigation” but instead “reflect”, this is very different. I would recommend the following questions: 1) is there genetic differentiation at fine scale in the Kerguelen? 2) is there association of genetic differentiation with environmental variables and/or geography? 3) can we detect footprints of introgression from Northern species and disentangle them from incomplete lineage sorting? 4) in this case, does introgression contribute to the fine scale genetic differentiation in the Kerguelen? 5) in this case, can we infer whether the heterogeneity in introgression is due to local adaptation or barriers to dispersal? (although not sure either you can address this last question).
Thanks for your suggestions. We followed them and re-write entirely this part. However, we changed the order of the questions by first asking whether we can detect introgression in the Kerguelen mussels. This is because we first need to present the phylogenetic position of the Kerguelen mussels among the Northern species, and highlights its divergence history, which was unclear before this study.

-L164-167: “more” than what?
We agree that these formulations were misleading. We rephrased the first sentence as follow “*the Kerguelen Islands harbor a Southern lineage of mussels, related to M. edulis*” (L119) and we removed the second one.

-L174: a map would be useful.
We now provide a map in Figure 1, and we refer to it along lines L135 - L136: “*We used samples collected from eleven localities in the Northern Hemisphere (Figure 1, Supp. Info. M&M and Table S1)*”.

-L179: how can samples be “representative” of a population? Do you mean non-admixed?
We meant that the samples we used have been previously established to be representative of monospecific panmictic patches. We now clarify in those terms: “*samples have been shown to be representative of monospecific panmictic patches of the Mytilus edulis species complex*” (L140 - 141).

-The Table and Figure legends are not clear enough: they should be understandable by themselves, while many abbreviations and population codes are not defined in the legends, genus names should not be abbreviated there.
We improved the legends as much as possible as you suggested. However, we did not redefine for each Figure/Table the population codes, as they are already described in Tables S1 and S3 (but we refer to those in the legends).

-L195-196: you cannot address this question
We replaced “*adaptation*” by “*micro-geographic structure*” (L154).

-L206: delete “any” and “retained” .
Changed.

-L210: I do not understand why only 30 were highly differentiated as all have been retained for being differentiated.. do you mean a kind of threshold? What threshold?
We used several criteria to select SNPs for the KASPar panel: (i) polymorphic within the Kerguelen, (ii) highly-differentiated between specific pairs of Northern populations, and (iii) passing the Illumina Assay Design Tool score. The combination of all three criteria was hard

to obtain, and so sometimes we had to choose SNPs not highly-differentiated in the Northern hemisphere, but still polymorphic in the Kerguelen and passing the Illumina Tool. We actually used these SNPs as background loci, which were compared to the highly-differentiated loci.

-L239: “artificial chromosome” is not the appropriate term.
Replaced by “*composite chromosome*” (L201).

-L259 and L377: The “Chilean” mussels have never been introduced.
Please, see our replies to Thomas Broquet’s comments above.

-P11 and P20: we really need to understand how introgression can be distinguished from incomplete lineage sorting: on what principle? Because this inference is at the core of your study, it is not sufficient to refer to the publication of the software, you have to convince here the reader that you have the power to reliably infer introgression. This issue should even be presented in introgression instead of adaptation.
We agree with you that this point should be convincing for the reader.
First, it is important to note that we relied on multi-locus inferences with *TreeMix* and ∂a∂i: the genealogical information across many independent genomic regions is combined to test for gene flow, and so inference is much more reliable than when using a handful of markers. This multilocus information is all the more important because, as highlighted by *Twisst*, the different parts of the genome have proved to capture different aspects of the divergence history.
Second, as it would have been out of the scope of the paper (and too long) to provide full explanations of the different methods, we preferred to return the reader to recent papers that illustrate them. In the Materials & Methods, we specified for *TreeMix*: “*TreeMix jointly estimates population splits and subsequent admixture events based on the F-statistics introduced by Reich et al. (2009), and commonly recognised as a valid support for admixture (e.g., in humans: Pickrell & Pritchard 2012, Wong et al. 2017)*”. For ∂a∂i: “*In complement to the TreeMix analysis, we used a model-based approach implemented in ∂a∂i v1.6.3 (Gutenkunst et al. 2009) to explicitly test for the presence of gene flow. This method has proven useful for distinguishing ancestral shared polymorphism between recently-diverged species evolving under strict isolation from a scenario including migration between them (e.g., in Arabidopsis: Hubert et al. 2014; sea bass: Tine et al. 2014; poplars: Christe et al. 2017; whitefish: Rougeux et al. 2017).*” And for *Twisst*: “*For each haplotype locus, the relationships between the Northern species and the Kerguelen 259 population were then quantified using Twisst, a tree weighting approach that has been successfully applied to Heliconius butterflies to evaluate the support of different phylogenies around colour pattern loci (Van Belleghem et al. 2017*).
Third, we emphasized the results obtained with the new *∂a∂i* inferences as those are very clear that the Kerguelen mussels experienced multiple rounds of admixture with the Northern species. We wrote: “*Our reconstruction of the divergence history with ∂a∂i consolidated evidence for admixture between the Kerguelen mussels and each Northern species, although pointed out to a periodic connectivity with several periods of admixture. In all three comparisons, the models without gene flow were consistently the worst supported (“Strict Isolation”, Table S5)*”.
Finally, we listed the multiple evidence of admixture at the end of the Introduction: “*we also robustly detected past introgression events between Northern and Southern mussels by: (i) testing for admixture with genome-wide allele frequency data, (ii) reconstructing gene*

genealogies at a small chromosomal scale and (iii) inferring their divergence history from the joint site-frequency spectrum".

-L278; BTGS and AIC are not defined.
We now defined BFGS as follow "*BFGS -Broyden–Fletcher–Goldfarb–Shanno- algorithm*"; and we replace AIC by "*Akaike information criterion*" throughout the manuscript.

-L365: the clade does not seem that divergent compared to others.
Agreed. We removed the word "divergent".

-L469: "adaptation in *M. edulis*": another example of unclear sentence where we have to guess what you mean: adaptation in M. edulis compared to other species as the sentence seems to say? Or local adaptation within *M. edulis*? Actually, most genes are "involved in adaptation", even housekeeping genes under purifying selection… in many other places, the term "selection" is used while you likely mean "positive selection" or "divergent selection" or "specific adaptation", this is different…
We removed this sentence.

- same comment L532 "selection in the island": compared to elsewhere or local adaptation?
We specified "*differential selection in the island*" (L438).

-L474: "from which they derive": incorrect wording: they do not derive from *M. edulis* but from a common ancestor, or I did not understand and the sentence is unclear.
You're right. We changed the sentence: "*These results suggest that the Kerguelen mussels have a genome of mixed ancestry, mainly dominated by M. edulis, from which they are closely-related, but with which they also have probably secondarily admixed*" (L409 - L411).

-L476: "negligible": so is it really reliable?
We totally reformulated this section, and discuss in more details the results of the different methods to detect introgression (L411 - L420).

-L499: awkward wording (a site cannot show genetic structure).
Corrected by "*genetic structure among several sites was detected*" (L427 - L428).

-L580 Looking at the map of the Kerguelen with the fractal coast, the genetic differentiation among sites does not seem that surprising, in contrast to what is said in the introduction about marine organisms? And it lets think that longitude is not the best variable to take into account geography in the analyses.. it should rather be the coastal rugged distance.
We acknowledge that geographical coordinates may not be the best proxy to estimate distance between sampling sites along the coast. Therefore, we were less affirmative in the Results and Discussion about the absence for a role of geography in the patterns observed. We wrote "*This suggests a limited effect of geography on the genetic structure of the mussels, although coastal distance would be a better proxy to account for spatial structure in the island*" and "*Another caveat is that spatial distance between sampling sites may be better described by oceanographic distance than geographic coordinates, and so we may be overestimating the influence of habitats.*".

-L582 and elsewhere: replace "*Macrocystis*" by "*Macrocystis* presence" otherwise the

sentences do not make sense. And actually, *Macrocystis* presence could also be linked to dispersal routes rather than indicating local adaptation of mussels?
We replaced "*Macrocystis*" by "*Macrocystis presence/absence*" throughout the manuscript. We think that *Macrocystis* kelps are locally and partially associated with one of the genetic backgrounds in the Kerguelen Islands, but this does not necessary mean there is direct selection on mussels.

-L621 and elsewhere : do not refer to panels without figure numbers.
Changed.

-L621 and 641: they are not "control" (for which treatment?), they are background loci.
We replaced "*control loci*" by "*background loci*" throughout the manuscript.

-L629: I would delete all reference to Glu-5' or most of it, this sounds really anecdotal.
As we argue above (see our reply to your comment "*L112-145*"), we think it is important to include Glu-5' in the study.

-L646-654: this should be introduction instead.
We removed this sentence from the revised manuscript.

-L674: this has not been shown, stochastic processes could also lead to such heterogeneity, or argue why not.
The reconstruction of divergence history with *∂a∂i* is precisely intended to model the effects of both genetic drift and migration on the site frequency spectrum. Based on model comparison, we then evaluated the likelihood of different models, including "divergence in strict isolation" and "divergence with migration". Moreover, we also modeled two classes of sites: those evolving under neutrality with a migration rate *m*, and those included in an interspecific genomic barrier with a null migration rate. As the best fits to our data were models with heterogeneity of migration rates (i.e. two classes of sites), we are confident that introgression occurs at variable rates across the mussel genomes.

-L679-680: not convincing, this can be incomplete lineage sorting.
While we agree that incomplete lineage sorting is substantial in *Mytilus* mussels (both ancient and ongoing ILS), we found several lines of evidence that admixture also contributes to the pattern, including by reconstructing gene topologies with *Twisst* (as illustrated by the gene genealogies on Figure 3 A2, B2 and C2).

-L688 and elsewhere: do not refer or figures and Tables in the discussion, the results should have been clear enough.
We removed the references to Figures and Tables in the Discussion. However, Figure S7 and Figure S8 are supplementary figures appearing only in the Discussion, and we used them to discuss specific points. So they were retained in the Discussion.

-L696-700: "relies on" would be more correct than "highlights".
Changed to "relies on" (L564) and "based on" (L568).

-L705: there is no such thing as "most significant" with a given significance threshold in statistics… replace by "strongest structure".

Thanks for spotting this mistake. We replaced "*most significant*" by "*strongest*" (L573).

-L714: I am not convinced the simple longitude analysis allows to say that you have controlled for spatial effects.
Please, see our reply to your comment "L580" above.

-L721: "substrata" is a Latin plural, use substratum or, better, substrate, as you used elsewhere.
Changed (L587).

-L765 and L773: I am not convinced this has been shown.
We tone down the references to adaptive introgression in this section.

-L785: evidence is never plural.
Changed.

-L788-L792: not sure this is the best conclusion given I am not convinced there is local adaptation.
We removed this part.

-L794: a conclusion is missing that would explain the interest of the study for scientists not working on mussels.
We added a more general conclusion: "*Overall, our work underlines the opportunity of using non-equilibrium introgression clines to assess genetic connectivity in natural populations and warns against systematically interpreting genetic-environment association as signal of local adaptation*".