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Response to Editor

Dear Dr. Jacob,

We have resubmitted a modified version of the manuscript entitled "Gene expression plasticity and frontloading promote thermotolerance in Pocilloporid corals" (https://doi.org/10.1101/398602), for recommendation by PCI Ecology.
We are grateful to the editor and the two reviewers for the work done on the two rounds of revision. We propose a revised version of the manuscript that includes notably new statistical analyses of the variables affecting microbiota composition, as well as a more detailed paragraph for discussion on functional analysis in the light of literature on corals. Since the first submission of this manuscript, the *Pocillopora damicornis* genome paper has been submitted to BioRxiv (https://doi.org/10.1101/698688) and is now cited in the present version (line 363).
You will also find below our responses (in blue) after each point that were raised by the reviewers. To ease the reading, the reviewer's comments have been numbered and reported in the revised manuscript to locate the corrections using the "search" command, rather than referring to line or page numbers that may differ according to the manuscript version.

We hope that the new version of the manuscript would be suitable for recommendation by PCI Ecology.

We look forward to hearing from you.

Yours sincerely,

Eve Toulza for all co-authors

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Dear Eve Toulza,
Your preprint, entitled Gene expression plasticity and frontloading promote thermotolerance in Pocilloporid corals, has now been reviewed. The referees' comments and the recommender's decision are shown below. As you can see, the recommender found your article very interesting, but suggests certain revisions. We shall, in principle, be happy to recommend your article as soon as it has been revised in response to the points raised by the referees.

Round #2

Author's Reply:

Decision
By Staffan Jacob, 2019-05-17 08:35
Manuscript: https://doi.org/10.1101/398602 version 1
Decision on manuscript

Dear Eve Toulza,
Following the submissions of your revisions, the two reviewers and myself agree to acknowledge helpful clarifications of several aspects of your manuscript. The reviewers still pointed out a number issues that should be dealt with, especially regarding the introduced concepts on the link between plasticity and adaptation and the way analyses were performed. I thus invite you to carefully use the reviewers' comments to revise your manuscript.
All the best, Staffan.

Review #1

Reviewed by Samuel Pichon, 2019-05-13 11:35
First, I am grateful to Kelly Brener-Raffalli and colleagues who submitted a revised version carefully considering all comments made by Dr. Jacob and the reviewers. They largely contributed to the clarification of the experimental design, reworked at the scales of species and primarily of genotypes for both corals and symbionts, refined the scope and importance of this study by nuancing the link between thermal regime and response to experimental stress in these different coral species. They also provided supplemental data that are important and helpful. I now found that the scientific community would benefit from reading this work.
We would like to thank again the reviewer for his helpful comments and suggestions that greatly improved the manuscript.

Minor comments or typos:
l. 57 a role for the phosphorylation of histone and DNA methylation
1.1. This has been added here in the abstract and mentioned in the modified paragraph for functional analysis (see also response 1.5).

e.g. l. 86, l. 279, l. 464, l.669 (and throughout the manuscript) may delete ";" before references and referencing could be improved

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1.2. This has been modified.

l. 133 “hyacinthus” instead of “Hyacinthus”
l. 141 can tolerate “symbionts” instead of “partners (bionts)”
l. 224 unit is missing for salinity
l. 284 space before comma
l. 408 missing space after “PSH05”
l. 434 “sign” instead of “signs”
l. 448 missing space after “explained”
l. 460 double space
l. 477 missing spaces
l. 507 place the second “genes after the figure
l. 542 “BLASTX” instead of “blastx”
l. 550 missing space after “272”
l. 615 missing space after the comma
l. 683 missing space between “a” and “more”
l. 857 “DESeq2” instead of “DEseq2”, missing space between “fold” and “changes”, space between “a” and “nd”
l. 860 missing space between “fold” and “changes”

1.3. Thank you very much for your attentive reading. These typos have been corrected.

Major comments:
Microbiota
I agree with the authors that the study on symbionts do not represent the main scope and results of this study. But in their Microbiome study published last year, they actually show that host genotypes and temperatures differently influenced Symbiodinium and bacterial microbiota. To my point of view, the authors in the present study could test the effect of host clonal line, Symbiodinium genotype, acclimation temperature and their interactions on the differential abundances of each OTU. The question behind this are: Is the host regulates its microbiota when “stressed”? What is the proportion of symbionts transmitted vertically? Microbiota can participate as genetic and non-genetic element in the host phenotypic plasticity.

1.4. This point was also raised by the second reviewer (see response to 2.5 and 2.10, 2.11 and 2.15). In the new version of the manuscript, we now provide statistical analysis of the effect of each variable (locality of origin, genotype for host clonal line and temperature treatment) on bacterial alpha diversity (using GMM instead of one-way ANOVA as requested by the second reviewer) as well as beta dissimilarity in composition (using MANOVA). Concerning *Symbiodinium* genotype, as it was completely confounded with population of origin (C in all NC samples, D in all Om samples), we did not test it specifically. The temperature treatment had no significant effect on bacterial (nor *Symbiodinium*) composition. We thus believe that this experimental design is not well suited to test the contribution of microbiota composition to host phenotypic plasticity and response to heat stress as we analyzed the changes visible in host transcriptome before the physiological collapse and probably before microbiota changes may occur.

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Discussion

I acknowledge the authors who streamlined the discussion, it helps to focus on the originality of the data. However, I think we now need a longer paragraph emphasizing on the functional aspects. Some aspects of the discussion, relative to other coral species could be expected. This would help to put these results in a wider picture.

1.5. The small paragraph summarizing the functional analysis has been developed with the most relevant elements from the literature as suggested.

Review #2

Reviewed by Mar Sobral, 2019-04-16 17:47

I already pointed out in my previous revision that this work is very interesting and important. The writing is also very good. But, although improvement has been made to the manuscript in comparison to previous version, I still disagree with a couple of important conceptual as well as analytical issues (that I had already pointed out in my previous revision). So, my recommendation is again a major revision. This I because I believe there is conceptual confusion that wont help understanding the implications of the work. Second, the analytical limitations preclude the exploitation of results to their maximum potential.

Thank you for pointing out the relevance of the study and the improvements that have been already made to the manuscript. We would also like to thank the reviewer again for attentive reading and useful comments. We now provide a new version of the manuscript after reworking the conceptual background, and running new statistical analyses for microbiota structure. We hope that the new version is more clear and statistically sound.

CONCEPTUAL ISSUES

Line 43-44. It is not possible to interpret this work in the context of differences between localities, and I , the other referee and the editor explicitly said in previous version. So, line 40-41 is not what is tested in this research. Same for lines 79-80.

2.1. We agree that we cannot interpret this work only in the context of thermal regimes of origin, and we are grateful for constructive comments in the two successive rounds of revision that greatly helped to refocus the conceptual background in line with the implications of the work. The abstract has been thus modified as suggested. We nevertheless discuss differences in thermal environments of origin as one of the factors affecting coral tolerance and response to heat stress. We thus argue that it is still relevant to present data from the literature that showed a link between thermal regime and tolerance to heat stress but we rephrased the sentence to avoid any ambiguities.

Line 83-94. In my opinion this is wrong, as I already explained in my previous revision. Refocuse it more in line of what you say in lines 94-97.

2.2. The paragraph has been streamlined and refocused to stick to the design of the study as suggested and one reference has been added (Hugues 2017).

Line 98-100. No, you cannot discriminate between both processes with molecular approaches.

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And in any case, both would be entangled. Also, you have not data to test whether local adaptation is going on etc.

2.3. The sentence has been modified to delete the link between molecular analysis and acclimations vs. adaptation processes and stick to coral response to heat stress.

Line 114-117. That is right, and that explains my two previous comments. Line 191-196. This is a good prediction to make, but a different story is to say that testing that is the purpose of the research (because in that case design would be not ok).

2.4. We agree that we are not in the perfect situation with two different yet undifferentiated populations from the same genetic background and living in contrasted thermal regimes to test these predictions. Nevertheless, we argue that different strategies may have been selected in these two populations from the same morphospecies that belong to the same functional group and that may be related to differences in temperature ranges as already reported in the literature. The sentence has been slightly modified to replace "promote the evolution" by "select for". Overall, to clarify the scope and importance of this study, we reworked at the scales of species and genotypes for both populations and greatly nuanced the link between thermal regime and response to experimental stress as acknowledged by the first reviewer.

ANALYTICAL ISSUES

Line 334, performing one-way anovas for alpha diversity is not correct here, as I said in my previous revision. You have 42 alpha values right? Therefore, including site, colony within site, treatment and treatment*site should be possible and they need to be factors explaining alpha diversity at the colony level. Maybe you can assess how stress affects beta diversity whether you asses beta diversity within colonies (among replicates) and then you can analyze it as a response variable including treatment, site and their interaction in the model (if I don't misunderstand u can have n=12 values of beta diversity, because you can asses it among replicates within colonies and different betas for different treatments.). If I am wrong, please explain why and make it clear in the manuscript that no such an analyses are possible to run.

2.5. We thank the reviewer 2 for this cognent proposition. As suggested, we ran a series of models including site, colony within site (as a random factor), treatment and treatment*site. We chose the best model for each alpha diversity metric (i.e. Shannon and Chao) according to the Akaike criterion and checked for possible significant effect of explanatory variables in these best models. We found that the site explained part of the overall variance for the Shannon index but none of these factors significantly explained the variance for the Chao index, confirming previous results. This is now included in the new version of the manuscript.

Concerning beta diversity, similar analyses cannot be run as we do not have individual values but a distance matrix. In the new version of the manuscript, we performed Multivariate Analysis of Variance (MANOVA) on sampling locality, genotype and treatment. This has been modified and clarified throughout the text.

As in the previous versions, whereas locality of origin and genotype were significantly correlated with alpha and beta diversity, temperature treatment had no effect on microbiota composition.

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MINOR COMMENTS

Line 160-161. You do not test the role of microorganisms for this, is misleading mentioning it here?
2.6. Yes it may be confusing indeed, we replaced "adaptability" by "response to stress" to stick to our experimental design.

L 238 and 244. You say 8 tanks per colony first, and later 4. I understand is 4.
2.7. You are right, there were 8 tanks in total, 4 per locality. This has been modified.

Line 388 and 392, change condition for "treatment".
2.8. This has been modified.

Line 414. Then, what is the name of this species? Again, this kind of means that design mixes species with localities, that is yet another reason NOT to interpret results in function of localities differences, but rather only in function of treatment differences.
The species definition in *Pocillopora* is very complex and relies on morphological criteria that do not completely match with molecular markers. Types 4 and 5 (represented in New Caledonia samples) consistently appeared as 'damicornis-like', while various morphospeciesincluding damicornis-like morphotypesare included in types 1, 3 and 7 (represented in Oman samples)(Pinzon et al. 2013). This lineage does not thus correspond to a peculiar species. We therefore indicated the Primary and Secondary Species Hypothesis to avoid confusion.

Line 417. Given this result is would make more sense to analyze either NC2 or NC3 all throughout the paper. It doesn't make a lot of sense to have a replicated genotype but no others. It is likely that differences between NC2 and NC3 can support some interpretations, in comparison with differences among other colonies (I am not thinking about anything in particular right now).
2.9. This is in fact also one reason to group all replicates for all colonies in the DGE analysis between temperature treatments for each locality. In the case of lower transcriptome variance between replicates in one group compared to the other (as it could have been the case with replicated genotypes), it would be expected to detect with more sensitivity differential gene expression between treatments and thus to artificially increase the number of DGE in NC. As we identified a much higher number of DGE in Oman colonies, we are thus confident that this higher transcriptomic plasticity reflects true biological differences with colonies from New Caledonia.

Lines 443-445. This needs to be tested trough stats. I had already pointed this out in my previous revision.
2.10. This has been tested using MANOVA on the dissimilarity matrix between pairs of samples (see also response to comment 2.5). This has been specified in this section of the text to clarify the significance of the observed grouping.

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Line 449-455. This analysis is not correct, as I pointed out in a previous comment.

2.11. We replaced one-way ANOVAs by GLM analysis (see also response to 2.5). The text has been modified accordingly.

Line 462-468. Why haven't you analyzed this at the OTU level? And just used "types"?
2.12. As the four OTUs represented only two different *Symbiodinium* clades (types) with very few polymorphism within each type, we choose to present the results at the type level after taxonomic affiliation. One single type was dominant within samples from each locality with no changes nor between in situ and experimental conditions, neither after temperature treatment. For Oman colonies, this also corresponded to the same dominant OTU (cluster 2). In NC samples, the proportion of the two OTUs (corresponding both to *Symbiodinium* type C) was different between the two genotypes but again no changeswere observed nor between in situ and experimental conditions, neither after temperature treatment (MANOVA p=0.859). We feel that the present version of the Figure 3 would be more clear, but we can prepare a new version at the OTU level (see below) and/or provide corresponding OTU table if the reviewer think it is more relevant.

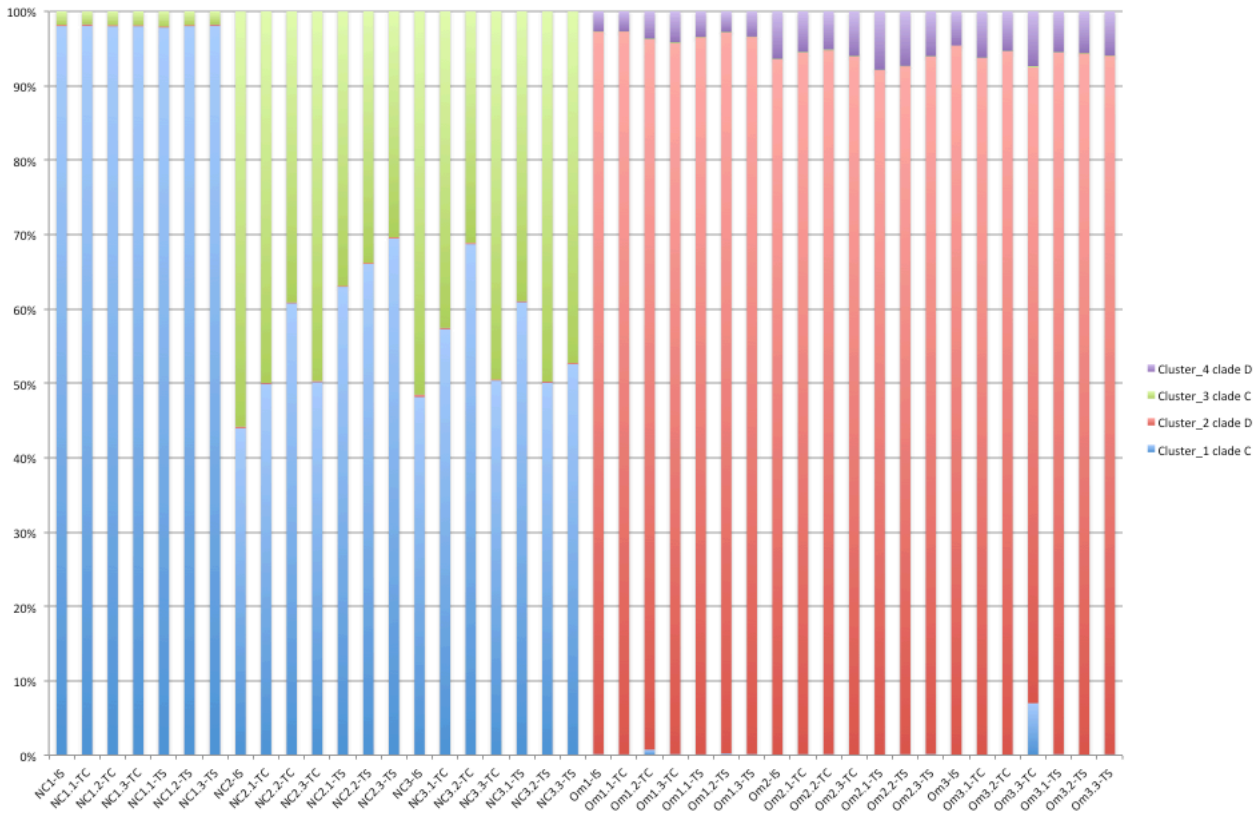

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Figure 7. I believe it is best to display it by colony. In the same colors and shades but making the differences between colonies.

2.13. Since the most important result in our study is the plastic difference in gene expression between colonies from the two localities (NC and Om) we believe this is most appropriate way to represent our results from the DAPC. In fact, each colony is represented by a vertical dash on the x axis. Representing each colony separately would require representing the DAPC using another dimension in the graphic. In this case the difference in plasticity between corals from different sites is not well illustrated visually. We thus prefer to keep the Figure 7 as it was in the previous version of the manuscript.

Line 536-538. Cool!

2.14. We also believe that the difference between colonies of the two localities in the magnitude of transcriptomic changes is a major result here, together with frontloading for some transcripts involved in response to stress.

Line 736-737. I remain unsure about these results as in previous versions.

2.15. We indeed could not identify a significant effect of treatment on bacterial composition (MANOVA p=0.761), whereas localities and genotypes had a significant effect (see also response to comment 2.5). For *Symbiodinium*, only one single clade was dominant in each locality with no differences between genotypes and no changes in response to heat stress (see also response to comment 2.12). We hope that the statistical analysis of the results is more clear in the new version of the manuscript and change “stable” for “similar” as only two points of the kinetics have been analysed here.

Line 749-751. Empty sentences as it is. Remove or develop if important.

2.16. It has been removed.

Line 754-757. This is not necessary to be evolutionary meaningful. As I had already pointed out in my previous revision with references included (non-genetic inheritance affecting evolutionary dinamics).

2.17. The work of Jablonka et al. on epigenetics priming and non-genetic inheritance has been discussed in the present version and additional references have been added. The conclusion has also been streamlined as suggested in the new version.

Line 819 Change line for row.

2.18. This has been modified.

Fig 6. Isn’t it misleading? Shouldn’t be all genes plotted and later, differences in density between quadrants could tell us whether there are more genes which behave similarly or differently between localities etc?

2.19. This analysis aimed at illustrating the higher differential expression levels for Om compared to NC among genes displaying the same pattern (over- or under-expressed in colonies from both localities). This result is also supported by statistical analysis. The sentence has been clarified.