June 05th, 2017

Professor Michael Singer
Academic Editor, *PeerJ*

Dear Editor,

We appreciate the time and efforts of the reviewers in this second review, as well as their thoughtful comments on the manuscript. As detailed below, we have addressed all concerns and suggestions pointed out by the Reviewer 1 and yourself. We hope you find this version acceptable for publication in *PeerJ*.

Sincerely yours,

Azucena Canto
Researcher of the Centro de Investigacion Cientifica de Yucatan (CICY)

On behalf of all authors

Responses to reviews

To help you follow changes made to the manuscript, our responses are given as sentences in blue below the passages corresponding to comments and suggestions (italicized text here) made by the reviewers. Line numbers refer to the revised version.

Editor's Comments

MAJOR REVISIONS

This new version of the manuscript is much improved, although it still needs some substantial work to make it ready for publication. I agree with the new comments and suggestions from reviewer 1, and I ask that you address each of these as thoroughly as possible. (I did not send it back to reviewer 2, who was generally positive the first time around.) In order to avoid another round of minor revisions after this one, please do try to have a native English speaker read the final draft of your revised manuscript.

As detailed below, we have addressed all concerns raised, and incorporated changes suggested to the manuscript. The language has been reviewed by the Elsevier Language Editing Services (see Elsevier letter below).

Reviewer 1 (Anonymous)

I suggest that the authors have an colleague who speaks English as a first language also read through the article, as there are still many grammatical issues throughout, especially in the newly added sections (e.g. lines 45-48, lines 221-233 etc etc).

Done. The manuscript has been edited by Elsevier Language Editing Services (see Elsevier letter below).

Experimental design

The new statistical analyses still require improvement before publication. Specifically, Figure 1 and the accompanying analysis or the rationale and interpretation still require improvement. Specifically, the goal of this analysis and its interpretation are unclear and I think perhaps incorrect. The current analysis suggests that sampling done at the drop level suggest that the community should have ~25 species and was nearly complete. By differentially pooling these data into matrices summarized by plant species (supplementary information), the authors suggest that increased sampling of plant species or genera would dramatically increase the number of yeast species in the community. However, this logic seems not quite correct and not justified in the manuscript. Instead, it is my understanding that the authors' goal was to compare diversity among plant species, which would require a different input matrix to iNEXT. If within-plant species diversity was of interest (which I think that it is), the input files would be either drop level or plant-level measures of yeast diversity from a single plant species which would be used for calculating sampling curves for each species. Individual curves from each species could be compared by examining overlap of confidence intervals and could address if different plant species host more or less diverse communities. But if I am incorrectly interpreting this figure and its goal is to highlight that communities are diverse, I think that the different sampling levels are unnecessarily confusing and generate misleading results due to sample pooling.

We are agreeing in that the rationale and interpretation of the analysis accompanying Figure 1 were not concordant with the goal stated in the text and that is why results, Fig. 1 and the respective discussion were confusing. The goal of our diversity analysis was to know how diverse the community of nectar-living yeasts is in a tropical host plant community following a hierarchical sampling design and comparing between different levels in that hierarchy. We have thoroughly revised and edited all those parts of the text related with this analysis to give a concordant context for the diversity analysis conducted. We think that our analysis of diversity is correct, the problem rather being that it was not concordant with the expressed objective. The specific question for the analysis has been thoroughly edited to clarify this point. We have also verified that results and their interpretation were concordant with the goal of the analysis. Changes have been incorporated in Abstract, Introduction, Results and Discussion sections (Lns 5-7, 14-17, 21-22, 63-65, 336-346).

In addition, more details are necessary to fully describe the regression presented in Table 3. What is a power regression and why was it used?

We use a power regression model because the relationship between response and explanatory variables follows a power pattern (e.g., the response variable is proportional to the explanatory variable raised to a power). A comprehensive explanation of what is a power regression model and why it was used to data analysis was added in the Materials and Methods section (Lines 225-236, 251-252). Table 3 was improved and now it shows a more complete information of the Type III least-square analyses and Akaike Information Criterion (AIC) values, degrees of freedom, which help to a better understanding of the models testing, the statistical significance of main factors and interaction term (see Table 3, title and values).

It is my understanding that a power regression had an exponential term in the regression?

It is correct; a power regression has an exponential term. In the Figure 3, power regression equations have been supplied with their respective exponential terms for regression relationship (yeast cell density versus sugar concentration).

The reference cited (Zahn 2010) does not mention power regression in the paper.

It was a misplaced reference; Zahn 2010 is the reference on which our Type III square sums are based. This reference has been now correctly placed (Line 247-248)

Was the 'total AIC ' the AIC of the model with no predictors included? For the model with interaction term, were previous factors (Yeast, Plant, Cell density etc) also included in the model? I believe that they should be but the df do not change as a result of changing predictors so perhaps the authors need to check the values in the table?

The "total AIC" corresponded to the AIC estimate when the model is saturated (i.e., all terms are included). The AIC analysis drops arguments from the full model one at a time and successively compares the original model to the reduced one. For the comparison between full model and model without interaction term, all previous factors were included. We have re-edited the Table 3 to clarify the changes in degrees of freedom and AIC values. We have also included brief information in the text about the way that AIC analysis works (Lns 251-252).

Validity of the findings

In addition, there is one thing that raised a question in my mind: nectar samples had very high cell densities (supplementary table 1), so it seems surprising that the authors only recovered between 1-5 colonies per plate (lines X_ , particularly if the total nectar drop was plated. Do the authors have any hypotheses to explain this? Also, other cell counts indicated extremely high cell densities in some samples but no colonies were recovered. Perhaps it the case that only a few (1-5) morphotypes were described per sample, but the total number of colony forming units was much higher?.

In some agar plates, a continuous mass of microbial growth was found, being difficult to count colonies; in other cases, very few colonies grew, therefore, for each agar plate we isolate the different morphotypes. We have clarified this important point in the Materials and Methods section (Lns 119-124).

The authors suggest that there is a yeast x plant species interaction in effects on sugars, but could there be an interaction between yeast density and plant species, where yeasts differentially

influence nectar sugars in some plant species and not others? This seems like a more appropriate analysis, since not all yeast species were observed in each plant species

The goal of this analysis was to address the main effect of yeast cell density on sugar concentration, taking into account that there are yeast species that are more frequent in some host plant species than in others. That is why we used the Type III square sums to examine the significance of each partial influence of yeasts on nectar sugars, that is, the significance of yeast cell density with all the other effects in the model. Given that if a significant interaction is present, the main effects should not be further analyzed, keeping the yeast cell density as main effect in the model.

Comments for the Author

Thank you for your hard work revising this manuscript. The manuscript is much improved and the addition of Figure 4 is welcome. These data are extremely valuable and should be shared with a broader audience. I encourage the authors to aim to improve presentation and rationale for the analyses, particularly Fig 1 and accompanying discussion (lines 327-329 and surrounding) and the new regression analysis as noted above.

Thank you for your comments. We have addressed as thoroughly as possible all suggestions and comments. We have edited the discussion to improve the analysis for yeast diversity. We have changed the suggested lines and surrounding to become a more concordant discussion (Lns 336-346).

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To whom it may concern

The paper "Nectar-living yeasts of a tropical host-plant community: diversity and effects on community-wide floral nectar traits" by Azucena Canto was edited by Elsevier Language Editing Services.

Kind regards,

Biji Mathilakath
Elsevier Webshop Support

(This is a computer generated advice and does not require any signature)