

Rebuttal letter

Editor comments (Paolo Giordani)

MAJOR REVISIONS

Please, when you will revise the manuscript, pay particular attention to the remarks by rev#2 about the use of the term “gene flow” and to recommendations by both reviewers who ask for a general improvement of the discussion.

> Dear Editor, please find enclosed our revised manuscript. We are thankful to both reviewers for their constructive criticism that has substantially improved our manuscript. Following their suggestions we have now improved our introduction and discussion, and carefully reviewed our Datasets and figures. Please find our detailed responses below.

Best regards,

Dr. Rodolfo Jaffé

Reviewer 1 (Anonymous)

Basic reporting

The study provides a comprehensive review of how landscape effect, particularly, anthropogenic disturbances influence the gene flow in tropical ecosystems. By analysing the studies across 157 tropical species, the authors conclude that the use of individual-based approaches rather than a community-level investigation is appropriate to understand the Landscape Effect on gene flow. Furthermore, the authors review the methodologies often used in the concerned field of investigation and suggest that next-generation sequencing and maximum likelihood-based analysis is appropriate to generate comparable results. In their conclusion, the authors highlight the lack of information for tropical species and emphasize that the future investigations must include tropical species across geography.

The topic of the investigation is definitely interesting, however, I feel that the paper does not introduce the subject adequately. As a result, the core question of the present investigation appears blurred. I have mentioned a few points below, which, I hope, would help to improve the manuscript and bring out the motivation for the investigation.

1. Authors need to expand on the Landscape Effect, with and without anthropogenic disturbances
2. Broadly discuss how the landscape effect influence gene flow using
3. Describe and link functional connectivity to the above discussions
4. Expand Isolation by Resistance, its mechanism and outcome in connection to the Landscape Effect
5. Optionally, you can introduce a flow chart describing the link across all that will be helpful in visualizing the importance of the present investigation

> We are happy to know the reviewer finds our study interesting and thank her/him for the constructive criticism. We have now followed her/his recommendation including a new paragraph in the introduction where we expand on landscape effects on gene flow, isolation by landscape resistance and functional connectivity.

Experimental design

The methods for literature survey and analysis seem fine to me. However, I have concerns regarding the study design and in formulating the objectives of interest. Please see the comments in "Basic reporting"

> **We have now clarified our main objective at the end of our introduction: “Aiming to unravel the main drivers of functional connectivity in tropical landscapes, here we compiled...”.**

Validity of the findings

The goals set out in the Introduction needs improvement for clarity
Similarly, bring a comprehensive outlook to the subject in the Discussion section

Moreover, the work, in its present form, seems incomplete and requires clarity on a conceptual platform to connect the ideas appropriately

> **We have now clarified our main objective at the end of our introduction (see previous comment) and improved our discussion following the suggestions made by Reviewer 2.**

Comments for the Author

The title seems quite uninformative. I suggest the authors modify the title to justify the objective of the study

> **We disagree and would like to keep our current title. The Editor and Reviewer 2 also seem to be OK with our title. We invite Reviewer 1 to watch the film by Woody Allen that inspired the title: <https://www.imdb.com/title/tt0068555/>**

Line 51 You may remove the 'comma' before ‘found’

> **Removed the comma as suggested.**

Line 55-68 The phrases ‘habitat degradation’ and ‘environmental change’ have been used in association with the biodiversity response. Do these phrases convey the same message or they underlie different concepts. Add or modify the sentence for clarity

> **We have now rephrased “environmental change” with “habitat loss and fragmentation” to improve clarity.**

Line 76-77 In the earlier paragraph, authors have described community- and population-level studies for biodiversity response to habitat degradation. However, here, they have mentioned population and individual level studies. Please clarify or modify!

> **We have now used the words “populations or individuals” when referring to units of analysis. We clarify that both units of analysis refer to population-level approaches.**

Reviewer 2 (Anonymous)

Basic reporting

Overall, this manuscript deals with a very interesting and timely topic that is cross-disciplinary and definitely within the scope of the journal. This specific topic has also not been reviewed before. The intro nicely outlines the motivation for the study. Indeed, the entire manuscript is very well written.

Experimental design

I found no major flaws in the analysis, but I think more insights could be gained from the analyses.

Validity of the findings

I cannot recommend to accept the manuscript in its current form, because it is not correct to talk about “gene flow” when the reviewed studies actually analyze genetic structure, and also because I found the discussion to be too general and not specifically related to the findings of the shown analysis. I provide details comment on these issues under point 4 of this review form and encourage the authors to resubmit the manuscript after revisions.

> **We are happy to know the Reviewer finds our study interesting, timely and well written, and would like to thank her/him for the constructive and detailed criticism. Below we reply to each one of the issues raised.**

Comments for the Author

Major comments

1) I do not like how the manuscript talks about “gene flow”, but actually compares measures of genetic differentiation and structure. Especially the population-based metrics (e.g., Jost D, FST etc.) are not only affected by gene flow, but also by drift, which is essentially a function of (effective) population size. This is well-known from basic population genetic theory, but the importance of this for landscape genetic inferences was recently highlighted by Prunier et al. - *Methods in Ecology and Evolution* “Contribution of spatial heterogeneity in effective population sizes to the variance in pairwise measures of genetic differentiation”). Similarly, individual-based metrics are not only influenced by gene flow, but also by mating schemes, space-use behavior etc., especially across small spatial scales. You need to explain this in the discussion of your manuscript, so that readers are not misinterpreting your results. I recommend to not talk about “gene flow” at all, but instead about “genetic connectivity” in a broader sense or even about “genetic structure”, and then explain what exactly this entails (i.e., gene flow and other processes).

> **We agree with the Reviewer on the importance of highlighting that population-level metrics like F-statistics are not estimates of gene flow *per se* but are primarily measures of the balance between genetic drift and migration. We have now stressed this out in a new paragraph in the discussion and also cited the mentioned work. Both population-level and individual-level genetic distance measures are influenced by life history and dispersal type, but unfortunately our dataset is insufficient to perform a formal meta-analysis that incorporates these traits (see comment below).**

We disagree on replacing the term “gene flow” with “genetic connectivity” or “genetic structure”. While genetic connectivity and gene flow are used interchangeably in the literature, the term “gene flow” is far more common (a Google Scholar search returned 537,000 results for “gene flow” but only 21,600 for “genetic connectivity”). Moreover, only the term “gene flow” was used in the original definitions of landscape genetics (see Table 1.1 in the Balkenhol et al. 2016 book). Genetic structure, on the other hand, may not necessarily comprise gene flow, as it can result from drift acting on isolated populations, adaptive processes or both. We have therefore maintained our initial wording, albeit emphasizing the limitations of indirect gene flow metrics.

2) One general concern I have is the combination of data/results from plant and animal species. I realize that it is currently difficult to draw cross-species conclusions, but I don't think it is correct to combine results from flying and non-flying insects, from plants and animals, and from terrestrial and aquatic systems without at least discussing the importance of different species traits for landscape genetic patterns. Also, you mention biases in previous studies, but you also have a strong bias towards insects in your quantitative analysis (Fig. 7 and 8, Data S2 and S3). You need to discuss this in much more detail. There is an interesting paper by Lino et al. that was recently published in *Mammalian Biology* (“A meta-analysis of the effects of habitat loss and fragmentation on genetic diversity in mammals”). It focused in genetic diversity and mammals, but you still might want to check out that paper and potentially include it as one of your references.

> **As we now highlight in our methods “... as in previous reviews (DiLeo & Wagner, 2016), small sample sizes did not allow performing a formal meta-analysis.”. While we only found 71 articles that met our search criteria (10 of which reported microsatellite-based IBR effects), DiLeo & Wagner (2016) analyzed a much larger dataset (541 papers) and still did not consider sample sizes were large enough to perform a meta-analysis. For instance, from the studies reporting microsatellite-based IBR effects, we found many categories represented by one or few observations (two birds, two fish and two mammal species; two organisms from aquatic ecosystems, two IBR estimates calculated using pairwise relatedness R_{ij} , two IBR estimates from Partial Mantel tests). Additionally, information on the dispersal ability or other life-history traits**

of the focus species is very poor and heterogeneous (Dataset S1), so we feel reluctant to perform quantitative analyses with our effect sizes (i.e. testing if effects sizes are different between taxonomic or functional groups). We have nevertheless included the requested information in Datasets S1 and S3 to prove our point, and also expanded our discussion to stress out the many sources of variation that could have influenced our observed effect sizes. We have also cited the recommended paper by Lino et al. (2018) in the introduction, when referring to the effects of habitat loss and fragmentation on genetic diversity, since this article does not address gene flow or genetic differentiation.

3) In addition to considering species traits, some more details on the studies are warranted. For example, in lines 163-164: I think in order to make sense of such counter-intuitive results, readers would appreciate more details on a) the study design employed by each study, especially concerning the spatial scale of analysis and the sampling scheme, and b) the study species (see above). For example, a small scale analysis on flying insects is much less likely to find an effect of habitat loss than a large scale analysis of ground beetles. You explain this for one of the studies (on army ants) later in the discussion, but it is important for all the studies you use.

> **We have now expanded our discussion to stress out the many sources of variation that could have influenced our observed effect sizes, including the resolution of the spatial data (grain size), the extent of the study area, sampling design, and time-lags in the responses to landscape changes. As mentioned above, information on the dispersal ability or other life-history traits of the focus species is very poor and heterogeneous (Dataset S1), and it was even worse for sampling scheme (most studied did not comment anything on the employed sampling scheme).**

4) Finally, I found the discussion to be too general. It is basically a list of things that should happen in landscape genetics (use of improved methods, use of NGS approaches, considering time-lags...). The need to consider all of this has been highlighted before and it is not clear exactly how tropical systems are different or how your study added to this existing knowledge. Thinking some more about the implications of species traits and study design might help you to get more info out of the analyses, but I already think there is a bit more in your data than you report. Isn't it interesting that the bias towards mammals is strongest outside of the Americas? Isn't it surprising that most amphibian studies use haplotype data? You could also discuss that several studies have used habitat suitability to estimate resistances, but suitability is often a poor descriptor of functional connectivity, so you could advise against this for future tropical studies. I found it surprising that no study has looked at road effects in reptiles, and no amphibian study assessed dispersal via waterways. I also think that a more detailed overview of study aims and outcomes would be nice. You mention these briefly in lines 224-229, but the info is missing from the results. Overall, I suggest to derive more novel insights from the results you have already obtained and can additionally obtain by considering species traits and study design more explicitly.

> **Following this suggestion we incorporated more details in our results and discussion, including new sections on the aims of the surveyed studies and the use of habitat suitability models. We nevertheless prefer to keep the focus of our discussion broad, stressing out common patterns or general gaps, instead of focusing on group-specific issues.**

Minor comments:

57: In the sentence "While these metrics underpin ecosystem function, they are not always be..." – either delete the "be", or change to "While these metrics underpin ecosystem function, they MAY not always be..."

> **Changed as suggested.**

119: Not sure why you are citing the Balkenhol et al. 2016 book here. Perhaps a better citation would be chapter 3 in that book (i.e., Waits & Storfer "Basics of population genetics: quantifying neutral and adaptive genetic variation for landscape genetic studies". (Also, the Balkenhol et al book is listed twice in the reference list (a + b)...))

> **We have now solved this referencing issue and replaced our previous citation with the Allendorf book.**

123: Fig. 1 does not show which studies are individual- vs. population-based.

> **We have now updated our map to show individual and population-level studies, as requested.**

139: Please explain why you did not perform a more formal meta-analysis.

> **We now wrote “... as in previous reviews (DiLeo & Wagner, 2016), small sample sizes did not allow performing a formal meta-analysis.”**

145: But looking at Figure 2 and Data S1, it really seems like the bias is mostly towards amphibians, while mammals, reptiles and insects are all rather similarly represented. I'd change your conclusions accordingly. Also see my comment on Fig. 1 concerning a mammal bias outside of the Americas.

> **Actually we found more papers focusing on mammals, but more species of amphibians were analyzed. We have now written “We recorded 154 target species belonging to eight major taxonomic groups, from which amphibians contained the largest number of species and mammals the highest number of papers”.**

146-148: I don't understand what this sentence means. Please reword to make it clearer.

> **Sentence has been reworded.**

165-166: It would be important to know whether studies tested for IBD separately, or after accounting for the effects of landscape variables. After all, straight-line and effective distances are often highly correlated and when testing for IBD separately, it will be supported, even if the actual driver is the landscape matrix.

> **We have now included this information in Dataset S3, as requested.**

170: Is “isolation by deforestation” the same as detecting a positive effect of forest cover on gene flow? If so, I would reword to “isolation by non-forest”, because non-forest is a pattern (just like elevation), while deforestation is a process.

> **Reworded as suggested.**

176: Most studies have focused on amphibians.

> **In fact more papers have focused on mammals, but they have studied less species than those focusing on amphibians.**

182: Citing Dyer 2015 here is not appropriate, because he actually argued that thus far, landscape genetics has not evolved into a truly distinct and independent discipline. Aside, I suggest to change “interdisciplinary discipline“ to „interdisciplinary field“.

> **We apologize for wrongly citing Dyer 2015.** We have now deleted this sentence.

188: Amphibians yes, but mammals not too much more than insects or reptiles.

> **Please see previous comments.** Most of the retrieved papers actually focused on mammals.

194-208: While I agree that the future will see many more landscape genetic studies using NGS approaches and resulting SNPs, this whole paragraph deals with a very general aspect of landscape genetics and is only marginally related to your own study. I suggest to shorten this and move it to the very end of the discussion.

> **We disagree. There is still a very large number of papers and research proposals employing microsatellites to address genetic diversity and gene flow patterns in tropical organisms. This needs to change, as sequencing costs are now similar to microsatellite development + genotyping costs, and the amount of information gained with NGS is orders of magnitudes larger and more**

accurate. May seem like common knowledge in developed countries, but in the tropics there is still a great resistance to NGS/genomic work, so we believe it is important to highlight this here.

211: Might be good to mention some of these improved methods, especially some that were not yet mentioned by Richardson et al. (e.g., commonality analysis – Prunier et al., *Molecular Ecology* (2015) 24, 263–283; extended causal modeling - Fourtune et al. *Am Nat.* (2018) 191:491-508). This is again something quite general.

> **We have now cited the suggested paper by Prunier et al. 2015, as the review by Richardson et al. Did not touch on multicollinearity or commonality analyses. Structural equation modelling (SEM) and path analysis are mentioned by Richardson et al. So we did not cite Fourtune et al. As our intention here is simply to stress out there are better methods than the Mantel test.**

213-214: This is incorrect. More than one variable can be partialled out in a partial Mantel test before assessing the effect of the focal variable. See e.g., the implementation of partial Mantel tests in r package ‘ecodist’.

> **We wrote “a maximum of two predictor variables that can be simultaneously analyzed” precisely referring to partial Mantel tests. To further clarify this we have now written “a limit of two predictor variables that can be simultaneously analyzed (in partial Mantel tests)”.**

215: But see Franckowiak et al. 2017 (*PLoS ONE* 12(4): e0175194) for a severe critique of model selection with pairwise data.

> **We do not intend to perform a review of analytical approaches in landscape genetics, as this has been done extensively already. We simply highlight that Mantel tests are still used very often, even though they are not the best methods, and that there seems to be a consensus on the use of MLPE regressions. We therefore do not cite Franckowiak et al. 2017, as this is a specific criticism against multiple regression on distance matrices, a method we do not discuss here (I actually commented on this manuscript suggesting the use of MLPE models: <https://journals.plos.org/plosone/article/comments?id=10.1371/journal.pone.0175194>).**

216: Add “models” after “population effects (MLPE)”

> **Done as suggested.**

223: Again, I don’t think the Balkenhol et al. 2016 book is a good reference for this statement.

> **We have now cited Collen et al., 2008 and Barlow et al., 2018.**

240: No, species with limited dispersal are not generally expected to be most impacted by habitat loss and fragmentation – it all depends on the scale of habitat loss, and species with high dispersal capability will also be highly susceptible to habitat loss. See also Evers and Didham (*Biol. Rev.* (2006), 81: 117–142 ; Fig. 2B) and (Mammalian Biology, A meta-analysis of the effects of habitat loss and fragmentation on genetic diversity in mammals).

> **Neither of these studies addresses landscape effects on gene flow. We have nevertheless highlighted that “Many sources of variation could have influenced the detection of landscape effects on gene flow, including species-specific differences in dispersal ability and reproductive systems, historical processes underpinning genetic differentiation, different sample sizes, the resolution of the spatial data (grain size), the extent of the study area, sampling design, and time-lags in the responses to landscape changes ...”.**

263: I wonder whether it is desirable / realistic to have one modelling framework for all landscape genetic studies. I feel that landscape genetic studies are too diverse for this in terms of spatial and temporal sampling scheme, scale of sampling, landscape and species characteristics, etc.

> **We disagree, as all these factors can be explicitly modeled and there seems to be a recent consensus in the use of mixed-effect MLPE models and model selection in landscape genetics:**

Jaffé, R., Pope, N., Acosta, A. L., Alves, D. A., Arias, M. C., De la Rúa, P., ... Carvalheiro, L. G. (2016). Beekeeping practices and geographic distance, not land use, drive gene flow across tropical bees. *Molecular Ecology*, 25(21), 5345–5358. <https://doi.org/10.1111/mec.13852>

Row, J. R., Knick, S. T., Oyler-McCance, S. J., Loughheed, S. C., & Fedy, B. C. (2017). Developing approaches for linear mixed modeling in landscape genetics through landscape-directed dispersal simulations. *Ecology and Evolution*, 7(11), 3751–3761. <https://doi.org/10.1002/ece3.2825>

Shirk, A. J., Landguth, E. L., & Cushman, S. A. (2018). A comparison of regression methods for model selection in individual-based landscape genetic analysis. *Molecular Ecology Resources*, 18(1), 55–67. <https://doi.org/10.1111/1755-0998.12709>

We have now cited these recent works and changed this sentence to further stress this point out.

Fig. 1: It looks as if the vast majority of studies outside of the Americas has focused on mammals. This is another bias you might want to report.

> We have now mentioned this pattern in our discussion.

Fig. 3: Need to mention that 2018 was not yet complete by the time you did this analysis

> We have now clarified this in the figure legend.

Fig. 4: A pie chart would be more appropriate for this figure

> We have now replaced our original figure with a pie chart, as suggested.

Fig. 5: Need to explain the abbreviations of the different methods

> We have now detailed full method names in the figure legend.

Fig. 7: It is not clear to me whether the significances shown by the different symbols are for the effects size (as shown in S3), or for the statistical tests in the original studies.

> Following this and the suggestion made below we have now included confidence intervals in Figs. 7 and 8. As stated in the figure legends, “The significance of IBR effects reported in the original articles is also highlighted.”

Fig. 8: Not sure that the ordering of this is very informative. Would it be better to order by species names, or even to move this mix of taxa to the supplement and here focus on a few species for which multiple landscape variables were tested? In the text, you use Fig. 7 and 8 to compare effects sizes for individual- vs. population-based studies, but for that it would be better to show individual- and pop-based results on the same panel...

> We prefer maintaining our original figure design, as population and individual-level studies are not directly comparable. In fact, as we state in our results, “individual-level studies (N = 14 effects; Fig. 7) showed larger effect sizes than population-level ones (N = 78 effects; Fig. 8).” Additionally, in the methods we clearly explained the criteria used to quantitatively compare landscape effects on gene flow across studies. These plots show all effect sizes from all studies that fulfilled these criteria. We would be reluctant to remove data or focus on an arbitrary subset of species.

Data S1: The column “N samples” sometimes lists just a number, sometimes it states “individuals” and sometimes “specimen”. Please explain these differences. Also, it would be important to know what level of analysis the studies used (pops or individuals), how many analytical units they had (a study with 600 individuals conducting population-based analyses with 3 populations has an effective N of 3) and whether the study was included in your quantitative analysis (because the citations are not listed in S3...). Some of this info is presented in S3, but it would be easier for readers to have it all in one table. Finally, some of the studies have been conducted in aquatic environments, but this is never mentioned in the main text.

> **All tables have now been carefully reviewed, new columns have been inserted (see comments above) and abbreviations clarified.**

Data S2: Please explain how “deforestation” is different from “% forest cover”

> **We have now clarified this at the bottom of the table.**

Data S3: Need to explain the abbreviations for the genetic distance metrics. What does “Pop_ind” mean? The s.e. are not shown in the Figures, but I think they should be used to calculate confidence intervals.

> **All tables have now been carefully reviewed, new columns have been inserted (see comments above) and abbreviations clarified. We have also included confidence intervals in the figures, as suggested.**