

July 7, 2018

Prof. Christopher Still

Department of Forest Ecosystems and Society, Oregon State University

321 Richardson Hall, Corvallis OR 97331-5752

Dear Prof. Christopher Still,

We would like to thank you for the opportunity to discuss our manuscript ID bg-2018-140 entitled 'Phylogeny of the *Stipa* and implications for grassland evolution in China: based on biogeographic evidence'. We are grateful to you and the third reviewer for constructive comments and thoughtful suggestions that are very helpful in improving significantly the quality of our manuscript. We have analyzed all the comments carefully. All major replies are described in detail point-to-point. Please let us know should you have any questions regarding the manuscript. We are looking forward to hearing from you.

Sincerely yours,

Qing Zhang

School of Ecology and Environment, Inner Mongolia University,

No. 235 University West Road. Hohhot, 010021, China.

Tel: +86-471-4992735

Fax: +86-471-4991656

Email: qzhang82@163.com

**Response to bg-2018-140 – RC3:**

Issue 1. *Line 32-35: There are a few confusing things in this statement. First of all, one cannot infer biogeographic processes based on molecular clocks/genetic information. A step - ancestral state reconstruction for geography, which the authors perform - provides this information, not the molecular clock or the genetic information itself. Next, what does isolation of organisms at a molecular level mean and how does one assess the importance of geologic events. This sentence would benefit from some editing and conceptual clarification.*

Response: We apologize for the confusion and agree with the reviewer for the point. We will modify this sentence based on clear concept and reasonable English style.

Issue 2. *Line 36: It is too strong to say that divergence times can be “basically confirmed” – they are inferred or estimated, based on some model of evolution and assumptions about molecular clocks.*

Response: Agreed. We will revise it as the suggestion “divergence times can be basically inferred”.

Issue 3. *Line 51: Suggest change of phrasing and clarification: “According to In relation to paleogeographical climate change: : :” What does paleogeographic refer to? There is nothing geographic in the statement being made here. Regional climate change in China?*

Response: We agree with the point and will revise it as “According to in relation to paleogeographical climate change, Chinese grasslands might first emerge in the late of the Tertiary Period. At that time, because of the uplift of the Qinghai-Tibet Plateau, the Himalayas Mountains blocked the warm moist air mass coming from the Indian Ocean, and the climate of the Mongolian Plateau became colder and drier. Drought-resistant grasses then emerged on the Mongolian Plateau about 7 million years ago during the late Tertiary Period”.

Issue 4. *Line 63-67: The rationale for this study is fairly clear, but I would suggest the authors moderate their language, especially with the use of “direct” evidence. Phylogenies and biogeographic models are built on a series of assumptions that enable us to infer the history of grassland expansion in relation to climate and landscape events; although these are often reasonable assumptions, this does not equate direct evidence. Direct evidence would comprise temporal and geographic series of grass fossils/biomarkers/etc., which is beyond the scope of this paper.*

Response: We agree with the reviewer and appreciate the valuable comment as follows “inference based on phylogenies and biogeographic models is not equally with direct evidence”. We will no longer use “direct”, and further ask for a native English speaker to check the revised manuscript.

Issue 5. *Lines 69-80: I am not familiar with the composition of grassland ecosystems in China – what do the authors mean, for example, by “constructive species in the typical grasslands”? And when the authors describe Stipa species as dominant, what does this mean (abundance, diversity)? For readers unfamiliar with Chinese grasslands or Stipa more generally, what is a “typical” grassland (line 74) and what is the broader context for Stipa evolution/biogeography? What is the basis for the statement that “the evolutionary processes of Chinese grasslands are closely related to the evolution of Stipa species”? Can the authors describe more about regional grassland communities? Otherwise, Stipa species are just a case study of one group of grasses, rather than a proxy for all grasslands.*

Response: We apologize for the confusion. We will add more information about Chinese grasslands and Stipa species as follows: (1) Constructive species is also called edificator, or edificator species. It is the most dominant species in a community, and also plays a significant

control role in community structure and function. *Stipa* species, as the constructive of grassland community, play very important roles in the total Chinese grasslands. However, due to the difference of precipitation and temperature, *stipe* species show obvious geographical replacement. Thus, the history of origin, divergence and expansion routes of *Stipa* species are an excellent proxy to reveal the evolution of Chinese grasslands. (2) Chinese grasslands contain as series of grassland types, and typical grassland is one of them. The typical grassland distributes with precipitation of 250 to 400 mm. Meanwhile, typical grassland reflects most obvious grassland feature with dominant drought-resistant perennial herbs.

Issue 6. *Lines 86-88: Please include references for BEAST and RASP.*

Response: Agreed. We will add references for BEAST and RASP.

Issue 7. *Line 139: Can you clarify how FigTree provided the divergence times for Stipa species. I was only aware that this program helped visualize trees.*

Response: We apologize for the confusion. We will add more information to clarify how FigTree provide the divergence times for *Stipa* species.

Issue 8. *Lines 147-148: Why did you assign a maximum number of distribution areas as 2?*

*Please explain your rationale – is that the maximum number of areas any given extant species occurs in?*

Response: Due to the difference in precipitation and temperature, the distribution of *Stipa* species show obvious geographical replacement. Considering that some *Stipa* species may be emergence on two consecutive areas, thus we assigned a maximum number of distribution areas as 2. The maximum number of areas any given extant *Stipa* species has been occurred in.

Issue 9. *Line 161: What is meant by “the divergence time of Stipa species” in this*

*sentence – I know what you mean when looking at the figure, but in the text it is unclear that this refers to the basal-most split in the studied clade. Since Stipa is a large, widely distributed group, what does this mean in the context of the whole group?*

Response: We also apologize for the confusion and agree with the point. Considering *Stipa* is a large, widely distributed group in the global and the number of *Stipa* species is only a part, we will revise this sentences as the suggestion.

Issue 10. *Line 162: I'm curious as to why the authors did not test for radiations/diversification rate shifts directly. If the primary evidence for an "explosive rapid radiation" is from visual inspection of the tree, I am somewhat unconvinced. It would be better to do a formal test for rate variation, such as using BAMM (Rabosky 2014 in PLOS One). If this kind of test is beyond the scope of the paper, I suggest reframing as an inference/hypothesis that could explicitly tested at a later date.*

Response: We thank the review for the constructive suggestion. We will add the BAMM analysis to verify explosive radiation according to the literature(Rabosky 2014).

Issue 11. *Line 168: I'm curious what the authors mean by isolated divergences – are they referring to a vicariance model? Can the authors clarify, and justify how they determined an event to be an isolated divergence event?*

Response: We apologize for the confusion. The isolated divergences referred to a vicariance model. In our study, we choose the GTR+G. model to infer the vicariance event based on software jModeltest. We will add more information in the methods part.

Issue 12. *Line 171: It is unclear what the authors are referring to – stronger interaction than what? What kind of interaction? This brings up another point – for readers unfamiliar with the landscape of the 7 regions, the topographic setting may be unknown and thus hard to know what the strength of the elevational gradient from the "top and bottom" of the mountains means. Please provide a little more information about the regions (perhaps earlier in the Introduction?).*

Response: We also apologize for the confusion. The "interaction" means gene flow. Stronger should be intense. The Qilian Mountains which are important distribution area of *Stipa*, include many mountain ranges with very different altitudes from about 3000 m to 5000 m.

The revised sentences as follows “There was intense gene flow between the top and bottom of the Qilian Mountains including many mountain ranges with very different altitudes from about 3000 m to 5000 m”. We will also provide more information about the region in the introduction part.

Issue 13. *Line 182: Can the authors please explain how Figure 4 was generated? Does RASP provide frequency estimates of event types, and from there, authors generated what looks like a kernel density plot? In Figure 4, there is a “Standard” and “Extinction” line; however, these are not mentioned in the text. Can the authors please elaborate? Furthermore, the colors in the figure do not match the legend provided, so it is difficult to align what is in the text with the figure. Finally, the three geologic events illustrated in this figure seem to occur instantaneously, when in reality these events likely took >1myr. Instead of drawing a single line, I suggest providing the age range of geologic events (similar to how it is presented in the text as a range and not a single date).*

Response: We are grateful the reviewer for the valuable suggestion and also apologize for the confusion. The Figure 4 is generated by BEAST software. We will add the details in the revised manuscript. Based on careful review, we also found the colors in the figure do not match the legend provided. We will modify the colors of four lines for a clear view.

Meanwhile, we also will provide the age range of geologic events as the text in the figure as the suggestion.

Issue 14. *Line 185-187: Can the author please clarify what is meant by “isolation events” – is this vicariance, founder event? Seems like the authors mean vicariance, but it would be helpful to be as clear as possible, and for the terminology in the manuscript to match that of the figures. Furthermore, I’m not sure that I see how the peak frequency (I’m assuming this is frequency, although the y-axis is not labeled) in dispersal and*

vicariance are “basically matched” in time - can the authors conduct a statistical test to verify this?

Response: We apologize for the confusion. The isolation events means vicariance event in our study. We will modify it in the entire manuscript including figures. Because of the mismatch of colors of Figure 4 and the legend as the comment 13, it is hard to see the peak frequency of the vicariance events. To our knowledge, there is no reasonable statistical test to verify the “basically matched”, it is inferred by the evolution process curve (Figure 4).

Issue 15. *Line 195-198: This is an incomplete sentence, please rewrite.*

Response: We apologize for the mistake. We will revise this sentence as follows “During the second and the third uplift periods of the Qinghai-Tibet Plateau, many species groups originated and differentiated”.

Issue 16. *Lines 212: Please include a reference for the changing climate conditions.*

Response: Agreed. We will add a reference for the change climate conditions as follows (Luo et al. 2016).

Issue 17. *Lines 214: I don't see this (short internodes) very clearly; again, this could be formally tested. Until it is tested, I am unconvinced that this is a clear indication of an explosive, rapid radiation, especially given the large error bars on divergence age estimates. I recommend that the authors moderate their statement – perhaps point to a suggestion of a radiation, but that this remains untested.*

Response: We also apologize for the confusion. As the reply to comment 10, we will add the BAMM analysis to verify explosive radiation according to the literature(Rabosky 2014).

Issue 18. *Lines 221: There are a few qualitative statements here that I think can be removed:*

*“Due to the crumpling effect of the uplift of the Qinghai-Tibet Plateau, the Tian Shan, Quilian, Altyn Tagh, and Kunlun Mountains all had large-scale elevation of fault-blocks, and many areas that were already elevated became medium-height mountains with*

*around 4000m height.”*

Response: Agreed. We will remove the qualitative statements of the sentences in the revised manuscript.

Issue 19. Line 225: *What do the authors mean here? I suggest rephrasing to say that geographic isolation was likely. “Obvious” here in and elsewhere in this paragraph is kind-of a loaded term, and I suggest avoiding it.*

Response: Agreed. We will revise “obvious” to “likely” as the suggestion.

Issue 20. Line 230/235: *Is there evidence, other than the assumed absence of physical barrier, for ecological and/or sexual speciation? Ancestral state reconstruction of geographic areas helps inform geography of speciation, but not necessarily mode of speciation.*

Response: We agree with the reviewer that ancestral state reconstruction of geographic areas helps inform geography of speciation, but not necessarily mode of speciation. In addition to physical barrier, species habitat and phonological differences are possible evident. We will add more information about this discussion in the revised manuscript.

Issue 21. Line 237-239: *This statement is too strong, better to be cautious and use language, such as we infer x or evidence supports y: : : I don't think we can know this definitively, even with a formal test for rate shifts.*

Response: Agreed. We will revise it as the suggestion.

Issue 22. Line 240-244: *This is an interesting idea. However, I am curious, if Stipa species have a high A+T content and A+T bonds are more prone to mutations, does this imply that the average evolutionary rate of herbaceous plants may be an underestimate of rates for the Stipa group? If so, how might that affect your findings? In general, a more developed discussion of the assumptions that went into the BEAST and RASP analyses would be good, as well as an explanation for the wide error bars on the*



*reconstructed phylogeny.*

Response: We thank the reviewer for the valuable suggestion. On one hand, we will adopt a rate of chloroplast evolution of Stipeae instead of average evolutionary rate of herbaceous plants to calculate the divergence times of Stipa species from this literature (Romaschenko et al. 2014). On the other hand, we will add some discussion about wide error bars on the reconstructed phylogeny of Stipa species.

Issue 23. *Line 246-247: It is a little unclear what the division between discussion sections 4.1 and 4.2 is, since geologic and climate history is brought up in 4.1 in relation the divergence dates and geographic expansions. This is up to the authors, but perhaps it would better serve the reader to include some of the background information about the landscape history of the Tibetan Plateau and regions of the study in the Introduction of the paper. Then, the authors could more freely discuss this history throughout the manuscript's discussion.*

Response: We appreciate the suggestion. We will revise it as the suggestion.

Issue 24. *Line 248: I suggest removing the line "During the developmental process of the whole geological history" since it is a little unclear what this refers to (e.g., the scope of geological history is far greater in space and time than what is explored in this study).*

Response: Agreed. We will remove the line "During the developmental process of the whole geological history".

Issue 25. *Line 266-268: Can the authors elaborate on how Oligocene faunal turnover (to a rodent-lagomorph dominated fauna) supports their inferred Miocene Stipa expansion?*

*It is still somewhat unclear, to me, how extensive Chinese grasslands were prior to divergence and expansion of the Stipa and/or whether Stipa are a major player in history of grassland expansion. Or, if they are an interesting group to study because of*

*their dominance (?) today and history in relation to more recent (e.g., Miocene) geologic/ climate events. I think this remains unclear throughout the manuscript – for example, in Lines 291-292, the authors surmise that the uplift of the Qinghai-Tibet Plateau and climate changes promoted the origin of grasslands, which appears to contradict an earlier origin inferred from faunal turnover and mentioned in Line 266. This confusion might be cleared up by clarifying early on the current state of knowledge (based on fossil evidence, other non-Stipa groups, etc.) and how Stipa specifically contributes to the grassland story in China – e.g., does it signal grassland expansion?*

Response: We appreciate the constructive suggestion and agree with the point. Considering the grassland emerged in Inner Mongolia Plateau at 33 MaBP (Meng and McKenna 1998) and Stipa species originated at 28 MaBP from our finding. It may be that Stipa species was not the most primitive species of the grassland. It may have evolved after the respective grasslands and subsequently invaded and became dominant. Thus, we will find more fossil evidence to prove the status of Stipa species in the history of grassland evolution. Otherwise, we will restate the goals of the study to focus on the evolutionary history of Stipa without the assumption that the history of Stipa is a good proxy for the evolution of the grasslands.

Issue 26. Line 284: *Suggest replacing “outbreak” with expansion, shift in ecological dominance, etc:*

Response: Agreed. We will replace “outbreak” with expansion as the suggestion.

Issue 27. *In general, the text in the accompanying figures is small and difficult to read.*

*Is it possible to enlarge the figures and figure text?*

Response: We apologize for the low quality figures. We will re-draw all the figures for enlarging the figures and figure text.

Issue 28. *Figure 2: There are very wide error bars on divergence times; this should be mentioned in the results and should be discussed in detail in the results and/or discussion. What contributes to wide error on divergence age estimates and how does this influence your interpretations of evolutionary processes? Stipa should also be capitalized in the Latin names.*

Response: Thanks the reviewer for the valuable comment. As the reply to comment 22, we will add some discussion about wide error bars on the reconstructed phylogeny of *Stipa* species. Meanwhile, we will modify *stipa* with *Stipa*.

Issue 29. *Figure 3: Can you make this figure larger? It is difficult to read as is, especially the ancestral states and landmark nodes on the phylogeny. Furthermore, the colors on the phylogeny seem to correspond with different regions. Can you color code the different regions on the map as well? Provinces appear to contain multiple biogeographic regions, so it is difficult to tell where the region boundaries are. Not necessary, but it might also help get the authors' message across if another panel is included with terrain, so that the readers can know where mountains ranges exist, etc. in relation to the biogeographic regions and inferred dispersal routes.*

Response: We are grateful for the constructive suggestion. We will re-draw the figure as the suggestion including enlarging the figure, coloring the map, deleting the province lines and adding the terrain.

Issue 30. *Figure 4: Please add y-axis and x-axis labels to this figure, and more detail as to how this figure was constructed. Are we looking at output from the RASP analysis? In addition, please change the colors of the curves to match those of the figure legend (for example, I cannot tell which curve is the extinction and which is the standard). In the figure caption, what does a time-geological time curve mean? Do the authors simply*

*mean event curves over geologic time from 30 Ma to present? And, is it necessary to use the terminology “time abscissas and range ordinates”? I think this is confusing, when, I believe, the authors are just describing x and y coordinates.*

Response: We apologized for the confusion We will re-draw the figure as the suggestion including adding x-axis and y-axis labels, changing the color of lines. A time-geological time curve means evolution process curve of *Stipa* species and corresponding geological events. As the reviewer elaborated, it is not necessary to use the terminology “time abscissas and range ordinates”, it is enough to describe x and y coordinates.

Issue 31. *Frequent typos: e.g., missing spaces between word and reference, missing punctuation; inconsistent pluralization of grasslands, area, etc.; “stipa” is lower case in the figures; comparative adjectives used without a comparison noun (e.g., lines 182-183*

*– “larger” should be “large” or the authors should state what the expansion is larger than); “MaBP” can just be “Ma”*

Response: We apologize for all the mistakes and appreciate the review for careful checking. We will read through the manuscript to correct all the corresponding mistakes.

## References

- Luo D, Yue JP, Sun WG et al. 2016. Evolutionary history of the subnival flora of the Himalaya-Hengduan Mountains: first insights from comparative phylogeography of four perennial herbs. *J. Biogeogr.*, 43(1):31-43.
- Meng J, McKenna MC. 1998. Faunal turnovers of Palaeogene mammals from the Mongolian plateau. *Nature*, 394(6691):364-367.
- Rabosky DL. 2014. Automatic Detection of Key Innovations, Rate Shifts, and Diversity-Dependence on Phylogenetic Trees. *PLoS One*, 9(2)
- Romaschenko K, Garciajacas N, Peterson PM et al. 2014. Miocene-Pliocene speciation, introgression, and migration of *Patis* and *Ptilagrostis* (Poaceae: Stipeae). *Molecular Phylogenetics & Evolution*,

70(1):244-259.