Sept 30, 2017

Tom Buckely

Chief Editor

AoB PLANTS

Dear Dr. Buckley,

We are delighted to hear of the decision to accept our manuscript (original reference number 18086) for publication in AoB PLANTS. We appreciate the prudent and constructive comments from the reviewers, and have accordingly taken care to incorporate their suggested modifications into the manuscript. Please see pursuant to this address our elaborated responses to each of their comments in turn. We feel that the manuscript has improved on account of these changes and other changes we have made which are detailed after the response to reviewer comments.

Sincerely,

Jeff Groh

Botany Department

University of British Columbia

September 29, 2018   
  
  
Dear Dr. Groh,   
  
Many thanks for submitting your manuscript to AoB PLANTS. The evaluations of two reviewers are shown at the end of this letter. Based on these assessments, I am pleased to accept your paper for publication in our journal, subject to a satisfactory revision being sent to us within 4 weeks.   
  
Please use the following link to submit a revised version:   
  
<https://aobplants.msubmit.net/cgi-bin/main.plex?el=A6Ef2wm7A5FBn3I4A9ftdQ4bk2RAmddxDAl6sXYWMAZ>   
  
When submitting your revision, please make all changes to the original manuscript using the track-changes feature of Microsoft Word. In addition, please include a detailed cover letter that (1) includes all text from the Associate Editor and the referees, (2) summarizes how you have changed the manuscript, and (3) is anonymous, to allow double-blind peer review, should the Associate Editor seek additional external reviews.   
  
Thanks again for submitting your manuscript to AoB PLANTS.   
  
Sincerely,   
  
Tom Buckley   
Chief Editor, AoB PLANTS   
  
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Referee #1 Evaluations:   
Recommendation: Minor changes needed   
  
Referee #1 (Comments for the Author (Required)):   
  
The MS in general is well written. It has a general interest and within the limits of the journal. I guess it will be cited because of its integrative approach to try to explain and successfully untangle the causes for the origin of an orphan hybrid populations of Aquilegia formosa x A. flavescens 

General comments   
The subject is of interest, it is well analyzed and studied, and the remarkably thorough sampling backs the results presented. Frequently, evolutionary conclusions are extrapolated from very poorly sampled case studies. In this MS, the integrative view helps a lot to understand the A. formosa and A. flavescens system and the nature of its Marble Range orphan hybrid population   
  
ABS   
L20-21. The sentence "The Marble..." is redundant with information already provided, please delete .

* Done.

INTR   
L29-34 Orphan hybrids shouldn't be confused with a putative hybrid ancestorship like in the case of Silversword alliance. I would delete this reference, otherwise all missing (extinct or unknown) parental species of present allopolyploids (and there are hundreds) would fit this definition, which means 90% of angiosperms btw.

* Done (and corresponding reference removed).

L34 Change "hybrids" to "hybrid populations".

* Done.

L42. Change "Alternatively " by "In addition". The explanation given doesn`t exclude the previous.

* Done.

L56 Change "system" by "species pair"

* Done. In addition, we altered the previous sentences in attempt to better reflect the conclusions of the research referenced therein.

L58 are they really sister species? Please cite phylogenetic evidence.

* A sister relationship between these taxa is supported by the most comprehensive phylogeny of *Aquilegia* to date (Fior et al. 2013 as cited in the manuscript). However, previous phylogenies suggest that other taxa are more closely related to *A. formosa*;these are taxa which, depending on the taxonomic view taken, could be treated as varieties of *A. formosa* or as separate species.To avoid possible contention, we avoid using the term ‘sister species’ and instead use ‘recently diverged’ or ‘closelyrelated’in the manuscript. We have nonetheless provided citations to support a close phylogenetic relationship.

L55-83. Three sentences start with "Yet", please enrich.

* These sentences have been minorly restructured.

L74-76. In modern practice, hybrids are better detected by genetic evidence than morphological traits Please reformulate the priority of the sentence. We don't perform paternity tests with morphometric studies.

* We have rephrased this sentence.

M&M   
L96 Change head to Natural History collections sampling. Hummingbirds were not studied at the Herbarium.

* We have changed this heading to ‘Museum collections sampling’ to encompass both the herbaria and the Beaty Biodiversity Museum.

L114-135. Site descriptions are excessively long. Please, be more direct (e.g. put estimated indiv. number between parentheses, or otherwise delete) or better, summarize all the information in an Appendix table.

* We have made all site descriptions much more concise.

L136-151: Consolidate "Sampling strategy" and "Field protocol" paragraphs.

* We have combined the material from these two sections into one.

L175 "Geographic analysis of phenotypic variation" seems a better description of what you do here.

* We have adopted the suggested heading.

L210. Genetic population analysis is ok, but could be much improved by including population sample sizes, which is a very important parameter to assess the credibility of the study, here instead of going to Figure 4 caption to get that information. Other important missing information is the ploidy level for A. formosa and flavescens. Are diploid both species? This should be referred either here or in the introduction.

* The ploidy of each species (they are both diploid) is now referenced in the introduction.
* We agree that sample sizes are necessary information for readers to quickly address the credibility of the study, and have included the total number of loci and individuals in the abstract to accomplish this. This information is then referred to under the heading ‘Molecular methods’ in line 195 of the revised manuscript. There we again mention the total number of loci and total number of individuals in the genetic analyses, and refer to the tables which contain more detailed information. (Note that tables 2 and 3 have been reversed from the previous manuscript as they now are referenced in a different order in the text).

RES   
The presentation is clear but somehow repetitive due to the arrangement chosen by the authors. It usually provide an emphasis in morphometric and nat hist data over genetic data. And I am definitely concerned about the way data are divided into "interspecific variation" and "intermediacy of Marble Range".   
It's been a long time since we know (thanks Arnold, Rieseberg) that hybridization doesn't mean intermediacy. Authors must avoid that impression from the Results section that among hybrids one should expect a between-parents intermediate placement for values for morphological, genetic and natural history traits.   
So, I suggest to delete " Interspecific differences", no need of separation between species and hybrid populations results. Further, hybrid data are better visualized in the context of parental values.

* This section has been re-titled as “Interspecific and hybrid variation”
* The material from the section previously entitled “Hybrid intermediacy” has been redistributed throughout this section so that the data on hybrid natural history, phenotypes, and genotypes are matched with corresponding sections describing the variation in the parental species.

L253-307 Except for this, natural history data and floral traits are well organized. Only nectar results could be more synthetic e.g. 298-307. I've been through this type of between-population variability as well, but I don't think it is a big deal. General values describe properly the interspecific variation.

* The section on nectar volume has been rewritten for a more concise presentation.

L308 Change "separation" by "differentiation"

* Done.

L321 Correct " than the of the" to "than that of the"

* Done.

L321-324 These lines are part of Dicussion, move to that section. /

* We understand the intent of the reviewer’s suggestion, but we mean for this (line 416 in the revised manuscript) to be a rather casual observation rather than a point of discussion. The focus of the discussion is intended to be on hybridization between the species, rather than the origins of floral divergence between them. For these reasons we think it is best to retain this sentence in its place.

L334-387 For the reasons I mentioned above, I 'd delete completely the "Intermediacy..." section and would distribute the Marble Range content right at the end of the correspondent section , i.e. Distribution and phenology, sepal color, floral form, nectar, genetic differentiation (including cpDNA haplotype variation),

* This has been done - see our response five comments previous.

DISC   
I believe that discussion is the best part of the work and I enjoyed reading it. It is focused, well-written, analytical in the treatment of the analyzed evolutionary evidence but synthetic in its conclusion. The integrative view helps to put together all the realm of data obtained

LIT CIT   
L558 Change Aquielegia by Aquilegia

* Done.

FIG   
Fig. 7 Red and orange colors indicating haplotype geographic provenance can't be easily distinguished at least on my screen or in the printed pdf copy.

* We have remade the figure using a more intense shade of red which hopefully contrast sufficiently with the pink color.
* Furthermore, additional samples have been included so this figure has been remade entirely. The conclusions remain the same.

The appendix is very informative and provides really useful supporting information. This is importat because of the integrative use of several methods.

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Referee #2 Evaluations:   
Recommendation: No changes needed   
  
Referee #2 (Comments for the Author (Required)):   
  
Review of "On the origin of orphan hybrids in Aquilegia (Ranunculaceae) and a cross-species spatial morphology gradient"   
  
In this article, the authors investigate a potential hybrid population between Aquilegia formosa and A. flavescens. This is notable because the population occurs in a region where A. flavescens is not present. The authors do extensive morphological measurements of pure populations and the hybrid to establish that it is indeed intermediate for most traits. Nuclear microsatellite analysis further support genetic intermediacey, while plastid sequence shows that the maternal parent of the population is A. formosa. The authors discuss possible origins of the population.   
  
This is a very thorough analysis of a hybrid population, I'm impressed by the amount of effort that went into this. For example, the use of RGB quantification of color rather than just visually-assigned color scores, is excellent. The phenotypic and genetic evidence is convincing of hybrid ancestry. Lastly, the writing is clear. With all this in mind, I recommend this paper be accepted. I have a few comments.   
  
-Did the authors try other values of K for structure? It'd be nice to see that K=2 was the best choice.

* Based on the reviewer’s suggestion, we conducted an exploratory analysis to determine the ‘optimal K value’ according to the commonly used Evanno method. It turns out that the optimal K value is 4. This is perhaps not surprising because we included 5 populations from different locations in the analysis, and there is naturally genetic structure at the population level. When K is set equal to 4, the hybrid population represents a separate genetic cluster, though evidence of admixture from parental populations is still present. While interesting, we have chosen not to include this analysis in the manuscript as (1) our intention with the STRUCTURE analysis was not to identify an unknown number of genetic clusters, but to explicitly test whether one group shows evidence of admixture from two other predefined groups, for which we have strong a priori information to specify, and for which STRUCTURE has a specialized mode of analysis. Furthermore, the assumption that different populations of the same species do cluster together was first verified before inclusion of the hybrids in the analysis. And (2) with respect to our research question (i.e. identification of hybrids), population structure within populations of the parental species more of a nuisance than a signal of interest.

What does the structure run look like without the location prior? Is the location prior used as a proxy for identified species (i.e. there are three locations, formosa, flavescens and hybrid) or by actual sampling location (i.e. there are 5 locations, Mt Kobau, Robert's lake, etc). As written it says it's the actual sampling location, but I wanted to be sure.

* In the previous version of the manuscript we used actual sampling locations as prior information (i.e. 5 groupings rather than 3). However, using the three categories (flavescens, formosa, hybrid) as prior information gave highly similar results.
* In the corrected version of the manuscript, we have increased the number of samples for the genetic analysis and taken a different approach with the STRUCTURE analysis which is detailed in lines 278 – 290 of the corrected manuscript. This approach is more directly suited to our research question.

-If the population was founded by long distance pollen dispersal from formosa, and is largely formosa genetically, would you expect it to be more inbred than it is? It's complicated by the hybrid ancestry, perhaps that's why the hybrid population doesn't seem to have more diversity than the others.

* We think the reviewer reversed the species here – we discuss possible pollen dispersal from *A. flavescens* and the structure analysis suggests higher ancestry from *A. flavescens.* But an interesting point is raised. Generally speaking, *Aquilegia* are predominantly outcrossing the hybrid population is very large and dense, so we wouldn’t expect inbreeding to be severe. But in the event of a rare dispersal event from *A. flavescens*, we might expect that the paternal gene pool was very limited, so there may have been inbreeding only with respect to the alleles of that species. This is hard to address with the resolution of our data set.

-I enjoyed the final section of the discussion and Figure 6, brought the discussion to a bigger picture.   
  
  
Specific questions for reviewing:   
  
1) Has the work been placed within a broad conceptual framework that integrates the relevant published literature and draws in the reader?   
-Yes.   
2) Are the specific research questions being addressed in the manuscript clearly articulated at the end of the Introduction?   
-Yes, the research question is very clear.   
3) Have the authors clearly and sufficiently described their statistical analyses, and do these analyses adequately address their focal hypotheses?   
-Yes, the statistics are very thorough.   
4) Have the focal research questions been evaluated rigorously and are the conclusions supported by the data presented?   
-Yes.   
5) Have the authors discussed the originality and significance of the work?   
(Papers will not be accepted or rejected based on these issues, but we want authors to discuss them.)   
-Yes.   
6) Does the manuscript title capture the concepts addressed in the paper and does it invite interest?   
-Yes. 

**Other changes**

* Additional genetic data have been added to strengthen our conclusions. Previously, we had 9 hybrid nuclear genotypes – we now have 29. The increased resolution supports our original conclusions. The data tables and figures corresponding to our genetic analyses have been updated accordingly.
* We revisited Mt. Kobau and Robert’s Lake and measured nectar concentration from these populations. These data are added into the results section and into figure 3. Additional text is added into the results section to describe these results, and the corresponding figure caption is adjusted to account for the added data.
* We have removed “cross-species morphology gradient” from the title, as we prefer the emphasis of the paper to be on the origins of the orphan hybrid population.
* We have taken the liberty to make several wording changes throughout the manuscript which improve clarity and precision of meaning.
* We corrected a couple of minor errors in our analysis of nectar volumes and traits which slightly adjusted some values in the test, but conclusions remain the same as in the previous version of the manuscript
* We added an additional paragraph to the discussion to more critically evalulate the evidence for different hypotheses on the origin of the hybrid population.
* We have added to the appendix a video clip of a female rufous hummingbird visiting a patch of *Aquilegia* flowers at one locality in the Marble Range.
* We added an additional analysis (described in line 225 of the revised manuscript), which is presented as an additional figure (Appendix S5) in the supplementary material.