

New insights into the population genetics of partially clonal organisms: when seagrass data meet theoretical expectations

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Review by Stacy A. Krueger-Hadfield

Arnaud-Haond et al. perform a meta-analysis using four species of seagrass, that vary along a continuum of asexual to sexual with corresponding patterns of departure from Hardy Weinberg and genotypic richness, in order to validate the relationship between the inbreeding coefficient F_{IS} and genotypic richness R . There were three main aims: (i) determining whether the estimate of genotypic diversity can provide informative information on clonal vs. sexual reproduction in species with a similar sampling scheme; (ii) does the departure from HWE reflect the distance from equilibrium and does the genetic composition of natural meadows provide a reliable estimate of relative clonal rates with species that invest differentially in sex vs. asex; and (iii) do the genotypic and genetic parameters provide congruent qualitative and quantitative estimates at both inter- and intra-specific levels?

There is a frequent observation of apparently high genotypic richness, suggesting lots of sex, but systematic departures from HWE in the direction of an excess of heterozygosity (negative F_{IS}). Importantly, the authors call for the use of F_{IS} rather than R if the aim of a study is to understand the importance of clonal reproduction. R is extremely sensitive to sampling. While the authors focus on seagrasses, for which there are an abundance of data, I would argue many other partially clonal taxa from pathogens to algae warrant similar approaches. We need to have a better theoretical and empirical framework with which to understand population dynamics, connectivity, and the evolutionary trajectories of important crops, ecosystem engineers, invasive species, pests, etc. etc. This paper builds on the work of (HalkETT *et al.* 2005; Arnaud-Haond *et al.* 2007) as well as the CLONIX consortium and is a necessary and important contribution to the literature on the population genetics of partially clonal organisms.

General Comments

First, this article was a pleasure to read. It was well thought out and constructed. In short, there is very little that warrants critical attention. There are few points that I think could be made clearer and more approachable to a more widespread audience, that I think critically need to understand the population genetics of partially clonal organisms. I have also addressed specific grammar issues in the specific comments.

In the first paragraph of the introduction, the authors discuss the lack of clear theoretical predictions. I wonder if another brief thought is needed here in particular the lack of a standardized format for metrics to use, data reported? As part of a recent meta-analysis, I was collating population genetic data from macroalgae, many of which are partially clonal, and this is indeed a problem across population genetic literature. I think this paper is *more* than just a meta-analysis and the relationship between F_{IS} and R . Thus, I think at some point the lack of standardization across studies and across taxa is warranted. That could probably be a paper in and of itself, but I would suggest the

authors acknowledge that that is also a problem that is on par with the lack of clear theoretical predictions.

In the second paragraph of the introduction, the authors discuss the fact that R has been used a proxy to infer the relative influence of sex vs. asex. In the red macroalgae for which population genetics have been studied in detail, *Gracilaria gracilis* (Engel *et al.* 2004), *Chondrus crispus* (Krueger-Hadfield *et al.* 2011; 2013), *Agarogphyton chilensis* (formerly *G. chilensis*; (Guillemin *et al.* 2008)), and *Agarophyton vermiculophyllum* (formerly *G. vermiculophylla*, (Krueger-Hadfield *et al.* 2016; 2017)), more than just R has been used to infer the influence of sex vs. asex. While the comparison of allele frequencies between free-living haploid and diploid stages is unique to haplodiplontic organisms, algal studies have used F_{is} (both single and multilocus estimates) and pairwise locus and multilocus estimates of linkage disequilibria. I admit that this work is my own and that of Myriam Valero and Marie-Laure Guillemin's lab. However, it might be worthwhile noting that partially clonal seaweed studies have not relied solely on R . I am curious as the introduction moves along in paragraph 4, do seagrass studies generally rely solely on R ?

When the four species of seagrass are introduced, I suggest the authors make a conceptual figure. As I mentioned above, this paper is incredibly important for conservation genetics, but also anyone doing population genetics. As a predominantly marine person, I think marine population genetics is in desperate need of these types of data. However, for non-seagrass researchers, it might be useful to have a conceptual figure in which there are sketches of the four species (also showing which ones grow faster, have higher shoot turnover) and below them is the continuum of sex to asex, R , F_{is} , etc. That would also make the paradox of high R , but significant departures from HWE also more intelligible. I suggest this figure also as a way for a new pop gen person, or someone who realizes partial clonality might be an important driver in their system or species, to be able to more easily wrap their minds around these concepts and metrics.

Specific Comments

I don't know if it is just the citation style chosen, but the in-text references are not always consistent throughout the text.

In order to avoid some confusion, I suggest you italicize R , genotypic richness.

Abstract

- Insert comma: "... coastal ecosystem, in terms ..."
- Insert comma: "... ecosystem services, but are ALSO declining..."
- Insert comma: "... sound management, conservation, and restoration..."
- Rephrase: "... of natural populations, severely limits our understanding..."
- Rephrase: "population genetic data" rather than population genetics data
- The sentence that starts with "Contrasting shoot life span" I wonder if this would make more sense to organize the seagrasses from *Z. noltii* as the sexual one with no or little departure from HWE and high genotypic diversity and move towards

asexuality. That makes more intuitive sense as sex is what more population geneticists and evolutionary ecologists would be familiar.

- Insert comma: "... at the species scale, but..."

Introduction

- Insert comma: "...collapse, shift, or expansion..."
- Insert comma: "... dynamics, and evolution of..."
- Rephrase: "...The combination of sampling bias seriously limits the interpretation of R^2 ..."

Methods

- In the *Z. marina* data, the Becheler citations are strangely formatted; same for Deikmann and Serrão 2012
- In the genetic and genotypic indexes ... you should use the word indices to refer to more than one index
- Perhaps define the with and without replicates (ramet vs. genet level)?
- I think something is missing in the sentence describing the R^2 and the F-test. R^2 is being used to assess the quality of the model and the F-test?
- The R^2 is written as R^2 and r^2 , be consistent

Results

- No specific comments

Discussion

- In the first paragraph, when using i.e. or e.g., these belong in parentheses
- The sentence that begins the third paragraph in the "Fitting empirical data to model predictions" is a run-on sentence. I suggest cutting up many of these sentences in this paragraph to short thoughts
- Insert comma: in the "Implications for understanding clonal vs..." insert a comma after selection on the second line; expansion on the 13th line

Conclusion

- This phrase is missing a word? "the mathematical hypothesis of greater deviations from HWE towards heterozygotes excess at higher levels of clonality" I think you mean greater deviation from HWE towards an excess of heterozygotes.

Figures

Figure 1 – I suggest making this a 'normal' boxplot and overlay the points from each of the studies. That is easier to visualize.

Figure 2 – If you make the conceptual figure suggested above, then you could use the line drawing or an image of each seagrass on this plot. It would be really helpful to keep all the species delineated in the reader's mind

Figure 3 – I do not know where you plan to submit this paper, but I would suggest color (maybe it is color and I printed in black and white?) for the different species. You could even assign them a color in the conceptual figure and that would help to

differentiate the tiny points. An alternative would be to make them transparent points that are all solid colors since there is a lot of overlap and the tiny points are hard differentiate.

Figure S1, S2, S3 – Same comments as Figure 1, 2, 3 respectively

References

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