

Dear PCI Editorial board, Dr. Emiliano Mora-Carrera, reviewer and Dr. Antoine Vernay, recommender,

We want to warmly thank you helping us to better communicate our results and messages in this new version of our manuscript. We considered all comments and made all the requested corrections. We hope this version will match the standard of PCI Ecology, and will be suitable for recommendation.

Sincerely,

The authors.

by Antoine Vernay, 07 Mar 2022 14:53

Manuscript: <https://doi.org/10.1101/2021.07.15.452457> version v1

Minor revision required

Dear authors,

This second round of your manuscript is now completed. The reviewer made a great job on this new version and encouraged you to improve it with small changes. Please consider the minor revisions asked by the reviewer. I think we are now close to the final version of your manuscript. If you have any questions, please contact me.

Best regard

Answer: we thank you again for leading this recommendation process and give a constructive direction to its whole. We hope this version will deserve your recommendation.

Reviewed by Emiliano Mora-Carrera, 04 Mar 2022 13:48

This is the second round of revisions of this manuscript. The main results are that: both floral morphs of Lgh do not have differences in pollen and stigma shape; there is evidence of LSI acting in Lgh, but only on the L-morph; outcrossed pollen is more successful than self-pollen at fertilizing the ovules due to faster pollen tube elongation. The authors use these results to explain the puzzling observation that the L-morph floral is more common in invasive populations despite being SI. This is the first detailed report of LSI in *Ludwigia* and confirms the presence of LSI in a third family within the Myrtales order.

In my opinion, the manuscript, specifically the Introduction and Discussion, has improved substantially by implementing the comments from previous reviewers. As indicated in previous revisions, the methods and results are thorough and sound, and deserve to be published. However, some minor changes in the manuscript need to be made. For instance, some typos (that I indicate above) need to be corrected. I made several suggestions throughout the manuscript in the hope that they increase its readability. In my opinion, most of the suggestions can be implemented quite easily, except for changes in the 2nd part of the Discussion. These of course are suggestions and their suitability should be discussed between the authors and the editor.

Having said the above, I suggest that this paper should be recommended for publication in PCI Ecology, provided the authors make the specific changes above.

Answer: we thank you for your constructive comments that again helped enhancing our manuscript. We also thank you for the clear and concise synthesis of our results in your first paragraph, that will help writing the recommendation we suppose, and helped us to refine our keywords and abstract.

Abstract

L28: Use 'among' or 'between', but not both.

Action: changed for “no matter the floral morph.”

L31: Maybe change "... questions on the distribution of this breeding system ..." for "... contributes a case of LSI in an additional family within the order Myrtales.", or something similar.

Changed.

Introduction

L47: Change 'promotes' for 'favors'. SI does not actively promote outcrossing (as in the case of heterostyly) but favors outcrossing.

Changed.

L49: Eliminate "and fertilization in particular combinations of parents".

Changed.

L53: Change "diversity" for "variation".

Changed.

L55: Eliminate "Characterizing the type of SI individuals develop in a species by".

Changed.

L56: Eliminate "first" or "essential". Keeping both is unnecessary.

Done.

L64-66: This sentence could be eliminated or moved to the first sentence of the paragraph.

That way the last sentence of the first paragraph would be related to SI, giving a natural flow to the first sentence of the 2nd paragraph.

Done.

L72: Eliminate "... , or a continuous variation of floral morphologies not correlated with compatibility."

Done.

L84: Change "occurring" to "it occurs".

Done.

L112: It would be good to also introduce and explain the other morph. For example "Conversely, in the S-morph ..."

Action: we removed this sentence, as it was a remnant of the previous version of the introduction, and is now disconnected from the following sentence. It now reads:

"The two floral morphs of Lgh develop bowl-shaped non-tubular flowers with two whorls of stamens of different heights. These two characteristics contrast with typical heteromorphic SI species that most frequently present tubular flowers and only one whorl of stamens (Barrett and Shore 2008; Cohen 2010; Barrett 2019)."

L119-120: Reduce "... successful invasive species may be mostly composed of individuals able to reproduce using self-fertilization when mating" to "... successful invasive species are the ones capable of self-fertilization".

Done.

Methods

L169: Eliminate "usually"

Done.

L170: Change 'Opale' to 'Opedal'.

Done.

Results

L271-278: Please provide the average herkogamy (+- SD).

Action: we added one sentence: "The means and standard deviations of measures of herkogamy (pistil length – stamen length in a same flower, Figure S1) for L-morph flowers outer whorls were of $+2.00 \pm 0.65$ mm and $+0.72 \pm 0.35$ mm for the inner whorls. In S-morph

flowers, they were of $+0.85\pm 0.64$ mm for the outer whorls and of -1.50 ± 0.43 mm for the inner whorls.”

Discussion:

368: Eliminate the "from" in the natural populations.

Done.

385: Change "present" to "displays".

Done.

387-389: Change 'heterostylous' to 'heteromorphic'.

Done.

L401: It is unclear what the authors mean when saying "genetic ancestry between individuals may be limited". I think the sentences can be understood without it. IF this information is important, then the authors need to explain in MS why this is important. One or two sentences should be enough.

Action: we removed as proposed.

L384-421: In general this section needs a bit more of work. It feels like the connection between LSI and its presence in HetSI is not clear and the idea goes back and forth, specially in the first three paragraphs of the section.

Action: we simplified and restructured this subsection into two subsections, and ideas into paragraphs in each new subsection.

L409: Authors mention 'GSI system' that has not been specified anywhere else in the manuscript. I assume this is gametophytic SI. If so just state it.

Changed.

L411: "..., most of the self-pollen" is imprecise. If you have the number (i.e., %90 of self-pollen tubes) it would be better to give specific information.

Action: it now reads: “In *Epilobium obcordatum*, another species of the Onagraceae family suspected to mate using an ovarian, LSI system (Seavey and Bawa 1986), 38.6% of the self-pollen tubes stopped before entering the embryo sac (Seavey and Carter 1996). However, contrary to our observations in Lgh, the rare self-fertilised ovules of *E. obcordatum* present substantial rates of post-zygotic failures resulting in an average of ~5% of viable seed set (Seavey and Carter 1994).”

L416: Eliminate 'other'.

Done.

L420-421: Eliminate this sentence or fuse it with the previous. Otherwise sound like repetition.

Action: it now reads as: “If confirmed, our results may add a third family developing an LSI system in the Myrtales order, calls for a potential reappraisal of the type of SI developed by *Oenothera organensis* and *O. rhombipetala* of the Onagraceae family as evidenced by Emerson, (1939) and Ball & Hecht (1965) and questions a possible wider occurrence of LSI in this order as reported in Gibbs (2014). “

425: Eliminate "at a low, stable rate". It is distracting in this sentence and it is imprecise. 'low' is a relative term, and is better used when comparing two different observations (e.g., lower than ... or higher than ...). A similar thing happen when using 'stable'.

Done.

L426: Eliminate "for some" and change "enabling" to "enables".

Done.

L425-428: This sentence has two main clauses (enables self-fertilization AND is present in multiple angiosperms) and is a bit hard to follow. I suggest the authors to split this sentence in two.

Done.

L430: I suggest to get rid of 'preferentially'. I think the sentence can be understood without it.

Done.

L431: Eliminate "some".

Done.

L459: Maybe add a sentence acknowledging that inbreeding depression could counteract the effect of selfing in local regeneration. A suggestion could be "Provided that inbreeding depression would not affect at later stages life cycle."

Changed.

L460: Maybe change 'regenerate' with 'establish'.

Changed for 'settle'.

L467: Change "..., better fitting Baker hypotheses." for ", providing support for Baker's Law.". Or if authors decide to keep it needs to be changed to "Baker's hypothesis".

Changed.

L471: Red 'e' in where.

Corrected.

L473-475: This sentence is hard to follow. Specifically, "... invasive populations worldwide in different ecological contexts ...". I suggest to get rid of everything after 'invasive populations'. Or break the whole sentence in two.

Action: it now reads as: "Concerning *Lgh*, we still lack of a clear picture of the respective importance of its reproductive modes in native and worldwide invasive populations in different ecological contexts. Especially, how its reproductive modes may explain and structure these monomorphic populations found along the recent European invasion front."

Fig2: Put '(b)' on the top-right corner for consistency with the other images.

Done.