

## Decision concerning your submission

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Dear Solenn Stoeckel,

Your article, entitled **Reproductive modes in populations of late-acting self-incompatible and self-compatible polyploid *Ludwigia grandiflora* subsp. *hexapetala* in western Europe**, has now been reviewed.

The referees' comments and the recommender's decision are shown below. As you can see, the recommender found your article very interesting but suggests certain revisions.

We shall, in principle, be happy to recommend your article as soon as it has been revised in response to the points raised by the referees.

When revising your article, we remind you that your article must contain the following sections (see our Guide for Authors in the Help section of the PCIEvolBiol website):

### 1) Data, script and code availability (if applicable)

- **Data, statistical scripts, command lines and simulation code must be made available to readers.** They should either be included in the article or deposited in an open repository such as Zenodo **with a DOI**. A perennial URL can be provided if no DOI is available; please note that GitHub URL are not perennial.
- **If deposited in an open repository, a reference to Data, statistical scripts, command lines and simulation code, with a DOI or a perennial URL, must be provided in the reference list and in the "Data, script and code availability" section**
- The "Data, script and code availability" section must clearly indicate **where and how** data can be accessed.
- Wherever possible, data, scripts and code should be provided in machine-readable formats. Avoid PDFs other than for textual supplementary information.
- Metadata should accompany the data, to make the data understandable and reusable by the reader.

### 2) Supplementary information (if applicable)

- Supplementary information (text, tables, figures, videos, etc.) can be referred to in the article. It must be available in an open repository (such as Zenodo, Dryad, OSF, Figshare, Morphobank, Morphosource, Github, MorphoMuseuM, Phenome10k, etc. or any institutional repository, etc...) with a DOI. A perennial URL can be provided if no DOI is available.
- A reference to the supplementary information, with a DOI or a perennial URL, must be provided in the reference list and in the "Supplementary information" section.
- List all documents attached to the manuscript as Supplementary Information in the "Supplementary Information" section.

### 3) Funding (mandatory)

- All sources of funding must be listed in a separate “Funding section”. The absence of funding must be clearly indicated in this section.

#### 4) Conflict of interest disclosure (mandatory)

- Authors should declare any potential non-financial conflict of interest (financial conflicts of interest are forbidden, see [the PCI code of conduct](#)).
- In the absence of competing interests, the authors should add the following sentence to the “Conflict of interest disclosure” section: “The authors declare they have no conflict of interest relating to the content of this article.” If appropriate, this disclosure may be completed by a sentence indicating that some of the authors are PCI recommenders: “XXX is a recommender for PCI XX.”

#### 5) Materials and methods (mandatory)

- Details of experimental procedures and quantitative analyses must be made **fully available** to readers, in the text, as appendices, or as Supplementary Information deposited in an open repository, such as Zenodo, Dryad or institutional repositories with a DOI.
- For specimen-based studies, **complete repository information** should be provided and institutional abbreviations should be listed in a dedicated subsection (if applicable). Specimens on which conclusions are based **must be deposited in an accessible and permanent repository**.

When your revised article is ready, please:

1) Upload the new version of your manuscript onto your favorite open archive and **wait until it appears online**;

2) Follow this link [https://evolbiol.peercommunityin.org/user/my\\_articles](https://evolbiol.peercommunityin.org/user/my_articles) or logging onto the PCIEvolBiol website and go to 'For Contributors -> Your submitted preprints' in the top menu and **click on the blue 'VIEW/EDIT' button at the right end of the line** referring to the preprint in question.

3) Click on the black 'EDIT YOUR ARTICLE DATA' button (mandatory step). You can then edit the title, authors, DOI, abstract, keywords, disciplines, and DOI/URL of data, scripts and code. Do not forget to save your modifications by clicking on the green button.

4) Click on the blue 'EDIT YOUR REPLY TO THE RECOMMENDER' button (mandatory step). You could then write or paste your text, upload your reply as a PDF file, and upload a document with the modifications marked in TrackChange mode. If you are submitting the final formatted version ready to be recommended, you should only add a sentence indicating that you posted the final version on the preprint server. Do not forget to **save your modifications by clicking on the green button**.

5) Click on the green 'SEND RESUBMISSION' button. This will result in your submission being sent to the recommender.

Once the recommender has read the revised version, they may decide to recommend it directly, in which case the editorial correspondence (reviews, recommender’s decisions, authors’ replies) and a recommendation text will be published by PCIEvolBiol under the license CC-BY.

Alternatively, other rounds of reviews may be needed before the recommender reaches a favorable conclusion. They may also reject your article, in which case the reviews and decision will be sent to you, but they will not be published or publicly released by PCIEvolBiol. They will be safely stored in

our database, to which only the Managing Board has access. You will be notified by e-mail at each stage in the procedure.

We thank you in advance for submitting your revised version.

Yours sincerely,

The Managing Board of PCIEvolBiol

## ***Revision round #2***

### **Decision for round #2 : *Revision needed***

I believe that the paper has improved significantly in this round of review and is ready to accept recommendation, pending a few minor changes and questions that I have. I would like the authors to review the suggestions and respond to the questions I have added as "Comments" in the attached Word document and to other more general questions that I plan to answer below, which will help me draft the future recommendation for the paper:

It is very interesting to discuss the origin of genetic diversity in these populations, since it seems that the success of colonization has to do precisely with maintaining the clonality of diverse genotypes, and could be key to undertaking a successful eradication plan. However, it is not clear to me what the real cause of this diversity is... I can guess, or speculate that it is due to the scarce outcrossing between different populations of L-morph, but also between populations of L-morph and S-morph... is this correct?... and this is possible thanks to the existence of S-morph, since if we eliminate these populations the diversity would decline until all of them were clonal... does this make sense?... it is like a system whose modes of reproduction feed back, thus maintaining a balance between diversity and clonality, something very successful for colonizing and invasive plants. If you can clarify this a little better in the discussion, without giving so much information, but developing the hypothetical cases more and ending with a proposal for eradication...what populations and how would these actions be undertaken?...it would be great.

I don't know if you have genetic structure analyses done, something like STRUCTURE to see recombination between populations. That would be great, but I don't know if possible, it would be very helpful. Well, anyway at least, I would receive your response so that I can make a good recommendation for your article. Thank you and congratulations! I think it's a fantastic job!

### **[Download recommender's annotations](#)**

by *Ines Alvarez*, 07 Aug 2024 13:19

Manuscript: <https://doi.org/10.1101/2024.03.21.586104>

version: 3

Dear Dr Ines Alvarez,

We warmly thank you for your constructive and pertinent returns and the incoming recommendation. We modified in accordance and try our best to answer your comments and clarify our manuscript. About the genetic diversity, we provide in Figure 5 (Minimum spanning tree of the genetic distances -number of different alleles- between LSI and SC individuals) a first view of the estimated coalescence between individuals with different morphs. We can't use Structure or derived method like Admixture as partially clonal populations don't match the criteria used for the convergence of the MCMC in these methods. A minimum spanning tree of the pairwise genetic distances are good visualization of the relationship between genotyped individuals. We see from this

tree that substantial admixture between the two morphs and quite diverse genetic origin(s) (different radiating branches). We are finalizing a manuscript tackling these specific questions (i.e., population genetic structure and the possible different origins and potential directions of the population expansions). Could you be interested to be recommender or reviewer for this next manuscript?

Do you mean by clonality ?. If so, It would be better to say : « When parents can yield multiple descendants **by clonality, they** may generate repeated genotypes in **different** populations »...did I understood well ?

Correct. Changed.

Not sure this is correct in English

Changed for “we don’t know yet if”

If I have followed the reasoning well...Every 1000 flowers there will be one that produce a selfed seed (1‰), so to have 1000 selfed seeds per m<sup>2</sup> it is needed to have 1 million flowers??. is that right?...if so, maybe just a brief sentence presenting the flowering of the species...for example, how many flowers per m<sup>2</sup> and how many times flowering occurs...500.000 flowers per m<sup>2</sup> twice a year?...this is a huge density of flowers, is this right?.

Right and thank so much for your vigilance! We made an error of a factor ten using the data published in Portillo-Lemus et al. 2022 (table S1, Peer Community Journal): seed set per meter square in L-morph: 104 seeds (a mean of 5.8 seeds per fruit); In S-morph populations: 34968 seeds per meter square (with a mean of 54 seeds per fruit thus per fecundated flower).

We corrected for “a hundred seeds”.

Still, there should be 999 times more seeds from crosses. Are there differences in germination rates between self-crossed and out-crossed seeds in these populations? How competitive are seedlings from one type of seed versus another?

Our question was on the importance of the rare but still significant number of selfed seeds in L-morph populations with only L-morph individuals, if they contribute to the demography and the evolutionary genetic of these populations.

About selfed seeds, see table S1 in Portillo et al. (2022, Peer Community Journal), selfed and outcrossed seeds presented the same germination rate (93%).

Answering myself to what I comment below...here you may say : « or alternatively, » instead of « and perhaps »...is this right ?

Correct. We changed for “or alternatively”

I don’t understand this reasoning...why is it expected the lack of compatible partners ?...could this be based on a result more than an initial hypothesis ?

We clarified. We found so far only one self-incompatible type in this species in France.

I see 8 population names underlined in Figure SI1, check !

Correct, we removed the underline of “Mazerolles Canal” population.

It seems to me a kind of circular reasoning...do you mean that all individuals of a L-morph population are incompatible ?, Are there no crossing at all ?

We found so far only one self-incompatible type in this species in France. Observing no or limited fruitsets with no or limited number of seeds in L-morph-only populations thus argue for the fact that floral morph corresponds the mating system as observed on the formally tested populations in Portillo-Lemus et al. 2022. We clarified by adding line 310: “All tested L-morph flowers expressed

an active LSI in western European populations, with only one self-incompatible type detected so far (Portillo-Lemus et al. 2022).”

It's hard for me to understand...could be better expressed ?... does this make sense : « ramets belong to genets that contains ramets » ??...I think I understand, but maybe better expressed using a couple of sentences ?...If I'm right each genet may include ramets from different populations, and N refers to the number of ramets per population belonging to a specific genet ??

First, mathematical subscript and superscript of the formula did a mess: we corrected. It is the number of ramets in genets having X or more ramets in the sampled genotypes. We didn't find a better sentence to express it, and this one is the common way to express it (see for example Arnaud-Haond et al. 2007 Box 4 in Molecular Ecology). Is it clearer?

I'd love to see this table, but i couldn't find it

This table is an excel file that is available in the Zenodo deposit

<https://doi.org/10.5281/zenodo.12760022>. Due to the fact that there are 53 populations (55 lines) and multiple population genetic indices (31 columns), we can't attach it in our previous submission. We compacted it and try to include it as a picture, hoping that the small font size will be readable.

Check values in figure and in figure legend. They are different

Check values, please

Check, please

Right. We corrected in accordance with the new figure in the legend and in the results.

This taxon is not mentioned in th intro, instead you mentioned Lpm...Maybe a brief comment in the intro about this ?...or simply say here that the unique chromosome number found (80) correspond unequivocally to Lgh without mentioning any other taxa ?

It was a point requested by reviewer#2 and #3, as they proposed that we may have *Ludwigia grandiflora* subsp. *grandiflora* in our dataset. As this is not the first time either a reviewer or a comment we had during conferences on this possibility, we believe that we clearly have to leave this sentence about this negative result, as requested by the two anonymous reviewers.

Where is found here Lgg and Lpm ?, would it be relevant to mention ?

We don't think we must mention that here to avoid confusion that our manuscript and its conclusions may also concern Lpm.

Are they native here ?...could be relevant

No, they are not native in north America. They probably originated from south to central America.

These are dominant markers, so could diversity be affected ?

Yes, we didn't detail too much on this point as, definitely, dominant markers are not appropriate to estimate heterozygosity in partially clonal and potentially inbred and selfed individuals, and thus to assess reproductive modes. That's why we developed our SNP set, which is one of the novelties bring by our work. The primers are listed on the Zenodo files and will now be usable by the community for any other question.

Which is the native range of this taxa ?...hmmm, I thought it was America, but i'm not sure...this is not mentioned in the intro !

Correct. We added this information line 244 that now reads:

*“Water primrose, Ludwigia grandiflora subsp. hexapetala (Hook. & Arn.) Nesom and Kartesz (2000), hereafter Lgh, is an insect pollinated, partially clonal, hermaphroditic and heteromorphic plant supposed to be native from central and south America.”*

The species has no interest at all...use an alternative word...like « benefit », tc., or rewrite, please  
Correct. We changed for “benefit”.

Please, indicate what do the numbers on the Y axe means, are these genotypes, proportions, etc?...  
Ahhh!, should be ramets, right?, please, clarify on the axe or in the legend  
Correct. We clarified this legend by detailing.

Could I have this file, please ?

Yes, we cannot attach it to the PCI deposit process, but it is available on zenodo public deposit.

Check values!, they are different in the figure

Corrected.

Why this section here ?...it is not cited anyplace...

We removed this part. We left this detailed reasoning previously to support our conclusion, and how we discarded alternative hypotheses to explain the genetic differences we observed between L- and S-morph populations were due to the effect of selfing in S-morph populations (rather than outcrossing or clonality in L-morphs).