

Dear PCI Editorial board, reviewers and recommender,

We want to warmly thank PCI editorial board, reviewers and recommender for allowing the reviewing process to continue and giving us the possibility to better communicate our results and messages in this manuscript, and to gain for the expert knowledge of the reviewers and recommender. We addressed all the comments of this round and the previous one, which greatly helped to enhance and clarify our writings. We regret Juan Arroyo decided to not participate in the recommendation process further, but we understand his decision and thank him for his contribution to introduce us the complexity of links between all the types of SI systems and floral heteromorphy. We remain available and open for any comments to improve our result presentation and writings.

Thank to reviewers' comments, we now wrote explicit perspectives and limitations of our current work for future studies to validate our initial discovery, and use appropriate semantics.

We definitely believe that our work and our results will interest a broad audience of botanists, evolutionists working on the evolution of breeding systems, ecologists working on breeding system during plant invasion (as *Lgh* is a good example that invasion may rely on reproductive mode rather than some limiting ecological conditions) and people studying this precise worldwide invasive species that has deep impact on the invaded ecosystems.

In the hope of future fruitful and relaxed exchange, and possibly on a fair recommendation, including, of course, the limits of our work, sincerely.

The authors.

Round #2

Author's Reply:

by Antoine Vernay, 29 Nov 2021 17:09

Manuscript: [10.1101/2021.07.15.452457](https://doi.org/10.1101/2021.07.15.452457) version 1

Major changes before taking a decision

Dear authors,

I carefully read your rebuttal letter about my first decision and I may make a decision "in hurry", as you suggested, therefore I asked the two previous reviewers to read again your reply. One reviewer declined the invitation to review your manuscript so I asked a third new one to do it. They now completed their reviews.

After reading their different comments, I think that the manuscript deserves some major changes again, especially because we need to read the entire manuscript (and not only your

rebuttal letter) with your edits relative to the first round of review, and now to the second. My main concern is about the use of "heterostyly" which can be misleading as a reviewer wrote. However, reviewers made several constructive comments to improve the manuscript.

In your next version, please reply to all comments from all reviewers. You wrote your disagreements with some comments but consider reformulate when your argumentation seemed to not convince everybody. Reviewers most often, suggest some ways to reply to their comments. I can ensure you that the reviewers and I, have spent considerable time on your manuscript and we debate a lot about it. We felt that all the comments were appropriate and deserve improvements in the text. You mentioned that semantic can be easily changed, which is certainly true, but that will change the message of your paper, therefore I thought it was not trivial change.

I hope you will accept to edit your manuscript accordingly. I will then make a new decision about it.

Best regards

Answer: We thank recommender for according this manuscript another round of review. We now get rid of any mention to heterostyly, and took into account for all comments, changing our writings and making manuscript modifications when requested.

Reviews

Reviewed by anonymous reviewer, 27 Nov 2021 23:00

I find that my suggestions for improvement have been mostly incorporated into the revised paper; as I declared in the previous round, I consider the topic of this paper adequate and interesting to be published.

Finally, (as the authors exposed) further work with this species will be necessary to really understand the role of self-compatibility (S-morph), self-incompatibility (L-morph) and vegetative reproduction in the evolution and stability of both morphs in natural and invasive populations.

Answer: We are grateful for your useful comments in the previous round of review that we now take in account in this new version of the manuscript.

Reviewed by Juan Arroyo, 18 Nov 2021 23:54

Dear PCI Ecology recommenders,

After reading the rebuttal letter by LO Portillo Lemus et al to my former review of their manuscript "Late-acting self-incompatible system, preferential allogamy and delayed selfing in the heterostylous invasive populations of *Ludwigia grandiflora* subsp. *hexapetala*" I consider that some of the most critical concerns I raised are still unresolved in their letter.

First, I would like to apologize if they feel that my review provoked a rude and hurried (their words) rejection or, more properly, an initial lack of recommendation. It was not my aim such a rudeness nor a rapid review. I read the manuscript several times and went to the relevant literature around the topic, including former published (that is, open to public scrutiny) work by the authors. It is my regular way to review.

Answer: We want to thank you again for your comments, and to have introduced us to the complexity of heterostyly, herkogamy, heteromorphy and related SI in plants. We still see approach and reverse herkogamy in our flowers. We understood that it's not heterostyly. We noted that you concluded your review with a positive recommendation, and we hope that our decision contestation that was written urgently, as requested by the editorial board, in the purpose to give a second chance to our manuscript within a delay of 4 weeks (while all authors were in a busy time) and without the possibility to submit a modified manuscript (PCI procedures) didn't hurt you.

We definitely recognize your knowledge and expertise in the topic of heteromorphy and SI in plants. We were greatly honored when we saw you accepted to review our work. We regret the final rejection decision ended our benefit of a relaxed reviewing process. We warmly thank you for the time you spent on our manuscript and all your comments. The definite rejection decision closed our possibility to submit a modified version, and we had first to contest the rude rejection before being allowed by editorial board and recommender to address properly your comments. We tried our best in this new version to take into account all the comments you made in the previous round of review.

Yes indeed, I consider the topic interesting of course. I have some doubts about its fitting to the scope of PCI Ecology, but this is subject to opinion which Recommenders and Managing Board should resolve, of course. My concerns were not about the semantics or the terms, and it is not a matter of being botanist, ecologist, or evolutionist. If I was very detailed in my report it was because I usually do that. I am or have been editor in other journals and know how frustrating can be a rejection based only in a short paragraph by an "established" reviewer. Although I have met similar cases as an editor, it is not common at all to re-submit the same manuscript after clear rejection. They have not corrected even those items where they offer a solution to concerns raised by at least one of the reviewers. It would have been good if at least these were addressed in a new version. This means that they prefer to put all time and effort in discussing the validity of the manuscript on very general grounds and opinions about the review process, which I think it is not the right place to do.

My main concerns were about the sampling design (how to know they were sampling different genotypes within a single highly clonal species is critical for this kind of study) and about stamen measurements, which are lacking and are necessary to determine heterostyly. If they do not want to speak about heterostyly, it is very easy to solve, just avoid it, from the very starting point: the title. This proper sampling of different genets is not addressed at all in their rebuttal letter. In fact, they answer to one of my concerns about this issue:

"Lines 169-179. The numbers of samples in this paragraph (which are large indeed) does not refers to how many individual plants (genets) and this is critical.

Action: We now added, in discussion, a new paragraph about this limitation of our work. "*Three style-polymorphic LSI Amaryllidaceae species, Narcissus tazetta L., Narcissus triandrus L. and Narcissus papyraceus also present similar pollen shape and papillae*

structure between floral morphs. However, their LSI systems are not related to the heteromorphy of their flowers, as crosses between individuals of the same floral type are fertile, and only strict self-pollination results into ovarian prezygotic pollen rejection (Dulberger 1964; Sage et al. 1999; Barrett and Shore 2008; Simon-Porçar et al. 2015). In the invasive Lgh populations we studied here, the genetic ancestry between individuals may be limited (Genitoni et al. 2021), and we didn't assess the genetic identity of the individuals we sampled along the 530km west-east transect of the Loire watershed. We were thus not able to identify if the LSI we identified in western European Lgh populations would be truly heteromorphic, or as in Narcissus sp., nonheteromorphic..”

Even if they aim to address in their study only incompatibility system, it is completely necessary to work with different genotypes. Incompatibility types, groups or morphs, if they prefer, are genetically based, thus the same genotype belong always to the same group. Since this is a problem of the design, I do not see how is possible to solve without a new sampling, or much easier, with a genetic screening of the plants used just to determine they are not clones. Random sampling of plants is fine for annual or perennial plants with no vegetative reproduction, simply by sampling a few meters apart; but this does not seem to be the case.

Action: we modified this point following the reviewer #2 proposal.

Just to be positive about the destiny of the manuscript, I prefer not to participate in the recommendation process further. I still think that there is an interesting point in their study (late acting self-incompatibility, in which I am not an expert). Finally, I have no objections, again, to accept a new case of heterostyly, when properly measured and analyzed. There are some recent cases in the literature which authors can check. This new case would much help in explaining the evolution of this breeding system, which usually provokes an outcrossing mating system in most of populations bearing it. I sincerely wish the authors are successful in their pursuits.

Juan Arroyo

Reviewed by Emiliano Mora-Carrera, 23 Nov 2021 13:36

In this manuscript, the authors make a comprehensive description of the self-incompatibility system in *Ludwigia grandiflora*. The main results of the manuscripts are that Ldh has two floral morphs that differ in the length of the style and that these floral morphs have differences in SI system. Specifically, the L-morph has a late SI system, whereas the S-morph is self-compatible. The manuscript has interesting results based on laboratory work and fieldwork, which are noteworthy and deserve to be published. However, in its current form, the manuscript has some important inaccuracies, and some changes need to be made. Mostly, I think there are some misuses of terms that could create confusion in the literature pertaining to the evolution of floral polymorphisms. If these inaccuracies are addressed, the manuscript has the potential to be recommended.

I went through the manuscript (first) and then through the authors' response to the comments of the Reviewers. I, however, see that despite the thorough response to the previous

Reviewer's comments, no changes were made in the original manuscript (or at least not in the one that was given to review [<https://doi.org/10.1101/2021.07.15.452457>]).

I attempt to provide constructive criticism, as asked by the authors, in the hope that the authors reconsider and incorporate the comments of previous Reviewers that could improve this manuscript. I will provide comments on the response from the authors (first) and then give specific comments on the current manuscript as it is now.

Answer: We warmly thank Reviewer#2 for his useful and constructive comments, that helped to enhance the clarity and the standard of our manuscript. We did not change the original manuscript when contesting the definite rejection decision because we were not allowed to submit a new version of the manuscript (even if we, indeed, may have done deposited a revised version of the manuscript to Arxiv in the quite-short delay of the 20 open-days allowed to contest the decision) and we had to wait before getting a decision from the editorial board and recommender to change our status from definite rejection decision to in review. We are now allowed by editorial board and recommender to take into account for the previous round of comments and this round into this new version of our manuscript. We apologize for this situation, and we hope this new version addresses all comments reviewers and recommender had.

Specifically, I agree that *Ludwigia grandiflora* does not have heterostyly. Although there is a difference in the style length among the floral morphs (supported by statistical analyses in this manuscript), this floral system may be considered a style dimorphic species (with approach and reverse herkogamy). This is because the distinctive character of heterostyly is not the difference in style length alone, but the case of reverse herkogamy. Therefore, differences in anther position and the measure of herkogamy should also be reported (as pointed out by Reviewer 2) if one is to study heterostyly.

Action: In order to study more precisely the style dimorphism in *Lgh*, we added measures of herkogamy in a dedicated part of the results (with one supplementary figure, and related method and statistical analyses sections) following Opedal (2018) recommendations. We also changed the results and discussion part accordingly to the constructive propositions of this comment.

We now cite Opedal (2018) and followed its recommendations in our measures and analyses: “Herkogamy is broadly defined as the spatial separation of stigmas ($x_{\text{♀}}$) and anthers ($x_{\text{♂}}$) within flowers or flowerlike inflorescences. Webb and Lloyd (1986) defined several classes of herkogamy, differing among other things in the degree of order in which pollinators contact floral organs. Species exhibiting ordered herkogamy can be further classified into those in which stigmas are contacted first by a visiting pollinator (approach herkogamy, $x_{\text{♀}} > x_{\text{♂}}$; stigmas positioned above or protruding beyond the anthers), and those in which anthers are contacted first (reverse herkogamy $x_{\text{♀}} < x_{\text{♂}}$; stigmas positioned below or behind the anthers). How to measure herkogamy depends on the functional question to be addressed.”

Citation: Øystein H. Opedal (2018). Herkogamy, a Principal Functional Trait of Plant Reproductive Biology. *International Journal of Plant Sciences* 2018 179:9, 677-687

In their response to Reviewer 1 (from now on R1), the authors indicate that the main aim of the manuscript is to identify the mating system of Ldh and not about invasion ecology. I agree with this. However, the authors stress invasion too much in the Introduction that may mislead the reader into thinking that the paper is about invasion ecology (which also happened to me). I would temper the use of invasion in the Introduction keep it in the Discussion section. In fact, the observation that the SI morph is the most common in invasive populations is intriguing that could be exploited in the Discussion rather than in the Introduction.

Action: To avoid such misleading, we changed multiple sentences in the introduction. However, we also had to take into account for the comment about the line 95 (see the comment below by the same reviewer), asking adding information about the origin of the invasive populations.

Given that a great deal of the manuscript is based on the idea of having different floral morphs, I disagree with the authors in the part that "we don't interest in heteromorphy and floral biometry" and in "Whatever, whether or not this plant is heteromorphic is not the purpose of this current manuscript and definitely doesn't matter to study.". The authors say, "If specialists can demonstrate this is not heteromorphy or something else, we would be interested to read the demonstration and explanation, out of an argument of authority about a reference that reviewed ... ". My assessment of the paper (and the reviews) is not that Ldh does not have heteromorphy (which it has). Still, this heteromorphy (i.e., floral dimorphism) is NOT heterostyly. In fact, I don't believe that R1 and R2 disagree in that there are two floral morphs. They, however, disagree with the misuse of the term heterostyly. Heterostyly has a precise definition (Barrett 2019). I think that the authors demonstrate, very convincingly, that the flowers of Ldh are dimorphic, and thus, the authors can keep their use of L- and S-morph if they define them as approach and reverse herkogamy, but not as heterostylous. As indicated by the authors, the elimination of the term 'heterostyly' will not change the results; rather, it will keep consistency in the literature of heterostyly. Many problems in the evolution of floral polymorphisms occur due to misuse of these terms. In fact, one part of the Discussion could address whether Ldh is heterostylous or not. Moreover, one of the interesting results is that different floral morphs are associated with different SI systems, and if this is an original observation from this manuscript, the authors should exploit this in the Discussion.

Action: We now removed all mention to heterostyly, and only mention heteromorphy. We also included the measure of herkogamy (new results and one new supplementary figure), allowing to define L- and S-morph as approach and reverse herkogamy. We also now better exploit in discussion the fact that different floral morphs are associated with different SI reactions in western-european populations. We also change the title of the manuscript in consequence.

I agree with R2 in that the possibility of vegetative reproduction should be at least acknowledged as an explanation of the fact that the SI morph (L-morph) is the most common in invasive populations. Authors do not need to make a whole discussion about it but only indicate that. Maybe supporting information from related taxa could be helpful.

Action: We now mention this point in material and method, since the first paragraph.

In general, it would be valuable if the authors acknowledge the limitation of their study, in Discussion, and propose experiments that could fill the gaps in our knowledge regarding the reproduction Ldh and the role of reproduction in invasive population of Ldh.

Action: we now included perspectives and explicit sentences about the limitations of our current work in the discussion.

Line 50: Get rid of "and beyond, and understand their phylogeny and evolution" since it adds nothing to the sentence and it is unclear. For instance, what is "beyond" angiosperms? It is unclear if the part that says 'their phylogeny' refers to angiosperms or to SI systems. In either case, SI is not essential to understanding the phylogeny of angiosperms. Moreover, one is interested in the 'phylogenetic distribution' of SI, not its phylogeny. I understand that the authors do not want to debate 'semantics' and 'terminology,' but some level of consistency should be kept for the scientific literature in order to avoid future confusion.

Action: We changed and clarified our sentence. It now reads: “*Moreover, characterizing the prevalence of SI systems developed in species, genera and families across the Angiosperms and across eukaryotes, will contribute understanding which biological, ecological and evolutionary features may explain the ubiquitous occurrence of mechanisms favouring allogamy across the tree of life (Charlesworth et al. 2005; Igic et al. 2008; Santos-Gally et al. 2013; Gibbs 2014a; Fujii et al. 2016; Grossenbacher et al. 2017; Barrett 2019).*”

Line 42: Mixed mating system is misused here and in several parts of the manuscript (Lines: 6, 42, 330, 419, and 423). Although there seem to be potential differences in the mating system among the population (and maybe even within), the mating system requires specific sets of experiments (pollen flow among individuals, estimation of outcrossing rates, etc.). Mixed mating systems are defined as outcrossing rates between 0.2 and 0.8 (Goodwillie et al., 2005).

Action: We removed the term “mixed mating” all along the manuscript and replaced by “breeding system” as proposed by reviewer#1 and #2.

Line 58: I think that the use of 'peripatric' is very specific in this part of the manuscript, so I would not use it here.

Action: we removed this term. The sentence now reads “*Breeding systems are indeed the main factor influencing the evolution of genetic diversity in populations and species with potential consequences for adaptation and speciation (Duminil et al. 2007; Ellegren and Galtier 2016).*”

Line 60: The authors already defined self-incompatibility as SI in the first paragraph. Use SI consistently throughout the manuscript, e.g., Line 359 and 363 and so on (except when one starts a sentence).

Action: we replaced self-incompatibility in full words by SI all along the manuscript, except when defining HetSI and LSI in the introduction.

Line 66: The authors define HetSI but then never use this acronym again. I would eliminate this acronym.

Answer: we now use it in the discussion.

Line 69: The part "spatial distancing of the anthers and stigma in the 3D architecture of a flower" says nothing about heterostyly. By eliminating the introduction about heterostyly, the authors can get rid of this part.

Action: we removed this part and also removed the mention to heterostyly. The sentence now reads: “*By contrast, heteromorphic self-incompatibility (HetSI) associates different compatibility between individuals with different floral morphologies, sometimes associated with additional features such as different patterns of spatial separation of anthers and stigmas (Webb and Lloyd 1986; Opedal 2018), differences in pollen sizes and shapes, and different lengths of stigmatic papillae (Darwin 1877; Barrett and Shore 2008; Iqic et al. 2004; Barranco et al. 2019; Barrett 2019; Matsui and Yasui 2020).*”

Line 73: add a comma after 'i.e.' as is done in other parts of the ms.

Changed.

Line 87: It is unclear to me why the use of 'literally' is important here.

Removed.

Line 91: *Ludwigia* needs to be italicized.

Done.

Line 93: Change "Water primrose ..." to "The water Primrose ..."

Changed.

Line 95: It would be great to know where does *Ldh* comes from (place of origin) and where it is invasive.

Note: Hereafter, we supposed Reviewer mentioned *Lgh* when writing *Ldh*, except if we miss some points.

Action: We added a sentence about the current prior knowledge on the origin of *Lgh*: “*This species is currently spreading out of south America into North America, Europe and eastern Asia (EPPO 2011, Portillo-Lemus et al. 2021).*” We however hope that it will not give readers the feeling that our manuscript stress invasion too much in the introduction which

may mislead the readers into thinking that the paper is about invasion ecology, even if this paragraph was precisely here to provide the context about this species.

Line 99: This is where the confusion is generated. Ldh is already defined as heterostylous in the manuscript based on Portillo-Lemus et al. (2021) when in fact, this has not been proven (see my comment above). In fact, the term used in that paper is "heteromorphic reproductive system," which is correct. Thus, I would suggest changing heterostyly to floral heteromorphism in all sections when discussing Ldh (e.g., Lines 346, 348, and 351 in discussion). Here the authors could define more clearly what they indicate as S- and L-morph.

Action: see answer below, we changed heterostyly by heteromorphy all along the manuscript. We also added in supplementary figure S1, and in the beginning of the results the measures of herkogamy for S- and L-morphs.

Line 135: Eliminate "... compared the results we obtained with other species, especially from the Myrtales order to ...". the comparison is irrelevant for the sentence.

Action: we eliminate the term "compared" and changed this sentence. It now reads: "*Finally, we discuss on the contribution of the patterns of SI reactions and breeding system found in Lgh with those already observed in other species, with a special focus on the Myrtales order that includes the Ludwigia genus.*"

Methods:

Line 215: Add space between the two paragraphs.

Done.

Results:

Line 252: Whenever p-value $\leq 10^{-15}$ just state p-value < 0.001 . If it has 10, 13, or 15 zeroes, it makes no difference.

Answer: It makes difference in regards with the number of observations, but we understand that reviewer mean that considering the classical confidence threshold 5% and 1% (which has no rational absolute support) and our dataset, this precision is not needed.

Action: we changed for the threshold of p-value requested by reviewer.

Line 258: "... and whatever the pollen origin." could be changed to "... independent of the pollen origin".

Changed.

Line 324: There seems to be a dash above a dot. Possibly due to a track change in Word.

Removed.

Discussion:

Line 330: The term of mixed mating system is misused. Mixed mating indicates that outcrossing rates are between 0.2 to 0.8 (Goodwillie et al., 2006).

Removed.

Line 335: The use of 'literally' is unnecessary.

Removed.

Line 340: Change 'all self-pollens' to 'all self-pollen grains'.

Changed.

Line 370: *Vochysia* should be in italics.

Done.

Line 377-385: It would be good if the authors point out the necessary experiments to determine 'reproductive assurance' in *Ldh*. For example, bagging experiments.

Action: we now added perspectives at then end of the discussion, saying that using genetic marker and parentage analysis will help to validate our first observations.

Table S1: In 3rd column it says 'fruitless' for some L-morph populations but then the fruit set is presented in the following columns which is contradictory information.

Removed.

Figure S1: There are two 'S-morph self' treatments. I wonder if one of them is meant to be 'L-morph self'.

Changed.