Peer Community In
Evolutionary Biology

Mixed reproduction modes explain a high genetic diversity in the invasive plant *Ludwigia grandiflora* subsp. *hexapetala* in western Europe

Ines Alvarez *based on peer reviews by* ***Rubén Torices*** *and 2 anonymous reviewers*

Solenn Stoeckel, Ronan Becheler, Luis Portillo-Lemus, Marilyne Harang, Anne-Laure Besnard, Gilles Lassalle, Romain Causse-Védrines, Sophie Michon-Coudouel, Daniel J. Park, Bernard J. Pope, Eric J. Petit, Dominique Barloy (2024) Reproductive modes in populations of late-acting self-incompatible and self-compatible polyploid *Ludwigia grandiflora* subsp. hexapetala in western Europe. biorxiv, ver. 4, peer-reviewed and recommended by Peer Community in Evolutionary Biology. https://doi.org/10.1101/2024.03.21.586104

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The introduction of *Ludwigia* species as ornamental plants in both North America and Europe dates back almost two centuries, during which time they expanded as naturalized and later invasive species in these territories (Dandelot et al. 2005, Okada et al. 2009). Repeated deliberate or non-deliberate introductions over time of this species complex that can hybridize has given rise to an evolutionarily complex scenario, which is compounded by the difficulty in delimiting some of these species and by the diversity of their modes of reproduction.

Dandelot (2004) and Dandelot et al. (2005) determined the presence of two *Ludwigia* taxa in France, *L. peploides* subsp. *montevidensis* (Spreng.) P.H.Raven (here after *Lpm*), and *L. grandiflora* subsp. *hexapetala* (Hook. & Arn.) G.L.Nesom & Kartesz (here after *Lgh*), based on their cytotypes (2n = 16, and 2n = 80, respectively) and without evidence of hybridization between them. Furthermore, despite a predominantly vegetative reproduction observed for both species, they differed in their breeding systems. While *Lpm* is self-compatible and produce a high number of viable seeds in all populations, *Lgh* is self-incompatible and its populations may drastically differ in seed viability (Dandelot 2004). Several years later, Portillo-Lemus et al. (2021) determined that the differences in seed production between some populations of *Lgh* are due to the existence of a

heteromorphic reproductive system in this taxon, involving a self-incompatible morph (long-style morph; hereafter L-morph), and a self-compatible morph (short-style morph: hereafter S-morph). Moreover, Portillo-Lemus et al. (2022) observed that self-pollen in the L-morph flowers stop growing lately (i.e., in the ovarian area) without fertilizing the ovules, concluding that a late-acting self-incompatible system (hereafter, LSI) is present in this morph.

At this point, it is relevant to understand the possible interactions between populations of different morphs in *Lgh*, and the implications that they may have on their expansive success in non-native areas in order to develop more effective management plans. To achieve this goal, Stoeckel et al. (2024) analyzed the population genetics in 53 *Lgh* populations in western Europe (without finding any *Lpm* population in the sampling area), 40 of which exclusively presented the L-morph and 13 the S-morph. This fact offered the opportunity to compare and interpret the differences between populations of different morphs in *Lgh*. Other previous works on genetic diversity of *Lgh* in peripatric or non-native areas pointed to a high clonality and an extremely low genetic diversity (Okada et al. 2009, Armitage et al. 2013), concluding in a monoclonal or few ancestral original clones for these invasive populations.

However, the investigations of Stoeckel et al. (2024) found a high genetic diversity in all populations of *Lgh* studied despite their predominant clonal reproduction. Interestingly, they found that sexual reproduction is also present, not only in the S-morph by selfing, but also in the L-morph, although limited and preferably by allogamy. They discuss the advantages and drawbacks of the different modes of reproduction observed in *Lgh* populations, the interactions among them, and the implications that both, the scarcely documented LSI (Gibbs 2014) and selfing, have in the reproductive success and in the maintenance of the high genetic diversity observed in *Lgh* in western Europe.

The contrasting results with the previous ones (Okada et al. 2009, Armitage et al. 2013) stress the relevance of using appropriate markers and analyses to assess the genetic diversity in autoploploid species, as well as the necessity of knowing the modes of reproduction in the populations studied for an optimal interpretation of the genetic metrics. The approach of the study by Stoeckel et al. (2024) had the challenge of having found suitable markers to deal with a taxon of complex origin such as *Lgh*, whose genome is compound by a set of autotetraploid chromosomes shared with *Lpm* and traces of ancient hybridizations of other diploid lineages (Barloy et al. 2024). Using RAD-Seq, Stoeckel et al. (2024) generated an original set of 36 polymorphic SNPs shared between *Lgh* and *Lpm* ensuring that these SNPs belong to the tetraploid part of the *Lgh* genome derived from *Lpm*. Another interesting contribution of this work is the exhaustive analysis of several genetic descriptors (indexes) and the interpretative guide they provide for each of them in relation to the different modes of reproduction of the study system. Finally, they propose a pair of very useful synthetic indices (i.e., clonality index and selfing index), since they allow to classify populations according to their levels of clonality and selfing.

Stoeckel et al. (2024) conclude the relevance that selfing and LSI populations, and the hybridization between them may have on the expansion and success of invasive plant species, and the necessity to know the modes of reproduction of these populations jointly with their genetic diversity in order to develop appropriate management plans. This study raises new questions such as the modes of reproduction and genetic diversity and structure have other *Lgh* populations, both invasive and native, and the dynamics of these populations under different future scenarios.

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Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2024.03.21.586104
Version of the preprint: 3

Authors' reply, 19 August 2024

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Decision by Ines Alvarez, posted 07 August 2024, validated 07 August 2024

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**

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2024.03.21.586104
Version of the preprint: 2

Authors' reply, 17 July 2024

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Decision by Ines Alvarez, posted 28 May 2024, validated 28 May 2024

Very interesting topic, but it needs a deep review and clarification of relevant issues

The manuscript deals with a very interesting topic in several areas. Knowledge about plant reproduction systems is crucial to understanding their evolution, population structure and demography. On the other hand, the invasion capacity of some species can be largely explained by their reproduction system. Therefore, this case of study is of great relevance and I believe it has great potential to be published.

However, the three reviewers who have worked on the manuscript agree on several aspects, to which I join as associate editor:

I think that the manuscript as a whole should be reduced to at least 1/3 of its original length, especially in the Abstract and the Introduction and Discussion sections. Additionally, the title should be changed to something more appropriate. In short, the manuscript requires in-depth reorganization and writing work to achieve a clearer and more synthetic text.

Regarding the content, the doubts raised by the anonymous reviewer are especially worrying and must be clarified in order to continue with the review process. The main one of all is about the identity of the system under study, since it could be two lineages instead of just one. This could be amended by checking the chromosome numbers of the two morphs in the case study. Another important question that this reviewer raises is about the selection of the SNPs used.

In short, it is important to take into account all these issues and others that the three reviewers who have handled this work consider in order to continue with the process.

Reviewed by Rubén Torices, 08 May 2024

Understanding the role of clonal reproduction and the reproductive system in structuring genetic diversity within and between populations is crucial for comprehending the diversity of a significant portion of today's

plant species. The analytical complexity increases substantially when dealing with a polyploid organism, as in this case. The study discussed in this article offers a valuable contribution, paving the way for future research on similar systems. It enhances our understanding of the synergistic effect on genetic diversity of two key components in many plants' reproduction: the capability for asexual reproduction and uniparental sexual reproduction. Given the ambitious scope of this work, it is understandably extensive. However, the introduction and discussion sections occasionally become very long, providing some details that are not directly related to the results of this study.

Below I answer all the questions posed and after the answers I have added some general and specific comments I have about the MS.

Title and abstract

Does the title clearly reflect the content of the article? [ ] Yes, [X] No (please explain), [ ] I don't know

The title is a bit vague. The work is very broad and it is certainly difficult to include a title that briefly and very precisely describes the content of the work.

Does the abstract present the main findings of the study? [X] Yes, [ ] No (please explain), [ ] I don't know

It does, but I believe that this abstract should be strongly reduced.

Introduction

Are the research questions/hypotheses/predictions clearly presented? [X] Yes, [ ] No (please explain), [ ] I don't know

Does the introduction build on relevant research in the field? [X] Yes, [ ] No (please explain), [ ] I don't know

Materials and methods

Are the methods and analyses sufficiently detailed to allow replication by other researchers? [] Yes, [X] No (please explain), [ ] I don't know

I believe this MS could benefit from a dedicated section in MM on the model species. While the introduction does contain an extensive paragraph about the species, it should be equally important to provide detailed information on the species, including its taxonomic status, major life history traits, and other relevant aspects.

Are the methods and statistical analyses appropriate and well described? [X] Yes, [ ] No (please explain), [ ] I don't know

Results

In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [X] Yes, [ ] No (please explain), [ ] I don't know

Are the results described and interpreted correctly? [X] Yes, [ ] No (please explain), [ ] I don't know

Discussion

Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [X] Yes, [ ] No (please explain), [ ] I don't know

Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [X] Yes, [ ] No (please explain), [ ] I don't know

General comments

1. Potential inconsistency between the number of genotypes and the rate of clonality

I found some of the results somehow contradictory. This study provided substantial evidence supporting a high rate of clonality. But at the same time, almost 60% of the individuals sampled were genetically different.

This makes me wonder whether these two outcomes are reconcilable. Given the observed high rate of clonality, would it not be reasonable to anticipate a higher frequency of clones?

2. The effect of the river basins on the genetic structure of this species.

As Ludwigia grandiflora subsp. hexapetala is an aquatic plant with a high clonal rate is expected that that the river basin might have a strong influence on how the genetic diversity is structured so that the expectation of genetic similarity should be higher within than between river systems. I wonder how this lack of independency between sampled sites was taken into account in both the statistical analyses and the estimation of genetic indices.

3. Statistical power of the developed SNP marker set

The authors argue that the statistical power of the developed SNP marker set to identify true ramets was exceptionally high given the low probability of two samples being identical purely by chance. However, these estimates assume panmixia which is somewhat unrealistic. To what extent can these expectations vary when mating is structured within populations or even within patches? As the interpretations of this study hinge on the reliability of these markers to distinguish true repeated genotypes, I believe that this potential bias should be addressed."

For instance, I question whether the statistical power to distinguish true ramets remains constant across populations, even when there can be significant variation in the mating systems, i.e., SC vs. SI populations.

Some other minor comments:

L31. I disagree with the use of acronyms for species names.

L490. SI4?

L556 Do you mean asexual or sexual dispersal propagules?

Reviewed by anonymous reviewer 2, 16 April 2024

I have reviewed the manuscript entitled "Reproductive modes of polyploid Ludwigia grandiflora subsp. hexapetala in western Europe: the effects of a late-acting self-incompatibility system and its absence on genetic diversity within populations" by Stoeckel et al. I enjoyed very much the authors' efforts to disentangle the genetic properties of plant populations made of individuals generated from different reproduction modes. In addition, the study organism has not a diploid genome, increasing even further the complexity of the system. Overall, I applaud the boldness of the authors and the skills developed here to face such a challenging study system.

The major problem of this manuscript is the style in which it is written. Sometimes, I felt like I was reading a methodological paper looking for a biological problem to stress its value, when in my opinion, it should be the other way around. Some symptoms of this problem can be found in the following issues:

1. The length of the sections, including a 2-page abstract, a 10-page introduction and a 14-page discussion, are disproportionate, which denote structural problems dealing with the contents.

2. Indeed, the introduction includes a series of theoretical paragraphs describing different biological topics of interest in this manuscript, whereas the discussion picks results one after another that are discussed in some cases out of context. Overall, it is hard to know and follow the rationale of the authors behind this confusing strategy.

3. The methods read like an endless list of methodological approaches that are not connected with the main questions, perhaps because such specific objectives, which should be tightly related to specific methods addressing them, are not well defined in the introduction.

Overall, I see a lot of potential in this manuscript, but the authors need to identify the major and specific goals of this study. Then, the authors will be able to select the appropriate theoretical background to end up in

the major and specific goals, which will define the set of methods required to address such goals. Finally, the discussion should also point to a given direction by interpreting the results in the context described in the introduction, as these two sections are tightly connected for the sake of coherence, readability and overall comprehension.

I do not provide comments on specific issues because I think that the authors have a major task ahead by reorganizing and rewriting the manuscript.

Reviewed by anonymous reviewer 1, 15 May 2024

The manuscript uses molecular markers to understand the reproductive system and clonal capacity of the invasive Ludwigia grandiflora subsp. hexapetala in Western Europe. To this end, the authors developed a set of SNPs from which they selected 36 and sampled a large number of populations to find out whether the two morphs described in the species differ in their vegetative and sexual reproduction. The topic is of great interest both to the field of reproductive biology and to the management of invasive species, which requires knowledge of their reproductive systems. The main results show that although cloning is the main form of reproduction, sexual reproduction is also present in the populations, which is different from the results obtained in other areas using other molecular markers. In spite of the interest of the topic, I have some important concerns about the manuscript.

In previous papers, the authors had described the presence of two stylar morphs in this species finding that they were interfertile, one being self-incompatible and the other compatible. This is a very unusual system for several reasons: differences in the reproductive system associated with the stylar morphs; the presence in one of them of the most unknown type of self-incompatibility of all those described (late-acting) and presence of only monomorphic populations. Because of the unusual and interesting nature of the system, I have read the authors' previous work, as well as others mentioned by them. These readings have raised doubts about the study system. The taxon is part of a polyploid, highly clonal complex that shows high rates of hybridisation. Some taxa in this complex are invasive in Europe. In that complex, L. grandiflora grandiflora and L. grandiflora hexapetala are very similar (according to Grewell et al., 2016) but differ in minor phenotypic differences and in chromosome number. These two taxa and others in the complex are interfertile and F1 hybrids are viable (Zardini et al., 1991; Grewel 2016). The study by Zardini et al. 1991 (Systematic Botany 16: 242-244) provides quantitative data indicating that L. grandiflora grandiflora and L. grandiflora hexapetala differ in both style length and flower size. The authors point out that the two morphs show differences not only in style length but also in flower size, which makes me doubt whether they are the same or different taxon. It is striking that the two morphs have not been described in their place of origin, despite extensive studies of the reproductive system of the complex. The absence of polymorphic populations is also remarkable. Finally, the authors described late-acting self-incompatibility in one of the morphs that is intra-incompatible. In species with LSI, plants may be intercompatible, as this system differ from the conventional SI systems associated with different floral morphs in the population (Gibbs, 2014 for a review). All these facts strongly suggest to me that they can be different lineages and not two morphs of a single taxon. Note that it is not uncommon for closely related species to interbreed, even if they are self-incompatible (e.g. Cistus).

Assuming the baseline situation of the two morphs, which was previously published by the authors, the data obtained could be of interest, as this is a taxon with a high invasive capacity in Europe, so knowing how it reproduces is essential for establishing eradication protocols. However, the first step towards good management of invasive species is proper identification. The authors have been working with Ludwigia for a long time, so these questions have probably already been raised. However, I think that in order to avoid any doubt for the reader (as was the case for me), some aspects should be made clear in the manuscript. Therefore, it would be necessary to check the chromosome number of the two morphs studied, because if they are different, the molecular analyses would have to be reconsidered. It is possible that the authors already have these data, so they can include them in a section on the species studied. However, they will need these data for some of the LS and SS populations studied.

Another concern is that throughout the manuscript the authors assume that all LS populations are self-incompatible and SS populations are self-compatible. They have only sampled 15 individuals/population (some of which are clones, so the number of genetically distinct individuals is smaller) in which they have observed whether are LS/SS and assumed differences in the reproductive system. I think this assumption could be made in the discussion (in the section starting on line 652) but not in the results. The difference in the reproductive systems of the morphs seems to come from an earlier study with hand pollination in only 7 populations. In species with polymorphism in the self-incompatibility system, variations between populations and even between individuals within a population are common. Therefore, if the authors only know the morph type, they should not assume the self-incompatibility system as they have not studied it in these populations. The results show that of the 53 populations studied, 40 have only LS plants and 13 have only SS plants, but by only looking at 15 plants per population (and some were ramets; e.g. one population had only 3 genets) they cannot assume that all plants in the population are similar. Therefore, I suggest that the results should be presented based only on what is known about the individuals studied (LS/SS), removing any reference to self-incompatibility until the discussion.

Another important concern relates to SNPs. The species described as 'alloautodecaploid' would more accurately be described as an allopolyploid with a complex origin. In addition, GISH (genomic in situ hybridisation) is useful but not the most appropriate tool for investigating such origins. For genomes with complex origins, comparison of genome assemblies is essential to draw accurate conclusions. My main concern is with the selection and validity of the SNP set. The study used RADseq from a pool of individuals from two species. This sampling design can be problematic for species with asexual reproduction. If all individuals in a population are clones, the result will only provide heterozygous markers for a single genotype. These markers may not be informative for other populations where different genotypes are fixed. In addition, the filtering steps to select high quality SNPs are not clearly described. A crucial filtering step is the removal of linked SNPs. If the SNPs in the final set are linked, the resolution is significantly reduced. There is also a lack of clarity regarding the selection of the "autotetraploid" part of the genome. The authors do not appear to have sequenced other species (apart from Lpm), so they cannot be sure that the markers are not shared with other related species.

Finally, the title does not reflect the content of the article because the authors did not specifically study the SC/SI systems of the populations studied. I find the discussion speculative in places (for example, the paragraph starting on line 783).

Minor

-Line 439. Change 99 individuals into 99 ramets

-Change reproductive insurance to reproductive assurance.

-Lines 491-505. It would be much clearer to the reader if these indices were in the form of a table separating the two morphs.

-Lines 645-650. Can the difference in the number of individuals sampled affect these results? 195 vs 600