Picking up the Ball at the K/Pg Boundary: The Distribution of Ancient Polyploidies in the Plant Phylogenetic Tree as a Spandrel of Asexuality with Occasional Sex

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Review timeline:

TPC2016-00836-LTE	Submission received:	Nov. 7, 2016
	1st Decision:	Dec. 8, 2016 *revision requested*
TPC2016-0836-LTER1	1st Revision received:	Jan. 5, 2017
	2nd Decision:	Jan. 28, 2017 *acceptance pending, sent to science editor*
	Final acceptance:	Feb. 15, 2017
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REPORT: (The report shows the major requests for revision and author responses. Minor comments for revision and miscellaneous correspondence are not included. The original format may not be reflected in this compilation, but the reviewer comments and author responses are not edited, except to correct minor typographical or spelling errors that could be a source of ambiguity.)

TPC2016-00836-LTE 1st Editorial decision – *revision requested* **Dec. 8, 2016**

Your submission has been evaluated by members of the editorial board as well as 5 expert reviewers in your field (there is no reviewer #3). The reviewers and editors are quite interested in publishing the piece and believe it will be a valuable contribution to the theories attempting to explain polyploidy. However, it was agreed that substantial revision will be required for acceptance. The reviewers were concerned that the piece appears to have been hastily written and lacks clarity, depth, and due consideration of the extensive literature on the subject matter in some respects. The main points for revision are to carefully consider the reviewer comments towards

i) improving the writing to deliver the arguments clearly (we recommend using a professional science editor to help with this),

ii) providing a more in depth consideration of apomixis, somatic asexuality, and the process of diploidization as related to the main thesis,

iii) consider also that for some (or even many) polyploids there is reasonably good documentation about their formation to think that there was no asexual interlude, for example, common wheat and Tragapogon.

iv) please improve the quality of the figure - submit a high resolution image; make good use of space and ensure that all elements are readily visible at printed size.

A revised submission will be re-reviewed, but will be sent back to only 2 (or 3 at most) of the original reviewers.

-- Reviewer comments:

[Reviewer comments shown below along with author responses]

TPC2016-00836-LTER1 1st Revision received **Jan. 5, 2017**

Reviewer comments and **author responses**:

<u>Reviewer #1:</u>

This letter addresses an issue involved with the abundant finding that there have been numerous polyploidy formation events in the evolutionary history of plants. Polyploids often have problems with the fidelity of meiosis so how could they be so successful in competition with diploid progenitors. Freeling provides a potential answer here: vegetatively propagating polyploids can survive and compete but will occasionally engage in sexual reproduction that will diploidize the pairing of chromosomes in meiosis. This is an interesting insight for the field of whole genome duplication in the evolution of genomes.

Point 1. The authors should expand more on the fact that almost all apomicts are polyploid with citations such as Apomixis in Plants by Asker and Jerling and Plant Breeding Systems by A. J. Richards. Indeed, almost all apomicts are facultative experiencing sex partially or cyclically. This fact strongly supports the theme of this piece. An example of cyclical diploid sexuality-polyploid asexuality as in Panicum and Dichanthium would be good to include.

RESPONSE: Such a useful review! The agamic asexual complex references are terrific. In general, apomixis is so complicated that a complete discussion could bog-down this essay. As you said, some extant polyploids clearly derive from populations, not individuals. I have now used the linkage between apomixis and polyploids in my argument, and also tried to treat the apomictic data with respect while, at the same time, suggesting that vegetative reproduction is a better model. I did not know the complicated, interesting population-level literature on apomixis, thank you, nor did I know that stem-to-bud reproduction research has been neglected. The A.J. Richards general reference was inspirational, and I didn't know that either of the two editions existed. Did you know that only the first edition covers vegetative reproduction? (And that coverage is so very superior (and unique, thoughtful) to any other I've been able to find: thank you.) These references and one quotation, are now used to document the general relationship between asexual reproduction, perenniality, water habitat, and polyploidy.

Point 2. Given the above, it is not clear whether polyploidy is a spandrel of occasional sex or whether facultative apomixis is a spandrel of polyploidy. Apomixis probably salvages triploids that experience meiotic failure. This paragraph could be deleted without loss of meaning or impact.

RESPONSE: I agree that apomicts probably salvage triploids that would otherwise fail, but triploids also derive from a wide-crossed diploid and tetraploid. I only mention triploids and paleohexaploidies now, and dropped the reference to triploids and apomixes as a "red herring". From an apomictic point-of-view, sure, apomixis may well be a spandrel of polyploidy. But the distribution of polyploidies in the phylogenetic tree of extant angiosperms is my problem and an all-plant problem. Apomixis is a more specialized developmental alternative and may well always dead end.

Point 3. The author might consider mentioning the work of the Bomblies lab on diploidization: PLoS Genetics 8: e1003093 and Curr Biol 23: 2151-2156. This work provided evidence that there is selection for diploidization.

RESPONSE: Not just that, but the tetrapoid was not "pre-adapted" to diploidize, and this team also has good idea of the genes involved. I used the Curr. Biology experiments. I also site the Bomblies lab review. Thank you.

Point 4. Also, the fact that apomicts are usually facultative would argue that the distinction between mutationist and selectionist might not be so absolute as implied here. Selection might act during the sexual cycles.

RESPONSE: No changes in response to this. The distinction between Selectionist and Mutationist is a distinction as to the type of theory that explains "the distribution of polyploids as in Fig. 1" Selection and mutation always happen, sexually and asexually. I now give my reasoning when I mention the Modern Synthesis. I know that not all biologists think this topic is even worth mentioning; I think this topic is fundamental and I hope to leave it in my essay. Yes, I do see that apomicts are usually facultative, but I don't see how to stress this without lending too much importance to apomixis as the sort of asexual reproduction that is likely to be protective given better vegetative reproduction strategies. I fear that discussion of specific sorts of asexuality will obscure the idea.

Reviewer #2:

Point 1. There are many reviews and commentaries addressing the phenomenon of polyploidy. While these reviews are interesting, original ideas and testable hypotheses are not common. We are still ignorant of why the angiosperm evolutionary tree is marked in many sites by genome duplication events. A common proposal, the Ohnoan view, is that polyploidy is good for the evolutionary potential of the genome and of the organism it affects. Another is that certain qualities related to hardiness and vigor favor the survival of polyploids in harsh environments. Freeling proposes a refreshingly different and interesting alternative: polyploidy (like the Seinfeld show) is about nothing. It is merely a spandrel, a non-structural ornamentation added post-facto to a construct of asexuality. It hitchikes along because it can and because it is protected.

RESPONSE: Just so, but once established, polyploidy—like all spandrels—is used for everything it's worth. The Ohnoan view now applies. But being used for now-important functions is not the same as understanding origins, and the PATTERN of polyploidies in Figure 1A.

Point 2. Neopolyploids are notoriously cumbersome at sex and specifically challenged by the need for precise pairing and partitioning of homologous chromosomes. For this reason, the mechanism proposed is plausible, interesting and well worth publishing. It should fertilize considerable thinking. It will be healthy for specialists as well as the initiated to consider the possibility that polyploidy is all about nothing.

RESPONSE: Thank you.

Point 3. Having said that, I am skeptical that hiding under the asexual umbrella is a common pathway for plant polyploids. First, I do not see what feature of the tree in Fig. 1A informs us about asexuality. Many such cases are well documented in non-angiosperms, but it is in the context of angiosperms that Dr. Freeling could expand a bit and clarify his thinking: yes, we know it can happen, but is there actually documented, large scale natural history supporting this for plants and how does it relate to the tree in Fig. 1A? Second, considering many current, presumably successful polyploids I see limited (not zero...) support for recurrent coincidence of asexuality and polyploidy.

RESPONSE: Accommodated. I do a better job now. New polyploids and asexuality are clearly linked. See the agamic complexes in panic grass genera, and the general linkage in A.J. Roberts. Once the hostile environment is past, the polyploid no longer needs protection, and once diploidized, the polyploid is now diploid and certainly doesn't need asexuality (although there are cases on earth today, of course). The general strategy of inferring the past based on what is happening now on the ground is, in my opinion, not logical. That is where I must part ways with Stebbins. What we need is some evidence from the fossil record!

Point 4. Consider, for example, wheat, cotton, coffee, utricularia, brassica polyploids, camelina, many wild species, etc etc.They are fertile and well-adapted polyploids for which I am not aware of asexual intermediates. Of course, these could all be dead ends (particularly those domesticated) and in ten million years only the rare asexuals will have formed persistent taxa.

RESPONSE: I made no changes directly in response to this comment. These successful polyploids are fully diploidized. For most of these species, humans positively selected them to be successful diploids. Some relatively (a few million years) recent diploidized polyploids may well have had a period of asexuality or some similar protective environment. My idea predicts that, given a few million years of excessive UV-B radiation, they would all be extinct. Now see the [section Observations on Extant Plants can be Off the Point] that specifically addresses this issue of over-reliance on the data from extant dead-end populations. Polyploids that are not diploidized are dead or on their way (Stebbins and everybody else, and logic) . The current research on diploidization is now reviewed.

Point 5. Yet, there is reasonable evidence that many current allopolyploids are as likely to produce fit progeny as comparable diploids. Furthermore, given that neoallopolyploid lineages go bad very fast and irreversibly (see Pires papers on polyploid ratchet and Henry et al. investigation of neo-A. suecica), the fortunate recruitment of pre-existing alleles that normalize meiosis, or their rapid evolution, is at least as plausible as the asexual umbrella.

RESPONSE: I do understand that 1) fortunate recruitment and 2) rapid mutation-fueled evolution will do the job. According to Bomblies on A. arenosa autotetraploid diploidization, it takes over 40 different gene mutations. The Henry paper, now cited, goes some way towards diploidization with a single gene, but not all the way. About the polyploid ratchet—I like the idea for understanding the allopolyploid B. napus' chromosomal structure. It involves sexual crosses among new diploids. This IS important, but comes after the question I am exploring here; why the pattern of polyploidies in Figure1A. I could not fit in this citation. Again, my topic is not to explain how polyploids happen or get stabilized: it is to explain the pattern of polyploidies in Figure 1A. All this diploidization and contemporary data discussions are probably off the point. I now add a short [section on this "Observations on Extant Plants can be Off the Point"].

Reviewer #4:

In the current letter/commentary, Freeling comments on ideas and results that have been put forward by the Van de Peer lab and colleagues, and in which it was argued that a large number of polyploidies or whole genome duplications (WGD) observed during the evolutionary history of different plant lineages are not random, but seem to coincide with the K/Pg extinction event, and as such correlate with periods of environmental upheaval or change. The argument here is that polyploidy can confer some sort of selective advantage in times of stress, and consequently, polyploids can outcompete their diploid progenitors. Freeling proposes that polyploidy is actually a spandrel (a by-product acquired along with different characters that have been selected for, and not of inherent adaptive properties) of occasional sex: the pattern of polyploidies arises naturally from the plant lifestyle of occasional sex, juxtaposed with the protective properties of asexual reproduction during extinction events characterized by radiation that might be expected to disrupt accurate meiosis.

Point 1. I have read this letter/commentary with great interest. Mike Freeling's papers and opinions are often thought provoking. At the same time, I found it difficult to assess the true value or merit of the paper, because of the sometimes biased citation of facts or pieces of information that fit the story, or help making the argument, while in many places, the story might be a bit more complex and the paper suffers from oversimplification.

RESPONSE: I now discuss the complex story involving apomicts and polyploidy in [a section on Details on the Origin of Paleopolyploids]. My aim is to present an idea. Whether it is correct or not is not for me to say. It will be proved correct or not. It's just an idea. The idea is simple.

Point 2. Also, I found the writing at times a little chaotic and not easy to understand (I guess particularly not for non-native English persons). At times the paper also gives the impression of being hastily written.

RESPONSE: No, this was not hasty. I see no evidence that my idea was understood. I sincerely hope that my revision does a better job explaining my idea. I spent a lot of time attempting to communicate the core steps necessary to "get" my idea.

Point 3. I have the following more concrete comments: Line 23 - 26. What is the point of this sentence about modern synthesis? What is the author trying to say here? What does 'step-by-step evolution without need for new mutation' mean?

RESPONSE: I have revised this sentence to better explain why. I restated the Modern Synthesis. Wikipedia has a great definition, "step-by-small-step" and without new mutations IS The Modern Synthesis, and that is right from Darwin himself. I didn't make that up. I now say that my idea does not fit within our current "Theory of Evolution", and that's why this topic is brought up.

Point 4. Line 31 - 33. Referring to Fig. 1, the author claims that 'how do polyploids fuel evolvability' is not the only question one could ask. Another question could be 'why are some polyploid lineages so hard to kill?' However, I'm not so sure some polyploids are 'hard to kill'.

RESPONSE: That polyploids are "hard to kill" is a restatement of the pattern of paleopolyploidies of Figure 1. That "hard to kill" conclusion <u>is the insight that begins this essay</u>. Notice that non-polyploids tend to go extinct in Figure 1A. So, how does new polyploidy help protect a lineage from extinction when new polyploids aren't fit?

Point 5. More and more, the current view is that (as also acknowledged by the author further in the manuscript) most often polyploidy is an evolutionary dead end and bound to be 'killed', unless the circumstances are such that polyploidy confers a selective advantage. Of course one can always turn around the argument, but is this backed up by (any) evidence?

RESPONSE: That [selective advatange] involves polyploids today. My question involves the pattern of polyploidies in Figure 1A. Everything else died off leaving only the polyploid lineage over and over again. That is the point of "hard to kill". [The evidence is shown in] Fig. 1A. The fossil record does not help, nor do observations of extant plants in the wild.

Point 6. The author goes on stating that 'if reproducing sexually, polyploids undergo inaccurate meiosis generating duplication/deficient gametophytes and seed. These characteristics should reduce fitness if the organism reproduces sexually. And yet, the polyploid lineages survive while, in almost all cases, all of its related lineages go extinct'. Although there might be some truth in this, I believe this to be a strong oversimplification.

RESPONSE: This is a restatement of the pattern of paleopolyploidies in Figure 1A. I'm restating, not simplifying.

Point 7. Furthermore, it has been shown that polyploidy often goes together with apomixis (asexual reproduction) or polyploids are formed by the successful merging of unreduced gametes. As a matter of fact, a more neutral theory (as also proposed by the Van de Peer lab) explains the increased formation of polyploids through the production of unreduced games as a consequence of inflicted stress on the plant (through for instance increased stress levels that occur during periods of change/extinction).

RESPONSE: No. Stress might well cause unreduced gametes and the production of polyploids, but the problem with polyploids is not their generation. Neopolyploids have unfit meiosis. I hope my revision is clearer.

Point 8. Line 41-44: 'Some or all mass extinctions are thought to result from increases in ionizing radiation - from a burst or ozone depletion - causing mutation, and breakage of DNA or microtubules.' The author here suggests that this would again cause deficient gametophytes because of problems in meiosis. Although, again, a possibility, is there any evidence for gamma ray bursts during the K/Pg extinction event directly affecting DNA or meiosis?

RESPONSE: No. I did not say this. Ionizing radiation causes broken chromosomes. Anything that disrupts the ozone layer will increase ionizing radiations. Gamma ray bursts would be a fine mechanism of mass extinction, but I found no good evidence for these astronomical events, nor do I say there is.

Reviewer #5:

The goal of this manuscript is to discuss novel reasons to explain the evolutionary success of polyploid plants, with reference to previous discussions of this observation (Comai, 2005; Van de peer lab, etc.).

Point 1. The author presents two negative points with respect to new polyploids to which their discussion is supposed to counter: "Each new polyploid individual has 1) a very low genetic diversity and 2) if reproducing sexually, inaccurate meiosis generating duplication/deficient gametophytes and seeds/kernels." It's not clear how the author counters this point with reference to asexual reproduction, as that would (for example) not lead to increased genetic diversity.

RESPONSE: Asexuals can do great with less genetic diversity. The idea is to survive first. Genetic diversity is undoubtedly a characteristic of most successful plant clades living today, but survival must come first.

Point 2. My main comment with regards to this manuscript is that it is quite vague, and in attempting to identify a general phenomenon to explain the persistence of polyploids, the author follow assumptions which are not applicable to many polyploid plant systems.

RESPONSE: The author disagrees. There is nothing vague about my idea. It is particularly specific and contained. You just don't understand it yet. It's just one singular idea. It will seem precise when you understand it, not vague. This revision is much more fact-based and less general. I hope the idea is not lost in the details. I had originally hoped that I could explain this idea in just a few sentences and let the reader do the subsequent thinking. Unfortunately, without convincing the average reader that I know my factoids about the modes of asexual reproduction evident among extant plants, I can't seem to communicate. The risk is that the simple idea will be lost, (but for many readers, there was no idea in the first place).

Reviewer #6:

In this Letter to the Editor, Michael Freeling presents an argument in favor of the hypothesis that the distribution over time of ancient polyploidies in plants is a spandrel of selection for primarily asexual reproduction in the plants concerned.

Point 1. Although I do see the point that asexual reproduction might have its advantages in an environment characterized by a high-intensity environmental stress factors interfering with normal meiosis, and that asexually reproducing polyploids that originate at or before the time point at which such an environment is established might benefit from this, I think the 'polyploidy is a spandrel of occasional sex' hypothesis advocated in this letter is overstretched and not sufficiently thought through. I find this letter lacking in depth and accuracy in some places, and in some sections, it is very hard to follow the argumentation.

RESPONSE: I want to make one thing clear. I am not "advocating" for the truth of my hypothesis. If a proof were possible and I could understand it, I would present the experiment and the data. I'm presenting an idea, pure and simple. I promise that, when you understand it, you will go "ah ha".

Point 2. line 16-22: Lohaus & Van de Peer (2016) do not conclude that a period of mass extinction increased the pressure of purifying selection, favoring polyploids. Instead, they argue that purifying selection should have been less effective in plant communities that were reduced in size during the K-Pg event, but that polyploids might have been better shielded against the ensuing increase in genetic load. The Lohaus & Van de Peer (2016) paper does not only cover the selectionist viewpoint, but also discusses neutral mechanisms that may lead to increased polyploid formation and establishment.

RESPONSE: I reread. You said it better than did I. I've now said something more accurate about what Van de Peer said. Thank you.

Point 3. line 33-38: I'm not so sure that each polyploid lineage can be traced back to one individual. In times of increased unreduced gamete formation due to heightened stress for instance, it is conceivable that multiple diploid individuals contribute to the same polyploid offspring population (I believe there is also some relevant literature on this subject, but can't remember exactly which papers to refer to).

RESPONSE: I have written a [new section] on this exact point. It is a complicated point. The agamic polyploid complexes of some apomicts do result in some genetic exchange between the polyploid and the diploid, so – in this case – it's not really one individual but a population. Keep in mind: there is no evidence that apomicts have ever survived long enough to initiate any lineage that was not a dead end. Vegetative reproduction is fundamental and often the reproductive meristem is underground.

Point 4. line 60-64 : I do not follow the argument that polyploidies might survive because they 'hide out' in the soma of asexually reproducing plants and are therefore protected by the heightened selection pressure during periods of mass extinction.

RESPONSE: The concepts of "hiding out" and "mutational hotbed" are now the topic of a new paragraph aimed at reinforcing the points necessary to grasp the spandrel idea using crystal clear text.

Point 5. Asexually reproducing organisms are subject to selection just as any other organisms, albeit in a different way. This also reflects on the argumentation from line 73 onwards, where an asexually reproducing polyploid is described as an entity undergoing 'relaxed selection', which I do not believe is true.

RESPONSE: You are wrong about that. Polyploidy is itself relaxing, and all obligate sexual genes are not only relaxed, they are "available for mutational experimentation" in a lineage reproducing without sex. This is more relaxed than a diploid under the sun. That's the point. All sorts of tries at diploidization post-polyploidy might be attempted, but they don't need to matter if the lineage is doing OK asexually. Understanding this "hiding out" as a hotbed of mutational creation is at the heart of this idea. Thus this new paragraph.

Point 6. The 'polyploidy is a spandrel of occasional sex' hypothesis illustrated in Figure 1B suggests that asexual reproduction in plants is a selected characteristic, which ultimately leads, as a spandrel, to survival of asexually reproducing polyploids after mass extinctions. The spandrel hypothesis however seems to suggest that all (or most) of the asexually producing plant lineages are polyploids or eventually polyploidize, which is not true (not even on Figure 1B).

RESPONSE: This is, I think, one of the over-arching issues of concern to most reviewers (TPC's and my own). See my new paragraph [Observations on extant plants can be off the point]. Thank you.

TPC2016-00836-LTER1 2nd Editorial decision – *acceptance pending* Jan. 28, 2107

We are pleased to inform you that your paper entitled "The distribution of ancient polyploidies in the plant phylogenetic tree is a spandrel of occasional sex" has been accepted for publication in The Plant Cell, pending a final editorial review by journal staff to address the following points.

.. the "supplemental information" should just be included in the main text. It seems pertinent enough and there really is no reason it should be "supplemental"; I don't think it will make the article too long. In addition, the article could still use some editing for organization and clarity, as well as journal style (also in agreement with Reviewer 4 comments on structure/headings). I also suggest breaking it up into more sections with more descriptive headings. I have attached an edited version of the manuscript with a number of suggestions along these lines.

-- Reviewer comments:

Reviewer #2: The author response to the reviews is semisatisfactory. I confirm my interest in Freeling's idea and the desire to see it published. I am slightly disappointed that Dr. Freeling thinks the idea is sufficient. A look at the nuts and bolts of current asexual polyploid systems does little to convince me that they are temporarily hiding while they are refining meiosis and become adept at it. Consider Spartina: Where is the selection for sexual reproduction? It has to be relaxed by definition. Will the retooling be faster than the dismantling by mutation? I would argue that successful polyploids are those that combine perfect characteristics from the start and are able to hit the ground running. But, all considered, this is a worthy opinion. Dr. Freeling has paid sufficient dues into the polyploidy secret society to be allowed visionary and messianic "beautiful ideas" without much else. We should read them, ponder them and criticize them if suitable.

Reviewer #4: The revised version of this paper, in which Mike Freeling proposes the 'idea' that ancient polyploidy might be, at least initially, nothing more than a spandrel because the polyploid can be hiding out within the soma of asexually reproducing plants during times of mass extinctions, is a great improvement over the original version. It was clear, as also acknowledged by the author, that initially, most reviewers - including this one - did not completely understand the hypothesis or 'idea', or at least had several additional questions that needed clarification. These have now been addressed and, at least to this reviewer, the idea presented by Freeling is now clear and makes actually lots of sense. I have gone over the revised version twice but have no major comments. This having said, I still find the structure of the paper a bit strange at places... it will be up to [the editors] how to best organize the overall structure of this essay. In conclusion, as I've said in my first review, Mike Freeling's papers and ideas are often though-provoking and very interesting, and this is no exception. He provides a new and refreshing (and now much more clearly explained) view on what might explain the specific pattern or clustering (in time) of WGDs at times of environmental upheaval or extinction.

Final acceptance from Science Editor **Feb. 15, 2017**