Corresponding author:
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May the 10th, 2022

Christoph Haag
PCI Evolutionary Biology Recommender

Dear Recommender,

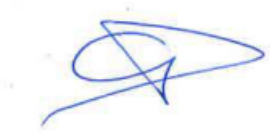
We enclose a newly revised version of our manuscript " Spontaneous parthenogenesis in the parasitoid wasp *Cotesia typhae*: low frequency anomaly or evolving process?".

We give below details about the changes and answers following your last remarks. The modifications in the text are highlighted in the "Revised Article with Changes Highlighted 2" file. We sincerely thank you for the time you took to review our article.

We hope that the changes made will be satisfactory for a recommendation in PCI Evol Biol.

Sincerely,

Claire Capdevielle Dulac



Dear Dr. Capdevielle Dulac,

I have read revised preprint as well as the response letter, explaining how you accommodated the points raised by the reviewers. I am satisfied that the revision successfully addresses these points (most of which were already minor) and am therefore happy to further consider your preprint for recommendation. That said, from my own reading, I have come across a number of additional points, which I would like you to consider in a further revision. I hope they will help to further improve the preprint.

Best wishes, and many thanks for submitting to PCI Evol Biol,

Christoph Haag

L. 55: add “than in others” after “in some taxa”

The change has been made.

L. 57-59: Perhaps add here (or elsewhere) that, in addition to egg development without fertilization, haplodiploids also have evolved spermatogenesis with aborted first meiotic division (e.g., Ferree et al. 2019. Sci Rep 9, 12194). In diploids, aborted (or suppressed) meiosis I is one of the possible thelytokous mechanisms leading to maintenance of centromeric heterozygosity or to 100% heterozygosity maintenance if recombination is suppressed. Adoption of the pathways for this type of modified meiosis (already present in males) for oogenetic meiosis may contribute to favouring evolution of telytoky in haplodiploids.

This indication and the reference have been added (lines 58-59).

Paragraph starting on L. 62 and elsewhere: the description of the different parthenogenetic mechanisms is now much improved. Still, a few further clarifications may be needed. In particular, I suggest using “Clonal apomixis” or “mitotic apomixis” instead of just “apomixis”: The reason is that the strict definition of apomixis also includes certain parthenogenesis modes that may be non-clonal (i.e., can lead to loss of heterozygosity): The term “apomixis” just means that there is no fusion of cells, so suppressed meiosis I and suppressed meiosis II are also apomictic modes of reproduction (“meiotic apomixis” in Archetti 2010). I know that the different terms are not always used in the same way and that there is a lot of confusion. But it’s perhaps better not to add to it. Also, the difference between “endoreplication” and “endomitosis” is unclear. They are probably the same, at least regarding their use here (also sometimes called “endoreduplication”), so it is better to use just a single term throughout. Finally, I agree that it is not needed to explain in detail the degree of heterozygosity loss or retention under the different mechanisms, but it would be good to provide a few references on the topic (sentence L.80-83).

We now refer to “clonal apomixis” and “endoreplication” in the text. References have been added line 84.

L. 200 and following paragraph: Define “parthenogenetic female” or probably better use “parthenogenetically produced females” (here and elsewhere in the manuscript), it is not much longer, but substantially more explicit.

The definition has been added line 201 and is also repeated in the result section (line 295). We preferred to keep the term “parthenogenetic female” for reading simplicity as it is very often used in the text.

L. 235 and L. 248: in each case, specify which or the two protocols.

The precision was added.

Fig. 1: I suggest “Clonal apomixis” and “E.g., mitosis” instead of “Apomixis” and “mitosis”. Note that also classical automixis with central fusion can result in fully clonal offspring if recombination is fully suppressed. Perhaps add this note to the figure legend.

The Figure 1 has been changed with “Clonal apomixis” and “E.g., mitosis”. Regarding your second comment, we prefer not to add this note. Indeed, even though we are aware that there are some exceptions, recombination is tightly linked to the meiotic process in the great majority of cases since it is involved in the pairing of homologous chromosomes and is used by the cell as a meiotic checkpoint. It seems to us that adding this note would complicate the legend too much for such a rare phenomenon.

L. 264: Here the formulation “parthenogenetic daughters” is particularly unclear: Does “females that produced parthenogenetic daughters” have the same meaning as “females that parthenogenetically produced daughters”? if so, the definition that they are the same should be given before.

The formulation “females that produced parthenogenetic daughters” has been replaced by “females that produced daughters parthenogenetically” and the definition of parthenogenetic daughters has been added line 211.

Table 1: The header of the last column is unclear (something wrong with the formulation “in the offspring presenting females”). Also, for the percentages (columns 3 and 5), should be specified “of …” (all females tested, all offspring) either here or in the table legend.

The header has been changed for “progenies containing females”. The percentages explanation has been added in the legend.

L. 305: Perhaps add “tested” to the end of the line.

The term has been added.

Table 3: “female sex-ratio” is unclear do you mean proportion of females among all offspring?

The precision has been added in the legend.

L 329 (twice): Here, probably “progeny” is meant instead of offspring (“an offspring” is one individual, 10 offspring 10 individuals, but a progeny is all of the offspring of a given individual)

We replaced the term offspring by the term progeny in the sentence.

L. 330: unclear if 773 males were only from the three progenies that contained at least one female offspring or the total across the 10 progenies.

The sentence has been reformulated to make it clear that the 773 males were obtained across the 10 progenies.

L. 339: perhaps better “along with 6653 males” instead of “for 6653 males”

We changed the sentence for “and 6653 males”, which seemed simpler.

L 339: “63 SNPs” instead of “63 SNP”.

Done.

L. 386: “fertilization” instead of “fecundation”

Done.

L. 397: N is probably the number of females tested not the number of offspring analyzed(?)

Yes it is, the change has been made.

L. 401: “virgin Makindu mothers” instead of “Makindu virgin mothers”

Done.

L. 417: “non-zero” instead of “not null”.

Done.

L. 428-430: Sentence unclear. What is meant by “in a common acceptation”? and isn’t that the definition for obligate parthenogenesis?

The sentence has been reformulated to make it clearer.

L. 442: low frequency thelytoky “appears to be” (instead of “may be”) and “rather than” instead of “but not”. You may also add that they had similar fertility as the sexually produced daughters.

Changes have been made and the precision has been added.

L. 445: “with an” adaptive benefit (instead of “due to its”)

Done.

L. 446: “confronted with” (instead of “confronted to”)

Done.

L. 448: “a honey bee” (instead of “an honey bee”)

Done.

L. 448-449: “egg-laying worker” (instead of “laying worker”)

Done.

Paragraph starting on line 489: I am not convinced that the results “strongly suggest” that two different mechanisms are at work. The low probability of single process was obtained under the assumption of Poisson-distributed numbers of crossovers. An alternative might be variable (overdispersed) crossover numbers (or locations) among different meioses: central fusion and suppression of meiosis I both result in fully clonal offspring (i.e., 100% heterozygosity retention, if no recombination occurs or if crossover locations are terminal to the last markers). Sure, all these meiosis occurred in F1 of crosses between two divergent inbred lines, so one doesn’t expect too much segregating variation. However, some segregating variation may persist, and crossover numbers (or locations) may also be plastic (as indicated by a single female that produced both daughters both clonally and non-clonally. The possibility that two different mechanisms are at work is interesting to discuss, but in my opinion, it is not needed to invoke any complicated mechanism such as inverted meiosis.

We tempered the comment indicating only that results “suggest” instead of “strongly suggest”. However, we maintain that it is difficult to simply explain the disruptive observation of zero recombination in 3 offspring over the 10 chromosomes while the number of recombination is comprised between 5 and 16 for the 6 other offspring. Regarding these 6 recombining offspring and the ~200 offspring obtained to build the genetic map (Benoist et al., 2020a), we noticed that recombination occurs in each meiosis. The location of the recombination is variable but the widespread genetic distribution of markers along the 10 chromosomes should have revealed at least some events. To maintain the discussion that two different mechanisms may co-occur, it seems necessary to show that other complicated mechanisms described in the literature are not congruent with our observations.

L. 512: What is the evidence that recombination rate was the same as under sexual reproduction?

The recombination rates were compared to the ones obtained when building the genetic map referenced in Benoist et al. 2020a from which the SNP markers of our present study were chosen.

L. 528: Reformulate the first sentence

The sentence has been reformulated.

L. 553: Meaning of “functional apomixis” unclear.

We changed the term to “clonal apomixis”.

L. 572: “parthenogenesis” (instead of “situations”).

Done.