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April the 8th, 2022

Christoph Haag
PCI Evolutionary Biology Recommender

Dear Recommender,

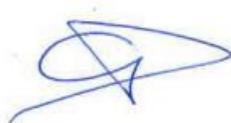
We enclose a revised version of our manuscript " Spontaneous parthenogenesis in the parasitoid wasp *Cotesia typhae*: low frequency anomaly or evolving process?".

We give below details about the changes and answers performed following your remarks and the ones formulated by the reviewers. The modifications in the text are highlighted in the "Revised Article with Changes Highlighted" file. We sincerely thank you for the useful comments that helped us improve our manuscript.

We hope that changes will meet your expectations. We thank you for your interest in our work for publication in *PCIEvolBiol*.

Sincerely,

Claire Capdevielle Dulac



Dear Dr Capdevielle Dulac,

Thank you for submitting your preprint "Spontaneous parthenogenesis in the parasitoid wasp *Cotesia typhae*: low frequency anomaly or evolving process?" to *PCI Evol Biol*. Your work has now been considered by two reviewers, whose comments are enclosed. As you will see, the reviews are largely positive, and, based on these reviews as well as my own reading, I am happy to further consider your preprint for recommendation. However, before reaching a final decision, I would like you to revise your manuscript according to the recommendations by the reviewers. Furthermore, from my own reading, I would also like you to consider the following points in your revision.

1. The general evolutionary implications of the findings, beyond those specific to the study system, should be explained in more detail, in my opinion. Indeed, both the description of the aims of the study (end of introduction) and the conclusions are highly system-specific, and a clear explanation of the broader implications are missing also elsewhere. To be honest, I would find it somewhat difficult to write a recommendation for a general evolutionary readership based on the current preprint. Nonetheless, there clearly is a potential to present a broader perspective on the results, and some elements are indeed present in various parts of the manuscript. In other words, it would be good to assemble these elements into some more explicit statements of the general evolutionary implications of the study.

The end of the introduction and the conclusion have been rewritten to enlarge the evolutionary perspective. We hope it will meet your expectations.

The figure explaining the different possible modes of parthenogenesis, as well as the corresponding text would need careful revision: (i) Several mechanisms of parthenogenesis are missing, (ii) apomixis is not necessarily equal to mitosis and is not the only way by which clonality can arise, and (iii) the genetic consequences of the different modes of parthenogenesis strongly depend on the amount of recombination. The whole topic is somewhat problematic as many different terms exist and because they are not always employed in the same way. In particular, from a cytological point of view, automixis is defined as normal meiosis followed by fusion of meiotic products (see for instance Archetti *J. Hered.* 2010). From a genetic point of view, suppression or abortion of one of the meiotic divisions is equivalent to central fusion automixis (suppression/abortion of meiosis I) or terminal fusion automixis (suppression/abortion of meiosis II). However, as there is no fusion in these cases, they are often regarded as apomixis ("meiotic apomixis" in Archetti 2010), especially by cytologists (the term "apomixis" means that there is no fusion). Furthermore, both suppression/abortion of meiosis I and central fusion automixis result in clonal offspring when there is no recombination as does also pre-meiotic doubling (or "endomitosis") if pairing occurs exclusively between sister chromosomes. These issues should be better reflected in the figure and in the interpretation of the results. Note also that inversed meiosis with terminal fusion is genetically exactly the same as normal meiosis with central fusion (see for instance Archetti 2022 *J Evol Biol.* 35:40–50). So perhaps it is not needed to complicate matters even more.

The text has been completed in the introduction (lines 62-84) to give a wider picture of the cytological mechanisms leading to thelytoky. We preferred however to leave the figure unchanged for simplification purpose, but we added a note indicating it was simplified to

focus on genetic consequences and referred to introduction for further details. We also modulate the conclusion about the mechanism (line 345-347 and 490-491)

2. Parts of the text, most notably in the discussion, could be streamlined. In particular, the discussion of issues not directly related to the results should be kept minimal. Also, please check the entire text carefully for minor language imprecisions.

Several parts of the discussion were shortened to focus on the interpretation of results from an evolutionary perspective.

3. “Presence of parthenogenetic females among the daughters of mated females”: In this section I did not understand why only mixed broods were considered. What was the reason for excluding female-only broods?

The text has been clarified (lines 245-248): no female-only broods were obtained. The progenies are either mixed or male-only. The last case corresponds to virgin females’ progenies that were discarded in this part focusing on mated females.

Best wishes,

Christoph Haag

Reviews

Reviewed by Jens Bast, 14 Feb 2022 13:50

In their study ‘Spontaneous parthenogenesis in the parasitoid wasp *Cotesia typhae*: low frequency anomaly or evolving process?’, Dulac et al use a nice study system to look at many angles of the occurrence of spontaneous parthenogenesis in two laboratory strains and one wild strain of a haplo-diploid hymenopteran. First, the occurrence of parthenogenetic females was counted, the fecundity of parthenogenetic mated females estimated, the mode of parthenogenesis (central fusion automixis) analysed and one possible cause for parthenogenesis surveyed (bacterial origin unlikely).

The ‘paradox of sex’ is one of the major enigmas in evolutionary biology and still not fully understood. While many studies focus on the fate of asexual lineages (such as deleterious mutation accumulation, etc.), few exist on the rate of transition to asexuality, its cause of origin and the mode of asexuality. How and how often incipient asexual lineages are generated can help to explain the predominance of obligate sexual species and the (maybe not so scarce as assumed) asexual lineages in nature. Thus, the study by Dulac et al is an important addition to the field.

In my opinion, the paper is well written, the exhaustive introduction is thoroughly researched and the elaborate discussion is well backed up by the results and potential shortcomings are explained. The methods seem elaborate and fitting.

However, I think the manuscript needs improvement and/or explanations on some points, mostly clarifications in the results part. My (minor) concerns and remarks are laid out below:

Line 93

What is meant by “...within species also varies between taxa”? Does it mean that a single species can feature mixed reproduction?

The sentence was changed to “The relative frequency of thelytoky and sexual reproduction within species also varies according to taxa” (line 100-101) for more clarity.

Line 273 ff.

The first part of the results was slightly confusing to me. It would be good to clarify what the Table 1 contents means (mostly the table header text can be improved). I had to write down as a sentence what I can see in the table: For example, out of 99 virgin females, 67 (68%) were able to produce females (that are thus produced parthenogenetically (here the term parthenogenetic females is used). The 99 virgin females produced 10882 offspring in total, out of which 10657 were males and 225 (2%) were females (parthenogenetic females).

Confusing are expressions like “offspring WITH parthenogenetic females” or “females PER offspring”, because it suggests (to me) that offspring produced offspring.

I suggest to rename the table headers to (of course you can choose something else): Total number of virgin females (N); Number of virgin females that produced female offspring (parthenogenetically); Number of males produced by all virgin females, Number of females produced by all virgin females; Mean number of parthenogenetic females produced per virgin female

Thank you for the suggestions that clarify the headers. Changes have been made following these proposals.

(Table 4 is much clearer in that respect)

I think another source of the confusion is this: In the description it says “‘N’ is the number of virgin females tested”, but “N” is just used interchangeably for “virgin females”. Maybe use “VF” instead, if the headers of the table get too big.

This comment is not completely clear for us. We hope the changes in the headers helped to clarify that point also.

Please state clearly in the main text somewhere that you use the ‘parthenogenetic female’ term as ‘parthenogenetically produced female’ throughout the manuscript to avoid confusion (it is hidden in the Table 1 description at the moment).

This statement has been added line 295.

What is the mean number of females produced by virgin females with parthenogenetic ability? It is clear that some produced none, but interesting would be if there is the ability and how many can be produced.

We changed the data presented in tables 1 and 4 to focus on the offspring presenting females: the mean number of parthenogenetic females is now calculated excluding the progeny of virgin females that produced only males.

Could the Kobodo lab strain have lost the (genetic) mechanism for the ability to produce parthenogenetic females through inbreeding?

Both strains (Makindu and Kobodo) have been submitted to similar inbreeding conditions and genetic drift pressure. Nonetheless, random process of genetic drift may effectively have led to the loss of the ability in one strain and not in the other. This is a likely explanation given that the natural population of Kobodo is still able to produce females through parthenogenesis process. This precision was added in the text (line 299-300).

Line 288 ff

Did you assess the ploidy of the Kobodo lab strain and Makindu wild strain? This is outside of the scope of the paper I guess, but just wondering, if genomes sizes between strains might be different.

The ploidy was only assessed on the Makindu lab strain. However, the different populations of *Cotesia typhae* are genetically very close (see Kaiser et al., 2017, Ongoing ecological speciation in *Cotesia sesamiae*, a biological control agent of cereal stem borers). A genome assembly of this species was obtained from a lab strain initiated with individuals from the Kobodo population. This assembly comprises about 187 Mb, which is smaller than the estimate obtained from flow cytometry. However, it's difficult to compare such estimates as genome assemblies generally miss heterochromatic regions and the genome sizes derived are often underestimated.

Line 299 ff

Are these females derived from Makindu wild strain or lab strain mothers? (Lab strain is explained in the Table 3 text)

This precision is indicated in Material and Methods section line 196 : "all coming from the Makindu laboratory strain, to determine their ploidy".

Did you also test the other strains?

The flow cytometry analysis was only performed on the Makindu strain for which we had females produced parthenogenetically.

Are the virgin parthenogenetic females able to produce daughters (and to what proportion / fecundity)?

We recently obtained data regarding this question, that we added in the paragraph concerning the fecundity assessment of parthenogenetic females (line 209-213 and 327-331).

Lines 319 ff

You state that these regions of maintained heterozygosity correspond to the centromeres (or “central parts of chromosomes” / “indicative of the position of the centromere”). While this seems logical, it is a bit of a circular argument. Were centromeres identified in previous studies?

Cytological preparations from male testis were produced by a colleague and confirmed the metacentric state of the chromosomes. The quality of the picture (pasted below) is not good enough to be included in the manuscript but the precision has been added in the figure caption line 360: “Given the metacentric nature of *C. typhae* chromosomes (C. Bressac, personal communication)”.

Lines 341 ff

Could you add the probability calculation to find purely heterozygous females to the methods or if it is an R script as supplementary or github (or zenodo)?

An R script used to estimate the probability of lack of recombination in 3 females is given as supplementary data with a simplified version of genotypic file.

Lines 345 ff

Table 4 Mean number for Makindu virgin females is different to the one in Table 1 (2.3 [1;8] vs 2.3 [0;8]). Lies the difference in “when present” and “all”?

The same result is now presented in both tables focusing on progenies containing parthenogenetic females (see comment above).

Lines 450 ff.

Just as a side note: Another ‘ecological explanation’ I quite like and seems fitting is the structured resource theory (Scheu & Drossel 2007 <https://doi.org/10.1098/rspb.2007.0040> ; Son et al 2012 <https://doi.org/10.1111/j.1420-9101.2012.02454.x> ; somewhat a background explanation Song et al 2011 <https://onlinelibrary.wiley.com/doi/10.1111/j.1600-0706.2011.19698.x>).

In this theory, asexuals have an advantage when resources are plenty and the carrying capacity is not maxed out. In other words: Sexual reproduction prevails in a world of structured resources in short supply. So, indeed studying the different *C. sesamiae* populations would be interesting.

Thanks for these interesting references. Son et al 2012 that combined previous studies for multicellular long-lived organisms such as metazoan was added in the discussion (lines 475-480)

Reviewed by Michael Lattorff, 14 Feb 2022 13:51

The manuscript “**Spontaneous parthenogenesis in the parasitoid wasp *Cotesia typhae*: low frequency anomaly or evolving process?**” by Dulac et al. submitted to bioRxiv and PCI Evolutionary Biology analyses the rare occurrence of thelytokous parthenogenesis by *C. typhae*. The authors present a range of experiments targeting both, wild and laboratory populations.

The manuscript is well written, the introduction is very clear listing all relevant literature. Material and methods are clearly described, but still can be improved. I wondered if this section could be structured slightly differentially. My suggestion is that general rearing, DNA extraction and genotyping are explained (the support tools you use), before coming to set-up of experiments, which are hypothesis driven.

The material and methods section has been reorganized following this proposal and seems indeed easier to follow. Thank you for the suggestion.

In the results section I suggest to use in addition to the number of individuals, the percentage as it is now presented in the tables, the 95% confidence intervals. This will make comparisons between different set-ups easier, especially as you have always different sample sizes. In most case you deal with binomial distributions (x females of a total N), for which you could calculate the 95% confidence interval for a binomial distribution.

The confidence intervals have been added in tables 1 and 4.

In the discussion I was missing reference to other systems. For example, in the honeybee, *Apis mellifera*, which is also a haplo-diploid Hymenopteran species, shows low levels of thelytokous parthenogenesis of queens, which is often around 1-2 %, rarely higher. It has been investigated since decades (Mackensen 1943 J Econ Entomol; Tucker 1958 Genetics; Gloag et al 2019 Apidologie; among others) and found that adverse environmental conditions can lead to that form of aberrant parthenogenesis, especially delayed egg laying of virgin queens and nutritional deprivation. I suggest to study those other models and see how these probably relate to the issue of thelytokous parthenogenesis in *C. typhae*.

Thank you for these references. They were added to the discussion (lines 426-428 and 445-447).

Furthermore, I wondered whether the size of the parasitized caterpillar could be a clue for the parasitoid to decide whether to lay male or female eggs. However, this is just a guess arising from gut feeling and just comes out of curiosity.

We only have preliminary results on mated females that suggest that the sex ratio of the progeny is independent of the size of the host. However, we don't have any data for virgin females.

Minor

issues

You should label figure panels (a & b) according to occurrence in the text. It seems that 1 B is cited before 1 A, thus I suggest to change A and B figures.

Both panels have been inverted in figure 1.

L223. The header Looking for thelytoky in mated females should be reworded. Sound a bit like slang language

Header has been changed to « Identifying the thelytoky phenomenon in mated females progenies » (Line 238)

L493 & L503 Cap bee, *Apis mellifera capensis*, it is the Cape bee...

The change has been made (lines 515 and 525).

L492-95 recombination rate in the Cape honeybee is reduced (these are thelytokous workers in comparison to their sexually reproducing mothers, the queens), you should always explain if something is reduced compared to what.

The sentence has been completed: "recombination in thelytokous workers is reduced by up to 10-fold in comparison to their sexually reproducing mothers in the Cape bee" (lines 514-517).

L507/508 insert reference for the statement that deleterious mutations are purged in haploid males

We added the following citations:

Hedrick, P. W., & Parker, J. D. (1997). Evolutionary genetics and genetic variation of haplodiploids and X-linked genes. *Annual Review of Ecology and Systematics*, 28(1), 55-83.

Henter, H. J. (2003). Inbreeding depression and haplodiploidy: experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa. *Evolution*, 57(8), 1793-1803.

