Peer Community In
Evolutionary Biology

The potential evolutionary importance of low-frequency flexibility in reproductive modes

Christoph Haag *based on peer reviews by* ***Michael Lattorff*** *and* ***Jens Bast***

Claire Capdevielle Dulac, Romain Benoist, Sarah Paquet, Paul-André Calatayud, Julius Obonyo, Laure Kaiser, Florence Mougel (2022) Spontaneous parthenogenesis in the parasitoid wasp Cotesia typhae: low frequency anomaly or evolving process? Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Evolutionary Biology. https://doi.org/10.1101/2021.12.13.472356

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Occasional events of asexual reproduction in otherwise sexual taxa have been documented since a long time. Accounts range from observations of offspring development from unfertilized eggs in Drosophila to rare offspring production by isolated females in lizards and birds (e.g., Stalker 1954, Watts et al 2006, Ryder et al. 2021). Many more such cases likely await documentation, as rare events are inherently difficult to observe. These rare events of asexual reproduction are often associated with low offspring fitness ("tychoparthenogenesis"), and have mostly been discarded in the evolutionary literature as reproductive accidents without evolutionary significance. Recently, however, there has been an increased interest in the details of evolutionary transitions from sexual to asexual reproduction (e.g., Archetti 2010, Neiman et al.2014, Lenormand et al. 2016), because these details may be key to understanding why successful transitions are rare, why they occur more frequently in some groups than in others, and why certain genetic mechanisms of ploidy maintenance or ploidy restoration are more often observed than others. In this context, the hypothesis has been formulated that regular or even obligate asexual reproduction may evolve from these rare events of asexual reproduction (e.g., Schwander et al. 2010).

A new study by Capdevielle Dulac et al. (2022) now investigates this question in a parasitoid wasp, highlighting also the fact that what is considered rare or occasional may differ from one system to the next. The results show "rare" parthenogenetic production of diploid daughters occurring at variable frequencies (from zero to 2 %) in different laboratory strains, as well as in a natural population. They also demonstrate parthenogenetic production of female offspring in both virgin females and mated ones, as well as no reduced fecundity of parthenogenetically produced offspring. These findings suggest that parthenogenetic production of daughters, while still being rare, may be a more regular and less deleterious reproductive feature in this species than in other cases of occasional asexuality. Indeed, haplodiploid organisms, such as this parasitoid wasp have been hypothesized to facilitate evolutionary transitions to asexuality (Neimann et al. 2014, Van Der Kooi et al. 2017). First, in haploidiploid organisms, females are diploid and develop from normal, fertilized eggs, but males are haploid as they develop parthenogenetically from unfertilized eggs. This means that, in these species, fertilization is not necessarily needed to trigger development, thus removing one of the constraints for transitions to obligate asexuality (Engelstädter 2008, Vorburger 2014). Second, spermatogenesis in males occurs by a modified meiosis that skips the first meiotic division (e.g., Ferree et al. 2019). Haploidiploid organisms may thus have a potential route for an evolutionary transition to obligate parthenogenesis that is not available to organisms: The pathways for the modified meiosis may be re-used for oogenesis, which might result in unreduced, diploid eggs. Third, the particular species studied here regularly undergoes inbreeding by brother-sister mating within their hosts. Homozygosity, including at the sex determination locus (Engelstädter 2008), is therefore expected to have less negative effects in this species compared to many other, non-inbreeding haplodipoids (see also Little et al. 2017). This particular species may therefore be less affected by loss of heterozygosity, which occurs in a fashion similar to self-fertilization under many forms of non-clonal parthenogenesis.

Indeed, the study also addresses the mechanisms underlying parthenogenesis in the species. Surprisingly, the authors find that parthenogenetically produced females are likely produced by two distinct genetic mechanisms. The first results in clonality (maintenance of the maternal genotype), whereas the second one results in a loss of heterozygosity towards the telomeres, likely due to crossovers occurring between the centromeres and the telomeres. Moreover, bacterial infections appear to affect the propensity of parthenogenesis but are unlikely the primary cause. Together, the finding suggests that parthenogenesis is a variable trait in the species, both in terms of frequency and mechanisms. It is not entirely clear to what degree this variation is heritable, but if it is, then these results constitute evidence for low-frequency existence of variable and heritable parthenogenesis phenotypes, that is, the raw material from which evolutionary transitions to more regular forms of parthenogenesis may occur.

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Reviews

Evaluation round #2

DOI or URL of the preprint: https://www.biorxiv.org/content/10.1101/2021.12.13.472356v4
Version of the preprint: 4

Authors' reply, 11 May 2022

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Decision by Christoph Haag, posted 25 April 2022

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”

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.

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).

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.

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

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.

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.

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.

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

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

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)

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.

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

L 339: “63 SNPs” instead of “63 SNP”.

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

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

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

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

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

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.

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

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

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

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

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.

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

L. 528: Reformulate the first sentence

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

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

Evaluation round #1

DOI or URL of the preprint: `https://www.biorxiv.org/content/10.1101/2021.12.13.472356v3`
Version of the preprint: 3

Authors’ reply, 08 April 2022

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Decision by Christoph Haag, posted 18 February 2022

Revise

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.

2. 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.

3. 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.

4. "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?

Best wishes,
Christoph Haag

Reviewed by Jens Bast, 14 February 2022

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?

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 partenogentically (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

(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.

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).

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.

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

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.

Line 299 ff

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

Did you also test the other strains?

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

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?

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)?

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"?

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.

Reviewed by Michael Lattorff, 14 February 2022

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. 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.

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 averse 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*.

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

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.
L223. The header Looking for thelytoky in mated females should be reworded. Sound a bit like slang language
L493 & L503 Cap bee, Apis mellifera capensis, it is the Cape bee...
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.

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