Peer Community In
Evolutionary Biology

The pros and cons of mating with strangers

Vincent Calcagno based on peer reviews by *Joël Meunier* and *Michael D Greenfield*

Salomé H. Clemente, Inês Santos, Rita Ponce, Leonor R. Rodrigues, Susana A. M. Varela and Sara Magalhães (2017) Despite reproductive interference, the net outcome of reproductive interactions among spider mite species is not necessarily costly. Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Evolutionary Biology. 10.1101/113274

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Interspecific matings are by definition rare events in nature, but when they occur they can be very important, and not only because they might condition gene flow between species. Even when such matings have no genetic consequence, for instance if they do not yield any fertile hybrid offspring, they can still have an impact on the population dynamics of the species involved [1]. Such atypical pairings between heterospecific partners are usually regarded as detrimental or undesired; as they interfere with the occurrence or success of intraspecific matings, they are expected to cause a decline in absolute fitness. The story is not always so simple however, and it might all depend on the timing of events and on the identity of the partners. Using the herbivorous mite *Tetranychus urticae* as a model, Clemente *et al.* [2] experimentally arranged matings with two other *Tetranychus* species that commonly share the same host plants as *T. urticae*. They carefully controlled the history of events: heterospecific matings could occur just before, just after, 24h before, or 24h after, a conspecific mating. Interestingly, the oviposition rate (total fecundity) of females was increased when mating with a heterospecific individual. This suggests that heterospecic sperm can stimulate oogenesis just as conspecific sperm does. Such a positive effect was observed for matings involving *T. ludeni* females and *T. urticae* males, but a negative effect is found in the interaction with *T. evansi*. Sex-ratio (fertilization success in those species) could also be impacted but, unlike fertilization, this occurred when the mating events were distant in time. This is is at odds with what is observed in conspecific matings, where sperm displacement occurs only if mating events are temporally close. Overall, the effects of heterospecific mating were quite variable and it is challenging to predict a single, general, effect of interspecific matings. The net effect will likely be context-dependent, depending on the relative frequency of the difference mating sequences and on how fecundity and sex-ratio contribute to overall fitness, both aspect strongly influenced by the population dynamics and structure.

References:

[1] Gröning J. & Hochkirch A. 2008. Reproductive interference between animal species. The Quarterly Review of Biology 83: 257-282. doi: [10.1086/590510](https://doi.org/10.1086/590510)

[2] Clemente SH, Santos I, Ponce AR, Rodrigues LR, Varela SAM & Magalhaes S. 2017 Despite reproductive interference, the net outcome of reproductive interactions among spider mite species is not necessarily costly. bioRxiv 113274, ver. 4 of the 30th of June 2017. doi: [10.1101/113274](https://doi.org/10.1101/113274)

Reviews

Evaluation round #2

DOI or URL of the preprint: **10.1101/113274**
Version of the preprint: 2

Authors' reply, 06 July 2017

We thank the recommender and the reviewers for this new round of review.

We double-checked the statistical analyses which allowed us to correct one mistake. In the discussion section, we added a paragraph on the effect on sex ratio. In the introduction we also added some dertails on our biological model.

We hope that this new version of the preprint is acceptable for recommendation by PCI Evol Biol.

Decision by **Vincent Calcagno**, posted 28 June 2017

Recommendation conditional on revisions

Dear authors, Thank you for revising your preprint. The reviewers and I are satisfied with your revisions and by your response letter as well.

I am therefore willing to recommend your preprint for PCI Evol Biol, once you will have double-checked the statistical points that one reviewer has raised: please provide degrees of freedom wherever appropriate and make sure there are no errors in the statistics reported.

Reviewed by **Michael D Greenfield**, 26 June 2017

This version is greatly improved in accuracy and clarity, in my opinion. But I offer a few points for future (eventual) improvement.

Removing the meta-analysis is most probably a wise move at this time, given the uncertainties involved in combining overall fecundity and fertility (sex ratio). That said, the overall positive or negative effect of inter-specific mating on fitness will depend on the two factors, and the influence of sex ratio will depend on population composition and dynamics. Thus, some discussion may be helpful, and it is hoped that an accurate method of combining the factors can be found.

The information on the species beginning on line 80 is helpful, and more would be better. Again, it is hoped that further information will be forthcoming so that the implications of interspecific mating in the field can be assessed in a more informed way.

I recommend posting the manuscript, and further input may lead to yet other refinements.

Reviewed by **Joël Meunier**, 22 June 2017

I have finished to read the corrected version of this manuscript and overall would like to thank them for their efforts. Having said that, I still have a few additional comments: 1) I did not manage to find any figure or table in the manuscript, which makes it relatively difficult to review. 2) Some of statistical values reported in the text are very similar across different test (e.g. L235 F(2,78) = 1.886, p = 0.158 and then L238 F(2,66)=1.886, p = 0.158), which calls for a double check of all the reported values. 3) Some reported statistical values are incomplete. For instance, it is important to give the df when a |t| value is reported.

Evaluation round #1

DOI or URL of the preprint: **10.1101/113274**
Version of the preprint: 1

Authors' reply, 09 June 2017

Dear Editor,
We herewith send you a revised version of our article entitled "Despite reproductive interference, the net outcome of reproductive interactions among spider mite species is not necessarily costly". Below, we present in detail how we have accommodated the (very useful comments) we have received.
All the best,
SM, on behalf of all authors **Recommender comments:**

I have read your preprint manuscript with great interest. It reports some intriguing results, such as increased fecundity following heterospecific matings. I got it evaluated by two expert reviewers, and based on this I believe I could recommend your work on PCI Evol. Biol.. However this would require you to prepare and upload a revised version that addresses the following concerns.

First, it is not clear what is the realized or expected frequency in the wild of the different events you are experimentally considering, i.e. the different mating sequences.
What are the existing premating barriers?
What is the typical number of matings experienced by females?
Such information should be very clearly provided to decide whether you are considering common events that have a fitness impact and whose frequency and consequences are potentially shaped by natural selection, or if you are considering more abstract postzygotic barriers and the level of isolation those species would experience in the case of hybridization.
R: We have information on mate choice between T. evansi and T. urticae (Clemente et al. 2016 Behav Ecol Sociobiol). While there is some assortative mating when T. urticae females or T. evansi males choose between conspecifics and heterospecifics, other choice experiments revealed no preference. We did not do this experiment for T. ludeni/T. urticae. In any case, all species mate with other, as shown in the current study. So, in sum, premating barriers are rather weak. Females become more reluctant to mate after the first mating, but they do remate. I'm not aware of any study that has evaluated the total number of matings experienced by a female. This would imply observing a female throughout her life, which is largely unfeasible... Again, I'm not sure that's fatally necessary, as long as we know that they do remate.

Second, the reviewers, and I had the same impression, were puzzled by your use of a metaanalysis statistical pipeline at the end of your study. As one reviewer states this is atypical in this experimental context, but more importantly it is not clear at all what this brings on top of the previous statistical analyses you are conducting earlier in the manuscript. Reanalyzing the same data, or presenting two sets of analyses for the same data, is not a good idea. As I understand the main reason you would want to use this metanalysis package is to get an idea of "combined effects". However, and I concur with reviewers on this point, it seems a bit artificial and akin to adding apples and oranges: brood size and

sexratio are two distinct components that enter fitness in a complex (and certainly context-dependent) way, so it does not seem possible to simply add them up with a 1:1 weighting. This seems too naive in this context. Furthermore, you could discuss such combined effects based on the simpler analyzes, with no need to compute precise (and oversimplified) quantities. I would have the same critics regarding the computation of a summary estimate over the different types of matings: as we do not know what is their relative frequencies (see the previous point) in the wild, it makes no sense to combine them additively with equal weighting in some sort of average. In terms of formatting, the metaanalysis also looks like an additional layer added on top of everything at the very end: it may be redundant or unnecessary, but it surely lacks sufficient explanation/justification. I personally think you should drop this entire analysis, unless you substantially rewrite the article so that it becomes obvious why you need it and what it does exactly (in which case it would probably replace several earlier analyses presented).

R: We opted for a reply to all points simultaneously because they affect each other. We actually totally agree with the concerns raised by the recommender and one reviewer concerning the meta-analysis. Because our data is quite comprehensive, we initially thought that having a statistic that summarized the results obtained would help interpreting such data. However, we now realize that the meta-analysis, by resting on several hidden assumptions, not the least that all events happen at an equal frequency, actually generates more problems than it solves. We thus opted for discussing the data as such, as suggested by the reviewers.

One reviewer also brought up several other concerns about the statistical results and the overall presentation that you would want to address. If you are willing to revise your manuscript, I will be happy to consider it for recommendation, and in any case I hope you will find these reports useful.

R: we have modified the presentation of the results and also removed post-hoc comparisons when the factor was not significant, indeed an error, thanks for pointing that out. **Reviewed by Michael D Greenfield, 2017-05-04 10:03**

In general, I found the article on reproductive interference in spider mites interesting and a report on a carefully conducted study. The topic is certainly important in evolutionary biology, and the authors present an unusual, if not novel, approach to the issue of hybridization between closely related species. Here are a few questions and points for further analysis and evaluations.

1) The authors use meta-analysis for arriving at an overall impact, positive or negative, of reproductive interference on a species by considering both the influences on fecundity and fertility (sex ratio). This is, of course, an appropriate method, but I do not understand how fecundity and fertility (sex ratio) can be combined, supposedly with equal weighting. The impact of the sex ratio should depend on various parameters of the population(s).

R: We have removed the meta-analysis

2) Are these species expected to encounter one another in the field to the extent that interspecific mating will actually occur? We need to know more about pre-mating barriers of all sorts.

R: We have added a few sentences on this in the introduction (lines 80-83).

3) More consideration might be given to the supposed effect of sperm on fecundity. Why would heterospecific sperm have a more stimulating effect than conspecific sperm? Some clarification is needed.

R: That’s indeed puzzling, but we can only widely speculate about it, and we do not have any particular hypothesis...

4) Overall, the study serves to highlight the impact of ’globalization’ (transport of host plants, etc.) and changing agricultural practices on the concept of ’species’.

R: Right. **Reviewed by Joël Meunier, 2017-05-04 10:03**

In this study, the authors investigate the outcome of heterospecific mating between a focal species (Tetranychus urticae) and two other species (T. evansi and T. ludeni) of mites. The results show that mating between T urtica and T ludeni do not produce any hybrids, whereas T urtica and T evansi do

produce hybrids. Overall, I liked this study. However, I have several concerns that should be addressed to increase the robustness of the results and improve the general clarity of the study.

Major concerns:

1. The study is overall very difficult to follow. The framework of the entire study is about reproductive interference, which is a very broad topic, whereas the study "simply" tests for mating incompatibilities between different species. This general framework is a bit misleading and does not perfectly fit with the experiments. This is, for instance, illustrated by the first experiment which only focuses on mating incompatibilities between two species and showing that there is one and thus that the three selected species are not ideal to test for reproductive interference the way they want to do it (see line 67-69).

R: Reproductive interference is indeed a general term, but actually most studies of it concern mating incompatibilities, so we think it may be better to maintain the term, as it is not incorrect and may attract a broader readership.

2. The material and method lacks numerous crucial information. For instance, the sample size is never mentioned! How many males and females did you actually test per treatment? On L178, it is written that you used groups of 15 females, but how many groups?? This is a crucial information, also for the meta-analysis part, as it is still unclear to me, as to why you use such a complex statistical process to address the question you are interested in. And can you really do such a large-scale statistical process with a small sample size?

R: We have now added sample sizes to the graphs.

3. I found the result part difficult to read as it is not connected to clearly formulated questions. The tables providing the stats are also unusual and therefore difficult to follow. I would suggest the author to add their statistical results to the main text (instead of the table) to help them better structuring this part.

R: Done.

Minor concernts:

L61-64: this is assuming that you are working on a species with multiple mating.

R: We added "in polyandrous species". Line 52

L70: "all possible effects": at least the ones you are raising above in the text.

R: we modified it to "by focussing on some event sequences only" Line 61

L83-88: Does it mean that a female with "good fertility" is supposed to produce females only? But, a female might "decide" to not fertilise her eggs in order to produce males (even if she has sperm stored in her spermatheca).

R: Right, sex ratio is never 0% males and generally varies with population structure. However, by comparing the sex ratio stemming from heterospecific matings and those from conspecific matings, we can assess the portion of males that results from disturbances due to the former.

L112: The last paragraph of the discuss lack clear predictions and a quick overview of the experimental design. As such, it is difficult to understand what the researchers have done in this study and to what extend it does answer their general question.

R: We have now considerably modified the last section of the introduction to account for this comment.

L127: the species was identified by analysing 50 females per population. Does it means that each population contains only a single species under natural condition? If yes, isn't it an issue for your study, as you are testing a pattern that is unlikely to occur in the field and thus for which selection is unlikely to have "naturally" operated?

R: Indeed, to form our laboratory populations, we used samples with a single species, to avoid contaminations. However, migration is high in this group, and species co-occurrence is frequent. Apart from the data available in the literature on this, we also often found them together in our sampling. Therefore, populations are not likely to be composed of a single species for long time periods.

L137: Do all the species and tested populations received the tetracycline treatment? This is manda-

tory to avoid any species-specific treatment that could have biased the results.
R: Yes, of course.

L222: Why no running a single statistical model in which you have the species of the focal female, and then a factors that says if the mate if homospecific or heterospecific?
R: The females have very different baseline fecundities, hence we opted for doing the analysis separately for females of each species, as we would have to separate them anyway to perform further analyses separately anyway...

L234: it is important to show the results of the block 1 only (at least at a supplementary material). Since the experimental design is not balanced at all between the blocks, it is at least important to provide all the available information for the reader to accept (or not) that it does not affect the robustness of your conclusions.
R: This is now done in the supplementary information.

L236-274: I am familiar with meta-analyses, but it is the first time I see it in this context. For now, it comes a bit out of the blue and I do not really understand why the results from your statistical models are not good enough to allow you drawing the same conclusions. Would it be possible to better explain why this statistical process is required to address this specific question? Tables: It is generally useless (if not wrong) to conduct pairwise analyses when the main factor is not significant. You did it several times.
R: We agree with this comment and have removed the meta-analysis (cf. our reply to the editor).

Decision by Vincent Calcagno, posted 04 May 2017

Revision needed

Dear author,

I have read your preprint manuscript with great interest. It reports some intriguing results, such as increased fecundity following heterospecific matings. I got it evaluated by two expert reviewers, and based on this I believe I could recommend your work on PCI Evol. Biol.. However this would require you to prepare and upload a revised version that addresses the following concerns.

First, it is not clear what is the realized or expected frequency in the wild of the different events you are experimentally considering, i.e. the different mating sequences. What are the existing premating barriers? What is the typical number of matings experienced by females? Such information should be very clearly provided to decide whether you are considering common events that have a fitness impact and whose frequency and consequences are potentially shaped by natural selection, or if you are considering more abstract postzygotic barriers and the level of isolation those species would experience in the case of hybridization.

Second, the reviewers, and I had the same impression, were puzzled by your use of a metaanalysis statistical pipeline at the end of your study. As one reviewer states this is atypical in this experimental context, but more importantly it is not clear at all what this brings on top of the previous statistical analyses you are conducting earlier in the manuscript. Reanalyzing the same data, or presenting two sets of analyses for the same data, is not a good idea. As I understand the main reason you would want to use this metanalysis package is to get an idea of “combined effects”. However, and I concur with reviewers on this point, it seems a bit artificial and akin to adding apples and oranges: brood size and sexratio are two distinct components that enter fitness in a complex (and certainly context-dependent) way, so it does not seem possible to simply add them up with a 1:1 weighting. This seems too naive in this context. Furthermore, you could discuss such combined effects based on the simpler analyzes, with no need to compute precise (and oversimplified) quantities. I would have the same critics regarding the computation of a summary estimate over the different types of matings: as we do not know what is their relative frequencies (see the previous point) in the wild, it makes no sense to combine them additively with equal weighting in some sort of average. In terms of formatting, the metaanalysis also looks like an additional layer added on top of everything at the very end: it may be redundant or unnecessary, but it surely lacks sufficient explanation/justification. I personally think you should drop this entire analysis, unless you substantially rewrite the article so that it becomes obvious why you need it and what it does exactly (in which case it would probably replace several earlier analyses presented).

One reviewer also brought up several other concerns about the statistical results and the overall presentation that you would want to address. If you are willing to revise your manuscript, I will be happy to consider it for recommendation, and in any case I hope you will find these reports useful.

Reviewed by Michael D Greenfield, 03 May 2017

In general, I found the article on reproductive interference in spider mites interesting and a report on a carefully conducted study. The topic is certainly important in evolutionary biology, and the authors present an unusual, if not novel, approach to the issue of hybridization between closely related species.

Here are a few questions and points for further analysis and evaluations.

1) The authors use meta-analysis for arriving at an overall impact, positive or negative, of reproductive interference on a species by considering both the influences on fecundity and fertility (sex ratio). This is, of course, an appropriate method, but I do not understand how fecundity and fertility (sex ratio) can be combined, supposedly with equal weighting. The impact of the sex ratio should depend on various parameters of the population(s).

2) Are these species expected to encounter one another in the field to the extent that interspecific mating will actually occur? We need to know more about pre-mating barriers of all sorts.

3) More consideration might be given to the supposed effect of sperm on fecundity. Why would heterospecific sperm have a more stimulating effect than conspecific sperm? Some clarification is needed.

4) Overall, the study serves to highlight the impact of 'globalization' (transport of host plants, etc.) and changing agricultural practices on the concept of 'species'.

Michael Greenfield CNRS Tours, France

Reviewed by Joël Meunier, 04 May 2017

In this study, the authors investigate the outcome of heterospecific mating between a focal species (Tetranychus urticae) and two other species (T. evansi and T. ludeni) of mites. The results show that mating between T urtica and T ludeni do not produce any hybrids, whereas T urtica and T evansi do produce hybrids. Overall, I liked this study. However, I have several concerns that should be addressed to increase the robustness of the results and improve the general clarity of the study. Major concerns: 1. The study is overall very difficult to follow. The framework of the entire study is about reproductive interference, which is a very broad topic, whereas the study "simply" tests for mating incompatibilities between different species. This general framework is a bit misleading and does not perfectly fit with the experiments. This is, for instance, illustrated by the first experiment which only focuses on mating incompatibilities between two species and showing that there is one and thus that the three selected species are not ideal to test for reproductive interference the way they want to do it (see line 67-69). 2. The material and method lacks numerous crucial information. For instance, the sample size is never mentioned! How many males and females did you actually test per treatment? On L178, it is written that you used groups of 15 females, but how many groups?? This is a crucial information, also for the meta-analysis part, as it is still unclear to me, as to why you use such a complex statistical process to address the question you are interested in. And can you really do such a large-scale statistical process with a small sample size? 3. I found the result part difficult to read as it is not connected to clearly formulated questions. The tables providing the stats are also unusual and therefore difficult to follow. I would suggest the author to add their statistical results to the main text (instead of the table) to help them better structuring this part. Minor concernts: L61-64: this is assuming that you are working on a species with multiple mating. L70: "all possible effects": at least the ones you are raising above in the text. L83-88: Does it mean that a female with "good fertility" is supposed to produce females only? But, a female might "decide" to not fertilise her eggs in order to produce males (even if she has sperm stored in her spermatheca). L112: The last paragraph of the discuss lack clear predictions and a quick overview of the experimental design. As such, it is difficult to understand what the researchers have done in this study and to what extend it does answer their general question. L127: the species was identified by analysing 50 females per population. Does it means that each population contains only a single species under natural condition? If yes, isn't it an issue for your study, as you are testing a pattern that is unlikely to occur in the field and thus for which selection is unlikely to have "naturally" operated? L137: Do all the species and tested populations received the tetracycline treatment? This is mandatory to avoid any species-specific treatment that could have biased the results. L222: Why no running a single statistical model in which you have the species of the focal female, and then a factors that says if the mate if homospecific or heterospecific? L234: it is important to show the results of the block 1 only (at least at a supplementary material). Since the experimental design is not balanced at all between the blocks, it is at least important to provide all the available information for the reader to accept (or not) that it does not affect the robustness of your conclusions. L236-274: I am familiar with meta-analyses, but it is the first time I see it in this context. For now, it comes a bit out of the blue and I do not really understand why the results from your statistical models are not good enough to allow you drawing the same conclusions. Would it be possible to better explain why this statistical process is required to address this specific question? Tables: It is generally useless (if not wrong) to conduct pairwise analyses when the main factor is not significant. You did it several times.