




# Peer Community In Evolutionary Biology

## Speciation in spider mites: disentangling the roles of Wolbachia-induced vs. nuclear mating incompatibilities

**Jan Engelstaedter**  based on peer reviews by **Wolfgang Miller** and 1 anonymous reviewer

Miguel A. Cruz, Sara Magalhães, Élio Sucena, Flore Zélé (2020) Wolbachia and host intrinsic reproductive barriers contribute additively to post-mating isolation in spider mites. *bioRxiv*, ver. 1, peer-reviewed and recommended by Peer Community in Evolutionary Biology.

<https://doi.org/10.1101/2020.06.29.178699>

Submitted: 09 July 2020, Recommended: 23 November 2020

### Cite this recommendation as:

Engelstaedter, J. (2020) Speciation in spider mites: disentangling the roles of Wolbachia-induced vs. nuclear mating incompatibilities. *Peer Community in Evolutionary Biology*, 100116. [10.24072/pci.evolbiol.100116](https://doi.org/10.24072/pci.evolbiol.100116)

Published: 23 November 2020

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Cytoplasmic incompatibility (CI) is a mating incompatibility that is induced by maternally inherited endosymbionts in many arthropods. These endosymbionts include, most famously, the alpha-proteobacterium *Wolbachia pipientis* (Yen & Barr 1971; Werren et al. 2008) but also the Bacteroidetes bacterium *Cardinium hertigii* (Zchori-Fein et al. 2001), a gamma-proteobacterium of the genus *Rickettsiella* (Rosenwald et al. 2020) and another, as yet undescribed alpha-proteobacterium (Takano et al. 2017). CI manifests as embryonic mortality in crosses between infected males and females that are uninfected or infected with a different strain, whereas embryos develop normally in all other crosses. This phenotype may enable the endosymbionts to spread rapidly within their host population. Exploiting this, CI-inducing *Wolbachia* are being harnessed to control insect-borne diseases (e.g., O'Neill 2018). Much progress elucidating the genetic basis and developmental mechanism of CI has been made in recent years, but many open questions remain (Shropshire et al. 2020). Immediately following the discovery and early study of CI in mosquitoes, Laven (1959, 1967) proposed that CI could be an important driver of speciation. Indeed, bi-directional CI can strongly reduce gene flow between two populations due to the elimination of F1 embryos, so that CI can act as a trigger for genetic differentiation in the host (Telschow et al. 2002, 2005). This idea has received much attention, and a potential role for CI in incipient speciation has been demonstrated in several species (e.g., Bordenstein et al. 2001; Jaenike et al. 2006). However, we still don't know how commonly CI actually triggers speciation, rather than being merely a minor player or secondary phenomenon. The problem is that in addition to CI, postzygotic reproductive isolation can also be caused by host-induced, nuclear incompatibilities. Determining the relative contributions

of these two causes of isolation is difficult and has rarely been done. The study by Cruz et al. (2020) addresses this problem head-on, using a study system of *Tetranychus urticae* spider mites. These cosmopolitan mites are infected with different strains of *Wolbachia*. They come in two different colour forms (red and green) that can co-occur sympatrically on the same host plant but exhibit various degrees of reproductive isolation. A complicating factor in spider mites is that they are haplodiploid: unfertilised eggs develop into haploid males and are therefore not affected by any postzygotic incompatibilities, whereas fertilised eggs normally develop into diploid females. In haplodiploids, *Wolbachia*-induced CI can either kill diploid embryos (as in diplodiploid species), or turn them into haploid males. In their study, Cruz et al. used three different populations (one of the green and two of the red form) and employed a full factorial experiment involving all possible combinations of crosses of *Wolbachia* infected or uninfected males and females. For each cross, they measured F1 embryonic and juvenile mortality as well as sex ratio, and they also measured F1 fertility and F2 viability. Their results showed that there is strong reduction in hybrid female production caused by *Wolbachia*-induced CI. However, independent of this and through a different mechanism, there is an even stronger reduction in hybrid production caused by host-associated incompatibilities. In combination with the also observed near-complete sterility of F1 hybrid females and full F2 hybrid breakdown (neither of which is caused by *Wolbachia*), the results indicate essentially complete reproductive isolation between the green and red forms of *T. urticae*. Overall, this is an elegant study with an admirably clean and comprehensive experimental design. It demonstrates that *Wolbachia* can contribute to reproductive isolation between populations, but that host-induced mechanisms of reproductive isolation predominate in these spider mite populations. Further studies in this exiting system would be useful that also investigate the contribution of pre-zygotic isolation mechanisms such as assortative mating, ascertain whether the results can be generalised to other populations, and – most challengingly – establish the order in which the different mechanisms of reproductive isolation evolved.

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## Reviews

### Evaluation round #2

DOI or URL of the preprint: <https://doi.org/10.1101/2020.06.29.178699>

Version of the preprint: version bioRxiv 2020.06.29.178699 - Posted July 05, 2020.

### Decision by **Jan Engelstaedter** , posted 09 November 2020

The authors have done an excellent job addressing the reviewers' and my own comments, and I find the new version to be much improved. In particular, I'm happy with the extended introduction providing background on the spider mite system and the improved discussion of pre-mating isolation. The summary sentences at the end of the Results subsections are also very helpful, and I really like the new figures with their consistent colour scheme. I only have a few more very minor comments:

- The first sentence in the Abstract seems to be its own paragraph, please merge the entire abstract into a single paragraph.
- It seems that some of the packages loaded and presumably used in the R scripts (e.g. car and lme4) have not been cited. Please make sure that all packages are mentioned in the methods with citations.

-l.721: Perhaps "narrows" -> "limits", or somehow rephrase this sentence? It's a bit hard to understand at first.

#### **Additional comment of the managing board**

We'll send you (today) instructions in a separate e-mail to format your article

## **Evaluation round #1**

DOI or URL of the preprint: <https://doi.org/10.1101/2020.06.29.178699>

### **Authors' reply, 31 October 2020**

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### **Decision by Jan Engelstaedter , posted 19 August 2020**

Wolbachia is a widespread maternally inherited endosymbiont of arthropods. These bacteria often induce mating incompatibilities between males and females with different infection status, which suggests that they may play a role in host speciation. In this article, Cruz et al. investigate the contribution of Wolbachia to reproductive isolation between different population of the spider mite *Tetranychus urticae*, relative to host-related incompatibilities.

Both reviewers commented positively on the experimental design of this study, and I agree that the experiments, based on a full factorial design testing all possible types of matings, are impressive in their scope and well suited to answering the questions posed. However, the reviewers also raise a number of issues that should be addressed in a revised manuscript. Most importantly, both reviewers suggest a thorough discussion of the role of pre-mating isolation in this system.

In addition, and related to this, I would also like see more context provided on the system in the introduction. The MD and FM types of CI are well explained but very little background information is given on the "biogeography" of these mites and their symbionts. E.g., do the green and red types co-occur symmetrically or only in allo- or parapatry? Is Wolbachia fixed in these populations or is there generally a polymorphism between infected and uninfected individuals? Are populations from the red and green type that are compatible infected with the same strain of Wolbachia, or are both uninfected? Given all that is known about this system, are the three populations chosen representative of the species (complex) as a whole?

Some other comments:

- Abstract: "However, most studies focus on closely-related populations of single species" - this sounds as if the authors are setting up the stage for a follow-up sentence like "Here, we instead do ...", but the next sentence doesn't quite live up to that expectation (partly because the study system is also a single species).
- Abstract: "these two sources": not quite clear which two sources are referred to because there's a sentence in-between, perhaps be more explicit here.

Fig.1 & 2: I was wondering why some of the crosses aren't shown here. Are those the ones that aren't as interesting for the questions asked, or is there another reason? Might be good to either add them or to mention why not in the figure legend.

Fig.1: I think the colour scheme used in Fig.2 is quite nice, perhaps the same scheme could be used for Fig. 1? (E.g., coloured circles above the plots instead of the numbers?)

Box S1 heading: I think it should be "relating to" instead of "relative to"

**Additional requirements of the managing board:**

As indicated in the 'How does it work?' section and in the code of conduct, please make sure that:

-Data are available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad or some other institutional repository. Data must be reusable, thus metadata or accompanying text must carefully describe the data.

-Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) are available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused.

-Details on experimental procedures are available to readers in the text or as appendices.

-Authors have no financial conflict of interest relating to the article. The article must contain a "Conflict of interest disclosure" paragraph before the reference section containing this sentence: "The authors of this preprint declare that they have no financial conflict of interest with the content of this article." If appropriate, this disclosure may be completed by a sentence indicating that some of the authors are PCI recommenders: "XXX is one of the PCI XXX recommenders."

**Reviewed by anonymous reviewer 1, 12 August 2020**

Some comments on "Wolbachia and host intrinsic reproductive barriers contribute additively to post-mating isolation in spider mites" by Miguel A. Cruz, Sara Magalhães, Élio Sucena, Flore Zélé (DOI <https://doi.org/10.1101/2020.06.29.178699>).

Cruz et al report here on a rather detailed and complete set of experiments aiming at assessing the respective contributions of Wolbachia-induced CI and other forms of incompatibilities on post-mating isolation between three populations of *Tetranychus urticae*. In fact, two of the populations are very close, and apparently not isolated at all, while the third is rather deeply divergent (over 5% divergence at the mtDNA level) and completely isolated. I do agree with the authors that teasing apart the effects of Wolbachia versus other causes in establishing reproductive isolation is important. I see, however, two limitations here, that the authors may want to discuss more specifically in a revised version of their manuscript. One is that the importance of pre-mating isolation is not known in this system, so that it is difficult to assess the actual contribution of post-mating isolation. The second is that isolation without Wolbachia seems so high already that it is difficult to derive conclusions with regard to the importance Wolbachia plays in current isolation or has played in establishing speciation.

Another general comment is that I found the Results section rather difficult to follow. It may help to guide the reader more gently through the results, possibly by asking / answering questions one at a time? In relation with this, it seems to me that useful take home numbers would be estimations of how much gene flow would be increased if one or the other causes of post-mating isolation were removed (assuming no pre-mating isolation). I suggest trying to derive such numbers from this analysis.

Below are some more detailed comments, that I hope will be found helpful. Some more details

L28: "polymorphs" => polymorphism ?

L30: "Moreover, whereas speciation induced by wCI..."; I think this idea could be made clearer by adding a few words: "Moreover, whereas speciation SOLELY induced by wCI may require very specific conditions, WOLBACHIA could STILL play a significant role in host speciation by interacting with other (intrinsic) isolation mechanisms."

L42: "However, in the latter scenarios..."; I think "however" could be removed.

L44: "additive or interacting effects"; but the words above "either a complementarity or a synergy" seem to indicate additivity in the first case and positive interaction in the second? So, in what sense have interaction's

not been studied?

L68: "variable levels of wCI"; it seems the abbreviation "CI" would be better suited in this context.

L69: "In spider mites, as in some other haplodiploid species...": is this part of the paragraph at the best possible place? The paragraph starts with specific statements on the study system, and we thus have these general statements on CI in haplo-diploids, without any specific information on *T. urticae*. Here we would expect some statements on whether CI leads for male development or female mortality in this species?

L79: "Wolbachia-induced CI (wCI)"; the abbreviation was defined earlier

L91: "COI mtDNA"; the COI difference is very large (over 5%) and falls largely over standards for conspecific populations. It would be of interest to know how much this translates into effective isolation. Is information available on how much the nuclear compartments differ?

L130: I suppose "quiescent females" in this context means "young and thus virgin females", that will produce only males? Please provide explanations for the non-specialist.

L152: could it be that female cryptic choices introduces some errors in this estimation, for example, a trend to fertilise fewer eggs in crosses between U females and I males would lead to more males being produced even though diploid zygotes are killed by CI?

L157: I would add something like the following sentence: "Cor is thus a measure of the excess in male production in comparisons with controls, taking a value close to 1 when this excess is maximal".

L197: "P-values were defined as..."; I don't understand this sentence and the meaning of these P-values. Could the authors clarify the rationale behind this test?

L230: "As F1 female fertility corresponds to their ability to lay at least one egg, and/or a normal number of eggs"; I don't understand this sentence very well; is it meant that the term fertility can mean both of these things (being fertile or sterile, versus being very much fertile or not?). This should be clarified; same comment on L257.

L245: "a single maternal chromosome"; so, these animals have only one pair of chromosomes? Was that mentioned earlier?

L253: "for males to mate"; isn't that a strange wording, considering that females do mate as well?

L274: "R statistical package"; I think "program" should be used rather than "package" (R includes many packages).

L312 "Overproduction of males (MD-type incompatibility)"; at the end of this section, at least after a 1st read, I must say it is difficult to have a clear view of what is causing this excess of males. I suggest adding some interpretations / hypotheses, rather than sticking to results only.

L35: again, "wCI" is used when it seems that the usual "CI" abbreviation would seem sufficient.

L425: "males mated with (and sons of) green females"; the bracket is not clear to me and does not read very well. Same comment L430 & 441 & 442.

L443: "cannot necessarily be explained"; I don't really understand this sentence, may be "necessarily" is superfluous?

One background issue that is not discussed is how much these different types of crosses could occur in nature, and thus what is the actual impact of the various post-zygotic problems seen here? Could it be that some of the post-zygotic barriers are simply not barriers because gene flow is prevented by other sources?

L453: "up to 2 times more"; some additional explanation of what this number means would help. Something like "how much gene flow would be increased if one or the other factor was removed" would be helpful.

L463: can you clarify what "unidirectional" means here?

L487: I don't think "Nevertheless" is useful here.

L524: "The combined effects..."; I am not at ease with the content of this paragraph. It is argued that one should try and quantify the respective contributions of CI and HI in restricting gene flow. If I understood well, it is seen that isolation would be complete even without Wolbachia. But still, it is argued that Wolbachia could play a critical role in isolation. The problem is that this Discussion faces a usual problem of interpretations of speciation processes: once several factors are at play, it is difficult to know which one actually mattered

initially...

## Reviewed by **Wolfgang Miller**, 17 August 2020

This is a carefully planned and painstakingly performed study aimed to estimate the contributions of Wolbachia-induced (wCI) and host-associated (HI) incompatibilities in the haplodiploid spider mite *Tetranychus urticae* at the post-mating level between sympatric populations differing genetically such as in coloration. By multi-factorial crosses within and between populations at different infection states they found that in this incipient speciation system HI dominates over wCI in effectivity (1.5 – 2), where wCI seems to affect mortality of female embryos but HI triggers symmetric F1 sterility and F2 hybrid breakdown in backcrosses. In addition, HI is causing the overproduction of sons in these incompatible crosses and not the Wolbachia endosymbiont. They further show that both mechanisms can act together synergistically and that possibly such cyto-nuclear incompatibilities might predate nuclear ones.

Hence, this elegant study has managed to dissect - for the first time to my knowledge - the different degrees of contributions of the two postmating isolation mechanisms acting in this system, i.e., cyto-nuclear vs nuclear-nuclear BDIMs.

Minor comments:

Line 130: please explain to non spider mite experts what is meant with “quiescent females”.

This last part is not meant as a critique: Although discussed later on, the design of this current study cannot rule out the possibility that not all females were inseminated by males in their crossing experiments and thereby the role of premating isolation is not yet fully determined. As nicely mentioned in the discussion, future cytological studies on early hybrid embryos will uncover additional mechanistic clues. In summary, however, this is a very important work that will further deepen our understanding in the short- and long-term dynamics of symbionts and their potential role in speciation, a highly exciting but still controversial subject in evolutionary and ecological biology.