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Caution Does Not Preclude Predictive and Testable Models of Cytoplasmic Incompatibility: A Reply to Shropshire *et al.*

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Scientists often face a dilemma: should they produce explicit, predictive models to explain a body of incomplete data, at the risk of missing some critical aspects, or should they accumulate additional observations, allowing more objective and realistic models to emerge. There is a genuine trade-off between these two positions, which tend to be given different weights by different scientific disciplines, from quantum physics to anthropology. The comments of Shropshire *et al.* [1], who we thank for having given attention to our recent Opinion paper [2], illustrate that, in the fields of molecular and evolutionary genetics, there are also different views on where one should stand with respect to this trade-off. Our colleagues argue that caution should prevent us from stating that cytoplasmic incompatibility (CI) induction and rescue most likely stem from a toxin-antidote (TA) system encoded by *Wolbachia* endosymbionts. We can only agree that caution is always advisable. However, the understanding of CI, with its long theoretical and empirical history, has, in our view, come to a stage where explicit and testable models can and should be formulated.

Let us first summarize the list of predictions and empirical data supporting our claim [2]. The TA model was first proposed as a theoretical possibility. It was later evaluated in light of a variety of empirical observations and found to be more parsimonious and flexible than other available explanations, some of which were similar to the models proposed by our commentators in their Figure 1, where a direct interaction between toxin and antidote factors

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is not predicted [1]. A potential *Wolbachia* CI gene was then identified in infected *Culex pipiens* mosquitoes through sperm proteomics, and this gene, later named *cidA*, happened to occur right next to another conserved gene in CI-inducing *Wolbachia* strains; this striking synteny was consistent with a putative TA-like genetic structure [3]. The two genes were subsequently found to encode proteins acting in a typical TA fashion in yeast that, importantly, were also shown to form a protein complex [4]. One of the two genes (the first in the putative operon, as is typical in TA systems) was confirmed to act as a necessary and sufficient antidote to CI in *Drosophila* embryos [5].

Arguably, some observations did not match the initial TA predictions: (i) the two gene products both appear to be required for CI induction in *Drosophila* [4,6]; and (ii) the putative toxin (CidB) was not the protein isolated from infected sperm [3]. We provided a reasonable explanation for the first apparent discrepancy, namely, that CidB may be toxic not only during the first embryonic mitosis in incompatible crosses, but also in maturing sperm and other cell types, in which the presence of CidA antidote would then be required [2]. This hypothesis does not exclude other possibilities; for example, the tightly bound CidA protein may be temporarily required for the delivery or packaging of CidB into mature sperm. The second discrepancy, the putative absence of CidB in sperm, was raised by our commentators as a major argument against the TA model [1]. In this case, however, absence of evidence should not be taken as evidence of absence. The original proteomic analysis was arduous and has not since been repeated; this study was based on evaluating specific SDS-PAGE gel slices that, it turned out, did not include any of the size of CidB [3]. Although both CidA and CidB are now known to be expressed in the infected insect testis as well as full body [6–8], the appropriate proteomic experiments, although difficult, should be performed to test whether the putative CI toxin does reside in the sperm of infected males, as predicted by the TA model.

Shropshire *et al.* also took issue with our proposals regarding how the CI genes should be named and whether the paired genes should be considered as part of an operon. Concerning gene names, the system we proposed was not directly connected to phylogeny but was instead oriented toward protein functions, namely deubiquitylase and nuclease. We see pros and cons in both approaches but, in our view, the accumulation of results supporting a causative link between these enzymatic activities and CI argues in favor of the function-based nomenclature. Biochemical data demonstrated deubiquitylase (DUB) activity in CidB, and a point mutation in the catalytic site of the DUB domain abolished CI in transgenic males [4]. Results with yeast expression of CidB corroborated this finding and similarly showed that mutation of a putative nuclease active site in CinB eliminates its toxicity. We are also aware that naming the first gene *'CidA'* when it is not itself a DUB, can in principle be seen as problematic. Yet, as noted by Shropshire *et al.* [1], it is generally acknowledged by microbiologists that such an approach is pertinent if the two genes 'govern related functions', which is precisely what we think they do, and as such, constitute an operon.

By contrast, Shropshire *et al.* argue that calling the CI genes an operon is premature because it is not clear yet that they are under a single promoter [1]. As previously emphasized [2], only one promoter has been found to date. However, even if two independent promoters were to be found, would this preclude use of the operon term and, more importantly, of the

operon concept? Considering the known bicistronic transcription of *cidA* and *cidB* [3,8] and the demonstrated functional link between these two genes, our current answer would be 'no'. We endorse the now widely accepted view that operons are best defined functionally; they may include more than one promoter and can sometimes have complex patterns of relative expression [9].

Lastly, our analysis of CI models not only relied on molecular data, but also explored the evolutionary implications of the TA model. We highlighted how much this model, in contrast to its competitors, predicts features of the complex *Culex* CI system, where the occurrence of multiple copies of the putative CI operon was anticipated and later observed [10]. Furthermore, we proposed that a TA system within a phage, where the CI genes usually sit, is reminiscent of other selfish TA systems in free-living bacteria, providing a possible hint about the evolutionary origin of CI. Many details about CI remain to be deciphered, but, in our view, the TA model, being both highly predictive and testable, represents a valuable working hypothesis. It will surely need to be refined in the future and may even be refuted, but in any case, we hope that this framework will spur more experimentation and thinking and, with them, a robust understanding of CI mechanism and evolution.

References

1. Shropshire JD et al. (2019) Models and nomenclature for cytoplasmic incompatibility: caution over premature conclusions. Trends Genet. 10.1016/j.tig.2019.03.004(in press)
2. Beckmann JF et al. (2019) The toxin-antidote model of cytoplasmic incompatibility: genetics and evolutionary implications. Trends Genet. 35, 175–185 [PubMed: 30685209]
3. Beckmann JF and Fallon AM (2013) Detection of the Wolbachia protein WPIP0282 in mosquito spermathecae: implications for cytoplasmic incompatibility. Insect Biochem. Mol. Biol. 43, 867–878 [PubMed: 23856508]
4. Beckmann JF et al. (2017) A Wolbachia deubiquitylating enzyme induces cytoplasmic incompatibility. Nat. Microbiol. 2, 17007 [PubMed: 28248294]
5. Shropshire JD et al. (2018) One prophage WO gene rescues cytoplasmic incompatibility in Drosophila melanogaster. Proc. Natl. Acad. Sci. U. S. A. 115, 4987–4991 [PubMed: 29686091]
6. LePage DP et al. (2017) Prophage WO genes recapitulate and enhance Wolbachia-induced cytoplasmic incompatibility. Nature 543, 243–247 [PubMed: 28241146]
7. Bonneau M et al. (2018) The cellular phenotype of cytoplasmic incompatibility in Culex pipiens in the light of cidB diversity. PLoS Pathog. 14, e1007364
8. Lindsey ARI et al. (2018) Evolutionary genetics of cytoplasmic incompatibility genes cifA and cifB in prophage WO of Wolbachia. Genome Biol. Evol. 10, 434–451 [PubMed: 29351633]
9. Güell M et al. (2011) Bacterial transcriptomics: what is beyond the RNAhoriz-ome? Nat. Rev. Microbiol. 9, 658–669 [PubMed: 21836626]
10. Bonneau M et al. (2018) Culex pipiens crossing type diversity is governed by an amplified and polymorphic operon of Wolbachia. Nat. Commun. 9, 319 [PubMed: 29358578]