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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 rather, should they accumulate additional observations, allowing more objective and realistic models to emerge.

25 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
30 argue that caution should prevent us from stating that 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.

35 Let us first summarise 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 wide variety of empirical observations and found to be more parsimonious and flexible than other available explanations, some of which were very similar to the models proposed in Figure 1 by our commentators, where a direct interaction between toxin and antidote factors
40 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,
45 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: (1) the two genes products both appear to be required for CI induction in *Drosophila* [4,6] and (2) 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. This hypothesis does not exclude other possibilities; for example, the tightly bound CidA protein may be temporarily required for 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 if the putative CI toxin *does* reside in infected males' sperm, 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 genes names, the system we proposed is not directly connected to phylogeny but is 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

70 nomenclature. Biochemical data demonstrated 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
75 by Shropshire et al, 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. As previously emphasized
80 [2], only one promoter has been found to date. But 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;
85 they may include more than one promoter and can sometimes have complex patterns of
relative expression [9].

Lastly, it is worth emphasizing that our analysis of CI models relied not only 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
90 complex *Culex* CI system, where the occurrence of multiple copies of the putative CI operon
was anticipated and later observed [10]. Further, we proposed that a TA system within a
phage, where the CI genes usually sit, is highly 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
95 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 this framework will spur more
experimentation and thinking and with them, a robust understanding of CI mechanism and
evolution.

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