

Dear Editor,

We would like to thank again both the reviewers for the work that they put in the review of this manuscript. We have made some additional changes following the suggestions of Ivan Junier, which we detail below.

- We have slightly modified the title so that it mentions the “Evolution of Bacterial Chromosome Organization”, as we think - in accordance with the reviewer - that this wording points more accurately to the genome evolution aspect of the manuscript. We have also slightly edited the abstract in the same spirit (pages 1-2, lines 10-16).
- Following the reviewer’s suggestion, we have added a sentence discussing the chosen value for the maximum supercoiling propagation distance in the Methods section (Experimental Setup subsection, page 35, lines 772-775).
- The question of the existence of several solutions for the system of gene expression levels seems to us slightly more complicated than stated by the reviewer, mainly because the system for which he expects two (or several) solutions at  $\delta\sigma_{env} = 0$  is not clearly defined. With  $\delta\sigma_{env} > 0$ , for evolved individuals, there are empirically two different stable states of gene expression levels depending on the value of each  $\sigma_{env}$ . By introducing some stochasticity by (for example) slightly tweaking initial gene expression levels, one could test the stability of the evolution of the system towards one or the other stable state, in each of the environments, for these individuals. With  $\delta\sigma_{env} = 0$ , the same perturbation could be evaluated for random non-evolved individuals (such as the initial population), but there is no evolved regulatory network in the genome of these individuals, so there is no reason to expect two stable states of gene expression to exist in that case. But it neither makes sense to evaluate evolved individuals at  $\delta\sigma_{env} = 0$ , as these individuals have evolved with  $\delta\sigma_{env} > 0$  (and have therefore evolved regulatory networks that respond to different nonzero values of  $\sigma_{env}$ ), nor to run the evolution experiment presented in the manuscript, as individuals would be unable to differentiate the two environments in that case. Nonetheless, we have added a discussion of possible stochastic versions of the model in the Discussion section (page 27, lines 562-566).

Best regards,

Théotime Grohens