Dear Editor,

We would like to thank both the reviewers for their thorough and insightful analysis of our manuscript. We hope that the changes that we have made in response to the reviews will make the manuscript clearer to the reader.

The changes are listed below.

Reviewer 1 (Ivan Junier)

A. 1. We agree with the reviewer that the biophysical model of gene expression as a function of supercoiling that we use in the model has many parameters, and that there is no absolute choice. Because this model is not meant to quantitatively represent the inherently stochastic nature of transcription in detail, but rather to provide a time- and population-averaged view of gene transcription levels, we chose to use an 'off-the-shelf' model of the transcriptional response to supercoiling that provides a good empirical fit to such data, that is, the model presented in El Houdaigui et al 2019 (https://doi.org/10.1093/nar/gkz300). Note that, in previous versions of our model (successively published in the Alife 2021 conference (https://doi.org/10.1162/isal_a_00434) and the Artificial Life journal (https://doi.org/10.1162/artl_a_00373)) we used respectively a completely abstract hyperbolic tangent function and a simpler biophysical model (using a single exponential) to represent gene expression as a function of supercoiling, and obtained qualitatively similar results to the current manuscript (to the extent of the analyses present in the former papers). Additionally, using a step function to model this response could induce numerical instability and thus possible convergence problems during the simulation, which are avoided here with the use of a continuous function. Nonetheless, if the reviewer deems it important to the quality of the message of the manuscript, we would be open to including an exploration of the effect of the biophysical parameters of the model in the manuscript.

2. As stated above, in this model, we chose not to model transcription in a time-explicit way, and thus not to explicitely model RNA polymerases. While RNA polymerases do act as topological barriers during transcription, many genes are not under constant transcription in bacteria (70% of *E coli* genes have a RNA/DNA ratio lower than 1, Deng et al. 2005 (https://doi.org/10.1111/j.1365-2958.2005.04796.x)) and it therefore seems empirically valid to us to model supercoiling as diffusing farther than 1 gene apart in the context of this model. We additionally ran simulations with interaction distances d_{max} smaller and larger than 5 kb. Larger interaction distances do not qualitatively impact the results, although the number of required genes for inhibition indeed somewhat increases (a median of 9 genes to inhibit *A* genes and 5 genes to inhibit B genes are needed in the opposite environments, each mean being higher than the respective median). On the other hand, values of d_{max} under 4 kb actually prevent the evolution of inhibition of *A* genes in environment *B* (data not shown); note that experimental data shows that supercoiling can in fact propagate at such distances on the chromosome, Moulin et al. 2005 (https://doi.org/10.1111/j.1365-2958.2004.04411.x)).

3. About the sensitivity of the model for low values of $\delta\sigma$: given our model, one could have expected that the supercoiling constraints due to local organization would precisely drown out too small values of $\delta\sigma$, and disallow for the evolution of environment-specific responses in this parameter range. This is why we mentioned robustness with regard to this particular parameter. On the other hand, a value of $\delta\sigma = 0$ seems difficult to interpret to us in the context of the experiment that we present in this manuscript, as the point of the experiment is to study environment-specific transcriptional responses. We nonetheless agree that a single-environment model would be interesting to study in further work, possibly focusing instead on the combinatorial exploration of the different regulatory networks that can be obtained with different local genes contexts.

4. We would like to thank the reviewer for his careful reading of the introduction and suggested improvements.

- line 27: we have rephrased the definition of supercoiling to mention the linking number.
- line 42: we have rephrased the description of topoisomerase function more accurately.
- line 50: we have rephrased this sentence to be more accurate.
- line 53: we included an explicit mention of polymerases and cited Deng et al. 2004.
- line 55: we have rephrased this sentence for the sake of clarity.
- about the TSC: we thank the reviewer for the reference to the 1989 Pruss & Drlica paper; we rephrased the sentence to be more precise that, to our knowledge, it is the term itself "transcription-supercoiling coupling" that was first used in the 2014 Meyer & Beslon paper.
- line 130: we rephrased the sentence to avoid the misunderstanding: in Peter et al. 2004, genes are found to form local clusters with similar SC responses (see Figure 6), but that are indeed spread around the chromosome.

B. We would like to thank the reviewer for the idea of considering gene triplets systematically in addition to pairs. We added a new paragraph and figure (figure 6 in the new version of the manuscript) describing our study of triplet frequencies in the model. Broadly, we agree that interesting patterns can also be seen at the triplet scale and not only at the pair scale. However, we interpret these observations as showing that these two levels act in synergy, rather than pairs or triplets being the phenomenologically optimal level at which to understand genome organization. This observation leads to the sub-sections that follow in the manuscript, which precisely tackle regulatory networks using two different approaches that by construction incorporate gene sets of all sizes. Nonetheless, we agree that increasing the number of different environments would certainly result in an even larger variety of favorable gene contexts of interest, although possibly at the cost of the a more difficult interpretation of these contexts.

C.
- line 17: we have rephrased the corresponding sentence.
- line 25: we rephrased the sentence to be more precise and explicitly mention plectonemes.
- line 30: we have rephrased the corresponding sentence.
- line 77: we have rephrased the sentence to be more precise.
- § from line 111: we contrasted transcription initiation versus elongation rates, with the help of the mentioned paper.
- line 138: we have rephrased the sentence to mention the larger number of species in the dataset.
- § from line 79: we thank Ivan Junier for mentioning this paper, which we added a mention to.
- line 177: we did not prove mathematically that the system has a single solution. However, our algorithm has always converged in practice. We added a mention to this in the Methods paragraph Computation of Gene Expression Levels (end of section 5.1).
- line 178: we have rewritten the first paragraphs of subsection 2.1 to present the evolutionary simulation and individual-level model in a clearer way, explicitly mentioning these assumptions.
- line 182-183: we did not run this particular experiment (varying mutation rates), but experience with other evolutionary simulations lets us make the following prediction: the rate of evolution scales with the mutation rate, until the mutation rate becomes too high for individuals to maintain information (here, in the form of their genome organization) from one generation to the next. In other words, we would expect to see the same qualitative result for a range of mutation rates, as long as we see fitness increasing over generations, until an error threshold is reached.
- line 184: this was indeed an oversight, we added an explicit mention of the number of genes of each individual.
- Figure 1 & 3: we have added the mention of the environment in all figures representing genomes, indeed clarifying the figures.
- Figure 4 & legend of Figure 4: we have rephrased the caption to clarify that there are two (shifted) horizontal axes, and replaced 'Random' by 'Random genome'.
- Page 11 and Figure 4: we have rewritten the first paragraph of the Evolution of Relaxation-Activated genes to make it clearer and more descriptive of the method used to generate the figure.
- observation: we have changed the title of this section to be more specific. Note that Figure 8 (7 in the first version) cannot by definition be obtained from random genomes, as we only consider

genes that act in accordance with their evolutionary target in the genome of evolved individuals.
- line 404-405: as the box plot at the right of figure 10 (figure 9 in the first version) shows, 75% of connected components have less than 10 genes in random genomes. Even though we agree that the network is circular by construction and strongly locally connected, this is thus not sufficient to create a single regulatory network in random genomes.
- line 515: we have rephrased for clarity.
- line 539: we have rephrased the conclusion to put more emphasis on the genome structure aspect of the work.

Reviewer 2 (Anonymous)

1. We have rephrased the corresponding sentence to make this clearer.
2. As suggested, we have added a sentence in the last paragraph of the introduction to mention possible outcomes of this line of research, broadly matching the points mentioned in the discussion.
3. We rephrased the first paragraph of section 5.2 (Evolutionary Model) to be more precise.
4. We have rephrased the Evolutionary Algorithm paragraph of section 5.2 to be more precise.
5. We have included explicit mention of the outliers in the figure legends.
6. We rephrased the first paragraphs of section 2.3 (and changed its title) to describe more accurately the algorithm presented in this section, and in particular the threshold in the text. As we do not consider expression levels but their binarized corresponding activation states throughout the manuscript, we decided not to include the suggested figures as we do not feel that they would provide additional clarity (but would be open to doing so if the reviewer believes it to be mandatory).
7. We've rephrased the legend of the figure to explicitly mention the box plots.
8. We thank the reviewer for spotting this error, the signs were indeed reversed in the text and have been corrected.
9. We added a sentence at the beginning of the Fitness paragraph of section 5.2 to make this clearer.
10. As for point 3., we've rephrased the initial paragraph of section 5.2 to be clearer.
11. We have striven to make the text itself clearer, but as for point 6., we can add a standalone diagram should the reviewer deem it mandatory.

Best regards,

Théotime Grohens