Note: Line numbers refer to the revised manuscript (version 3) on bioRxiv. A separate track changes version is provided for review.

Dear authors,

First, I'd like to apologize for the time it has taken to provide you with feedback. Securing reviewers and navigating through the holiday season and personal commitments resulted in some delays. Nevertheless, I've managed to secure three expert reviews, which have provided valuable insights to improve your manuscript.

Our response: Thank you very much for evaluating our manuscript and providing constructive feedback.

As you will discover from their comments, all three reviewers agree that your manuscript has merit but also important shortcomings. In particular, all agree that the introduction lacks focus and does not naturally converge to the questions addressed by the manuscript. They've also highlighted the need for a more exhaustive explanation of the model, particularly regarding the simulations' crux. I therefore encourage you to give more detail regarding the genotype-to-phenotype relationship and how mutations translate into phenotypic variation, as these aspects seem particularly relevant to understanding your results.

Our response: Thank you for these suggestions. We have completely rewritten the Introduction to make it more streamlined. We have also added information on the mutation rates and effects which was previously only in the table to the main text and reworked the methods based on specific comments of the reviewers. We also add Fig. S4 which shows how mutations change the dispersal phenotype for evolved reaction norms in both GRN and RN models.

Reviewer 3 also raised concerns about several citations, and I echo their criticism. I additionally draw attention to the relevance of Ezoe, H., Iwasa, Y. *Evolution of condition-dependent dispersal: A genetic-algorithm search for the ESS reaction norm*. Res Popul Ecol 39, 127–137 (1997). This paper looks at the evolution of a dispersal reaction norm to local competition, and compares the results coming from an optimality mathematical argument and those from an evolutionary simulation of a neural network. There are therefore many similarities with the current study and it is imperative that you engage with this literature.

Our response: Thank you for highlighting this. We have added the reference (lines L57–59 and L 325–332).

Additionally, the discussion on current theory of the evolution of reaction norms to social traits appears somewhat superficial (e.g., only one broad and non-technical review on adaptive dynamics is cited on l. 328). There are many mathematical results at our disposal to understand the evolution of reaction norms (see citations below for examples), and I think the Discussion section could do a better job at explaining how the use of GRN may help understand adaptation beyond these results.

Our response: Thank you very much for pointing this out. We have added some of these references to lines L 295–301 of the General Discussion.

I hope you'll consider addressing these comments in a revised manuscript. Please provide a point-by-point response to the reviewers' comments with your revision.

All the best,

Charles Mullon.

• Metz, J. A., & Diekmann, O. (1986). The dynamics of physiologically structured populations. Springer.

• Gomulkiewicz, R., & Kirkpatrick, M. (1992). Quantitative genetics and the evolution of reaction norms. Evolution, 46, 390-411.

• Avila, P., Priklopil, T., & Lehmann, L. (2021). Hamilton's rule, gradual evolution, and the optimal (feedback) control of phenotypically plastic traits. Journal of Theoretical Biology, 526, 110602.

• Parvinen, K., Dieckmann, U., & Heino, M. (2006). Function-valued adaptive dynamics and the calculus of variations. Journal of Mathematical Biology, 52, 1-26.

• Durinx, M., Metz, J.A.J. & Meszéna, G. (2008). Adaptive dynamics for physiologically structured population models. J. Math. Biol. 56, 673–742.

• Gomulkiewicz, R., Kingsolver, J. G., Carter, P. A., & Heckman, N. (2018). Variation and evolution of function-valued traits. Annual Review of Ecology, Evolution, and Systematics, 49, 139-164.

*by **Charles Mullon**, 26 Feb 2024 16:22*

Manuscript: **https://doi.org/10.1101/2023.07.18.549508**

version: 2

Review by Arnaud Le Rouzic, 01 Feb 2024 08:00

Review of "A gene-regulatory network model for density-dependent and sex-biased dispersal evolution during range expansions" by J.N. Deshpande and A. Fronthofer, BioRxiv 2023.07.18.549508 for PCI Evol Biol #749.

This is a theoretical study focusing on the evolution of the plastic response of dispersal as a consequence of population density. Dispersal is a key life history trait for most organisms, as it allows the species to colonize new patches and to avoid extinction. It also promotes gene flow. Yet, knowing when and how much to disperse is a tricky question, as dispersal is generally associated with some risks, and leaving a comfortable patch for the unknown is not

necessarily a good strategy. Furthermore, studying the evolution of dispersion-related genes is complicated, since the dispersal rate that maximizes the population survival is not necessarily the same as for the most successful allele. Here, the authors address the question of how the optimal dispersal strategy should depend on the population density; the benefits of dispersing being indeed much larger when competition is tough compared with the situation where many resources are available. The authors also address the question of optimal male vs female dispersal, and the consequences of a change in the availability of new patches.

The approach here is genuinely original. Instead of setting up a model where the plastic response (dispersal rate as a function of the population density) is a parameterized function, the authors assume that this behavioral trait results from the expression of several underlying genes, and simulate the evolution of a small gene network in a dynamic metapopulation setting. As a result, the plastic response is not expected to follow a pre-defined shape, which greatly limits the possibility that the model results are accidentally constrained by some modeling choices.

Here, the authors show that the plastic response of dispersal follows closely the theoretical expectation from the "RN" model by Poethke and Hoverstadt 2002: there is a density thershold below which dispersal should be avoided, and the optimal dispersal rate raises rapidly above the threshold before stabilizing. Yet, simulations also show the plastic response in the gene network simulations is featured by a large amount of cryptic genetic variation, which ensures a rapid genetic response when conditions change (typically, when new patches become abundant). The authors also found that, in their setting, males should disperse more than females, especially when the risk of local extinction is low.

Overall, there are several interesting points in this manuscript.
(i) using a flexible, but biologically realistic gene-network based genetic architecture to study the evolution of the shape of a reaction norm for a behavioral trait seems very relevant.
(ii) the studied trait (dispersal as a function of population density) has some non-trivial evolutionary properties, and deserves attention.
(iii) the conclusions of this work are compared with existing theoretical predictions, which is reassuring when models involve complex pieces of simulation software.

Our response: Thank you for a positive evaluation of our manuscript.

This study also has shortcomings. Some point that could be possibily fixed are listed below:

1. I am not completely sure that the introduction converges naturally towards the questions 1) and 2). I was rather unsure to understand what the paper was about before the very end of the introduction, and I think it would probably help to achieve faster a focus on specific questions. In addition, I found it difficult to find a natural connection between the different sub-questions (sex-specific behavior, shape of the reaction norm, and rate of expansion) beyond the fact that they are related to the evolution of dispersal.

Our response: We have reworked the Introduction. We now briefly introduce the literature on density-dependent and sex-biased dispersal. We explain (lines L 33–51) the theoretical expectations for density-dependent dispersal under equilibrium metapopulation conditions (e.g. Poethe & Hovestadt, 2002) and state that these plastic responses must have

underlying molecular mechanisms (lines L 60–63). We then propose the Wagner model as a potential candidate to model dispersal plasticity, outlining its advantages (lines L 65–75) especially in understanding adaptation in novel environmental conditions. We then briefly summarise that range expansions can serve as a case study for such novel conditions (lines L 79–88).

2. I was confused for a long time (and I am still a bit unsure) about what the authors wanted to test exactly when comparing simulation results to the RN model. If the existing theoretical results are derived from first principles, any mismatch between these predictions and the simulations could only mean that the model implemented in the simulations did not match the assumptions of the mathematical model (unless there was a mistake when deriving this model). In other words, the RN model predicts "with certainty" the optimal reaction norm. There would be several reasons for the GRN model not to converge to the optimum; for instance, the genetic architecture might not be flexible enough to give this precise shape. Another possibility could be that Darwinian selection may not be powerful enough to precisely shape the plastic response (because some environments are too rare to induce enough selection pressure, or because of genetic correlations with other selected characters). Yet, any of these observations should not dismiss the RN model, because the model prediction about the optimal reaction norm would still be valid. A similar question arises with the sex-specific model; here, there is little doubt that sex-specific responses should arise in parallel in both GRN and RN models, what was the authors' expectations and what would have been the conclusion in case of mismatch?

Our response: We apologise that we were not clear. We do not intend to invalidate the optimal reaction norm model and we agree that any mismatch could be for all the reasons mentioned. The comparison is in order to answer the following questions:

1) Do we obtain the theoretically expected reaction norm from a more molecular mechanistic approach?

2) What are the conditions under which optimisation fails and why?

We have re-written the Results and Discussion section in order to reflect this more clearly. Specifically, we highlight that the extent of match between the GRN model and the RN model is greater for conditions with greater variation in population density (i,e., higher extinction probability and dispersal mortality) as seen in the histograms (Fig. S1). Further, we now add figures (SI Fig. S2 and S6) in the Supplementary materials that shows the distance from the theoretically expected optimum. We find that the greatest mismatch in the GRN model is found at low dispersal mortality when the strength of selection on dispersal is low.

3. A central point of this work is to compare the GRN predictions to the theoretical RN model, but the way results are presented does not make it easy to evaluate to what extent these results match.

3a: figures 2 and 3 display the mean +/- sd for the RN model, and individual phenotypes for the GRN model. I may have missed something, but I am not sure to understand why both are not represented in the same way. The full reaction norm could easily be computed for GRN genotypes (by changing the environment), or the simulated dispersal for the RN model could be restricted to the environment in which genotypes were sampled. Overall, it is

indeed possible to eyeball that both results seem to match, but it is impossible to assess to what extent they do.

Our response: We now add SI Fig. S2 and S6 showing the mean distance to optimum as a function of normalised population density. This is discussed explicitly in lines L 203–220. Specifically, this figure indicates that the mismatch is greatest for conditions in which dispersal mortality and extinction probability are low.

3b: the introduction mentions that alternative shapes for the plastic response were proposed in the literature. Even if the fit to the RN predictions seems convincing, I wonder if e.g. a sigmoid response would not be at least as convincing.

Our response: The goal of this study is not to compare fit of different RN shapes, This was done in Hovestadt et al. (2010). But rather to standardise the GRN model against the RN model. We have carefully rewritten the Introduction and Results and Discussion to avoid this misunderstanding.

3c: a substantial part of the results is devoted to the difference between the GRN model and the RN predictions in terms of genetic variance in the population. Even if the authors make a convincing argument about the higher evolvability in the GRN architecture, I was not sure that the mutational variance in both models was calibrated. Indeed, how much single mutations affect the phenotype remains quite arbitrary in these models (mutational targets, such as W, V, or Cthresh, are not expressed in the same dimensional units). A larger effect of mutations would generate a larger mutational variance, and as a consequence, a larger genetic variance at equilibrium. Here, the authors state in Table 1 that mutations change the genotype with a standard deviation sigma_m, but it is unclear whether this sigma_m is the same across models. Does it mean that mutations affect W, V, and Cthresh with the same standard deviation? This could generate drastically different mutational variances on the dispersal itself, and thus different evolvabilities. I assume that it would be reasonable to calibrate the mutational effects in the different models before drawing conclusions on evolvability, by e.g. adjusting sigma_m so that the mutational variance of dispersal would be identical in some arbitrary standard conditions (e.g. d=0.5 at hat_N?).

Our response: Thank you for this suggestion. It is not very straightforward to maintain similar mutational effects on the phenotype in the two models because how mutations impact the plastic response at the phenotypic level may also evolve during the simulations for both the models. In the RN models, the position of the threshold and population density impacts mutation effects. Here is a very rough calculation that shows the change in dispersal phenotype corresponding to a positive change in the threshold C_thresh.

$$\Delta d = \begin{cases} 0 & 0 \leq \frac{N_{x,y,t}}{\hat{N}} < C_{thresh}, 0 \leq \frac{N_{x,y,t}}{\hat{N}} < C_{thresh} + \Delta C_{thresh} \\ 1 - \hat{N}\frac{C_{thresh}+\Delta C_{thresh}}{N_{x,y,t}} & 0 \leq \frac{N_{x,y,t}}{\hat{N}} < C_{thresh}, \frac{N_{x,y,t}}{\hat{N}} > C_{thresh} + \Delta C_{thresh} \\ \hat{N}\frac{\Delta C_{thresh}}{N_{x,y,t}} & \frac{N_{x,y,t}}{\hat{N}} > C_{thresh}, \frac{N_{x,y,t}}{\hat{N}} > C_{thresh} + \Delta C_{thresh} \end{cases}$$

So , we expect that for population densities below the threshold, the trait remains unchanged unless post-mutation the same density is now above the new threshold. If both pre and post mutation, the density is above the threshold, then we expect that the trait will decrease with population density. And finally, if the population density is below (above) the threshold and after mutation it goes above (below) it, then the dispersal trait will increase with the population density. Obviously this is not so straightforward in the GRN model.

Thus, we took a numerical approach that can be described as follows: we took 1000 samples of individual GRN and RN genotypes at the end of 2000 generations. Since the mutation effect we describe is a per locus mutation effect, we mutated the same fraction of loci for each model and added a mutation effect drawn from a Gaussian with mean 0 and sd =sigma_m=0.1. For each individual, we calculated the difference between the perturbed and unperturbed phenotype at different population densities. We repeated this 10 times since we were sampling. We plotted this sensitivity to mutation of the trait in the RN model and GRN model for all population densities in SI Fig. S4 (SI Fig. S8 for DDD + sex biased). In the RN model, change in dispersal phenotype results from the shape of the function and in the GRN model from the ecological parameters. Thus, we would expect that even if in the RN model, we had allowed for larger changes in the threshold, the exact pattern across parameter space (i.e., dispersal mortality and extinction probability) in the GRN model would not be reproduced by the RN approach.

4. In figures 4 and 5, the speed at which the species expand in the available patches depends on the genetic architecture. In many panels, the range expansion seems rather linear, with a different speed from the initial generation. As far as I understand, the authors' interpretation is that this is due to different evolvabilities of the dispersion reaction norm, but I do not understand why these differences do not always build up progressively (as in panels 4C or 4F, for instance). My interpretation of panels e.g., 5A and 5B is that the dispersion itself differs between models from generation 0, which does not seem to be the case in the corresponding panels 3A and 3B. Am I missing something here?

Our response: If there is enough standing genetic variation, this can be spatially sorted rather early in the range expansion process, thus, the difference between the two models need not build up. This indeed seems to be the case, since we see in Figs. S3 and S9 that there is greater variation for dispersal before range expansions begin in the GRN model. Moreover, Fig. S11 shows that greater dispersal does evolve in the GRN model relative to the RN model.

5. In the range expansion simulations, the genetic (and phenotypic) composition of populations probably varies as a function of time and distance from the origin. Figures 4 and 5 focus on the expansion, i.e. the alleles "surfing" the expansion wave, but what about the other parts of the landscape? Are central populations affected by the expansion? Does the increased dispersion at the edges of the species range generates some maladaptive gene flow towards saturated parts of the landscape?

Our response: There is possibly maladaptive gene-flow towards the range core from the front. However, since we focus on expansion dynamics, it is not very relevant to the present study. Since the manuscript is already quite complex, we prefer not to add this aspect.

A few minor issues:
* Introduction, lines 34 or 37 for instance, but at other places as well: the literature review at present tense ("They find that ...") sounds slightly confusing.

Our response: We have changed sentences reviewing the literature to past tense.

* Two first paragraphs of the introduction (up to line 64): the review is sometimes not very precise. For instance, it is said that some authors have studied or discussed the effect of different factors, but no indication about the conclusions of these studies.

Our response: We have completely reworked the Introduction. We now explicitly state the mechanisms for the evolution of density-dependent and sex-biased dispersal that are relevant to understand our study (lines L 33–51)

* Line 100: is sexual dimorphism a special case of plasticity? According to the point of view, one could indeed consider that sexual dimorphism is just a consequence of the presence/absence of sex-specific hormones. However, in many organisms, sex determination is genetic, and sexual dimorphism would be a clear case of genetic determinism of the phenotype.

Our response: We have now removed reference to sex hormones. The main point is that the GRN receives some cue that indicates the sex of the organism. This does not depend on how sex is determined in the system.

* Methods, line 129: What is the influence of the second (y) dimension of the landscape? If the same results were obtained with a one-dimensional landscape, is it really necessary to have the y dimension? And if y matters, why only 5 elements in the grid?

Our response: We are looking at range expansion along the x-direction only, thus, we model a relatively narrow landscape. We do not analyse this aspect in detail here, but other work that we are currently conducting is focused on landscape topology. From this work, we can infer that, as long as we do not change the variation in connectivity our results should not change qualitatively. Clearly, what will change is that, the more connections exist on average, the slower range expansions will be. While it is certainly interesting, we prefer not to add this level of complexity, especially since we are comparing scenarios where the landscape context is invariant.

* I might have missed it, but I could not find the information that alpha and lambda_0 were chosen so that hat(N) = 100 (if my calculation is correct).

Our response: We have added this information to the caption of Fig. 2 and 3.

* In equation (3), the star is generally not considered as a correct mathematical symbol for multiplication.

Our response: Sorry, we missed this. We have corrected the equation.

* Figures 2 and 3, why are datapoints discretized on the x axis? If my understanding is correct, these data point should come with any value of x.

Our response: Since the plastic response is numerically calculated (rather than having an analytical expression) by iterating the gene-regulatory network, we need to discretise the population density values to calculate the plastic response. In any case, we now also represent the plastic response from the GRN model as lines with transparency weighted by occurrence of population density.

* Caption of figures 2 and 3: I am not sure that the acronym ES was defined before in the text (does it stand for environmental susceptibility?).

Our response: We apologise, it stands for evolutionarily stable. We have added this information to figure captions.

* Line 252: this sounds like discussion material, and not results.

Our response: Based on this and another reviewer's comment, we have moved this to the General Discussion (see lines L 364–370).

* Line 266-274: I am not familiar with the problem of sex-related dispersal; I am quite convinced that the authors are correct but I confess I did not understand why dispersal was male-biased in the model. In particular, I was confused that from fig 3, it seems that male dispersion is quite constant across the different panels. Therefore, it seems more natural to say that females disperse less than males (especially when epsilon = 0), since the evolvable parameter seems to be female dispersal.

Our response: This is possibly a confusion arising from terminology. Male-biased dispersal means that males disperse more than females. Consistent with predictions of previous work (Gros et al., 2009), we find that males always disperse more than females. We explicitly define male bias in lines L 48–51 of the Introduction.

* The color/line code was completely different between figures 2/3 and 4/5, I found it slightly disturbing.

Our response: We have changed the colours in the figures based on this suggestion.

Review by anonymous reviewer 1, 19 Jan 2024 15:55

In this study, Deshpande and Fronhofer investigate the evolution of the density dependent dispersal reaction norm under two models that assume different genetic architectures for it. In the first model (the "RN" model), the reaction norm is assumed to be a threshold function, the shape of which has been derived from first principles by previous authors. There is no dispersal below a threshold density, and a saturating increase in the probability of dispersal above it. Here, only the threshold value evolves, and the overall shape of the reaction norm is fixed. The second model considers a more complicated, mechanistic genetic architecture for the norm, that makes no assumption regarding its shape (the "GRN" model). Instead, the reaction norm emerges from a gene regulatory network inspired by the Wagner model. The network behaves analogously to a recurrent neural network, that takes either the local population density or local population density and the sex of its bearer as input, processes those inputs, and outputs a dispersal probability. In this second model, the weights, slopes and thresholds of all the genes in the network evolve. The authors first consider equilibrium metapopulation dynamics, and compare the reaction norm evolving under the GRN model to the one obtained under the RN model (which is known to be the optimal one). They find that the GRN is able to generate the optimal reaction norm with reasonable accuracy over the population density values that frequently occur in the simulations, even with a small number of genes in the intermediate layer (n=4). They then let the population undergo range expansion, and show that the GRN leads to more efficient colonisation than the classical RN model because the GRN retains large amounts of standing variation for densities that do not occur under equilibrium conditions, allowing it to react more efficiently to selection. Overall, I found the study to be quite interesting but I think it has a few shortcomings, and could be strengthened in a few ways. Please find my comments below.

Our response: Thank you for a positive evaluation of our manuscript.

Introduction The Introduction is very long and difficult to follow. I think it could be much shorter and to the point. Here is a few suggestions.

Our response: We have re-written the Introduction based on your suggestions. We removed information that was not required based on the comments below.

- The introduction contains a lot of information that is not directly relevant to the study being presented, which causes the reader to be confused as to what the study is going to be about. For example, the sentence starting on line 25, about dispersal not being random and being associated with other traits does not seem useful to me in the context of the study. Similarly, I did not get the point of the discussion of the Harman (2020) on lines 34 to 38. The discussion of Li and Kokko (2019) on lines 38 to 41 is not helpful either, because the authors are not addressing any of the problems identified in this previous study.

Our response: Thank you for this suggestion. We have removed some of this information and mentioned these studies more clearly. In lines L 35–51, we now briefly review theoretical expectations for density-dependent dispersal and sex-biased dispersal along with reasons why they evolve that are relevant to understand our study.

- The text is repetitive on a few occasions. For instance, the first paragraph finishes with the statement that the mechanisms underlying dispersal plasticity have not been modelled, and

the second paragraph begins with 4 lines (46 to 50) essentially repeating the same thing. The paragraph on lines 64 to 72 says the same thing again: a lot of work has been done on density-dependent reaction norms, using different approaches, but none of the previous studies have considered the underlying mechanisms.

Our response: We have now removed the discussion of genetic architecture in this part of the Introduction.

- The Wagner model should either not be explained in detail in the introduction (leaving explanations for the methods section), or the description given on lines 74-76 should be expanded and made less abstract.

Our response: We have removed the sentences explaining the Wagner model (see lines L 60–65) from the Introduction.

Model description - l. 129: Mention at this point that each cell of the grid corresponds to a patch where the population follows a Beverton-Holt model.

Our response: We have added this information to lines L 109–110.

In fact, it might be better to introduce the landscape in one sentence, and then rather than describing the approach taken to analyse the model (which can be done in the "Analysis" part at the end), immediately explain the life cycle.

Our response: In lines L 115–124 we now have only the description of the landscape.

- l. 131: typo "plasticity"

Our response: We have corrected this.

- l. 137: explain what "periodic" boundary conditions are.

Our response: Based on the suggestion of another reviewer, we have replaced “periodic” with “toroidal” for clarity (line L 121).

- The authors need to give the readers more explanations on the Wagner model, especially since it is the heart of the paper. The authors should walk us through eq. (2) and how an iteration of the network happens

Our response: We have rewritten the section on the Wagner model (see line L 152–177)

. - l. 194-195: What does discarding the GRNs that do not reach fixation mean? Are the corresponding individuals killed? How should we interpret this biologically?

Our response: We have clarified that individuals with GRNs that do not reach a fixed point equilibrium die (see lines L174–175. This is a standard assumption in many GRN models (e.g. Siegal and Bergmann, 2002).

Results - Based on the visual inspection of Figures 2 and 3, the authors claim that the GRN is able to accurately produce the optimal reaction norm. While it does seem to perform

reasonably well for some parameter values, comparison of the fit of a cloud of points with a curve is usually not a very good way of representing goodness-of-fit. Why not average the norms produced by the 1000 GRNs shown, and plot this averaged norm as a second curve? This would give a much more convincing depiction of how well the network actually performs. It could be interesting to do this over the whole range of densities and not just the ones that most frequently occur, as it could help to prove the next result on the network performing well only at the densities that actually occur in simulations.

Our response: Based on this suggestion, we now assess the quality of fit captured as the mean distance from optimum for individual plastic responses calculated both from the RN and GRN model (see SI Fig. S2 and S6). Thus, we can compare the quality of fit of the GRN model against the expectation set by the variation in the RN model. From this figure, we see that indeed the quality of fit of the GRN model is not the same across parameter space. Particularly, low dispersal mortality and extinction probability lead to lower quality of fit. While this was implicit earlier in the subsections describing range expansions, we have made this more explicit in lines L 205–220.

- This latter point is intuitive, but could be made in a more convincing way. For instance, the histograms showing the occurrence of the densities could be moved to the main text, accompanied by a plot showing (for instance) the variance in the dispersal probability given by the 1000 networks as a function of the frequency of occurrence of the considered density. I expect lower variance to be found where the network was able to optimise

Our response: This is a good idea but it does not quite work. The reason for this is that the phenotypic variation as a function of population density also depends on the value of dispersal probability. We have added plots (Fig. S3 and S7) that show phenotypic variation maintained at the end of the equilibrium metapopulation phase.

. - The network seems to be performing much better for some combinations of ϵ and μ than others, but the authors do not discuss this.If it is possible, I would be curious to get some explanation as to why that is.

Our response: We now make this more explicit in lines L 205–220 of the Results and Discussion. Specifically, low dispersal mortality and extinction probability prevents optimisation. Fig. S2 makes this point.

- Have the authors tried to use networks with different numbers of genes involved, to see how it affects accuracy for instance? On that note,you thought why did the authors decide to stop iterating the network at I=20?

Our response: We based the decision to iterate up to I=20 based on Draghi and Whitlock (2012). Since we already explore many other aspects, we do not add simulations exploring the number of genes. We had tried this with a previous version of the model (looking at 2 and 3 genes) and did not find an effect on the optimisation. If required, we can re-do these simulations with the updated model.

- The part of the results on range expansion could be improved: the authors have not really proven what they claim to be the mechanism (high standing variation for some population densities) producing faster expansion in the GRN model. Perhaps a plot showing the

phenotypic variance over the range of population densities, as suggested above, could be useful.

Our response: We already had a figure in the SI showing variation in the GRN model across all population densities (Fig. S9 in the revised version). But based on your suggestion we also added Figure S2 to the SI which shows phenotypic variation as a function of population density for different dispersal mortality and extinction probability. This figure shows that the phenotypic variation maintained in the GRN model is greater for low dispersal mortality and no patch extinctions. Also, at high dispersal mortality and extinction probability, phenotypic variation is similar between the two models.

Review by anonymous reviewer 2, 23 Feb 2024 08:37

In this manuscript, the authors investigate the evolution of dispersal plasticity under equilibrium and range expansion regimes, with an individual-based meta-population model. To encode plasticity, they use a Wagner-like evolvable genetic regulation network (GRN) that senses the local population density and the sex of the individual. Through numerical simulations, the authors suggest that after long-term evolution at equilibrium (20,000 generations), GRNs evolved a plastic response similar to previous analytical predictions. During range expansion, GRNs allow a faster population dispersal than hard-encoded plastic responses.

I think that the authors addressed a very interesting question, by using a more complex genotype-to-phenotype map to evolve dispersal plasticity.

Our response: Thank you for a positive evaluation of our manuscript.

However, there are several issues in the manuscript that need to be solved before considering it suitable for publication:

1) The structure and writing of the manuscript need improvement. The manuscript does not maintain a clear track of the question, and the reader can become lost in literature and general considerations that are sometimes out of context (see my comments below). Conciseness must also be improved.

Our response: We have rewritten the Introduction and addressed the specific comments raised below.

2) The presentation of the model lacks clarity. Some features, such as population dynamics in each patch, sexual reproduction, and the mutational process, are poorly explained. I personally struggled to understand the detail of the model.

Our response: We have added lines L 136–138 that explain the population dynamics (logistic growth) that the model produces and the expected equilibrium density (line L 138). We have added lines L 142—150 explaining the mutational process in more detail.

.

3) I also believe that the results are not presented in a convincing manner:

- At equilibrium, the comparison between GRN and RN relies solely on graphical interpretation. While this approach could be acceptable, commenting on hardly readable scatter plots is insufficient to demonstrate the similarity to RN curves. There are sometimes a large number of outliers, casting doubt on the results (e.g., the left column of Figs 2 and 3). The minimum requirement would be to plot a running mean/median/mode and perform some statistical or fitting tests to convince the reader.

Our response: We have re-written the Results and Discussion section lines (L 205–220) to clarify that indeed the quality of the fit between the GRN and RN model is not the same for all parameter combinations of dispersal mortality and extinction probability. We also add a supplementary plot Fig. S2 and S6 that show the distance from optimum in both models to demonstrate this point. We comment that the fit between theoretically predicted reaction norm and the GRN model is greater for higher extinction probability and dispersal mortality, in other words, when strength of selection on dispersal plasticity is the highest.

- At range expansion, the authors extensively interpret the results without substantiating their claims with additional simulation insights. On this point, I believe that the mutation rate plays an important role, and the values used should be discussed, at least in the Methods section.

Our response: We have now added two plots in the SI to support our previous verbal arguments. In Fig. S3 and S7, we show that phenotypic variation is greater in the GRN model than the RN model at low dispersal mortality. This implies that there is greater standing genetic variation for dispersal at the beginning of range expansions in the GRN model for those conditions. We also explicitly mention mutation rates and effects (line L 142–150) in the methods. Additionally, we also explore how perturbations impact GRNs vs. RNs (Fig. S4 and S8), which clarifies that mutation effects are comparable between the two models at high extinction probability and dispersal mortality, but greater in the GRN model at low dispersal mortality.

To conclude, I find the question and the methodology used interesting, and I believe the authors have the opportunity to improve their manuscript and strengthen their results with additional simulation insights (I am thinking about the male-biased dispersal and/or why GRNs spread faster than RNs). I certainly do not suggest re-running entire simulation campaigns but rather attempting to support some of the authors' claims with mechanistic simulation insights

Our response: On the point of male-biased dispersal, the reasons for why it evolves under random mating have already been shown in Gros et al., (2009). Thus, we do not see any reason to re-run these simulations. On the point of the phenotypic variation, we have now added plots (Fig. S2 and S6) to the SI which backs up our claim about the differences in phenotypic variation maintained in the GRN vs. RN models.

I believe that if the authors can address the issues mentioned above, this could make a valuable contribution to PCI EvolBiol.

Detailed comments:
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- General: check for spelling and conciseness.

Our response: Thank you for pointing this out. We have reduced some text based on your suggestion.

Abstract:

- Abstract (lines 2 -> 9): Improve the writing of this section. Should we distinguish evolution rate from plasticity evolution rate? What is the interplay?

Our response: The abstract has been re-written.

- Abstract: Introduce one sentence to tell why GRN are biologically relevant, compared to RN (as extensively discussed in the introduction).

Our response: These lines in the abstract state the biological relevance of understanding molecular mechanisms underlying dispersal plasticity : “While optimal dispersal plastic responses have been derived from first principles, the genetic and molecular basis of dispersal plasticity have not been modelled. An understanding of the genetic architecture of dispersal plasticity is especially relevant for understanding dispersal evolution during rapidly changing spatial ecological conditions such as range expansions.”

Introduction:

In general, prefer past form to present form, and focus more on the question (for example, it should be clear from the beginning that the GRN senses sex and/or population density, nothing more).
- l.22 remove "(evolution)" and "(ecology)", repetition of the previous line.

Our response: This has been rephrased.

- l.26 remove "now".

Our response: This line has been removed.

- l.55, l.59: "fragmentation": I would avoid discussing this notion, as it is not explored in the manuscript.

Our response: This has been removed.

- l. 73-89: shorten this section. Btw, if I am not wrong, this is not exactly a Wagner model (where the genotype is a single weight matrix).

Our response: Indeed it is not the model proposed by Wagner but one in which modifications are made to model plasticity and quantitative traits (Draghi and Whitlock, 2012).

- l. 91: "at the molecular level": a weight matrix is already a significant abstraction of molecular processes.

Our response: We agree. In line L 65 we now clarify that the GRN model is likely still highly simplified compared to reality.

- l. 100: "organism's internal state": only individual sex is being sensed here.

Our response: We have removed this (see line L 98).

- l. 101-112: the approach and bibliography are somewhat mixed up here.

Our response: The bibliography sentences have been moved above (see lines L 76–88)

- l. 113: "concretely": Avoid, as it should be clear to the reader already.

Our response: We have removed this word.

Model description:

- l. 122: focus on the question, the inputs are not external cues and internal states, but individual's sex and population density, i.e. two values.

Our response: The section describing the GRN model is now re-written without referring to more general applications of the model (see line L 152–177).

- l. 137: "periodic" -> toroidal?

Our response: We have changed periodic to toroidal for clarity (see lines L 121).

- l. 138: Does the simulation automatically stops when the border of the grid is reached?

Our response: Yes, this is when the simulations stop. We have added the line “Range expansions stop when the expanding population reaches the end of the boundary of the landscape in the x dimension.” for clarity (see lines L 123–124).

- l. 145: "eight nearest neighboring patches": Moore neighborhood

Our response: We have added this information (see L 131).

- l. 152-159: Population dynamics are not clearly explained here.

Our response: Thank you for pointing this out. We have now mentioned that this model exhibits logistic growth (line L 136) and have stated the equilibrium density that can be expected from this model of population growth (L 138).

- l. 160: "The parental generation then dies and is replaced by the offspring": Generations are non-overlapping.

Our response: We have re-phrased this sentence (L 149) for clarity: “Generations are non-overlapping, therefore, the offspring generation replaces the parental generation.”

- l.168-173: Could be removed (hence the "More concretely")

Our response: We have removed this phrase.

- l.178-188: Same problem here, too many general considerations.

Our response:m We have re-written the methods to remove these general considerations (line L 153–177).

- l. 194: "discard": how is it implemented in the simulation? Does the individual die?

Our response: Yes, that is what happens in the simulations. Sorry we were not clear. We have rephrased this as : “Individuals with GRNs that do not reach steady state equilibrium at this point die (Wagner, 1994)” in lines L 174–175.

- l. 217-218: "mu = 0.01, 0.1, 0.3" ==> mu \in {0.01, 0.1, 0.3} and elsewhere (\in LaTeX math symbol).

Our response: Thank you. We have changed this everywhere.

- l. 217: "dispersal costs" ==> dispersal mortality?

Our response: We have changed this everywhere.

Results:

- l. 222: "emerge from cellular and molecular processes": I would nuance with "can indeed emerge from a more complex genotype-to-phenotype map".

Our response: This line has been removed from the Results and Discussion,

- l. 228: "Fig. 2 shows" ==> cf. to my main comment, the figure alone is not convincing enough.

Our response: We have added Fig. S2 to the supplement which shows the quality of optimisation for the values of dispersal mortality and extinction probability that are explored in the model. The quality of fit is greatest at high dispersal mortality and patch extinction probability.

- l. 231-235: not clear.

Our response: This subsection has completely been re-written.

- Fig. 2: cf. my main comment. Especially for left-bottom panels containing GRN outliers.

Our response: We have now added lines L 205–220 stating conditions of dispersal mortality and extinction probability in which the GRN model performs better. We also add Fig. S2 and Fig. S3 to the SI which show the distance from optimum and phenotypic variation maintained in the GRN model for different combinations of dispersal mortality and extinction probability.

- l. 246-256: perhaps this part should belong to the discussion.

Our response: We have moved this to the discussion (see lines L 364–371).

- Fig. 3: same problem here. Moreover, it would be nice to have simulation mechanistic insights to better understand the male-biased dispersal.

Our response: Thank you for the suggestion. Since mechanisms leading to male biased dispersal have already been worked out in another paper that we cite (Gros et al., 2009) we do not repeat this.

- l. 266-274: perhaps this part should also belong to the discussion.

Our response: We have shortened this part (see L 238–250).

- Figs. 4 and 5: There is no legend to interpret the colors. Letters sometimes overlap with curves.

Our response: We have increased the size of the legend saying GRN and RN and removed the letters.

Discussion:

- l. 335: "robustness": I would avoid this notion and stay focused on the question.

Our response: This line has been removed.

- l. 344: "genotype-to-phenotype (GP)": this term is introduced too late in the manuscript.

Our response: This information is there in the Introduction (lines L 61).

- l. 346: "While empirical evidence supporting our work is scarce": another reason to seek more insights from the simulations.

Our response: We have reformulated the discussion and are now confident that the insights from our work are stated more clearly.