Dear Dr. Barrett,

Editor in Chief, Proceedings of the Royal Society of London B: Biological Sciences

Thank you for inviting us to submit a revised version of our manuscript (RSPB-2018-0230: “Urban spandrels: The roles of genetic drift, gene flow and natural selection in the formation of parallel clines”) to Proceedings B. We are grateful for the constructive suggestions offered by the reviewers. We have carefully considered and responded to each comment, which has resulted in a number of improvements. The revised manuscript is stronger and clearer as a result of these changes and we anticipate the revisions will increase the impact of our paper with Proceeding B’s broad readership.

Our detailed responses are provided below. We abbreviated some of the reviewers’ longer comments while making every effort to retain their main points. Our response to each comment is in **bold**.

We hope that you find our revised manuscript acceptable for publication in Proceedings B. We are happy to accommodate further suggestions for improvement.

Thank you again for the thoughtful appraisal of our work.

Sincerely and on behalf of all authors,

James Santangelo

**Referee: 1**   
  
Comments to the Author(s)   
Review for RSPB-2018-0230   
‘Urban spandrels: The roles of genetic drift, gene flow and natural selection in the formation of parallel clines’   
  
  
The paper is well-written, clear and very interesting.  It should encourage ecologists to be more careful in their interpretation of observed phenotypic clines, even when they are repeated across environmental gradients, and should also help to understand the forces triggering genetic and phenotypic clines along environmental gradients, and not only urbanization ones. The epistatic determination of HCN phenotype in white clover provides a good example of how ‘simple’ genetic mechanisms can have strong consequences on the formation of clines.  I guess the authors will have a lot more to explore using their model(s) and I look forward to read the following papers investigating some of the numerous questions arising from this one.   
  
I think this paper fits the expectations of Proceedings of the Royal Society London B: Biological Sciences in terms of scope, quality, originality and impacts for the scientific community.   
I recommend major revisions because several points must be clarified along the manuscript and because the discussion needs to be improved.   
  
I also joined this review as a Microsoft word file.   
  
**My main concerns are the following:**

1) Although titled “Urban sprandels”, this paper does not address urban-specific questions but, as explained L79-91, use urbanization as an ‘excellent large-scale replicated system to understand how adaptive and non-adaptive evolutionary processes contribute to the formation of parallel clines’. The biological model (the white clover) and the previous papers published by some of the authors on this species justify the use of an urbanization gradient as an environmental background for their model, but their methodology and findings are, in my sense, larger than that. Does this kind of modeling approaches have already been applied to non-urban environmental gradients?

Consider replacing ‘Urban sprandels’ by the term used in the conclusion: ‘Contemporary sprandels’, or something like ‘Modern sprandels’.

**Thank you. We agree with the reviewer that the impact of this work extends beyond urbanization gradients and can be applied when examining the evolution of parallel clines along any environmental gradient. We have therefore replaced the title “Urban spandrels” with “Modern spandrels”, as suggested by the reviewer.**

2) The authors justified why selection strength (on HCN) change linearly along the urbanization gradient, but they should also justify why population size are expected to increase linearly from the city center to rural areas (for DS1) or why the colonization of new habitats should come from rural to urban areas (DS2). I think that this is crucial in order to allow the comparison between simulated and empirical clines (i.e. Fig. S10).

**Thank you. In the case of DS1, we modelled a linear decrease in *N* with increasing urbanization for simplicity. A non-linear change in the population size across the landscape would simply have resulted in non-linear changes in the frequency of HCN across space, which would have (1) been inconsistent with the linear clines observed across cities (i.e. from Thompson et al. 2016), and (2) added an additional complication to the manuscript, which on serves to illustrate that a gradient in *N* can lead to the formation of clines. We have specified in text that the decrease in *N* with increasing urbanization was modelled for simplicity (LXXX).**

**For DS2, we modelled colonization from rural to urban as this is a biologically realistic scenario given clovers history of being seeded into agricultural pastures and the direction of observed clines in nature. However, we emphasize that the direction of colonization (in the absence of selection) does not influence our main point that colonization through founder events can results in the loss of cyanogenesis and the evolution of repeated clines in HCN. In fact, we reversed the direction of colonization (urban to rural) when examining the formation of clines when drift and selection are in opposition (text S1b). We have added numerous sentences explaining our motivation in the electronic supplementary materials describing DS2 (LXXX – XXX).**

3) I am glad that the authors proposed an alternative drift scenario (DS2) with no direct assumption on the carrying capacity along the distance to the city core. Drift scenario 2 does not make assumption on the carrying capacity of cells along the urbanization gradient but rather rely on founder effects. These two approaches are very interesting and I am surprised that they generate similar results. In the simulations using DS2, did the urban-most populations reached the carrying capacity (N=1000)? if yes, at which generation? Please see my comment (about L131) for methods. 

**Thank you for this comment. The urban-most population always reached carrying capacity soon after colonization. In addition, the effects of drift on allele frequencies are determined by the effective population size (*Ne),* which in a population of fluctuating size, is determined the strength of population bottlenecks or founder events (e.g. Vucevitch et al. 1997. *Evolution*). In other words, the allele and phenotype frequency of the urban-most population would be driven primarily by its size upon colonization, and not whether or not it reached carrying capacity. Nonetheless, we have added text to the supplementary materials describing the methods for DS2 detailing (1) the number of generations required for the landscape to fill under different bottleneck strengths and (2) the number of generations for the urban-most populations to reach carrying capacity under different bottlenecks strengths (LXXX – XXX). We have also added a supplementary figure (figure S2) illustrating these results.**   
  
**Introduction**

4) Please define ‘parallel clines’ (L58) as soon as it is named.

**Thanks for the suggestion, it’s now done.**  
  
5) L94: ‘They found that HCN defended genotypes were less frequent in urban populations in 3 of the 4 cities examined [23].’ In figure S10, regrouping refs [23] and [26], only 7 out of 26 cities show significant positive clines…

**While the reviewer’s assessment of the number of significant clines across refs [23] and [26] is correct, we were initially motivated in this work only by the 3 or 4 clines observed by Thompson et al (2016, i.e. ref [23]) since Johnson et al. (2018, i.e. ref [26]) was not yet published and in fact is part of the same issue as the current paper. Thus, in the intro (LXXX), we only cite ref [23] for context and not to provide a thorough description of phenotypic clines in HCN across all cities for which there are data (i.e. refs [23] and [26]). However, we do discuss our results in the context of both refs [23] and [26] in the discussion (LXXX – XXX), as it is clear our results have an impact on the evolution of HCN clines beyond those reported in ref [23], as the reviewer pointed out.**   
  
**Methods**

6) At the first read, it is difficult to understand how the model works: how CYP79D15 and Li genes determine HCN value? Line 99 and figure 1 give useful information on how HCN frequencies are determined but a brief explanation in the first paragraph of the methods, might be useful. Or maybe simply adding Fig S6 (c) in the manuscript.

**We have added text to the first paragraph of the methods detailing how the frequency of HCN is calculated from the frequency of alleles at underlying loci (LXXX – XXX).**  
  
7) L122: Define ‘CYP79D15 and Li’

**We have defined *CYP79D15* and *Li* as the loci that underlie the production of HCN (LXXX – LXXX)**  
  
8) L131, about drifts scenarios 1 and 2, the authors wrote: ‘Both scenarios were simulated independently and produced qualitatively similar results.’   
I am not convinced. It is too difficult to compare the results between drift scenarios 1 and 2 based on available figures and ESM.   
Maybe an additional figure, in ESM, could be added to allow an easier comparison of the two models’ output?

**Thank you. We have added an additional supplementary figure (figure S1) showing the overlap in the distribution of slopes from simulations implemented with drift scenario 1 and drift scenario 2. We have reference this figure in text (LXXX).**   
  
9) L132: Why did you choose to present drift scenario 1 rather than drift scenario 2?

**We chose to present drift scenario 1 (DS1) because it is simpler, and the results of both scenarios were qualitatively similar (see response above). Because explaining the methods and results of DS1 was easier and required less text, we decided to focus on DS1 in the main text and report DS2 in the online supplementary materials.**  
  
10) L180: Add the symbol of the selection parameter (‘s=’ ?) as in L158.

**Done.**

11) I would have preferred to see Table S2 directly in the manuscript rather than in the ESM.   
Maybe giving a name to each model would facilitate to do the link between table S2, the text and figures.   
  
**We would also have liked to place table S2 in the main text but unfortunately length restrictions have prevented us from doing so. However, we have added the drift scenario to the captions of all figures to ease interpretation (see response to point #25).**

**Results**

12) L244: ‘Drift scenario 1: Gradient in carrying capacity across the landscape’   
I understand it corresponds to the partitioning of the ESM additional texts, but it stands alone in the manuscript, which is perturbing.

**We have removed this subheading from both the methods and the results.**  
  
13) L258-260: ‘In contrast, the mean strength of clines at each of the two unlinked loci (i.e. CYP79D15 and Li) was consistently zero under even a strong gradient in drift, independent of levels of gene flow.’   
I am not sure to understand this statement… the whole paragraph (L255-263) could be made clearer. 

For a single neutral locus drift will increase the frequency of a given allele as often as it will decrease the frequency. And the probability of fixation for a given allele is simply equal to its initial frequency. Therefore, it is a core prediction of drift that the average change in allele frequency across multiple independent populations is zero even if there is substantial drift within each population. **We have added a sentence clarifying this result (LXXX – XXX). In addition, we have added an additional supplementary figure (figure S10), which is referenced in text (LXXX), to show that drift generates equal proportions of positive and negative clines at the single loci underlying HCN production, thereby resulting in a mean slope of 0 when averaged across all clines, as shown in figure 2b.**

14) L269-270: Homogenize (‘(s=0)’ or ‘when s=0’) 

**Done.**

**Discussion**:   
  
15) L311-312: ‘Subsequent work has shown that clines in the frequency of HCN are common, with higher frequencies occurring in warmer and drier habitats [23,33–37].’   
Urban areas are often warmer and drier than adjacent rural areas because of urban heat island effects. HCN should consequently be more frequent in urban areas?

**The reviewer is correct that urban areas are often warmer than surrounding rural regions and we would thus predict higher frequencies of HCN in urban populations. In fact, this was an initial prediction of Thompson et al. (2016), although they found the opposite pattern. We have added text to clarify that the observed urban-rural cyanogenesis clines are actually consistent with the known agents of selection acting on HCN, since selection on HCN in urban population appears to be driven by winter conditions, rather than summer conditions (LXXX -XXX).**

16) L312-314: ‘These patterns are thought to reflect the benefits of producing HCN in warmer environments where herbivores are more common’. Ok. Give a reference. What means ’more common’? More abundant? Higher diversity of species?

**We have revised this to (changes italicized): “in warmer environments where *herbivore damage is greater*” (LXXX). We have also provided references.**   
  
17) L311-315: Please, make this part clearer. 

**We have clarified the text in this section (see responses above).**   
  
18) L351-356: Seven out of 26 cities showed significantly positive clines in HCN. I think it would be interesting to discuss the possible reasons why 19 cities do not demonstrate significantly positive clines. Drift may not have the same impact on HCN frequencies depending on the structure of the suitable habitat along the urban gradient. Thus, it would be interesting to investigate the link between the availability or structure of habitats along the urban gradient and the slope of the cline in HCN. I do not ask the authors to perform such analyses here, but discussing it might give rise to interesting questions and perspectives. 

**Thank you. We have added text describing how urban fragmentation is expected to reduce the availability of suitable habitat and reduce local populations sizes, although corridors that facilitate gene flow may slow the loss of diversity and reduce the amount of genetic and phenotypic differentiation (LXXX – XXX).**

19) L359-372: If HCN, like many other traits, is likely to be affected by urbanization- or climat-induced changes (in the case of white clover, affecting herbivores prevalence and therefore selection on HCN), then the latitudinal position of cities might affect the formation of clines.

**We agree with the reviewer that the latitudinal position of cities is likely to influence whether or not they show clines. However, this would only influence clines that are formed by selection since it is putative selective agents that are varying with latitude and affecting the formation of clines. Latitude is not expected to influence variation in the strength of drift across urbanization gradients since as the reviewer point out in their other comments, drift is influenced primarily by landscape-levels factors that affect the size and connectivity of populations. Because our manuscript involves primarily understanding the effects of drift on the formation of clines rather than variation in specific selective agents, and because we are limited to 10 manuscript pages of text, we have opted not to discuss how latitude might influence variation in the selective agents responsible for producing clines. However, we agree with the reviewer that a discussion the landscape characteristics that influence the propensity for drift to influence clines is warranted and we have included text to this effect throughout the discussion (see responses to points #18 )**

20) L359-372: The authors do not discuss how the characteristics of cities could affect the formation and the shape of phenotypic clines along urbanization gradient. Cities are not only identical replicates, they regroup specific characteristics (micro-climate, UHI effect, habitat structure (e.g., quantity and quality of green spaces)) that should be taken into account when investigating parallel clines as they might generate strong variability in observed phenotypic patterns along the urban gradient.   
  
**Thank you. We agree with the reviewer that this is an important consideration and have added text describing how variation in landscape characteristics between cities may influence their propensity of drift the generate clines (see response to point #18)**

21) L382-387: Exotic species often undergo founder effects during both introduction and secondary spread and alien/invasive populations should therefore be highly susceptible to drift. In my sense, the effective size of populations along the urbanization gradient is more important than the exotic status of the species itself… In addition, in my experience (with ants), some native species can have bigger populations in urban environments than in rural or semi-natural adjacent ones. The common observation that exotic species are often urban specialists or at least urban tolerant species might explain why some species do not suffer from drift in urban populations (population size does not decrease with increasing urbanization). It would be interesting to run drift scenario 2 with inverse colonization dynamics (from urban to rural), because exotic species are more likely to be introduced in or near urban cores and then spread to adjacent rural areas. (I suppose it is not the case for white clover as it seems to have been introduced outside its native range for agricultural purposes. ?). 

**We agree with the reviewer that it is ultimately the effects of urbanization of reducing effective population sizes (*Ne*) that are important in determining the extent to which drift will alter allele frequencies in cities. The argument we are making in this paragraph is that native species may be more susceptible to decreases in *Ne* than exotics, and the limited data that is available suggests this could be true. While it’s true that exotic species often undergo drift due to founder effects upon introduction, this would generate differentiation relative to populations in the native range and not necessarily across an and urban-rural gradient if the species is tolerant to urbanization. We agree that modelling the expansion from urban to rural would help address the likely colonization dynamics of exotic species, which as the reviewer points out are more likely to be introduced in cities. However, in the absence of selection, reversing the colonization dynamics will generate the same results as those presented in the paper but of opposing direction (i.e. more negative than positive clines). Nonetheless, we do present results for inverse colonization dynamics in the presence of selection, which yields interesting results since drift and selection are in opposition in these simulations (see LXXX – XXX).**   
  
22) L393-394: Please, provide a reference. 

**We did not provide a reference because we are specifically referring to the heuristic comparison of the effects of urbanization on native vs. non-native species. However, we realize this may not have been clear and thus we have modified the statement accordingly (LXXX)**   
  
23) L395: ‘Predictions on the effects of urbanization on the strength of genetic drift should therefore be based at least in part on the natural history of species being studied.’   
And also on the structure of populations along urbanization gradients, which is linked to landscape characteristics such as the structure of the preferred habitat for each species, which is linked to the characteristics of each city (e.g., age, size, urban shape; see Forman, R. T. (2014). Urban ecology: science of cities. Cambridge University Press. Around page 78). The authors should discuss the potential role of cities’ characteristics (especially land cover structure along the gradient) on the emergence of clines induced by drift, especially when using drift scenario 1. 

**Thank you. We have added text describing the effects of landscape features within cities on the strength of drift in urban populations. Please see response to point #18.**

24) L414-416: ‘We simulated cases where genetic drift leads to the formation of clines on contemporary time scales under non-equilibrium conditions as these are the conditions most likely to occur in urbanizing areas’. Give a reference to this statement please.

**We have removed this statement.**

**Comments for Figures:**   
  
General observations:   
25) All figures are useful and give a lot of information. They may be easier to understand if the caption specified the model used or if table S2 was directly added in the manuscript.

**Unfortunately, we are at the page limit and cannot place table S2 in the main text. However, we have added the drift scenarios to the captions for each figure, as suggested by the reviewer.**

26) Figures representing the proportion of significant slopes are not homogeneous. In some figures (fig.3 c and d and FigS4 b, points are linked by lines while in other figures, they are not. Is this made on purpose or not? 

**Thank you. We have added line to all figure for consistency.**

27) Figure 2: Please, improve caption for (c). 

**Done.**

28) Table S1:   
‘Proportion of alleles sampled when founding new populations. Lower proportions result in stronger effects of drift. This is equivalent to manipulating the number of individuals sampled to form new populations.’   
Why not sampling individuals then? It would be more realistic.

**We sampled alleles rather than individuals as this is common practice in population genetics simulations. Sampling individuals would require generating genotypes from allele frequencies and keeping track of two biallelic loci. However, the same information is obtained directly from allele frequencies and knowledge of the extent of linkage disequilibrium in the population. Because, we have shown that LD is unimportant in this case (text S5, figure S10), it is mathematically and computationally more effective to sample allele frequencies.**

**Referee: 2**

**Comments to the Author(s)**

This paper nicely explains how traits resulting from epistatic interactions can evolve in a non-random direction when influenced only by the stochastic drift process. The authors explicate this process using the cyanogenesis polymorphism in Trifolium, but the same process will apply to any epistatic trait. The study models how drift can result in the increasing loss of HCN along a cline of increasing drift and elucidates how the resulting cline in the trait could be misinterpreted as selection. They also model selection on cyanogenesis and show that with strong selection gradients, the slope of the cline can be greater than expected under drift. The authors compare the results of their simulations to empirical work from their team on the evolution of cyanogenesis in rural-to-urban clines in cyanogenesis. They conclude that adaptation likely has an influence, but the evidence is not rock solid. This modeling process has allowed them to identify the data required to confirm whether drift can account for rural-to-urban clines in cyanogenesis in future studies.   
  
This paper is a little gem. There are many facets that shine, including the wonderful explanation of how epistatic traits evolve under drift and the utility of studies of urban environments to understand the evolutionary process. It is well organized and wonderfully presented. I have reviewed a lot of papers in my career, and it is extremely rare to come across one for which I have no constructive criticism.

**We thank the reviewer for their kind assessment of our work.**