**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’.   
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).   
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.   
  
  
**Introduction**

Please define ‘parallel clines’ (L58) as soon as it is named.   
  
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…   
  
**Methods**

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.   
  
L122: Define ‘CYP79D15 and Li’   
  
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?   
  
L132: Why did you choose to present drift scenario 1 rather than drift scenario 2?   
  
L180: Add the symbol of the selection parameter (‘s=’ ?) as in L158.   
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.   
  
  
**Results**

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.   
  
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.   
  
L269-270: Homogenize (‘(s=0)’ or ‘when s=0’)   
  
**Discussion**:   
  
L311-312: ‘Subsequent work has shown that clines in the frequency of HCN are common, with higher frequencies of HCN 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?   
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?   
  
L311-315: Please, make this part clearer.   
  
  
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.   
  
L359-372: Some points I would have liked to see discussed in this section:   
1) 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.   
2) 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.   
  
  
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. ?).   
  
  
L393-394: Please, provide a reference.   
  
  
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.   
  
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.   
  
**Comments for Figures:**   
  
General observations:   
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.   
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?   
  
Figure 2: Please, improve caption for (c).   
  
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.

**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.