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January 29, 2018

Dear Dr. Barrett,

Please find enclosed our manuscript entitled, “**Urban spandrels: the roles of genetic drift, gene flow and natural selection in the formation of parallel clines**”, to be considered for publication in *Proceedings of the Royal Society London B: Biological Sciences* as an Original Article. Our article is intended as a contribution to the special issue entitled: “**The Evolutionary Ecology of City Life**” guest edited by myself, Marc T. J. Johnson and Ruth Rivkin. Urban environments offer the opportunity to study the role of adaptive and non-adaptive evolutionary processes on an unprecedented scale. While the presence of parallel clines in heritable phenotypic traits is often considered strong evidence for the role of natural selection, non-adaptive evolutionary processes can also generate clines, and this may be more likely when traits have a non-additive genetic basis due to epistasis. In this paper, we use spatially-explicit simulations modelled according to the cyanogenesis (HCN) polymorphism in white clover (*Trifolium repens*)—a two-locus, Mendelian-inherited trait with an epistatic genetic basis—to examine the formation of phenotypic clines along urbanization gradients under varying levels of drift, gene flow and selection.

This research makes several novel contributions to our understanding of how genetic drift, gene flow and natural selection interact in the formation of clines:

* We demonstrate that gradients in the strength of drift across a landscape result in consistent phenotypic clines with lower frequencies of HCN in strongly drifting populations, giving the misleading appearance of deterministic adaptive changes in the phenotype.
* We show that while selection generates stronger phenotypic clines than drift alone, the presence of gradients in the strength of drift can constrain the ability of selection to generate phenotypic clines in HCN.
* Despite the stronger clines generated by selection, we show that there is substantial overlap in the strength of clines simulated under drift alone and those observed across multiple urbanization gradients, suggesting drift can generate clines as strong as those observed across cities

We believe that our manuscript will be of interest to *Proc B’s* broad audiences. Our paper has been formatted to *Proc B’s* guidelines and includes 26 pages of text (excluding references), 3 figures, and online supplementary materials with 2 tables, 10 figures and 12 pages of supplementary text. This manuscript represents our original research and it is not currently being considered for publication elsewhere. All authors contributed substantially to the manuscript, have read and approved the final submission, and declare no conflicts of interest. On behalf of all authors, I thank you for your attention to our submission.

Sincerely,

James S. Santangelo