**Outline for simulations manuscript**

**Introduction:**

* *Paragraph 1:* What are clines and what are their purpose
  + Topic: Clines are a change in the frequency of alleles, phenotpyes, or genotypes over some spatial extent (Haldane 1948; Endler 1977).
  + Clines are of continued interest to evolutionary biologists as they can help us disentangle adaptive (i.e. deterministic) from non-adaptive (i.e. stochastic) evolutionary mechanisms and inform speciation processes (Takahashi 2015).
  + The relative roles of deterministic vs. stochastic processes continues to be of central importance in evolutionary biology (Losos 1998; Simões 2008; Travisiano 1995).
* *Paragraph 2*: Parallel clines are the hallmark of adaptation: contrast parallel clines vs. single (i.e. unreplicated) clines
  + Topic: Parallel clines are considered strong evidence for the role of adaptive evolution (Stock et al. 2015; Samis et al. 2012; Huey 2007; Gilchrist 2001) especially clines in quantitative traits (Samis et al. 2012).
  + However, non-adaptive processes such as drift and spatially restricted gene flow (Vasemägi 2006), in addition to founder events and unequal sampling from ancestral populations (Keller and Taylor 2009) can generate phenotypic clines.
  + Disentangling the relative importance of stochastic and deterministic forces is essential prior to invoking the role of selection in generating adaptive phenotypic clines (Colautti and Lao 2015).
* *Paragraph* 3: Clines in complex traits (e.g. epistasis) may more readily evolve via neutral processes. Other ecological processes may also generate clines (e.g. inbreeding at range margins resulting in loss of heterozygosity)
  + Topic: Non-adaptive processes may more likely generate phenotypic clines in traits with complex genetic architectures (e.g. epistasis).
  + For two-locus traits with epistasis, stochastic processes (e.g. drift, founder events) can lead to predictable changes in the frequency of a phenotype within populations.
  + Cite loss of S morph in *Eichhornia* as example (e.g. Husband and Barrett 1992a; 1992b; Barrett et al. 1989; Barrett et al. 2009).
  + Therefore, stochastic processes can lead to deterministic outcomes.
* *Paragraph 4*: Where do we study clines
  + Topic: Clines are studied latitudinally, altitudinally and across habitat types (e.g. urban vs. rural).
  + Clines often occur with demographic correlates – population size, inbreeding, founder events, stability, connectivity. DRIFT
  + In some cases we can study parallel clines; cities are a great place to study clines — parallelism
* *Paragraph 5*: Clover and urban-rural clines in cyanogenesis. Brief history of clover as a model system testing for clines. Urban-rural clines in cyanogenesis.
  + Topic: The purpose of this paper is to examine under what conditions non-adaptive processes such drift and gene flow can lead to phenotypic clines in traits with complex genetic architectures. Clover is a classic system for studying clines.
  + Thompson et al. (2016) recently detected parallel clines in the frequency of hydrogen cyanide (HCN) — an antiherbivore defense — across independent urbanization events; three out of four sampled cities show decreased HCN with increasing urbanization.
  + HCN is the result of two, independently assorting Mendelian genes and plants require a functional (i.e. dominant) allele at each locus to produce HCN.
  + Given that the loss of the dominant gene at either locus results in the loss of HCN, the frequency of HCN in populations in more likely to decrease than increase when stochastic forces are the only ones operating (Fig. 1: 3D allele freq. plot).
* *Paragraph 5*: Research questions
  + In this paper, we seek to address the following specific questions:
    1. How do genetic drift, migration, and selection interact to influence the formation of urban-rural clines in HCN?
    2. Is the formation of urban-rural clines in HCN contingent upon the colonization history of white clover in cities (i.e. urban to rural, rural to urban, always colonized)?
  + We then parametrize the simulations using estimates of contemporary migration rates and Ne obtained from macrosats to infer the likelihood that observed clines in HCN are the result of stochastic vs. deterministic processes.

**Methods**

* Section 1: Structure of simulations
  + *Paragraph 1*: Overview
    - Spatially-explicit, individual-based simulations coded in Python.
    - Allow independent control over stochastic and deterministic parameters across the landscape (Table 1: Parameters with meaning and ranges)
    - Life cycle of a generation in simulations (Fig. 2: Life cycle)
    - Model where we sample alleles from infinite pool (*What is this called?*)
  + *Paragraph 2*: Within population dynamics and drift
    - Logistic population growth
    - Population created in adjacent cells with some probability that depends on the population’s size (linear relationship, Equation)
    - Drift primarily controlled by the strength of the bottleneck during founder events. Stronger bottlenecks = stronger drift.
    - Drift can also be controlled either by varying *K* across the landscape (Alleaume-Benharira 2006).
    - Show how varying *K* or bottleneck proportion influence *Ne*, calculated as the harmonic mean of census population sizes over time (Wright 1938, supplementary figure)
  + *Paragraph 3*: Simulating migration
    - Migration declines with distance based on exponential distribution (Kimura and Weiss 1964). Makes migration like stepping-stone model since most migration occurs among populations close in space. (Equation)
    - For each population, allele frequencies are calculated based on Wright’s (1931) continent-island model (Equation), where *m* is the average proportion of immigrants arriving into the resident population across all existing populations (Equation), weighted by their population sizes (Equation).
  + *Paragraph 4*: Selection
    - Two-locus selection model (Likely multiple equations here)
    - Each cell in the matrix is given a selection coefficient (*s*). Selection declines linearly with increasing distance from the cell with the strongest selection, simulating an environmental gradient.
    - Selection acts independently on the dominant alleles of both loci
* Section 2: Cases examined in the paper
  + *Paragraph 1*: Overview
    - Ran 1000 simulations for all cases below
    - Number of generations for case 1 = 250, all others = 1000
  + *Case 1*: Matrix filled entirely
    - All cells initialized with populations
    - No creation or bottlenecks.
    - No population growth.
    - *K* varies across the landscape resulting in lower *Ne* inside cities.
    - Models a simple scenario where clover was always present but has reduced effective population sizes in urban environments
    - Vary migration as well.
  + *Case 2*: Drift through founder events and migration
    - Independently vary the strength of population bottlenecks and migration.
    - From results above, select interesting values from each and vary them interactively.
  + *Case 3*: Allele frequency variation
    - Vary frequency of dominant alleles at both loci in the first population interactively
  + *Case 4*: Selection
    - Under most interesting scenarios from above, add selection of varying maximum strengths.
    - Under panmixia, how strong does selection have to be to generate phenotypic clines? (*Should this be a question of the paper?*)
  + *Case 5*: Colonization history
    - How do above results change if initial population starts rural and colonize urban, start urban and colonizes rural, or was always present across the matrix (like case 1 but with selection).
* Section 3: Analyses
  + Response variables:
    - Mean strength of clines across all simulations
    - Proportion of significant positive or negative clines (Figure 3: Mock cline figure with description of positive vs. negative clines).
    - Look for differences based on confidence interval estimation.
    - Something about matrix filling and how data was subset for analyses (last generation only)?

**Results:**

* Present key results that mirror the cases identified in the methods section.
* Discussion as it relates to cities