**Methods**

*Overview of simulations*

To examine the formation of spatial clines in HCN, we created a series of spatially-explicit simulations in Python 2.7 to track the frequency of HCN within populations through time and across space. We represented a transect from urban to rural habitats as a one-dimensional, linear matrix with 40 cells, consistent with the number of populations sampled across cities by Thompson et al. (2016), where each cell (hereafter patch) represents a patch of suitable habitat that can support a population of *Trifolium repens*. These simulations allowed for fine scale, independent control of both stochastic and deterministic parameters important for varying and maintaining the frequency of *CYP79D15* and *Li*—and thus HCN—in patches distributed across the landscape (Table 2). The order of events in the simulations are as follows: (1) Local reproduction and population growth, (2) selection, (3) migration, (4) colonization (Figure 3C). We first explored two broad colonization scenarios (Figure 3A and 1B, described below), which differ in how they manipulate the amount of genetic drift acting within populations. We use these two scenarios to produce a total of eight simulated cases (Table 2): six of these explore the effects of drift, migration, and selection on the formation of phenotypic clines in cyanogenesis while two examine effects of drift and initial allele frequencies on clines. In the interest of space, we exclude allele frequency variation simulations and focus on drift, migration and selection. Thus, in all simulations reported here, the initial frequency of both dominant alleles was fixed at 0.5, which produces the strongest cyanogenesis clines (see supplemental results: “Effects of initial allele frequency variation on cyanogenesis cline formation”).

*Drift scenario 1:* *Gradient in carrying capacity across the matrix*

The first scenario represents a case where clover populations were initially similar but increased fragmentation associated with urbanization reduced urban population sizes and increased the strength of drift. We imposed a gradient in the carrying capacity (*K*) of populations across the matrix, thereby placing an upper-limit on the population size (*N,* Figure 2A). Drift is expected to be greatest in populations with the smallest carrying capacity and this method has been used in other agent-based simulations exploring the effects of drift, gene flow, and selection on patterns of local adaptation (Alleaume-Benharira et al. 2006). We first simulated a scenario where *N* is assumed to be greatest in rural populations (maximum *N* = 1000) and decline linearly with increasing urbanization (Figure 2A). We simulated multiple minimum urban population sizes (minimum *N =* 10; 100; 500; 1000 representing strong to no drift gradient, respectively) to examine how gradients in drift of differing strength influences the formation of HCN clines. All 40 populations were initialized—and remained— at carrying capacity; thus, population growth is irrelevant in this first case. These simulations were run for 500 non-overlapping generations. Note that in other simulated cases that manipulate drift by imposing a graident in carrying capacity as described here, the minimum population size was fixed at 10, representing a strong spatial gradient in drift.

*Drift scenario 2:* *Colonization and founder events*

In the second scenario, the simulations begin with a single rural population at carrying capacity and adjacent patches are colonized toward the urban end until all patches contain populations (Figure 2B and 2C). There is no gradient in carrying capacity in this scenario; rather, the strength of drift is manipulated by varying the strength of founder events, determined as the proportion of alleles sampled from the parent population (i.e. smaller proportion = stronger founder event). We initially simulated 10 different founding proportions (0.01; 0.02; 0.035; 0.05; 0.075; 0.1; 0.2; 0.5; 0.75; 1.0) to explore the formation of clines under a broad range of serial founder events. To optimize the number of simulations performed for later cases, we chose three founding proportions from among the 10 above: 0.01, 0.2, and 1.0, representing strong, intermediate, and no effects of drift through founder effects, respectively. These were chosen as they sufficiently capture the variation in the effects of founding events on the formation of clines.

In our simulations, the probability that a population colonizes an adjacent patch depends on its size: this probability is 1.0 for populations at carrying capacity and decreases linearly with decreasing population size. Because founder events reduce the size of newly formed populations, serial founder events would result in populations becoming rapidly extinct (or exceedingly small), preventing the colonization of new patches. We therefore implemented a model of logistic population growth allowing populations to grow every generation until they reach carrying capacity. Under this model, a population of size 10 takes 27 generations to reach a carrying capacity of 1000 (growth rate [r] = 1.5). Simulations were run for 500 generations beginning when all patches on the landscape contained populations.

*Selection*

We used two-locus selection models to explore the effects of selection in generating and maintaining cyanogenesis clines (Kimura 1956; Lewontin and Kojima 1960; Felsenstein 1965). Selection favoured either cyanogenic (i.e. HCN+) or acyanogenic (i.e. HCN–) genotypes, depending on the population’s position in the landscape matrix. This model represents a case where selection favouring HCN+ genotypes in rural environments changes gradually along an urbanization gradient until HCN– genotypes are favoured in the urban core, consistent with the phenotypic clines reported by Thompson et al. (2016). For each simulation, we defined a maximum strength of selection that favoured HCN+ genotypes in the rural-most population and HCN– genotypes in the urban-most population. The selection coefficient varied linearly across the matrix such that HCN+ and HCN– genotypes had equal fitness in the central population of the landscape (i.e. population 20, Figure 3). In all simulated cases, we simulated 10 different maximum selection coefficients (0; 0.001; 0.0025; 0.005; 0.0075; 0.01; 0.025; 0.05; 0.1; 0.2) to explore the formation of clines across a fine-scale range of selection strengths. In two cases (Table 2), drift was strongest in rural, rather than urban, populations. The multiple selection coefficients explored here thus enabled us to identify the strength of selection necessary to counteract the loss of HCN due to drift and examine the formation of HCN clines under opposing forces of drift and selection.

When selection acts on two or more loci, linkage disequilibrium (LD) may accumulate as genotypes with particular allele combinations are favored, resulting in gamete frequencies that differ from their expectation based on allele frequencies (Lewontin and Kojima 1960). However, given that the *CYP79D15* and *Li* loci are unlinked (REF NEEDED), theory predicts that free recombination (recombination fraction = 0.5) between these loci would limit the accumulation of significant LD even under selection (Felsenstein 1965). Simulations exploring the build-up of LD under varying selection regimes acting for or against cyanogenic genotypes confirmed that even strong selection (*s* = 0.1) results in little accumulation of LD (see supplementary materials: “Effects of selection on linkage between *CYP79D15* and *Li*”). We therefore ignored the effects of LD in our simulations and gamete frequencies each generation were thus calculated directly from allele frequencies, with recombinant gametes being produced with equal frequency (0.25) from heterozygous genotypes.

*Migration*

In all simulations, we varied the amount of migration between populations across the matrix to explore the effects of gene flow on the formation of clines due to drift and selection. We modelled migration according to a modified version of Wright’s island model (Wright 1943). Specifically, the frequency of the dominant allele (e.g. *CYP79D15*)in population in the next generation ()is given as:

where is the frequency of the dominant *CYP79D15* in population in the current generation, is the weighted-mean immigration rate from all populations into population and is weighted-mean frequency of the dominant allele in the current generation for population ’s migrant pool, averaged across all other existing populations, respectively. Migration is assumed to decline linearly with increasing distance between populations such that there is effectively no migration between populations 1 and 40 of the matrix. Migration rates and dominant allele frequencies were weighted by population size such that larger populations contributed more migrants to the migrant pool. Specifically, the weighted-mean immigration rate from all populations into population was calculated as:

where is the realized migration rate between populations and , based on the distance between them, is the size of population , and in this case the number of populations minus one (i.e. 39) since populations do not exchange migrants with themselves. Similarly, the weighted-mean dominant allele frequency for population ’s migrant pool was calculated as:

where is the frequency of the dominant allele in population . Assuming no LD (see selection above), we performed the above process separately for both dominant alleles (i.e. *CYP79D15* and *Li*). For scenario (1) described above, we simulated 13 migration rates (*m* = 0; 0.001; 0.0025; 0.005; 0.01; 0.02; 0.035; 0.05; 0.1; 0.2; 0.35; 0.5, 1.0) to explore the full range of migration rates that can influence the formation and maintenance of clines via drift. Note that these values represent the maximum proportion of alleles exchanged between populations, which occurs among adjacent populations. To minimize the number of simulations performed in scenario (2), we simulated three migration rates: *m* = 0, 0.01, and 0.05, representing no, low, and high migration, respectively, and corresponding to levels of gene flow that resulted in substantial decreases in the strength of clines in scenario (1). We used these same migration rates in all additional simulated cases that explored the combined effects of drift, selection and migration, regarless of how drift was manipulated (Table 2).

*Analyses*

We performed 1000 iterations for each of the simulated scenarios listed in Table 2. For each iteration, we ran a linear regression using within-population HCN frequency as the response variable and distance from the urban-most population (i.e. patch 40) as the predictor. For simulations involving complete colonization of the landscape (i.e. drift scenario 1 above), this regression was performed using HCN frequencies at generation 250, consistent with the age of many large north American cities. Note however that our results are no contingent on the generation chosen for analysis as any generation between 50 and 500 produces qualitatively similar results. For simulations involving serial founder effects (i.e. drift scenario 2 above), we ran this regression in the first generation after the entire matrix became filled with populations. Once again, the generation chosen for analysis has no qualitative effect on our results. In both cases, each regression can have one of three possible outcomes: (1) A positive cline, representing significantly (*P* < 0.05) higher rural than urban HCN frequencies. These clines are consistent in direction with the urban-rural cyanogenesis clines reported by Thompson et al. (2016); (2) a negative cline, representing significantly higher urban than rural HCN frequencies, and (3) no cline (i.e. *P*  > 0.05). For each simulated scenario, we report the proportion of significantly positive and negative clines in addition to the mean slope across all 1000 iterations. We explore how these proportions and the mean slope are affected by varying levels of drift, migration and selection.

**Table 2:** Parameters used in our simulations and the evolutionary mechanism they control.

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| **Parameter** | **Description** |
| Maximum migration rate | Determines the maximum proportion of alleles (CYP79D15 and Li) exchanged between any two populations. The actual proportion depends on the distance between populations (see text) |
| Maximum carrying capacity | Determines the carrying capacity of the largest habitat patch on the landscape (rural-most or urban-most population). |
| Minimum carrying capacity | Determines the carrying capacity of the smallest habitat patch on the landscape (rural-most or urban-most population). |
| Founder proportion | Proportion of alleles sampled when founding a new populations. Lower proportions results in stronger effects of drift. |
| Maximum population creation probability | Maximum probability that a new population is created. Actual probability depends on the populations size such that larger populations have a greater probability of creating new ones. Value is fixed at 1.0 so that populations at carrying capacity are guaranteed to found new populations. |
| Maximum selection coefficient (smax) | Maximum strength of selection acting on cyanogenic or acyanogenic genotypes. Actual strength of selection depends on a population's position in the landscape matrix. |
| Frequency of dominant CYP79D15 | Initial frequency of the dominant allele at the *CYP79D15* locus. |
| Frequency of dominant Li | Initial frequency of the dominant allele at the *Li* locus. |
| Intrinsic rate of population increase | Intrinsic growth rate parameter used in logistic equation of population growth. Fixed at 1.5. |
| Number of generations | Number of generations to run simulations once all patched on the landscape have been colonized with populations. Fixed at 500 |
| Number of iterations | Number of iterations to run to run for each simulated scenario (see Table 2). Fixed at 1000. |



**Figure 2:** Diagrammatic representation of simulations examining the effects of genetic drift, gene flow and selection on spatial clines in HCN. We manipulated the effects of drift in two ways: (A) By creating a spatial gradient in carrying capacity (*K*) across the linear matrix, thereby placing an upper limit on the population size (*N*) in each population. For most simulations (see Table 2), population size was greatest in the rural-most population (*N* = 1000) and declined linearly to the urban-most population (*N* = 10). In this case, all patches (separated by solid vertical lines) started with populations at carrying capacity in generation one (represented by grey filling of patches). (B) Through serial founder events during the colonization of the urban environment, beginning with a single rural population at carrying capacity. Populations could only colonize adjacent patches and the proportion of founding alleles was varied to control the strength of drift (i.e. lower proportion = stronger drift). (C) Schematic of the order of events during simulations of drift scenario 2 (i.e. B, numbers represent order of events). Boxes represent a single population as it proceeds through the simulations. Upon colonization, populations first grow according to a logistic growth model (growth rate [r] = 1.5). Populations are then subject to selection, followed by migration. Every generation, we track the frequency of dominant alleles at both loci underlying HCN production (i.e. *CYP79D15* and *Li*) and the frequency of HCN within each population in the matrix.



**Figure 3:** The strength of selecting favoring cyanogenic (i.e. HCN+) or acyanogenic (i.e. HCN–) genotypes depended on the population’s position on the landscape. We first defined a maximum selection coefficient (-*s* to *s*), which favoured HCN+ genotypes in rural populations and acyanogenic HCN– genotypes in urban populations. The selection coefficient varied linearly across the matrix such that HCN+ and HCN– genotypes had equal fitness in the central population of the landscape (i.e. population 20).

**Table 2:** Details for all eight cases we simulated exploring the combined effects of drift, migration and selection on the formation of clines in cyanogenesis. Rows highlighted in grey implement drift scenario 1 and do not include colonization dynamics. Rows in white implement drift scenario 2 and include colonization through serial founder effects.

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| **Mechanisms explored** | **Drift controlled by** | **Parameters controlling drift** | **Initial allele frequencies‡** | **Selection coefficient** | **Migration rates** |
| Drift and migration | Spatial gradient in *K* | Max K = 1000 (Rural);  Min K = 10; 100; 500; 1000 (Urban) | *CYP79D15* = 0.5;  *Li* = 0.5 | None | 0; 0.01; 0.05 |
| Drift and migration | Spatial gradient in *K* | Max K = 1000 (Rural);  Min K = 10 (Urban) | *CYP79D15* = 0.5;  *Li* = 0.5 | None | 0; 0.001; 0.0025; 0.005; 0.01; 0.02; 0.035; 0.05; 0.1; 0.2; 0.35; 0.5 |
| Drift and migration | Serial founder events | **Founder proportion:**  0.01; 0.02; 0.035; 0.05; 0.075; 0.1; 0.2; 0.5; 0.75; 1.0 | *CYP79D15* = 0.5;  *Li* = 0.5 | None | 0; 0.01; 0.05 |
| Drift , migration, allele frequency | Spatial gradient in *K* | Max K = 1000 (Rural);  Min K = 10 (Urban) | *CYP79D15* = 0.1; 0.5; 0.9.  *Li* = 0.1; 0.5; 0.9. | None | 0; 0.01; 0.05 |
| Drift , migration, allele frequency | Serial founder events | **Founder proportion:**  0.01; 0.2; 1.0 | *CYP79D15* = 0.1; 0.5; 0.9.  *Li* = 0.1; 0.5; 0.9. | None | 0; 0.01; 0.05 |
| Selection and migration | None | None. All population with contstant K = 1000 | *CYP79D15* = 0.5;  *Li* = 0.5 | 0; 0.001; 0.0025; 0.005; 0.0075; 0.01; 0.025; 0.05; 0.1; 0.2 | 0; 0.01; 0.05 |
| Selection, migration and drift† | Spatial gradient in K | Min *K*: 10 (Rural)  Max *K*: 1000 (Urban) | *CYP79D15* = 0.5;  *Li* = 0.5 | 0; 0.001; 0.0025; 0.005; 0.0075; 0.01; 0.025; 0.05; 0.1; 0.2 | 0; 0.01; 0.05 |
| Selection, migration and drift† | Serial founder events | **Founder proportion:** 0.01; 0.2; 1.0 | *CYP79D15* = 0.5;  *Li* = 0.5 | 0; 0.001; 0.0025; 0.005; 0.0075; 0.01; 0.025; 0.05; 0.1; 0.2 | 0; 0.01; 0.05 |

† Modified drift scenarios. Refer to gradient in drift running from urban (weak drift) to rural (strong drift) rather than rural-urban, as simulated in other scenarios. These are used to explore drift-selection balance.

‡ Where multiple allele frequencies are specified, these were crossed factorially.