**Online supplementary materials for: Urban spandrels: the roles of genetic drift, gene flow and selection in the formation of parallel clines.**

**Contents of online supplementary materials:**

* Supplementary text:
  + Effects of initial allele frequency variation on cyanogenesis cline formation
    - *Drift scenario 1:* *Gradient in carrying capacity across the matrix*
    - *Drift scenario 2:* *Colonization and founder events*
  + Effects of selection on linkage between *CYP79D15* and *Li*
* Supplementary tables SX – SX
* Supplementary figures SX – SX

**Effects of initial allele frequency variation on cyanogenesis cline formation**

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

The initial frequency of both dominant alleles influenced the formation and strength of phenotypic clines in HCN. The strongest clines (β = 0.006) occurred when the frequency of both dominant alleles (i.e. *CYP79D15* and Li) was 0.5 (Figure SXA). The weakest clines occurred when the frequency of one or both dominant alleles was low (i.e. 0.1; 0.0005 < β < 0.002) whereas clines of intermediate strength occurred when either or both alleles were at high frequency (i.e. 0.9; 0.003 < β **<** 0.005; Figure SXA). These results hold regardless of migration rate; increasing migration reduced the strength of clines, regardless of initial allele frequencies (Figure SXA).

The proportion of significantly positive clines was always greater than the proportion of negative clines, independent of initial allele frequencies. The greatest frequency of significantly positive clines peaked at 30% when the frequency of both dominant alleles was 0.5, followed by cases when one or both alleles were at low frequency (i.e. 0.1; 11 < % < 16) and finally by cases where one or both alleles were at high frequency (i.e. 0.9; 16 < % < 22; Figure SXB). Significantly negative clines were rare and only arose when the frequency of one or both dominant alleles was high (Figure SXB).

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

****

**Figure SX:** Effects of initial frequency of both dominant alleles (*CYP79D15* and *Li*) on (*a*) the mean strength of clines across 1000 simulations and (*b*) the proportion of significantly positive (open triangles with dashed line) and negative (black inverted triangles with solid line) clines. Simulations were run under a strong gradient in drift, manipulated by imposing a gradient in the maximum size of populations: rural populations were large (*N =* 1000) while urban population were small (*N =* 10). In (*a*), we examined the mean slope of clines under no (open circles with dotted line), low (grey square, with dashed line), and high (black diamonds with solid line) migration. In (*b*), positive clines reflect significantly (*P <* 0.05) less HCN in urban populations relative to rural populations while negative clines reflect the opposite. All points represent means or proportions ± 95% confidence intervals.

**Effects of selection on linkage between *CYP79D15* and *Li***

We performed a small-scale simulation to examine the build-up of linkage disequilibrium (LD) between *CYP79D15* and *Li* when selection is acting on HCN. We initialized a single population at with the frequency of both dominant alleles set to 0.5. From these allele frequencies, we calculated the frequency of all 16 possible diploid genotypes, assuming Hardy-Weinberg equilibrium. We then subjected these genotypes to selection, which acted against (negative selection coefficients) or in favor of (positive selection coefficients) cyanogenic genotypes. From the selected genotypes, we calculated the frequency of gametes, where heterozygotes were assumed to produce equal frequencies (i.e. 0.25) of all 4 possible gametes given the absence of physical linkage between *CYP79D15* and *Li* (i.e. recombination = 0.5). We calculated linkage disequilibrium as: r2 = D2 / (p*CYP79D15* × q*CYP79D15* × pLi ×qLi), where D represents the coefficient of linkage disequilibrium and is a function of gamete frequencies in any one generation. In the denominator, p*CYP79D15*, q*CYP79D15*, pLi, and qLi represent the frequency of dominant (i.e. p) and recessive (i.e. q) alleles at *CYP79D15* and *Li*. Thus, r2 is a measure of LD that accounts for allele frequencies and has a value of 1 when loci are in complete LD and 0 when they are in equilibrium (i.e. independent of one another, REF). Gamete frequencies in following generations were then calculated from selected genotypes and this process was repeated recursively for 500 generations, allowing us to track the build-up of LD due to selection for cyanogenic and acyanogenic genotypes.

Our results show that the build-up of LD is minimal and decays rapidly over 500 generations (Figure SX). Even under strong selection (e.g. -0.1), r2 reaches a maximum just under 0.0005, which is sufficiently close to zero to consider the loci in linkage equilibrium for all practical purposes. Given these results, we ignored the build-up of LD due to selection in our simulations.



**Figure SX:** The build-up of linkage disequilibrium (LD) between *CYP79D15* and *Li* due to selection acting on cyanogenic white clover genotypes. Negative selection coefficients represent selection acting against cyanogenic clover genotypes while positive coefficients represent selection favouring cyanogenesis. Selection causes minimal build-up of LD between *CYP79D15* and *Li*, which decays rapidly over 5000 generations.