**Results**

*Drift scenario 1: Gradient in carrying capacity and migration*

In the absence of selection and gene flow, imposing a gradient in carrying capacity across the landscape lead preferentially to the formation of positive clines (i.e. less HCN in urban populations). The mean slope of clines across 1000 simulations was always positive in the presence of spatial drift gradients, although the mean slope became gradually weaker with increasing minimum urban population size (Figure 4A). Similarly, positive clines occurred with much greater frequency under strong drift gradients; negative clines only occurred at the highest minimum population size and occurred at the same frequency as positive clines in the absence of spatial gradients in drift (Figure 4B).

Migration reduced the mean slope of clines and the proportion of significantly positive clines. Under a strong spatial gradient in drift (minimum *N* = 10), the strongest clines in the frequency of HCN occurred with little to no migration while increasing migration reduced the mean slope of clines to near zero (Figure 4C). As expected, the mean strength of clines at each of the two unlinked loci (i.e. *CYP79D15* and *Li*) was consistently zero, independent of migration rate. Finally, increasing migration generally reduced the proportion of significantly positive clines and negative clines only occurred when migration was highest (Figure 4D).

*Drift scenario 2: Colonization and founder events*

Serial founder events during the colonization of urban populations lead overwhelmingly to the formation of positive clines, although the results are more complex than those under a spatial gradient in carrying capacity. With no or low migration, the strength of clines was highest with an intermediate founder size during colonization (founder proportion = 0.2, Figure 5A), and declined as the founder size increased or decreased from this point. However, high migration eliminated this effect, instead leading to a gradual decrease in mean cline strength with reduced strength of drift (Figure 5A). Similarly, the proportion of significantly positive clines peaked when the founder proportion was 0.1 and decreased as the strength of drift increased or decreased from this point (Figure 5B). In contrast, the proportion of negative clines increased gradually with increasing founder size.

The peak cline strength and proportion of significantly positive clines at intermediate founder effect strengths can be best understood by exploring the dynamics of HCN loss as the landscape is colonized. When founder effects were very strong (e.g. founder proportion = 0.01), HCN was lost so rapidly during colonization (Figure 6A) that resulting reductions in HCN were not linear, and clines were therefore only weakly positive (β = 0.003, Figure 6B). In contrast, when founder effects are absent (e.g. proportion of founding alleles = 1.0), HCN is never lost from the matrix (Figure 6A) and clines are very weak as the frequency of HCN shows little change across space (β = 0.0009, Figure 6C). However, when founder effects are of intermediate strength (e.g. proportion of founding alleles = 0.2), HCN is maintained for longer during colonization (Figure 6A) and its frequency changes substantially across space, resulting in stronger positive, linear clines (Figure 6D).

*Selection and drift (scenario 1)*

In the absence of drift, selection influenced the formation of spatial clines in HCN. Independent of migration rate, increasing the maximum strength of selection increased the mean strength of clines across 1000 simulations (Figure 7A) and the proportion of significantly positive clines (Figure 7B). Nonetheless, increasing migration reduced the effects of selection, leading to weaker clines for a given selection coefficient (Figure 7A). Importantly, clines formed by selection (Figure 7A) are consistently stronger than even the maximum strength of clines formed by drift, regardless of whether drift is manipulated by varying the maximum population size (Figure 7A) or through serial founder effects (Figure 7A).

In the presence of an opposing drift gradient, selected generated weaker clines for all but the strongest selection coefficients and negative clines were more common when selection was weak. When the strength of selection is less than 0.005, the mean slope of clines is negative for all migration rates (Figure 7C), consistent with gradients in drift preferentially generating clines in HCN (see scenario 1 above and Figure 7). Similarly, *s =* 0.005 represents the threshold where the proportion of positive and negative clines are approximately equal (Figure 7D). Below this, drift dominated making negative clines more common and s > 0.005, the selection dominated and the proportion of positive clines rapidly increases to fixation (Figure 7D). Finally, in the presence of drift, the strength of selection where 100% of clines are positive is 5x stronger (*s* = 0.05, Figure 7D) than in the absence of drift (*s =* 0.01, Figure 7B).



**Figure 4:** Spatial gradients in drift—controlled by varying the minimum *urban* population size across the landscape matrix—influenced (A) the mean strength of clines across 1000 simulations and (B) the proportion of significantly positive (open squares) and negative (filled diamonds) clines. When there is a strong gradient in drift (minimum *urban* *N* = 10), migration influenced the mean strength of HCN (filled diamonds) clines, but not clines in *CYP79D15* (open triangle) or *Li* (grey inverted triangle) (C). Similarly, (D) migration influence the proportion of significantly positive (open squares) and negative (filled diamonds) clines. All points represent mean or proportions ± 95% confidence intervals.



**Figure 5:** Serial founder events influenced the mean strength of clines and the proportion of significantly positive and negative clines. (A) Shown are the effects of serial founder effects—controlled by varying the proportion of founding alleles used for population colonization—on the mean strength of clines across 1000 simulations under three migration rates: no migration (*m* = 0, open circle), weak migration (*m* = 0.01, grey square) and strong migration (*m* = 0.05). (B) Serial founder events influence both the proportion of significantly positive (open squares) and negative (filled diamonds) clines. All points represent mean or proportions ± 95% confidence intervals.



**Figure 6:** The strength of founder events influenced the pace at which HCN was lost from populations during colonization and ultimately the strength of phenotypic clines in HCN. (A) Proportion of 1000 simulations where HCN is lost (i.e. frequency = 0) for each population in the landscape (i.e.1 to 40) under strong founder effects (proportion of founding alleles = 0.01, grey squares), intermediate founder effects (proportion = 0.2, black circles) and no founder effects (proportion = 1.0, open triangles). Also shown are linear regressions of mean within-population HCN frequency across 1000 simulations against a population’s position in the landscape matrix for (B) strong founder effects, (C) no founder effects, and (D) intermediate founder effects.



**Figure 7:** Selection influenced the formation of spatial clines in HCN in both the absence (A and B) and presence (C and D) of opposing gradients in drift. Selection favours HCN+ genotypes in rural populations and HCN– genotypes in urban populations. In (C) and (D), we imposed a spatial gradient in carrying capacity such that the minimum *rural* population size was 10. As such, the stochastic loss of dominant alleles in smaller rural populations is countered by their higher fitness. In both the absence (A) and presence (C) of an opposing drift gradient, selection influenced the mean strength of clines across 1000 simulations under no (*m =* 0, open circles), low (*m =* 0.01, grey squares) and high (*m =* 0.05, black diamonds) migration, although this effect was reduced in the presence of drift. Similarly, selection influenced the proportion of significantly positive (open squares) and negative (filled diamonds) clines in the both the absence (B) and presence (D) of an opposing drift gradient. All points represent mean or proportions ± 95% confidence intervals.