**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, peaking at 0.0066 in the presence of a strong drift gradient (minimum *urban N* = 10) and becoming gradually weaker as the gradient in drift was reduced (Figure 4A). Similarly, ~28% of clines were positive under strong drift gradients whereas negative clines were absent; negative clines only occurred at the highest minimum *urban* population size (*N*  = 1000) where the absence of a gradient in drift resulted in both positive and negative clines occurring at a frequency of ~3% (Figure 4C).

Migration reduced the mean slope of clines and the proportion of significantly positive clines. Under a strong spatial gradient in drift (minimum *urban N* = 10), the strongest clines (mean slope ≈ 0.006) in the frequency of HCN occurred with little to no migration while increasing migration reduced the mean slope of clines to near zero (Figure 4B). As expected, the mean strength of clines at each of the two unlinked loci (i.e. *CYP79D15* and *Li*) was consistently zero under even a strong gradient in drift, independent of migration rate. Finally, the frequency of positive clines peaked at ~36 % when *m =* 0.01 and decreased to a minimum of ~14% with increasing migration whereas negative clines peaked at ~4% at only the highest migration rates (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 peaked at ~0.006 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 from ~0.0016 when drift was strong (0.01 ≤ founder proportion ≤ 0.035) to ~0.0002 when drift was weak (founder proportion = 1.0, Figure 5A). Similarly, the proportion of significantly positive clines peaked at ~75% when the drift was strong (founder proportion = 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 from ~17% to ~25% as the strength of drift decreased.

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*

We next investigated the influence of selection on the formation and strength of clines in HCN, first in the absence of drift and then in the presence of an opposing gradient in the strength of drift. 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 7C); in the absence of migration, selection increased the strength of clines from zero (*s =* 0) to 0.037 (*s* = 0.2) and the frequency of significantly positive clines from 2.7% (s = 0) to 100% (*s* ≥ 0.025). 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 4A) or through serial founder effects (Figure 5A).

We next explored the effects of selection at generating clines in HCN in the presence of an opposing drift gradient. Given that stronger drift in urban populations preferentially leads to positive clines (see above), we were interested in understanding the extent to which selection can counter the formation of clines under drift. Thus, we imposed a spatial gradient in carrying capacity such that the minimum *rural* population size was 10 while selection favours HCN+ genotypes in rural populations and HCN– genotypes in urban populations. As such, the stochastic loss of dominant alleles in smaller rural populations is countered by their higher fitness. In the presence of an opposing drift gradient, selection generated fewer and weaker clines for all but the strongest selection coefficients; in the absence of migration, the mean slope of clines became positive and positive clines were more common only when the selection coefficient was greater than 0.005 (Figure 7B and 7D). In contrast, negative clines were more common when selection was less than 0.005, consistent with gradients in drift preferentially generating clines in HCN (see scenario 1 above and Figure 7B and 7D). Thus, *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 when s > 0.005, selection dominated and the frequency of positive clines rapidly increases to 100% (Figure 7D). Finally, drift increased the strength of selection necessary to produce 100% positive clines by 5×; in the presence of drift, 100% of clines were positive when *s =* 0.05 (Figure 7D) whereas in the absence of drift, 100% of clines were positive when *s =* 0.01 (Figure 7C).



**Figure 4:** Spatial gradients in drift—controlled by varying the minimum *urban* population size across the landscape matrix—influenced the formation of clines in HCN. (A) The mean strength of clines in HCN across 1000 simulations. (B) 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) The proportion of significantly positive (open triangles) and negative (black inverted triangles) clines. (D) When there is a strong gradient in drift (minimum *urban* *N* = 10), 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 on the mean strength of clines across 1000 simulations under three migration rates: no migration (*m* = 0, open circles with dotted line), low migration (*m* = 0.01, grey squares with dashed line) and high migration (*m* = 0.05, black diamonds with solid line). (B) Serial founder events influence both the proportion of significantly positive (open triangles) and negative (black inverted triangles) 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 C) and presence (B and D) of opposing gradients in drift. Selection favours HCN+ genotypes in rural populations and HCN– genotypes in urban populations. In (B) 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 (B) of an opposing drift gradient, selection influenced the mean strength of clines across 1000 simulations under no (*m =* 0, open circles with dotted line), low (*m =* 0.01, grey squares with dashed line) and high (*m =* 0.05, black diamonds with solid line) migration, although this effect was reduced in the presence of drift. Similarly, selection influenced the proportion of significantly positive (open triangles with dashed lines) and negative (black inverted triangles with solid line) clines in the both the absence (C) and presence (D) of an opposing drift gradient. All points represent mean or proportions ± 95% confidence intervals.