**Discussion**

In this paper, we build off of recent empirical work identifying the presence of parallel urban-rural clines in the frequency of hydrogen cyanide (HCN) across eastern North American cities (Thompson et al. 2016; Johnson et al. 2018, *this issue*). We used spatially-explicit, agent-based simulations to test the hypothesis that parallel clines in non-additive traits—of which HCN is an example—can arise due to gradients in the strength of genetic drift. We found that independent of how drift is manipulated (e.g. through serial founder events or a gradient in maximum population size), drift lead overwhelmingly to the formation of positive clines and steeper drift gradients generated more strongly positive clines, although these effects decreased with increasing migration. However, clines formed by drift alone were substantially weaker than those generated by selection, suggesting an upper limit to the strength of phenotypic clines in non-additive traits due to drift. Finally, when selection operated counter to the prevailing drift gradient, stronger selection was required to generate clines as strong as those observed in the absence of drift. Below, we begin by discussing the relevance of our results for the evolution of cyanogenesis clines in white clover. We then suggest that urban environments—in combination with knowledge of the genetic architecture of focal traits—can provide the replication necessary for understanding the contributions of drift, selection, and gene flow to the evolution of clines in non-additive traits. We end by more broadly discussing the role of various evolutionary mechanisms in the formation of phenotypic clines.

*Evolution of cyanogenesis clines in Trifolium*

Understanding geographical variation in allele and phenotype frequencies often provides insight into the evolutionary mechanisms structuring patterns of genetic variation in natural populations. In white clover, pioneering work by Hunor Daday identified broad-scale latitudinal clines in the frequency of HCN across multiple continents (Daday 1954a, 1958) and altitudinal clines across the central European Alps (Daday 1954b). This work has since been extended and clines in the frequency of HCN appear quite common, with higher frequencies occurring in warmer and drier habitats (de Araújo 1976; Ganders 1990; Kooyers and Olsen 2012, 2013; Kooyers et al. 2014; Thompson et al. 2016). These patterns are thought to reflect the benefits of producing HCN in warmer environments where herbivores are more common, the cost of producing HCN in frost-prone habitats, and the benefits of producing cyanogenic glycosides in regions subject to moderate drought stress (Daday 1965; Kakes 1989; Kooyers and Olsen 2013; Kooyers et al. 2014). Thus, investigations into the mechanisms structuring spatial variation in HCN frequencies have focused largely on selection (but see Kooyers and Olsen 2012; Johnson et al. 2018, *this issue*), despite the genetic architecture of HCN making populations particularly susceptible to loss of HCN via drift.

The presence of repeated correlations between environmental variables and phenotype frequencies is often considered strong evidence for the role of natural selection in generating adaptation. However, clines may also form via neutral processes (Vasemägi 2006; Colautti et al. 2010) and this may be exacerbated in traits with a non-additive genetic basis (e.g. epistasis). For example, we found that gradients in the strength of drift from rural (weak drift) to urban (strong drift) populations preferentially lead to urban populations having lower HCN frequencies, consistent in direction with phenotypic clines in HCN observed across cities (Thompson et al. 2016; Johnson et al. 2018, *this issue*). Thus, when a trait’s genetic architecture predisposes populations to evolve in a particularly direction under the effects of drift alone, drift must be rejected as a null hypothesis prior to invoking the role of selection in generating adaptive differentiation. Observing more clines in natural populations than would be expected under drift alone is one way to address; null distributions for the proportion of clines expected under drift can be generated through simulations and compared to the actual proportion observed in nature. Ideally, simulations would be parameterized with empirical estimates of migration rates and effective population size to provide system-specific estimates of expected proportions. Unfortunately, we lack these data for white clover populations making it difficult to compare the clines observed across cities to the proportions estimated in these simulations.

While drift preferentially generated positive clines in HCN, the mean strength of clines formed by drift was substantially lower than those involving selection. This occurred because although strong drift in urban populations drives HCN frequencies downward, the weak drift in rural populations and the absence of selection results in rural HCN frequencies fluctuating around their initial frequency. In contrast, simulations involving selection favoured HCN+ genotypes in rural populations and HCN– genotypes in urban populations, resulting in a steeper gradient in phenotype frequencies between urban and rural populations and thus stronger clines. Given this, we were interested in comparing the magnitude of slopes from our simulations to those observed across cities for which there are data (Thompson et al. 2016; Johnson et al. 2018, *this issue*). Since cities varied in transect lengths, it was necessary to standardize distance values by placing them all on the same scale, resulting in distance values that vary between 0 (urban) and 1 (rural) for all regressions (see supplementary material: “Comparison of standardized slopes from simulated and empirical data”). //// . The distribution of observed slopes overlapped completely with the distribution of simulated slopes, suggesting drift can generate clines as strong as those observed across cities. However, the absence of decreases in neutral genetic diversity in urban populations suggests that genetic drift is not an important mechanism structuring urban-rural phenotypic clines in HCN (Johnson et al. 2018, *this issue*). Similarly, the absence of clines at neutral microsatellite markers in North American clover populations suggests that clines are adaptive (Kooyers and Olsen 2012), at least in the invaded range. Thus, while there is overlap in the strength of observed clines and those simulated by drift, additional evidence from neutral markers strongly suggests that many clines in cyanogenesis are adaptive.