Does Urbanization Drive Adaptive Evolution in White Clover?

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Introduction

The white clover (*Trifolium repens)* is polymorphic for cyanogenesis; the production of hydrogen cyanide (HCN) following tissue damage. The clover cyanogenesis polymorphism arises from presence/absence of two cyanogenic components, Linamarin/ cyanogenic glucosides (encoded by *Ac/ac* gene) and their hydrolyzing enzyme, linamarase (encoded by *Li*/li gene). Thus, plants with at least one dominant (functional) allele at both *Ac* and *Li* are cyanogenic (*AcLi*).

Therefore, white clover cyanogenic polymorphism is a model system that could be used to understand how adaptive variation can be maintained within and between populations of white clovers. ***Our objective this summer was to research how urbanization affects the cyanogenic trait in white clovers, across a latitudinal cline, and provide a baseline information for researchers to study the adaptive evolution of organisms.***

Materials and Methods

We sampled 30 White Clover populations along an urbanization gradient transect in each of Columbus, Cincinnati, Peoria, Naperville, Davenport and Cedar Rapid, all were chosen on a latitudinal basis (see Fig. 1). To estimate proportion of cyanogenic vs acyanogenic plants in natural populations of *T. repens,* we screened each plant for the presence /absence of HCN using the Feigl–Anger assays - employing a color change upon reaction with copper ethylacetoacetate (Fig. 2).

The acyanogenic plants do not have the ability to produce cyanide by themselves, We screened all acyanogenic plants in each population for either Ac or Li, (to identify acLi, Acli and acli cyanotypes) with exogenous Linamarin and Linamarase.

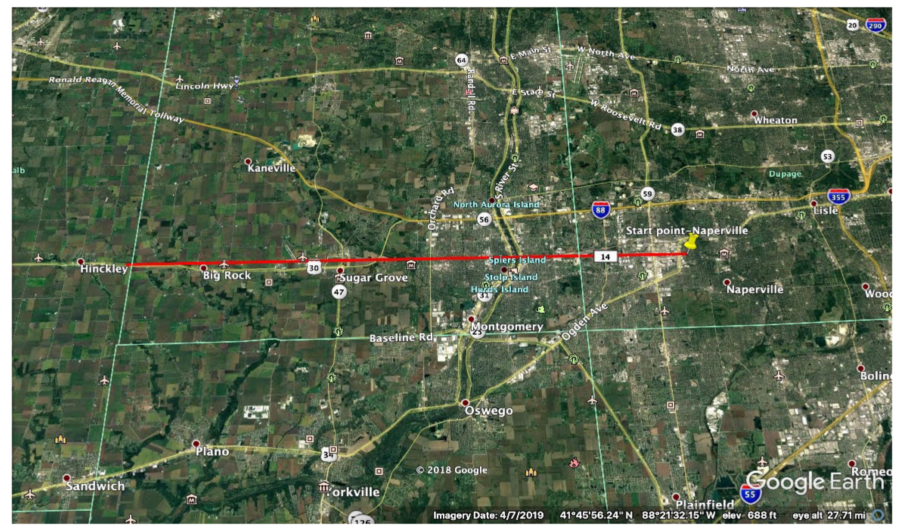

Figure 1. An approximately 30km transect of Naperville, starting in the urban areas and ending in the rural areas.

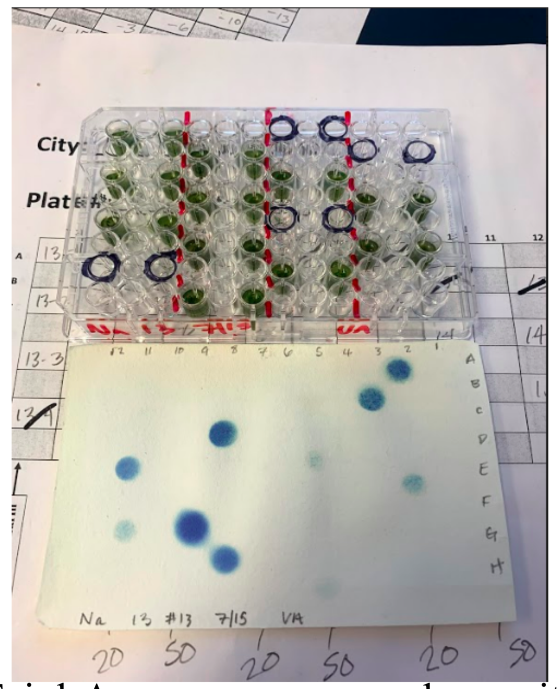

Figure 2: Feigl-Anger assay paper along with associated template sheet showing a blue circle over top of the wells containing cyanogenic plant samples (HCN+, positive).

Results and Discussion

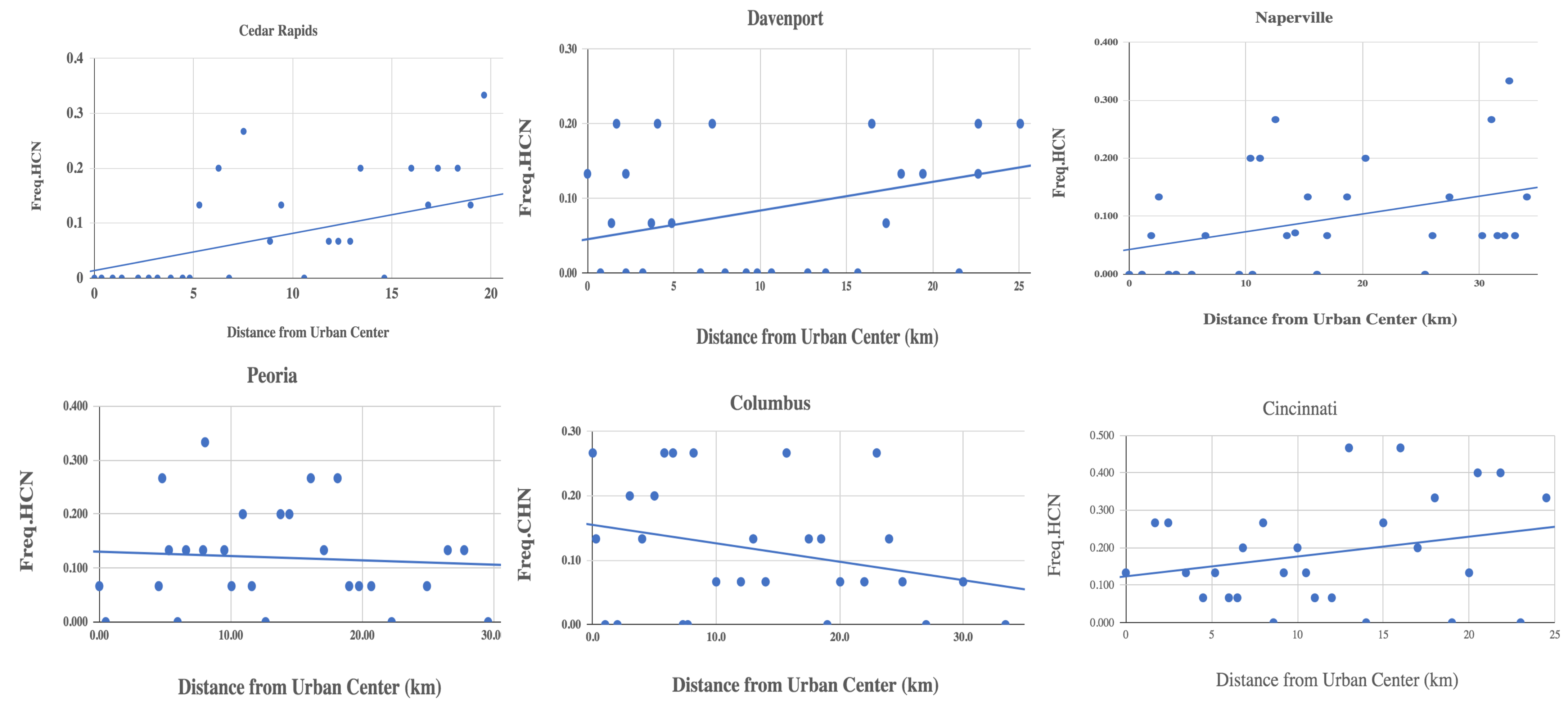

Figure 3. The relationship between the frequency of plants producing hydrogen cyanide (HCN) and distance from urban centers across six cities.HCN frequency indicates the frequency of functional alleles at Ac and Li, which are both required to produce HCN.
In the upper panel; Cedar Rapids (r = 0.5, p = 0.0087), Davenport (r= 0.4, p=0.049), Naperville (r = 0.4,p = 0.042), the frequency of cyanogenic plants within populations decreased towards the urban center.
In the lower panel; Peoria (r = 0.10, p > 0.05), Columbus (r = 0.10, p > 0.05), Cincinnati (r = 0.3, p > 0.05) we did not observe significant correlation between frequencies of cyanogenesis and urbanization gradient.

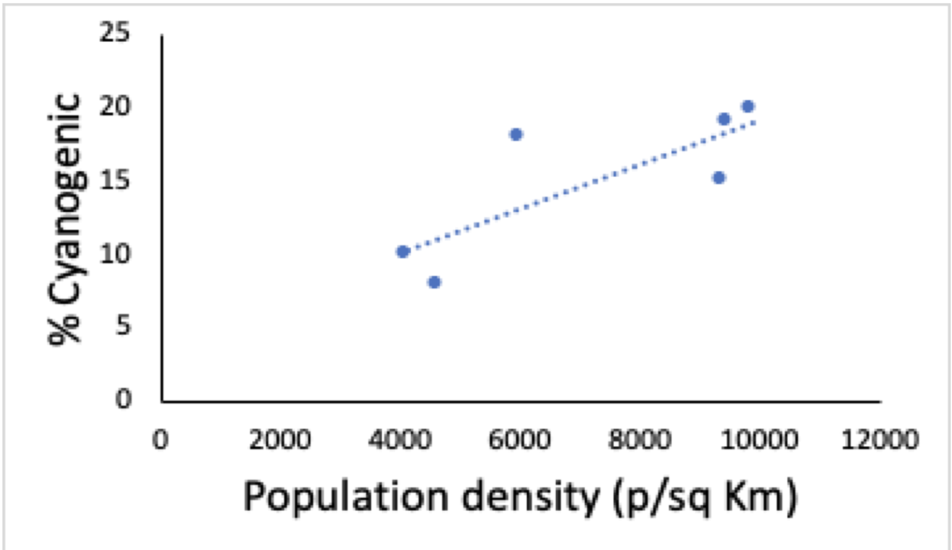

Figure 4. Relationship between population density of each city and frequencies of cyanogenesis in clover populations (r^2= 0.64; $p < 0.05$).

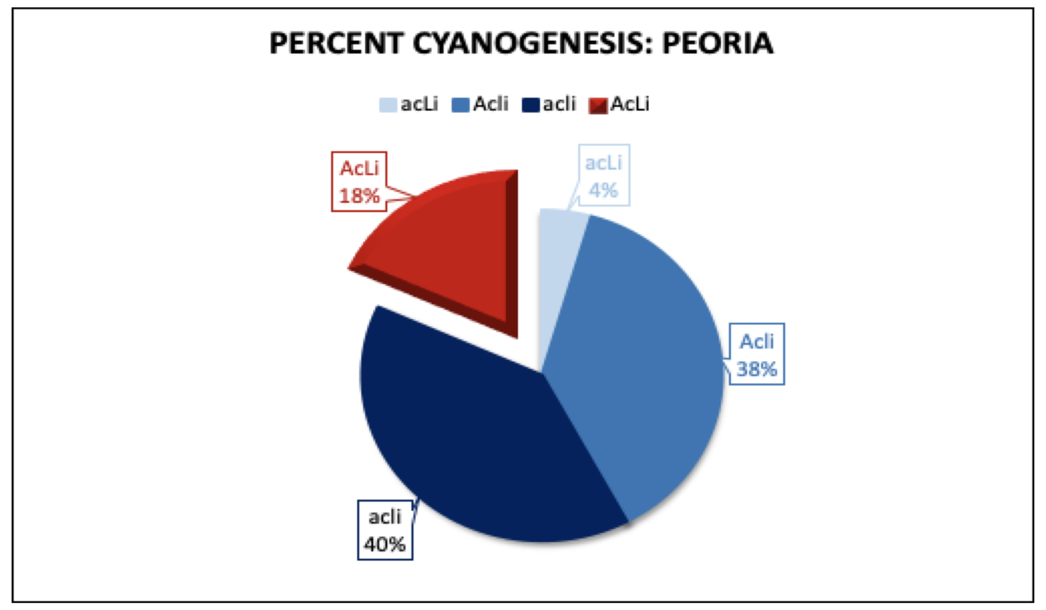

Fig. 5 Cyanotype frequencies within Peoria's urban and rural populations. *acLi* = Plants with Linamarase, *Acli* = plants with Linamarin, *acli* = plants lacking both components.

Conclusions

Overall, this study supports the notion that insect herbivory is of higher prevalence in rural environments and therefore renders a higher frequency of cyanogenesis in rural areas, due to the previously indicated defense mechanism against predators (Sun et al., 2018).

In the urban areas studied, we did not observe significant adaptive evolution, but instead unique features (such as population density, climate, landscaping, etc.) (Fig. 4) of an individual city could influence the strength of selection imposed by urbanization.

To further understand the effects that urbanization has on the cyanogenic trait of white clovers, more cities and populations and/or traits must be studied.

Among the three acyanogenic cyanotypes, weak frequency is apparent for the acLi cyanotype within both urban and rural populations (Fig. 5) However, both *Acli* and *acli* cyanotypes showed an increase in frequency within rural and urban populations.

Thus, our findings showed that the production of the cyanogenic component, Linamarase, (in acaynogenic plants) comes at an energetic cost to the plant, and cyanogenic glucosides, which are predominant in acyanogenic plants, could act as a buffering mechanism against drought-induced nutrient stress (Kooyers et. al., 2012).

Cited Literature

Sun, Zhanmin, et al. "Biosynthesis and Regulation of Cyanogenic Glycoside Production in Forage Plants." *Applied Microbiology & Biotechnology*, vol. 102, no. 1, Jan. 2018, pp. 9–16.

Kooyers, Nicholas J. and Olsen, Kenneth M. "Rapid evolution of an adaptive cyanogenesis cline in introduced North America white clover (*Trifolium repens* L.)." *Molecular Ecology (2012)* 21, 2455-2468.

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