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### **Assessing the Role of Quantitative Variation in the Adaptive Cyanogenesis Polymorphism in White Clover (*Trifolium repens*)**

A major goal of evolutionary biology is to understand the underlying genetics of ecologically adaptive traits. The polymorphism for cyanogenesis (the production of hydrogen cyanide in response to tissue damage) that occurs in *Trifolium repens* is a useful model system for understanding how an adaptive polymorphism is maintained in natural populations. However, previous studies have examined this polymorphism solely in qualitative terms, i.e., the presence or absence of the underlying cyanogenic components, which may lead to inaccurate conclusions regarding the targets of selection and mechanisms of adaptation. Here we develop a semi-quantitative measure of HCN production and use this method to investigate both how quantitative variation in cyanogenesis correlates with gene copy number variation at two underlying cyanogenesis genes, and how quantitative variation is distributed geographically. We use a pedigree of molecularly well-characterized individuals to correlate variation in quantities of two cyanogenic components (cyanogenic glucosides and their hydrolyzing enzyme) with gene copy number variation at their two corresponding loci (*CYP79D15* and *Li*). Further, we examine quantitative variation in HCN production in individuals collected across a latitudinal transect in the central US to provide insight into the mechanism of local adaptation in wild populations. This study finds that quantitative variation in both the enzyme and the cyanogenic glucosides is highly heritable and that gene copy number has additive effects on HCN production. Furthermore, there are significant differences between populations in HCN production, but the production levels do not entirely fit with the clinal distribution predicted by allele frequencies alone. These results reveal subtleties in selection pressures that are not apparent through qualitative phenotype analysis alone, and they suggest that the target of selection may be more complicated than formerly assumed in the cyanogenesis system.