

New Mexico is host to numerous specialized environments, supporting a diverse flora able to survive in the harshest of habitats. One such habitat is the island-like outcrops of gypsum that occur throughout the Chihuahuan Desert that contain over 200 endemic species¹. My master's project focused on two species in the evening primrose family that are restricted to gypsum deposits, causing them to occur in a naturally patchy, fragmented distribution throughout southeastern New Mexico. In the face of increasing anthropogenic habitat fragmentation, studying the current genetic structure of naturally isolated populations of species like *Oenothera gayleana* and *O. hartwegii* subsp. *filifolia* may inform us of the future consequences of fragmentation on other species.

However, fragmented plant populations usually maintain some degree of connectivity via pollen transportation by insect pollinators, which may vary depending on the pollinator. Two major pollinator guilds in the southwestern United States are bees and hawkmoths, which display several major differences in foraging preferences and behavior^{2,3}. Some of these traits may impact the size of their associated pollen neighborhood and the extent of gene flow between populations they visit⁴. Bees are diurnal foragers, and most species forage close to a nesting site⁵. Between floral visits, they will often groom pollen from their bodies, storing it on their hind legs, which can reduce pollen carryover between plants⁶. Most solitary bees rarely forage over 1 km from their nesting site⁵. In contrast, hawkmoths are night-time foragers, searching for nectar in pale, tubular flowers that often emit a strong, sweet scent⁷. They do not groom themselves of pollen and do not nest; they are mobile pollinators that have been reported to transport pollen up to 32 km in a night⁸. Due to these differences, it is expected that a species primarily pollinated by bees will experience reduced gene flow when compared with another primarily pollinated by hawkmoths. Reduced gene flow has a number of consequences, including genetic differentiation between populations, inbreeding depression, and eventual speciation.

The frequent co-occurrence and similar life-history traits convinced me to use two species in the Onagraceae family in my master's thesis work, completed in 2015. I compared the population genetic structure of the recently recognized *O. gayleana* (bee-pollinated) with *O. hartwegii* subsp. *filifolia* (hawkmoth-pollinated). I collected DNA from eight sites where they co-occur to determine the effects of primary pollinator on the amount of gene flow and consequential genetic differentiation between populations at both a local and regional scale. My data support that the shorter average foraging distances of bee pollinators does lead to greater genetic isolation between populations than the long distance foraging ability of hawkmoths, but only at a large, regional scale across the Chihuahuan Desert. Close, nearby populations, like those found in the Yeso Hills region of southeastern New Mexico, showed little genetic difference when compared. This suggests that pollinators may connect populations across the landscape at a larger spatial scale than estimated foraging distance would predict.

That study sparked my curiosity about other forms and reasons for genetic differentiation, including multiple colonization events per spatially distinct population. Colonization events can be determined by examining chloroplast (plastid) DNA, inherited through the maternal line in plants and dispersed via seeds. Unlike nuclear DNA, which reflects both maternal and paternal lineages and is contained in the individual's chromosomes, plastid DNA represents only the maternal line and is haploid. If there are multiple haplotypes (identities of plastid DNA) present in a population, it is likely that multiple colonization events from seed dispersal occurred, which may influence within-population genetic diversity and differentiation from other populations.

Funding from the Native Plants Society of New Mexico provided the specialized supplies needed to genetically analyze the chloroplast DNA from these eight populations. Procedures

were completed at the Chicago Botanic Garden with the help of a volunteer, Eileen Sirkin, over the summer of 2016. The identification of three plastid microsatellite markers was completed with a CEQ 8000 Genetic Analyzing System 9.0. Fifteen individuals from each population (240 individuals) were genotyped, which necessitated ~3 plates of 96 wells. Buffer solution, gel solution, and ladder were all required to run the CEQ 8000 Genetic Analyzing System, and purchased with the generous grant provided by the Native Plants Society.

Although the genotyping of the individuals is now complete, we are still in the process of interpreting the results. Preliminarily, we can determine that *O. gayleana*, the bee-pollinated species, appears to have fewer haplotypes across all eight populations. *Oenothera hartwegii* subsp. *filifolia*, the hawkmoth-pollinated evening primrose, may have had a greater number of maternal lines contributing to each population, which may explain other differences between the two species. We will continue to work on furthering our understanding of plastid diversity and how it relates to nuclear diversity. Plastid DNA represents a whole dimension of genetic diversity that is largely understudied and may represent another method of conserving genetic lineages and differences¹⁰. In addition, we hope the results will increase our understanding of the unique gypsophilic flora and how species spread across the habitat islands of gypsum deposits in the Chihuahuan desert that decorate the landscape of New Mexico.

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