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A comparison of genetic diversity within and among Michigan populations of Pitcher's thistle (*Cirsium pitcheri*)

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ABSTRACT:

Pitcher's thistle, *Cirsium pitcheri*, is a threatened species endemic to Great Lakes Dune ecosystems that has suffered greatly from shoreline development and recreational use (Harrison 1988, USFWS 2002). Previous genetic studies on the species have suggested that there is little gene flow between populations and high inbreeding within populations. I sampled four populations representing a total shoreline distance of approximately 1070 km in attempts to 1) further understand of the genetic health of the species; 2) to see if any historical factors that have influenced Pitcher's thistle movement and dispersal can be inferred; and 3) to see if a wide sampling range reveals markedly more genetic variation for the species than a limited one. Four microsatellite loci were examined, and a loose grouping structure emerged between the northern and southern sample sites.

INTRODUCTION

Pitcher's thistle (*Cirsium pitcheri*) is a threatened endemic plant of Great Lakes shorelines. It is one of many dune species in decline. Others include the dwarf lake iris, Houghton's goldenrod, the piping plover, and the Lake Huron locust (USFWS 2002). While Pitcher's thistle occurs in dune and grassland habitats on Lakes Superior and Huron, most occurrences are on Lake Michigan, particularly its northern and eastern shorelines (Guire and Voss 1963).

Changing shoreline dynamics and extensive coastal disturbances from development, recreational foot traffic, and off-road vehicle (ORV) use have been the primary causes for Pitcher's thistle's decline (Harrison 1988, USFWS 2002). The species flourishes in a moderate disturbance regime where dunes are not yet fully stabilized by other pioneer species, but excessive foot traffic and other frequent disturbances exceed the threshold of what this species can tolerate (McEachern et al 1994). Additionally, construction of shoreline features such as groins and jetties often cause rapid shoreline erosion that eventually claim prime Pitcher's thistle habitat (Dobberpuhl and Gibson 1987, McEachern et al 1989). Pitcher's thistle typically occurs in swales immediately landward of the foredune (*i.e.* the most shoreward dune in a dune system) and on any successive dunes. Destabilized patches with approximately 30% groundcover and 70% open sand tend to be this species' preference (McEachern 1992).

An allozyme study by Loveless and Hamrick (1988) suggests that Pitcher's thistle is a genetically depauperate relative originating from *C. canescens* that reached the Great Lakes region sometime in the late Wisconsin glaciation. The close relatedness of these species has also been proposed on morphological and cytological grounds (Moore and Frankton 1963; Ownby and Hsi 1963). In addition to delineating the most likely origin of Pitcher's thistle, Loveless and Hamrick (1988) also noted that populations from Mackinac Straits area (Emmet and Mackinac

counties, Michigan) were more genetically distinct from other groups, even more so than comparisons between other northern populations and southern populations. This is consistent with the former isolation of the straits region during the Lake Chippewa and Nipissing stages (Hansel et al 1985; Schaetzl et al 2002). Gauthier et al. (2010) noted genetic diversity in Pitcher's thistle to be greater than what was observed by Loveless and Hamrick but nonetheless low. They also found population differentiation to be high, suggesting low gene flow among populations and high inbreeding within populations (Gauthier et al. 2010). Additionally, Gauthier et al. hinted at a correlation of isolation by distance in the species. Earlier research along the southern shores of Lake Michigan has suggested similar relationships, and like Gauthier et al., there was more observed variation than Loveless and Hamrick (1988) and greater diversity among states than within states (K. Havens, Chicago Botanical Gardens, unpublished data; USFWS, 2002).

It is unclear what the predominant factors influencing this species' genetic diversity are and how modern changes to Pitcher's thistle population structure due to loss of habitat and extirpation of some populations are likely to affect its population dynamics. While some ecological traits such as wind-dispersed seeds and pollen, and an inability to reproduce asexually promote species to greater genetic diversity, other traits such as its ability to self pollinate could potentially reduce diversity (Keddy and Keddy 1984, Loveless 1984, Gauthier et al. 2010). Shoreline development has likely stifled the linear contiguity of Great Lakes dune ecosystems and may lead to further genetic isolation amongst Pitcher's thistle populations (Trizio et al. 2005). Examining genetic variation within and among populations of Pitcher's Thistle offers the two-fold benefit of evaluating the genetic health of populations and highlighting historical factors that have influenced the species. This information improves our understanding of the

species and can be used to establish better-informed guidelines for management (Keller and Waller 2002, Avise 2009, Knowles 2009).

I will further investigate the effects of distance and isolation on Pitcher's thistle populations by examining two populations along the perimeter of Lower Michigan (Allegan and Cheboygan counties), one from Charity Island (Arenac County), and one from the Upper Peninsula (Mackinac County) (Fig. 1). Sampling over a relatively long distance will allow me to test if increasing geographic distance and insular isolation reveals more nuanced genetic structure and more genetic variation than has been previously observed. My genetic analyses will also potentially add insight into the factors that have influenced Pitcher's thistle distribution and evolution. A more detailed picture of the isolation occurring in the straits region will likely be clear after a modern genetic analysis. I predict greater similarity between the Allegan County and Charity Island sites compared to the Cheboygan and Mackinac county sites given the former isolation of the straits region. Given the larger distance my study will cover compared to Gauthier et al. (2010) and the more powerful genetic analysis I'm using compared to Loveless and Hamrick (1988), I predict to see greater levels of divergence amongst Pitcher's thistle populations than previously observed, but low within-population diversity—particularly on Charity Island where isolation appears to be greatest

METHODS

Study Area

Sites were selected in Allegan, Cheboygan, and Mackinac counties and on Charity Island (Arenac County) (Fig. 1), and samples were collected during June and July 2011. When distance between populations is measured linearly along shorelines (Fetzner and Crandall 2003), this

selection scheme represents about 1070 km between Oval Beach and Charity Island. I sampled plants opportunistically along informal transects until tissue samples from 20 individuals were obtained at each location (Gauthier et al. 2010). When plants were tightly clustered together, I only sampled tissue from one individual in the group. Upon collection, tissue samples were stored in zip-lock bags with silica gel where they remained at room temperature in the lab until DNA extraction. For extraction, tissue samples were ground using liquid nitrogen, and the Qiagen DNEasy Plant minikit protocol for total DNA purification was followed (Qiagen Inc. 2006).

DNA Analysis

Microsatellites were used to analyze the nuclear genome because of their high rate of polymorphism and their co-dominant inheritance (Gauthier et al. 2010). Four loci were examined using primers originally developed for other *Cirsium* and optimized for use in Pitcher's thistle by Jeremie Fant (Chicago Botanical Gardens). For each PCR reaction I used, one μL of total genomic DNA was added to: 18.25 μL dH₂O, 2.5 μL reaction buffer, 2.0 μL dNTP, 0.125 μL of the primer that accepts the fluorescent tag, 0.25 μL of the remaining primer, 0.375 μL fluorescent dye, and 0.125 μL *Taq* polymerase. Polymerase chain reaction (PCR) thermocycler parameters were 5 minutes at 94 °C, 30 cycles of 30 seconds at 94 °C, 30 seconds at 72 °C, and 30 seconds at 49 °C, and a final extension step of 45 minutes at 72 °C (Gauthier et al. 2010).

For tissue samples that failed to amplify well using the above protocols, I used a touchdown PCR protocol (D. Wcisel, Grand Valley State University, personal communication). For one reaction, 14.5 μL dH₂O, 5 μL reaction buffer, 2 μL MgCl₂ 2.5 μL dNTPs, 0.1 μL of fluorescent tag-accepting primer, 0.2 μL of remaining primer, 0.5 fluorescent tag, and 0.2 μL *Taq* polymerase were used. The PCR thermocycler parameters were 10 minutes at 94 degrees C,

10 cycles of thirty-second intervals at 94 degrees C, 60 degrees C, and 72 degrees C, with the 60 degree C interval dropping by 1 degree C with each successive cycle, 25 cycles of thirty second intervals at 94 degrees C, 50 degrees C, and 72 degrees C, followed by a final step of 10 minutes at 72 degrees C. Gel electrophoresis was conducted on all PCR products to determine quality of amplification.

All successful PCR products were analyzed on an Applied Biosystems genetic analyzer, and the resulting fragment data was examined using Peak Scanner 1.0 (Applied Biosystems, Inc). Pairwise population differentiation (F_{ST}) indices were calculated per Weir and Cockerham (1984) using Genepop (Rousset 2008). To examine within-population genetic diversity, number of fixed loci and proportion of private alleles were also calculated (Cole et al. 2003, Gauthier et al. 2010)

RESULTS

You need to begin with the results of your PCR amplification for the various sites. The reader needs to know to what degree your PCR amplifications were successful. This provides the basis for the number of samples that are being used to conduct the pairwise statistic. If we do not know how many samples are being analyzed in the pairwise, we do not have a basis for evaluating the relevance of the statistic.

Pairwise population differentiation indices were fairly high (Table 1). Mackinac county and Grass Bay had the lowest pairwise F_{ST} (0.0833) followed by Oval Beach and Charity Island (0.1049). Grass Bay and Charity Island had the highest pairwise F_{ST} (0.3158). Nine alleles total were identified across the four loci, and none of them were private. Two sites (Oval Beach and Charity Island) had fixed alleles at two loci each—*caca04* and *caca20* for Charity Island and

caca20 and caca23 for Oval Beach (Table 2). None of the loci showed significant linkage to one another.

DISCUSSION

The pairwise F_{ST} indices suggest that gene flow between the sites in this study is very limited. This is not surprising considering the distance between collection sites. On the contrary, it does not seem that geographic distance is strongly correlated with increasing differentiation. While it's not surprising that the pairwise index between the Grass Bay and Mackinac County sites was low given the sites' proximity to one another, it is surprising that the second lowest pairwise F_{ST} was between Oval Beach and Charity Island—the two most distant sites when using measures of linear shoreline distance. This pattern in the indices suggests a loose grouping of the two northern sites and the two southern sites—an inference that conforms with the observation of Loveless and Hamrick (1988) that Pitcher's thistle from the straits region appear to have a distinct genotype compared to other areas. Genetic data from more sites outside the straits region will be needed to confirm this whether this observation is valid.

While it has been proposed that dispersal be thought of as confined to a linear system for shoreline dune species (Molins et al. 2009), the implication in my data that Pitcher's thistle may be broken into northern and southern groups suggests that some alternative form of dispersal not confined to shoreline movement to be presently influential or to have been influential in the past. If Pitcher's Thistle is indeed a derivative species of *Cirsium canescens* as Loveless and Hamrick (1988) suggest, then perhaps there were two historic colonization routes for Pitcher's thistle in Michigan—one via the Upper Peninsula, and one from the south that moved up the lower peninsula. More data from additional loci and collection sites will be needed to develop this claim.

The allele frequencies at each locus suggest that Charity Island has the least within-population genetic diversity while the Mackinac County site has the most. This is not particularly surprising given Charity Island is an island population fairly distantly isolated from the mainland in the Saginaw Bay (Johnson et al. 1999). The Mackinac county site, on the other hand, is within one of the largest and most intact critical dune habitats remaining in Michigan today (MNFI 2011).

My study found less genetic differentiation than Gauthier et al. 2010. On average, my F_{ST} indices were lower, and there were no private alleles confined to any of the sites I sampled. Despite these indications, I suspect the appearance of lower population differentiation in my study to be the result of my limited number of sample sites and loci examined.

My hypothesis that the two Pitcher's thistle populations from the Mackinac straits area would be most similar to one another was supported, but my hypothesis that my sampling methodology, which represents a long shoreline distance, would yield greater measures of population differentiation, was not supported. In both cases, more sites and more loci need to be sampled in order to more completely understand the overall genetic and phylogeographic picture for this species. Additionally, many essential statistical measures, such as F_{IS} indices, isolation-by-distance regression analyses, and observed and expected heterozygosities need to be calculated for this data set and any data obtained in the future. Without these analyses, the amount of inference that can be done is severely limited.

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Figure 1. Map showing Pitcher's thistle collection sample sites.

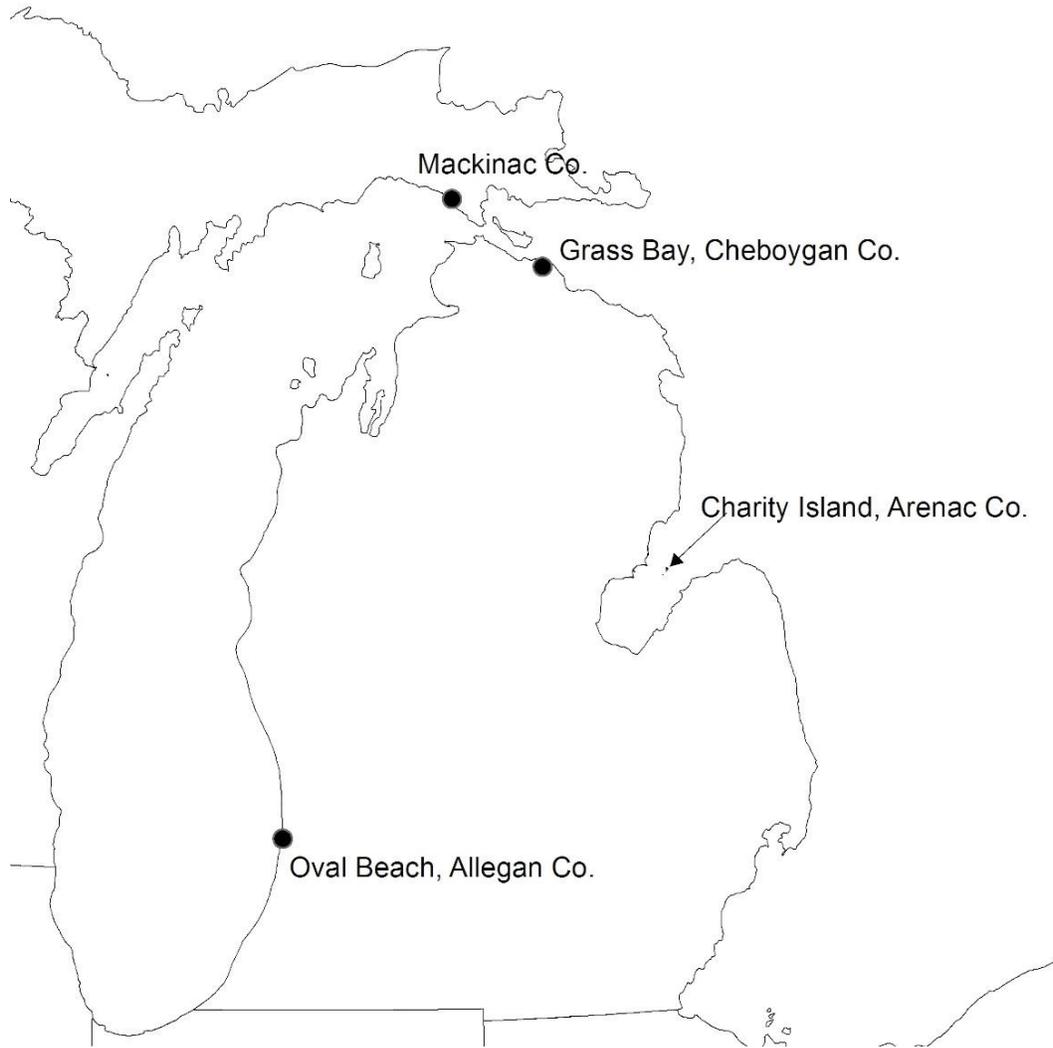


Table 1. Pairwise F_{ST} indices for all populations averaged across all loci.

Population	Mackinac County	Charity Island	Grass Bay
Charity Island	0.2192		
Grass Bay	0.0883	0.3158	
Oval Beach	0.2082	0.1049	0.2666

Table 2. Mean pairwise F_{ST} indices and number of fixed loci in each population

Population	Mean F_{ST}	Number fixed loci
Mackinac County	0.1719	0
Charity Island	0.2133	2
Grass Bay	0.2236	0
Oval Beach	0.1932	2

