Bridging the generation gap: Temporal variation in genetic diversity in naturalized and restored populations of the rare plant, *Cirsium pitcheri*

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Introduction:

Restoration succeeds only if gene flow is maintained within the population, both spatially and temporally. Restoration must mimic nature in predicting and maintaining changes in genetic trends. New alleles must be introduced into small populations, because they are subject to genetic drift, a random increase or decrease in alleles, which can weaken the population. In this study, ten populations of Pitcher's thistle, *Cirsium pitcheri* (Asteraceae), of the Lake Michigan coast were studied for indications of temporal and spatial gene flow between the populations, as well as internal genetic drift. The samples were collected in 1997 from Wisconsin, Indiana, and Michigan, and in 2003 from the same occurrences with an addition of the restored population of the threatened *Cirsium pitcheri* in Illinois State Beach. This study aims to discover familial ties between populations of C. pitcheri that inhabit the lake coast and assess genetic diversity in newly restored *C. pitcheri* in Illinois. *Cirsium pitcheri,* a truly rare plant, inhabit lacustrine dunes [4] , due to human interference were extirpated from dunes of Illinois in early 1900's[1]. *Cirsium pitcheri* is listed as a threatened species by the federal government [6].

Materials & Methods:

Figure 1. Pitcher's Thistle. Michigan, Upper Peninsula.

Cirsium pitcheri samples came from ten separate sites of four states: WI, MI, IN & IL. Gel electrophoresis was used to determine the amount of DNA in extracted samples and genes were amplified with microsatellites primers and PCR. Six primer pairs (1, 4a, 5, 7, 17 & 20) were used to identify alleles, which were genotyped by Beckman Coulter CEQ 8000. FSTAT was used to determine the F_{st}, population structure, and F_{is}, the inbreeding coefficients.

Results:

C. pitcheri populations that are spatially isolated like Warren Dunes and Saugatuck of MI show very little gene flow, with F_{st}, a measure of population structure of 0.2 and above as seen in Table 1. Indiana populations, however, show that there is very little difference between their neutral genes, meaning that plants must be exchanging genetic information and crossbreeding to be so similar. The seeds and pollen seem to have considerable success in crossing over between Indiana's *C. pitcheri* populations, as most population structure values are less than 0.2. Wisconsin, with all F_{st} values more that 0.37, is geographically isolated (as seen on Figure 2) and has very little chance of exchanging genes with Illinois or the Indiana dune thistles. Geographic isolation and small population size could be causing the Wisconsin thistles to inbreed and reject any new genes.

	WD	SA	KR	UN	BB	DA	WB	MB
Michigan								
Warren Dunes (WD)								
Saugatuck (SA)	0.2609							
Indiana								
Kemill Road (KR)	0.2604	**0.0571**						
Unknown Blowout (UN)	0.3308	0.2228	**0.1427**					
Big Blowout (BB)	0.3437	0.3268	**0.2043**	**0.1199**				
Dune Acres (DA)	0.3405	**0.1807**	**0.0841**	**0.1052**	0.2279			
West Beach (WB)	0.3344	0.3677	0.2156	**0.1003**	**0.1361**	**0.1798**		
Miller Beach (MB)	0.4599	0.3024	0.2618	**0.0972**	**0.0935**	0.2803	**0.1901**	
Wisconsin								
Sheboygan (KA)	0.3782	0.327	0.4105	0.4987	0.4603	0.5311	0.5973	0.4142
Illinois								
Illinois State Beach	0.2225	0.1988	**0.1271**	**0.1873**	0.2669	**0.0631**	**0.1607**	0.3063

Table 1. Table of comparison of population structure among individual populations.
0 = identical genes 1 = absolutely different genes

Results Cont'd:

The FSTAT analysis of pair-wise comparison of the two generations of 1997 and 2003 populations showed that temporal genetic drift occurred in about one third of the Indiana's studied dune thistle populations. The *C. pitcheri* collected from the same occurrences about five years apart were supposed to represent two generations of same thistles. Over span of one generation, in *C. pitcheri* 4-8 years, Warren Dunes of MI has changed its frequency of neutral alleles (F_{st} = 0.4) meaning that it is experiencing gene drift due to small population size (Table 2). But high F_{st} could be caused by sampling error. Wisconsin's Sheboygan (2003) *C. pitcheri* population is very similar to the previous generation; it is not likely to have much gene flow as it is geographically isolated, inhabiting a northern coastal part of Wisconsin.

		WD (2003)	SA (2003)	KR (2003)	UN (2003)	BB (2003)	DA (2003)	WB (2003)	MB (2003)	KA (2003)
MI	WD 1997)	**0.3951**								
	SA (1997)		0.0656							
IN	KR (1997)			**0.2801**						
	UN (1997)				**0.5549**					
	BB (1997)					0.1236				
	DA (1997)						0.0694			
	WB (1997)							**0.4454**		
	MD (1997)								**0.2713**	
WI	KA (1997)									0.0729

Table 2. Comparing the change between two generations. 0 = no change. 1 = complete disparity.

		Illinois
MI	WD 1997)	0.2225
	SA (1997)	0.1988
IN	KR (1997)	**0.1271**
	UN (1997)	**0.1873**
	BB (1997)	0.2669
	DA (1997)	**0.0631**
	WB (1997)	**0.1607**
	MD (1997)	0.3063
WI	KA (1997)	0.4216

Table 3. Comparing Illinois restored *C. pitcheri* to the populations of MI, IN & WI.

C. pitcheri from Illinois show little disparity in neutral genes when compared with the Indiana, which is expected since Illinois was reseeded with Indiana and Wisconsin dune thistles. Wisconsin's *C. pitcheri* are not as successful as Indiana's thistles in affecting the allele distribution of the Illinois *C. pitcheri* (Table 3). This could be from any number of reasons stemming from the fact that Indiana's thistles may germinate earlier or produce more flowers on one separate occasion, thus having greater reproductive success, that can skew the allele frequency of the next generation in its favor.

F_{is}, or the Weir & Cockerham inbreeding coefficient, showed that the Illinois (F_{is} = 0.39) and Wisconsin (0.23) populations are inbreeding. *C. pitcheri* that inhabit Indiana costal dunes are showing low levels of inbreeding (Table 4).

	Michigan		Indiana						Wisconsin	Illinois
	WD	SA	KR	UN	BB	DA	WB	MD	KA	IL
F_{is}	**0.097**	**0.434**	-0.081	-0.127	-0.133	0.117	-0.027	**0.566**	**0.23**	**0.338**

Table 4. Inbreeding coefficients for all ten populations. 0 = cross-breeding, 1 = completely inbreeding.

Discussion:

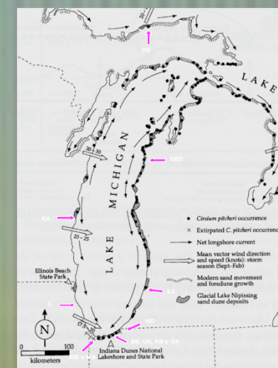

Figure 2. Map of *C. pitcheri* sample sites[5].

"Distance matters"

Gene movement for *C. pitcher* plants is largely dependent on the distance that the seed or the pollen can travel, since *Cirsium pitcheri* do not reproduce vegetatively[2]. As geographical isolation increases, a reduction in both seed dispersal and pollen flow will result in decreased gene flow between populations [3]. Decrease in gene flow could mean gene drift or inbreeding. That explains why the neutral alleles of Indiana *C. pitcheri* are so similar – the sample sites are clustered together. But due to gene flow, there is no inbreeding. Wisconsin is largely isolated, much like Illinois from Indiana and Michigan, so it lacks genetic diversity.

Conclusion:

This ongoing study will continue to focus on restoring the native *Cirsium pitcheri* to the coastal dunes of Illinois. Population genetics was used to select the best candidates for the first generation of *Cirsium* [5], but this newly established population is inbreeding, which is not bad, but if it leads to inbreeding depression, these isolated plants could not survive. If a population to be restored, gene flow must be maintained. Perhaps, more seedlings from Michigan and Wisconsin should be introduced into the Illinois State Beach colony to increase genetic diversity.

Figure 3. Flowering *Cirsium pitcheri*. The color of blooms can range from yellow to light pink [5].

Courtesy of Smithsonian Institution.

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