Checking for Inbreeding Depression in *Cirsium hillii*

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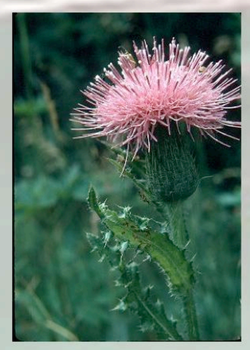
Introduction

Hill's Thistle, *Cirsium hillii*, is a rare thistle, endemic to the Midwest. The destruction of its prairie habitat throughout the Midwest has diminished this species to less than 200 distinct populations; few enough that it was a candidate for federal protection. Though the US Fish and Wildlife Service decided not to list *C. hillii*, it did note in a 1997 report that "it is clear that the biology and ecology of this species is rather poorly known. Thus, management attempts will need to be coupled with long-term biological monitoring programs." (Penskar 1997) The research conducted by the Chicago Botanic Gardens is serving exactly those purposes. Prior data gathered by the Plants of Concern program revealed that local *C. hillii* populations are not reproducing successfully. Only a small portion of the plants flower in any given year, and even in good years the majority of the seeds are not viable. If the cause can be accurately determined, it may possibly be remedied. *C. hilli* is able to reproduce vegetatively, and so many populations may have a low effective population size (Ne) and consequently inbreeding depression might be the cause of the low reproductive success. The genetic studies were conducted to determine the proportion of individuals derived from clonal versus sexual reproduction, as well as determine how much genetic diversity existed within these populations.

Figure 1: Flowering *C. hillii*



Methods

Monitoring

Seven local populations of *C. hillii* have been monitored and tracked over the past five years as part of the Plants of Concern program. For this study, monitoring data was collected for 2005, and to replicate a viability study conducted in 2004, all known flowers were covered with mesh bags, once the flowers had died, to monitor seed set. The mature flowers were collected a few weeks later, and all of the seeds were counted by hand. Based on data collected in 2004 large, plump seeds weighing at least four milligrams were deemed viable. The vast majority of the non-viable seeds were so shriveled as to be unmistakable for viable seeds (see figure 2). Unlike 2004, the seeds were not cut open to check for proper development. All plant materials were returned to their respective sites after measurements were concluded.

Genetic Analysis

The samples for the genetic study of the *C. hillii* population were collected from random plants in each of the seven monitored plots. Only the samples from the plot in the largest, most fecund population and from one of the smaller, reproductively less successful populations have been analyzed to date.

	Average # seeds 2004	Average % viable 2004	Average # seeds 2005	Average % viable seeds 2005
Site 1	164	10.9%	207	1.0%
Site 2	80	0.0%	0	0
Site 3	174	16.9%	183	0.6%
Site 4	67	6.0%	201	0.6%
Site 5	125	24.2%	0	0
Site 6	0	0	143	0.4%
Site 7	0	0	0	0

Table 1: Seed production data

DNA Extraction

Standard kits proved insufficient for cleanly extracting *C. hillii* DNA. Instead, the samples were prepared using the CTAB protocol with extra phenyl-choloform washes, and a subsequent spermine cleaning. The purified DNA was analyzed using standard PCR techniques with inter-simple specific repeat primers 816, 817, 826, and 827.

Results and Discussion

The 2005 seed output was markedly lower than the 2004 seed output in every population (see Table 1). However, average plant size increased. Considering that 2004 was a poor year reproductive year for *C. hillii*, which could make the poor seed set in 2005 seem quite alarming. Although the drought this year almost certainly contributed to the low fecundity observed. The average plant size actually increased in 2005 compared to 2004. For instance, the mean diameter increased from 21cm to 32cm, an increase of 60%. The mean size of each plant's longest leaf also increased, albeit by 42%. The standard deviations of these measurements increased even more- by 44% and 129% respectively. Combined with the limited number of flowering plants this year, this suggests that the drought may have restricted the growth of many individuals, while causing others to direct their limited resources into vegetative growth instead of flowering. Hopefully this means that they will be in a better position to flower next year. The decreased levels of viable seed can also be attributed to the drought. With fewer potential mates, the few plants that did flower may have been forced to self-fertilized.

An AMOVA analysis using Hickory, a Bayesian analysis software, an Fst (θ B) of 0.0761. These results suggest that there is little difference between the two populations as far as the amount of genetic variability they contain. Hickory calculated an inbreeding co-efficient (Fis/*f*) of 0.6264 (sd 0.2357), suggesting a high level of inbreeding. However, these results need to be interpreted with some caution due to nature of dominant markers. Nonetheless, this value is consistent with what we might expect for a small isolated population of a vegetatively reproducing species such as *Cirsium hillii*. The data further indicate that plants which had been tagged as clones (due to their proximity to each other) are not always in fact genetically identical.

Figure 2: One viable seed and several non-viable seeds

Figure 3: Range of *C. hillii*

Ramifications for future management of *C. hillii*

If our conclusion of inbreeding depression being a problem for local *C. hillii* populations holds true, a possible solutions for restoring their reproductive success would include introducing new genetic material. Local populations are not in any way connected, but artificial transfers of pollen and seed could restore healthy genetic diversity. Since the two populations examined to date do not display significant differences from each other, pollen and seed from another source might need to be identified. Such undertakings would need to be monitored carefully. Other studies of *Cirsium* species in the UK has identified a decline in seed set near the edge of its range (Jump et al 2003) which was not fully explained by reduced genetic variability (Jump et al 2003). Since Illinois is at the edge of *C. hillii*'s range, this effect maybe be observed (See figure 3).

Acknowledgments

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Literature Cited

Jump, Alistair, and F. Ian Woodward. 2003. Seed production and population density decline approaching the range-edge of *Cirsium* species. *New Phytologist* **160**: 349-358.
Jump, Alistair, F. Ian Woodward, and Terry Burke. 2003. *Cirsium* species show disparity in patterns of genetic variation at their range-edge, despite similar patterns of reproduction and isolation. *New Phytologist* **160**:359-370.
Penskar, Michael. Rangewide status assessment of *Cirsium Hillii* (Canby) fern.. USFWS, 1997. http://www.largocanyon.org/science/Cirsium/techreps/hillii/c-hillii.htm