The little outplanting that could: Genetic variation in a micropropagated introduction of the Cumberland sandwort

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Abstract

The use of reintroductions as a means of plant conservation is not without its share of controversy, with much criticism centered around the ability to capture adequate amounts of genetic diversity in the reintroduced population. In this study, we examined the amount of genetic diversity in a tissue culture propagated outplanting of the rare, endangered Cumberland sandwort (*Minuartia cumberlandensis*) relative to its natural parent population and the micropropagated source population, using SRAP markers. We found that all three populations were similar to each other (θ = 0.10, 95% credible interval [0.03, 0.22]), and that the outplanting was reaching levels of genetic diversity found in the original wild population just 8 years since establishment. This represents a potentially successful introduction using in vitro methods that has overcome low levels of founding genetic diversity, and could serve as a model for future plant introductions.

Introduction

- *Minuartia cumberlandensis* (Wofford & Kral) McNeill, commonly known as the Cumberland sandwort, is a federally endangered perennial found in sandstone rockhouses of the Cumberland Plateau of southern Kentucky & northern Tennessee (Wofford & Kral 1979).
- As an ex situ conservation measure, the Cincinnati Zoo & Botanical Garden's Center for Conservation & Research of Endangered Wildlife (CREW) Plant Research Division developed a protocol for the micropropagation and cryopreservation of *M. cumberlandensis* using seeds sent from The Missouri Botanical Garden, originally collected from Hazard Cave at Pickett State Park, in Tennessee (Pence et al. 2011).
- On August 27, 2005, 63 plants from 7 genetic lines propagated through tissue culture at CREW were planted out in a sandstone rockhouse of suitable habitat containing no prior population of *M. cumberlandensis* in Daniel Boone National Forest (DBNF) in Kentucky (Pence et al. 2011).
- As of 2013, the DBNF population appeared healthy, having more than doubled to over 150 individuals which were flowering and producing seedlings. Having such a small number of founding individuals & particularly genotypes makes the DBNF population an unexpected success, according to a recent review of plant reintroduction successes (Godefroid et al. 2011).
- We sought to quantify levels of genetic variation and genetic similarity between the microproagated introduction and it's original source population. We expected to find a lower amount of genetic diversity in the Daniel Boone National Forest (DBNF) outplanting population than in the Hazard Cave source population, and a high similarity between the DBNF population, Hazard Cave population, and the original tissue culture samples at CREW.

Figure 1. An example of the typical sandstone rockhouse habitat of *M. cumberlandensis*. The Hazard Cave population is located behind the fence on the left side of the picture.

Methods

- Leaf samples were collected from 30 individuals at the Daniel Boone National Forest outplanting, 30 individuals at the Hazard Cave source population, and 4 of the micropropagated source genotypes still maintained in tissue culture.
- DNA extractions were carried out using a modified CTAB protocol (Doyle & Doyle 1987).
- SRAP markers were used for the genetic analysis. SRAP markers are sequence-related amplified polymorphism markers which target open reading frames and coding regions of DNA (Robarts and Wolfe 2014). They are non-specific, reproducible, dominant markers widely used in agriculture and horticulture. A single forward primer can work with multiple reverse primers.
- Sets of 4 forward and 4 reverse primers were screened together for a total of 16 primer combinations, of which 4 were chosen for their variability and legibility, producing 12 scoreable bands.
- PCR was run using modified SRAP cycling conditions according to Li & Quiros (2001), and products were visualized on agarose gels & scored by hand.
- Descriptive genetic statistics (Table 1) and a principal coordinates analysis (Figure 2) were generated using GenAlEx 6.5 (Peakall & Smouse 2012).
- Estimates of population differentiation, f & θ, were generated using Hickory (Holsinger & Lewis 2003).

Table 1. Descriptive statistics for *Minuartia cumberlandensis* based on SRAP data. Shown are the sample sizes (*N*), mean values for the number of different alleles (*Na*), the Shannon's information index (*I*), and percentage of polymorphic loci (*P*).

Population	N	Na	I	P (%)
Hazard Cave	30	2.000	0.524	100.00
Tissue Culture	4	1.333	0.272	58.33
DBNF	30	1.667	0.453	83.33
Mean	**64**	**1.667**	**0.416**	**80.56**

Results

- Genetic diversity (Na) was highest in the Hazard Cave population, followed by DBNF and the tissue culture population (Table 1).
- Shannon's information index was highest in the Hazard cave population and lowest in the tissue culture population (Table 1).
- The inbreeding coefficient was high (f = 0.553, 95% credible interval [0.043, 0.979]).
- Population differentiation was low, but significant from zero (θ = 0.100, 95% credible interval [0.028, 0.222]).
- A principle coordinates analysis reveals little differentiation between populations (Figure 2).
- 3 genotypes were common between Hazard Cave and DBNF, and 1 genotype was common between DBNF and 2 tissue culture samples.

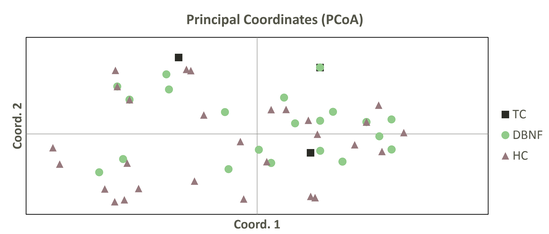

Figure 2. Principle coordinates analysis of the remaining tissue culture plants (TC), the Daniel Boone National Forest outplanting (DBNF), and the Hazard Cave original source population (HC).

Figure 3. *Minuartia cumberlandensis* in bloom.

Discussion

- As expected, genetic diversity was highest in the natural Hazard Cave source population and lowest in the remaining tissue culture samples.
- Somewhat surprisingly, genetic diversity in the Daniel Boone National Forest population was much closer to the Hazard Cave population than the tissue culture population from which it was created.
- The low fixation index and the scatter in the principle coordinates analysis indicate that there is not substantial differentiation between all three populations.
- The inbreeding coefficient and multiple common genotypes between DBNF and the other 2 populations indicates a large amount of inbreeding in the DBNF population, as expected given the artificial bottleneck.
- Eight years since the initial outplanting of 63 individuals from 7 genotypes, the DBNF population appears to be approaching levels of genetic diversity on par with the natural source population.
- This research describes a case study of the successful establishment of a rare, endangered plant species in the wild using micropropagation as a plant conservation measure.
- This analysis will also help to inform future outplantings and reintroductions for *M. cumberlandensis*, in addition to serving as a case study for a successful reintroduction of a rare, endangered plant.
- Future research will integrate additional samples from Hazard Cave and Daniel Boone National Forest, another natural Tennessee population, and cryopreserved samples representing more of the original micropropagated genotypes.

Acknowledgements

We wish to thank David Taylor for his assistance in monitoring and counting the Daniel Boone National Forest population since 2005, and for his assistance in the 2013 sampling. We also thank Madison Smith and Omer Donmez for their work as field research assistants, the Tennessee Department of Environment & Conservation Division of Natural Areas for permits, and the University of Cincinnati and Cincinnati Zoo & Botanical Garden – CREW's Eisenberg Fellowship for funding.

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