A comparison of genetic diversity within *ex situ* collections of *Brighamia insignis*

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Introduction

Brighamia insignis, or *'ōlulu* in Hawaiian, is an endemic succulent, historically known from Kaua'i and Ni'ihau, and has been listed as federally endangered since 1994 (as cited in [2]). In fact, the National Tropical Botanical Garden (NTBG) reports that the species has declined to only **ONE** naturally occurring wild individual (as cited in [2]).

Figure 1: *Brighamia insignis* grown on the cliffs of Hawai'i. (Picture from Center for Plant Conservation (CPC) webpage).

Prior to the decline of *Brighamia insignis*, the uniqueness of the species had captivated the attention of collectors, and the species has successfully propagated in *ex situ* collections worldwide (e.g. botanic gardens, universities, private collections) [2].

In 1998, isozyme analyzes were conducted to demonstrate NTBG collections were illustrative of the levels of genetic diversity found in the wild, which indicated that the *ex situ* individuals were genetically representative of natural populations [1].

Purpose

This study focuses on examining individuals of *Brighamia insignis* from different *ex situ* collections for polymorphic loci using inter-simple sequence repeat (ISSR) markers in order to determine if there is sufficient genetic diversity in these collections. Such information can help with the restoration efforts and reintroduction of the species back into the wild.

QUESTION:

Is there sufficient genetic diversity in *ex situ* collections of *Brighamia insignis* to help support a natural population for the reintroduction of the species back into the wild?

Hypothesis

If an *ex situ* collection was made from multiple individuals of *B. insignis* collected over multiple years, then that collection will have more genetic diversity than collections created from single individuals at a single time.

Variables

Independent Variable: *Ex situ* collections of *Brighamia insignis*
Dependent Variable: Number of bands produced

Materials and Methods

Figure 2: 96 *Brighamia* tissue samples from 24 different *ex situ* collections. (Photo credit: Kevin Cheng)

Figure 3: Preparing a PCR plate using a pipette. (Photo credit: Seana K. Walsh)

Brighamia DNA extractions were prepared ahead of time using the CTAB method. DNA samples were tested using 6 ISSR primers. Each primer was tested twice in a 15µl/rxn. Each individual was run twice on a 3% agarose gel and only clear reproducible bands were scored for polymorphisms.

Results

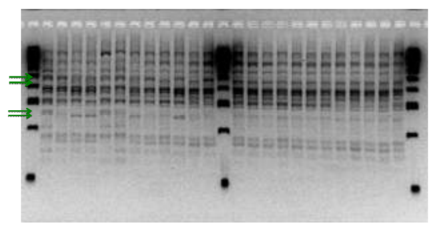

Figure 4: Example of gel (primer 841, rows G & H) showing diversity. (Photo credit: Kevin Cheng)

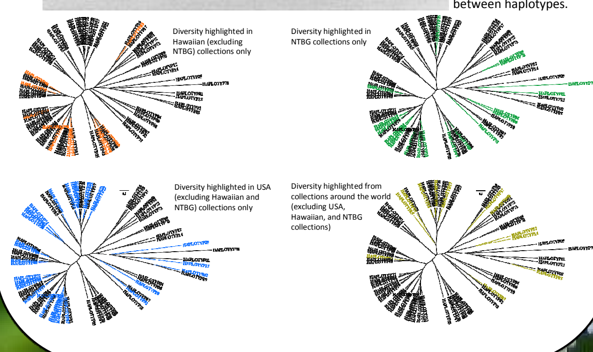

Figure 5 (below): UPGMA genetic distance trees that display how distal the genetic relationship is between haplotypes.

Percentage of Genetic Diversity Accounted for in Various Ex situ Collections

Source of Variation	Percentage of Variation
Among collections around the world	7.27
Within ex situ collections in Hawai'i, excluding NTBG	3.42
Within NTBG collection	89.31
Total	100.00

Table 1: Results from the AMOVA test show that 89.31% of genetic variability was found within the NTBG collection.

Table 2: The pairwise F_{ST} values that display the differences between groups of collections due to their genetic structure. All differences are statistically significant with the exception of the difference between NTBG and *ex situ* collections in Hawai'i.

For the analysis, the NTBG *ex situ* collection, which is the most comprehensive collection of *B. insignis* in the world, was compared with the other collections in Hawai'i and collections from around the world. All 96 samples were scored for 24 polymorphic loci, with 78 distinct genotypes present.

Results from the AMOVA test show that 89.31% of genetic variability was accounted for within the NTBG collection, 3.42% was accounted for in other *ex situ* collections located in Hawai'i, and the remaining 7.27% of genetic diversity was found in additional collections around the world.

Conclusion

The current hypothesis is supported because the *ex situ* collection at NTBG represents over 89.31% of the known genetic diversity within the species (Table 1). Despite the remaining 10% of the genetic variability being found in other collections, which were statistically different based on the F_{ST} values (Table 2), the comprehensive NTBG collection still has the most genetic diversity when compared to any other collection of *Brighamia insignis.*

Potential sources of error may involve plant misidentification, insufficient aspiration when pipetting, and scoring a band different than the one assigned.

The results show that NTBG and Hawaiian collections combined possess sufficient genetic variability for the creation of diverse plant material that could be used for the reintroduction of the species back into the wild (Table 1). However, some genotypes found in collections within the continental USA and international collections do represent unique genotypes that may be useful for augmenting genetic diversity (Figure 5). Additional studies to confirm these results may involve the use of more individuals from a variety of collections and the use of more extensive DNA methods such as whole genome sequencing.

Works Cited

[1] – Gemmill, C. E.C., Ranker, T.A., Ragone, D., Perlamn, S.P., Wood, K.R. (1998). Conservation genetics of the endangered endemic Hawaiian genus *Brighamia* (Campanulaceae). *American Journal of Botany, 85(4),* 528-539.

[2] – U.S. Fish and Wildlife Service (USFWS). (2007). *5-Year review of Brighamia insignis (olulu).*

Background photo is of *Brighamia* from Cal Poly Plant Conservatory website.

SAFETY: One of the risks involved with the experiment include the use of SYBR® Green I Nucleic Acid Gel Stain. This product does bind to DNA, which raises concerns of mutagenicity. Although developed to be a safer substitute to ethidium bromide, gloves, goggles, and a lab coat were worn to protect the experimenter from a potential mutagen. In addition, electrical equipment for running gel electrophoresis may produce electrical shocks. Safety efforts for protection included keeping the voltage boxes away from a water source, using dry gloved hands to connect the leads one at a time, and shutting off the power before removing the gel. Another safety concern may be photographing the gel with UV light; the gel was placed in a chamber and the door was closed prior to UV light emitting. Gloves were worn at all times.

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