GENETIC DIVERSITY ASSESSMENT OF EX SITU COLLECTIONS
OF ENDANGERED QUERCUS HINCKLEYI

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Premise of research. Conservation of imperiled plant species can benefit from ex situ collections, including the living collections of botanic gardens and arboreta. Such living collections should strive to capture and maintain the genetic diversity found in the remaining wild populations. Ex situ collections may also harbor genetic variability that has been lost from wild populations. Quercus hinckleyi is an extremely rare (International Union for Conservation of Nature and Natural Resources [IUCN] Critically Endangered) oak known from a few sites in Presidio County, Texas. It is the only native US oak protected under the Endangered Species Act. Here we evaluate the genetic variation of the Q. hinckleyi ex situ metacollection relative to that of the remnant in situ population.

Methodology. We apply microsatellite genotyping to Q. hinckleyi sampled from plants growing in ex situ collections (N = 22, from nine gardens) and compare the results with genetic studies of the in situ population. We compare allelic diversity, genotypic diversity, and population structure.

Pivotal results. The ex situ metacollection is small but has high allelic diversity and captures about 57% of the allelic diversity of the wild population. Ex situ plants contain 22 new alleles that contribute 13% of the species’ total allelic diversity. All ex situ individuals have unique genotypes, none of which were found in situ, and thus they comprise 15% of the species’ genotypic variation. The ex situ plants align with only one of three genetic clusters identified in situ, demonstrating an important gap in the ex situ metacollection.

Conclusions. The ex situ collection of Q. hinckleyi, while small, is genetically diverse and provides additional allelic and genotypic diversity not currently existing in situ. However, a significant portion of genetic diversity is lacking from ex situ plants, with the metacollection likely derived from only one of the in situ clusters. Collecting seed from the other clusters is a high priority.

Keywords: ex situ conservation, endangered oaks, conservation genetics, metacollection, microsatellites, Quercus hinckleyi.

Introduction

Ex situ conservation is an important complement to in situ conservation, especially when species are extremely rare and/or threatened in their natural habitats by anthropogenic disturbance, climatic extremes, or stochastic events. Plants can be maintained ex situ as seeds, frozen embryos, tissue culture, or living plants in botanic gardens or germplasm repositories. These are usually kept as a safeguard against extinction but also allow for scientific study of rare species that may improve conservation strategies. Ex situ collections also provide opportunities for the public to learn about conservation issues and plant ecology and evolution (Oldfield 2009; Cavender et al. 2015).

Genetic diversity has long been used as a principal goal to guide the organization and maintenance of such collections (Marshall and Brown 1975; Guerrant et al. 2004), but as yet we know very little about how much intraspecific genetic variation actually exists in most ex situ collections. Genetic diversity is essential because it allows species to adapt to environmental change, including extreme weather, global warming, changing precipitation, pollution, and disease—it is often referred to as “evolutionary potential” (Barrett and Schluter 2008; Morikawa and Palumbi 2019). High genetic diversity is also correlated with higher fitness and lower inbreeding depression, which is especially important in captive populations (Reed and Frankham 2003). Genetic diversity can also support greater species richness and ecosystem resilience, especially in foundation species like trees (Raffard et al. 2019).
In many cases, we can predict that genetic diversity ex situ is relatively low because of small sample sizes. Most species are conserved in relatively low numbers (often with only one accession, e.g., one population), while ideal sampling would require dozens of individuals to maintain sufficient diversity (Beckman et al. 2019). Several recent studies have shown that ex situ genetic diversity is influenced by the strategy used to sample seed from wild populations, with lower ex situ genetic diversity when fewer populations are visited and when there are fewer accessible plants producing seed. In addition, the species’ biology, especially pollen and seed dispersal, influences how much genetic diversity is contained in ex situ collections and how it is distributed within and among ex situ collections (Guerrant et al. 2004; Hoban and Strand 2015; Hoban 2019, 2020).

Approximately 10%–15% of all plants cannot be maintained as seeds because of physiological or ecological factors, and this percentage is even higher, approximately 30%, in threatened tree species (Pence 2011; Wallace 2015; Wyse and Dickie 2017; Wyse et al. 2018). Such species must be kept as living plants in ex situ collections, where the number of individuals may be limited by available space as well as the time and resources needed to catalog and care for them. Oak species are included in this non-seed bankable or “exceptional” group (Beckman et al. 2019). Acorns do not maintain viability indefinitely, so seed banks are not an option. Growing them out periodically to collect fresh acorns is also difficult because of long generation times (typically 30+ yr), while the “species purity” of the acorns used would be difficult to maintain because of the propensity of oaks to hybridize (often, gardens contain many species of oaks). Other options for plant preservation, such as in vitro or cryopreserved collections, have also proved difficult when applied to oaks (Kramer and Pence 2012). Attempts at somatic embryogenesis are complicated by high tannin levels (Park et al. 2016; Martínez et al. 2017), and cryopreservation of embryonic tissue has been shown to negatively impact DNA methylation, which is important in growth regulation and differentiation (Nuc et al. 2016). For these reasons, ex situ living plant collections as repositories of genetic material are especially important for the conservation of threatened oak species.

Quercus hinckleyi is a rare International Union for Conservation of Nature and Natural Resources (IUCN) Critically Endangered species and is the only native US oak protected under the Endangered Species Act (Beckman et al. 2019). It is likely one of the most endangered plant species in the United States; it is found almost exclusively in Presidio County in West Texas along the Rio Grande River. The Chihuahuan Desert ecosystem where it is found extends into Mexico, but to date there have been no confirmed reports of Q. hinckleyi there. The species was more prevalent in a mesic period that occurred more than 10,000 yr ago, but as the environment has dried, its range has been reduced (VanDevender and Spaulding 1979; VanDevender 1990; Nixon et al. 1997). Reproduction is both sexual and clonal. Sexual reproduction appears to be restricted in parts of its habitat (Backs et al. 2015). It currently is thought to have approximately 200 individuals in a small, fragmented range threatened by human impact and climate change (Jerome et al. 2017). According to a conservation gap analysis (Beckman et al. 2019), the highest threat to Q. hinckleyi is its limited range, followed by habitat disturbances that may impact reproduction, such as mining or roads; landscape shifts due to global warming; and potential loss of genetic variability. Expansion of ranching or tourism in nonprotected areas and recreational use of Big Bend Ranch State Park, a protected area, are also concerns. Clearly, any environmental impacts within the range of the plants that reduce their numbers or cause more habitat fragmentation could be fatal to the species.

A previous genetic study of Q. hinckleyi used DNA microsatellites to characterize genetic diversity and clonal growth in the in situ population (Backs et al. 2015). Genetic diversity, as measured by number of alleles, heterozygosity, and allelic richness, was high for the wild plants, and there was no evidence of inbreeding. Despite the dangerousely low remaining number of Q. hinckleyi, the species harbors allelic diversity and heterozygosity comparable to that of more widespread oaks (Ashley et al. 2015; Backs and Ashley 2016; Pohjanniemies et al. 2016; Ashley et al. 2018; DiPietro et al. 2020). However, the genetic analysis also revealed a high level of clonal growth, including identical clones separated by up to 30 m. Clonal identification showed that the number of unique genotypes in the remaining natural population was only 123 (of 204 plants sampled), indicating that the in situ population is even smaller than previously thought, at least in terms of effective population size. Clonal growth was particularly prevalent at two sites where a sample of 58 “individuals” comprised only seven clones (Backs et al. 2015). While the propensity of Q. hinckleyi to have clonal growth has enabled it to maintain genetic diversity even as its range has contracted and its population has been greatly reduced, it ultimately does not allow for genetic exchange and adaptive evolution.

Two genetically distinct clusters were reported in the extant population (Backs et al. 2015). One of the clusters is located primarily around Shafter, Texas, but members of this cluster were also identified in samples collected about 60 km away in Big Bend Ranch State Park. Individuals of the second cluster were all collected at other sites in Big Bend Ranch State Park. Hybridization with closely related congeners, common in oaks, does not appear to be an immediate threat to Q. hinckleyi because of the spatial isolation of the remaining plants, although introgression into a small population of Q. pungens was detected (Backs et al. 2016).

Our goal in the current study is to determine to what extent combined ex situ collections, that is, metacollections (Griffith et al. 2020), of Q. hinckleyi can aid in its conservation. We address three questions: (1) Given the propensity for cloning in Q. hinckleyi, do ex situ trees harbor unique genotypes currently not identified in situ? (2) How well do ex situ collections represent the genetic diversity of the in situ population? (3) Do ex situ trees belong to one or both of the two genetic clusters identified in the field? By answering these questions, we can identify collection gaps for Q. hinckleyi, if they exist. Furthermore, we can determine whether ex situ metacollections comprise additional genetic resources that could be used to supplement in situ genetic diversity.

Methods and Material

Study Species

Quercus hinckleyi C.H. Mull. (Hinckley’s oak) is a shrublike species that grows to approximately 1 m in height with multiple ramets. It is protected under the Endangered Species Act (Beckman et al. 2019) and is also listed as threatened by the state of Texas (Texas Parks and Wildlife Department 2020). Because it
is a federally endangered species, a recovery plan has been developed (Kennedy and Poole 1992; USFWS 2009). Previous genetic studies based on in situ (wild) plants have addressed some of the conservation questions in the plan: the genetic viability of the remaining plants, the types of reproduction and their contributions to the remaining populations (Backs et al. 2015), and an assessment of threats to species integrity due to hybridization (Backs et al. 2016).

Ex Situ Sampling

Our study is based on ex situ plants growing in botanical collections in the United States and Europe. We collected samples from ex situ sites by identifying all known ex situ collections of the species using the Conservation Gap Analysis Program of Native U.S. Oaks initiative by Morton Arboretum (Beckman et al. 2019) and with the assistance of Botanic Gardens Conservation International PlantSearch (http://tools.bgci.org/plant_search.php), which is a global database of botanic gardens in 126 countries. Through this search, we found *Q. hinckleyi* in nine collections, with one to five individuals per collection, for a total of 22 individuals; all were included in this study. We are not aware of any other ex situ *Q. hinckleyi* growing in botanical collections. Three to five whole green leaves were collected per plant and shipped either in silica or on ice to the Morton Arboretum. The leaves were kept at −80°C both on arrival and at the Ashley Lab at the University of Illinois at Chicago, where DNA was extracted and genetic analysis completed.

DNA Extraction and Microsatellite Analysis

To the extent possible, materials and instruments used for genetic DNA extraction and microsatellite analyses were the same as those used in the earlier study of in situ *Q. hinckleyi* (Backs et al. 2015). This was done to ensure that scored alleles and genotypes were comparable between the in situ and the ex situ samples. Approximately 20 mg of sample was homogenized to a fine powder and used for genetic DNA extraction using DNaseasy Plant Kit (Qiagen, Hilden, Germany). DNA was quantified using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE). The same eight microsatellite loci and associated M13+ forward primer, reverse primer, and fluorescent primer were used. It has been shown that using different fluorescent primers for analysis of a given locus can alter allele sizing (Sutton et al. 2011). PCR amplification followed the same protocol for the in situ studies and is described in Abraham et al. (2011), and PCR products were genotyped using ABI 3730 DNA Analyzer and GeneScan 500 LIZ Size Standard (Applied Biosystems, Waltham, MA). This was the same size standard used in the earlier work, again to maintain consistent results. For this study, genotypes were scored on Applied Biosystems Peak Scanner Software version 1.0.

Data Analysis

To ensure that comparisons were valid, software tools and procedures used for the current analyses were the same as those used in the studies of the in situ *Q. hinckleyi* (Backs et al. 2015, 2016). We first tested for clones in the ex situ samples themselves, as well as between them and in situ individuals, using the R package allelematch (Galpern et al. 2012). Clones in the prior study had been collapsed to 123 unique multilocus genotypes (UMGs) from an initial 204 ramets collected (Backs et al. 2015), and UMGs were used for further analysis.

To examine the genetic diversity of the ex situ samples, we ran statistical analyses using GenAIEx version 6.502 (Peakall and Smouse 2006, 2012). This provided the numbers of alleles, expected heterozygosity (*H*), observed heterozygosity (*H*), fixation indexes (Fst), and numbers of private alleles by locus and population. Allelic richness (*R*), the number of alleles per locus ranging from 7 to 16, with a mean of 12.75 per locus, comparable to the in situ samples. Alletic richness was comparable to that of the wild population (table 1). Interestingly, the ex situ samples had 22 alleles that were not identified in in situ samples. Given that there was a total of 143 alleles found in in situ samples, the ex situ samples provide an additional 13% (22 of 165) of allelic diversity to the species. Genetic variation as measured by expected and observed heterozygosity indexes in the ex situ samples was comparable to that found in the wild.
of genetic distance also show the closer affinity of the ex situ set of individuals to cluster 1 than to cluster 2 (table 1). Measures of genetic distance also show the closer affinity of the ex situ set of individuals to cluster 1 than to cluster 2 (table 1). The G_{ST} between ex situ and cluster 1 is 0.027 and between ex situ and cluster 2 is 0.042 (Bonferroni-corrected \( P = 0.001 \)). The \( K = 3 \) results indicate subpopulation structure within cluster 1, separating the ex situ and Shafter individuals from the Big Bend Ranch State Park individuals (fig. 1A). Because of the close genetic similarity between the ex situ samples and those near Shafter and because the Shafter population is the most accessible population, we examined the data to see whether any of the ex situ samples could be offspring of the seven clones we found at Shafter. Two of the ex situ samples shared at least one allele at every locus with a Shafter clone, indicative of a parent/offspring relationship. These two plants are reported to have been collected from the Shafter site and were apparently grown from an acorn produced by one of the clones that we previously identified in situ.

### Discussion

As ex situ collections increasingly support the conservation efforts of imperiled plant species, it becomes imperative to evaluate how well such collections capture the genetic makeup and diversity of wild populations (Oldfield 2010; Cavender et al. 2015; Fant et al. 2016). To capture a full range of a species’ genetic diversity, including adaptive traits, ex situ sampling should be from across its native range (geographic location coverage) and the ecological zones in which it is found (Environmental Protection Agency level IV ecoregions; Khoury et al. 2015; Maschinski et al. 2019). High genetic diversity will increase the usefulness of a collection for restoration, reintroduction, or assisted migration; the number of source plants is correlated with replanting success (Godefroid et al. 2011). According to a recent analysis of US oaks (Beckman et al. 2019), *Quercus hinckleyi* is a species of greatest concern. In this study, which looked at risk of extinction, vulnerability to climate change, and presence of species in ex situ collections, only two oaks ranked higher in demographic vulnerability indicators. Conservation recommendations for *Q. hinckleyi* are land protection, propagation programs, and wild collection and/or ex situ curation.

Here, we evaluated the ex situ metacollection of *Q. hinckleyi* to assess its genetic variability and determine how well it captured the genetic makeup of the remaining remnant wild population. Since previous work had evaluated all known wild individuals (Backs et al. 2015), a precise comparison of in situ and ex situ populations was possible. Ex situ conservation is part of a set of options that includes hand-pollination and the translocation of individuals that may help in the protection and management of *Q. hinckleyi*.

We first address genotypic diversity and its implications. The metacollection that we compiled consisted of 22 individuals from nine botanical collections. Microsatellite genotyping revealed that all ex situ plants were genetically unique. None of the 123 clones identified in field sampling was represented ex situ, which is perhaps expected since the likely source of ex situ plants was acorns. Given the high level of clonality reported in the field, the ex situ collection adds a significant amount of genotypic variation to the species overall (−15%). This additional diversity will be valuable for future conservation plans, which may include controlled breeding, reintroduction, or translocation. Recently, successful propagation of *Q. hinckleyi* through excised root suckers has been reported (Black 2019). This method is simple and relatively noninvasive and, combined with testing for clones, suggests a good starting point for developing a breeding strategy for *Q. hinckleyi*.

### Table 1

Descriptive Statistics

| Locus | \( N \) | \( \bar{N} \) | \( H_o \) | \( H_e \) | \( F_{IS} \) | \( R_s \) |
|-------|-------|-------|-------|-------|-------|-------|
| Cluster 1 |
| Q1/5  | 20    | 10    | 0.90  | 0.799 | -0.127 | 9.68  |
| Q110  | 18    | 11    | 0.833 | 0.861 | 0.032  | 11.00 |
| Q1    | 21    | 9     | 0.667 | 0.734 | 0.091  | 8.91  |
| Q9    | 19    | 10    | 0.684 | 0.842 | 0.188  | 9.89  |
| QM69  | 20    | 11    | 0.850 | 0.864 | 0.016  | 10.78 |
| MSQ4  | 19    | 15    | 0.789 | 0.907 | 0.130  | 14.73 |
| Q15   | 21    | 12    | 0.762 | 0.793 | 0.039  | 10.86 |
| MSQ13 | 19    | 15    | 0.895 | 0.880 | -0.017 | 14.53 |
| Mean  | 19.6  | 11.6  | 0.798 | 0.835 | 0.044  | 11.30 |
| Cluster 2 |
| Q1/5  | 101   | 15    | 0.901 | 0.892 | -0.010 | 11.54 |
| Q110  | 101   | 14    | 0.752 | 0.875 | 0.140  | 9.40  |
| Q1    | 102   | 10    | 0.588 | 0.600 | 0.019  | 5.15  |
| Q9    | 93    | 16    | 0.871 | 0.886 | 0.017  | 11.94 |
| QM69  | 101   | 11    | 0.881 | 0.843 | -0.045 | 9.10  |
| MSQ4  | 101   | 17    | 0.673 | 0.878 | 0.224  | 10.75 |
| Q15   | 101   | 15    | 0.881 | 0.878 | -0.003 | 10.71 |
| MSQ13 | 102   | 18    | 0.922 | 0.818 | -0.126 | 10.19 |
| Mean  | 100.3 | 14.5  | 0.809 | 0.832 | 0.027  | 9.85  |
| Ex situ |
| Q1/5  | 22    | 7     | 0.773 | 0.812 | 0.048  | 6.99  |
| Q110  | 22    | 11    | 0.500 | 0.810 | 0.383  | 10.03 |
| Q1    | 22    | 13    | 0.909 | 0.832 | -0.093 | 11.69 |
| Q9    | 22    | 13    | 0.909 | 0.848 | -0.072 | 11.87 |
| QM69  | 22    | 11    | 0.727 | 0.692 | -0.017 | 10.02 |
| MSQ4  | 22    | 15    | 1.000 | 0.900 | -0.111 | 14.12 |
| Q15   | 22    | 16    | 0.909 | 0.913 | 0.005  | 14.99 |
| MSQ13 | 22    | 16    | 0.955 | 0.879 | -0.086 | 14.33 |
| Mean  | 22.0  | 12.9  | 0.835 | 0.839 | 0.007  | 11.75 |

Note. \( N_0 \) = number of alleles; \( H_o \) = observed heterozygosity; \( H_e \) = expected heterozygosity; \( F_{IS} \) = fixation index (GenAlEx 6.502); \( R_s \) = allelic richness (FSTAT 2.9.4).
Next, we observed that the allelic diversity of the ex situ samples is comparable to that of the wild population (table 1). However, the ex situ plants did not capture a high proportion of the in situ alleles. Our estimate of 57% of alleles is lower than all but one value reported in a recent review (Griffith et al. 2020) in which most ex situ collections contained more than 80% of in situ alleles. Importantly, however, the ex situ collection harbored alleles (22) that were not currently found in the in situ populations, presumably because of losses in the wild occurring since the specimens were collected or because some individuals were not sampled in our in situ survey. The range of *Quercus hinckleyi* is small but covers remote and rugged terrain, so some plants might have been missed.

It does appear that the ex situ collections harbor unique genotypic and allelic diversity, supplementing what exists in the wild at this point in time. They are good candidates for use in propagation programs. Given that *Quercus hinckleyi* is well adapted to warm and xeric environments, the ex situ metapopulation could also be used for genomic investigations of genes associated with these adaptations. When cultivated in a more mesic climate, *Quercus hinckleyi* grows into a small flourishing tree (Black 2020). The acquisition and then loss of plastic adaptive traits may also make it a good candidate for epigenetic research (Bräutigam et al. 2013). Findings from such studies could have important implications for other oak species in the face of rapid environmental change.

Provenance information was available for only a few of the ex situ samples. Some of the more recently collected samples have recorded latitudes and longitudes, but some have none at all, and some have only general locations. Interestingly, one of the ex situ specimens is listed as coming from Brewster County, Texas, and two others as coming from Presidio/Brewster, although no coordinates are given. It is therefore unclear whether these originated in Big Bend Ranch State Park, which spans the Presidio County/Brewster County line; another location in Brewster County; or, indeed, Presidio County. Certainly, these areas should be explored for additional individuals, and any future sampling should include the waypoints of collection sites.

Our analysis of genetic structure provides additional information about the ex situ collections. Previous work showed that the remaining in situ population comprises two genetically distinct clusters (Backs et al. 2015). All of the ex situ samples fall within genetic cluster 1, as shown by analyses with STRUCTURE, PCoA, and the differentiation indexes $G_{ST}$ and $D_{JOST}$. In the current study, we found further substructure within genetic cluster 1,
which provides genetic evidence that the acorns propagated for ex situ collections were collected near Shafter since the ex situ samples cluster with these plants. At this site, individuals of *Q. hinckleyi* occur close to a main road and are much more accessible than at the sites in Big Bend Ranch State Park. The only four ex situ individuals with waypoints came from a site near Shafter, and two of the ex situ individuals appear to be offspring of clones growing at this site. Thus, the ex situ collection has sampled only one of the three genetic groups that exist, and our results highlight this deficiency in the ex situ sampling. To address this geographic and genetic gap and capture potential adaptations, as well as alleles in the ex situ collections, future collections should target the areas where the cluster 2 individuals occur and sites in Big Bend Ranch State Park where the cluster 1 individuals occur.

Various studies have recommended minimum sample sizes, from fewer than 15 to more than 1000, for ex situ collections to maintain the genetic diversity of a species (see table 1 in Hoban and Strand 2015). Empirical work (Hoban et al. 2020) and simulations (Hoban 2019) have started to better resolve this minimum number, and these studies suggest that if sampling were ideal (from many maternal plants in all known locations), a minimum sample size of approximately 100 plants might be suitable for this rare species. On the basis of this past work and the genetic assessment in this article, the current size of 22 is clearly inadequate and needs to be increased to capture genetic diversity. This is difficult as the species rarely produces acorns. An alternative would be propagation of vegetative stems or root suckers (Black 2019), which would efficiently capture existing genotypic diversity. Of course, for species to survive in the long term, effective population sizes need to be greater than 500 to allow species to adapt (Jamieson and Allendorf 2012), meaning that both the in situ and ex situ population sizes of *Q. hinckleyi* must be increased.

We used microsatellites in this study to assess genetic diversity ex situ. Microsatellite markers are long-established genetic markers that reflect overall genetic diversity, allow for assessing the number of unique genotypes, track parent-offspring relationships, and identify inbreeding. Other studies have used chloroplast sequences (Christe et al. 2014) or RADseq (Bragg et al. 2020). Because *Q. hinckleyi* is so rare, it is possible that only one or very few chloroplast sequences would be found, so this approach would not be advised. RADseq or other broader genome sampling approaches (GBS, ddRAD, etc.) might provide additional resolution, although microsatellite and RADseq data are often correlated and RADseq analysis is more expensive (Hoban et al. 2014; Fischer et al. 2017; Zimmerman et al. 2020). All of these approaches target neutral genetic variation. Future research might focus on genomic regions with known function, such as through target capture using recently developed oak genomic resources (Lesur et al. 2018), which would provide insight into important adaptive variation. Evaluating candidate genes may be more fruitful than correlation approaches such as gene-environment association (GEA) studies because *Q. hinckleyi* occurs in a small number of locations in a relatively homogeneous environment,

![Principal coordinates (coord.) analysis of *Quercus hinckleyi* in situ (cluster 1 and cluster 2) and ex situ plants. Principal coordinates 1 and 2 account for 6.89% and 6.21% of the variation, respectively (GenAlEx 6.502). A color version of this figure is available online.](image)

### Table 2

|                      | Cluster 1 | Cluster 2 | Ex situ |
|----------------------|-----------|-----------|---------|
| Cluster 1            | ...       | .033      | .027    |
| Cluster 2            | .431      | ...       | .042    |
| Ex situ              | .333      | .528      | ...     |

Note. Pairwise estimates of genetic differentiation: $G_{ST}$ (Nei 1973) above diagonal, $D_{JOST}$ (Jost 2008) below diagonal.
conditions that would result in low power for GEA studies (Loottersho and Whitloch 2015; Hoban et al. 2016).

A final note regards the use of ex situ samples for conservation management. As noted above, ex situ individuals could produce seed for conservation reintroduction to nearby sites or assisted migration to sites that may be more suitable in the future. To complete such actions, more information, such as on environmental tolerance (e.g., ecological amplitude, niche breadth, etc.), is needed than is provided by genetics. Environmental niche models could be built using in situ Q. hinckleyi individuals and potentially using botanic garden individuals to describe the realized and fundamental niches of Q. hinckleyi (as in Vetaas 2002). Long-term data on Q. hinckleyi in botanic gardens are also informative—locations where Q. hinckleyi has failed to establish in cultivated conditions reveal where the species cannot survive. Experimental treatments could be possible in some botanic gardens (as in Ebeling et al. 2008), but considering the species’ exceptionally low numbers, such experiments (requiring large numbers of replicates) may not currently be the best use of rare seed production.

In summary, in this study we found that currently only 57% of Q. hinckleyi alleles are conserved ex situ. This conclusion assumes that no other populations exist in the wild, and continued surveys are warranted to determine whether this is the case. We recommend that the ex situ metacollection be expanded to at least 100 individuals, as recent research has recommended (Hoban 2019; Hoban et al. 2020). We also found, using PCoA and genetic clustering methods, that all current ex situ individuals appear to have been sampled from only one in situ genetic cluster. It is especially urgent to sample from the unsampled clusters. We also found that 13% of the species’ alleles and 15% of its genotypes are found only ex situ. Efforts should be made to reintroduce this genetic diversity back into the in situ populations. Finally, botanic gardens could use our findings to illustrate the value of their collections and to educate and inspire conservation action for this species “on the brink” of extinction.

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