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Comparing Genetic Diversity in Three Threatened Oaks

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Abstract: Genetic diversity is a critical resource for species' survival during times of environmental change. Conserving and sustainably managing genetic diversity requires understanding the distribution and amount of genetic diversity (in situ and ex situ) across multiple species. This paper focuses on three emblematic and IUCN Red List threatened oaks (*Quercus*, Fagaceae), a highly speciose tree genus that contains numerous rare species and poses challenges for ex situ conservation. We compare the genetic diversity of three rare oak species—*Quercus georgiana, Q. oglethorpensis*, and *Q. boyntonii*—to common oaks; investigate the correlation of range size, population size, and the abiotic environment with genetic diversity within and among populations in situ; and test how well genetic diversity preserved in botanic gardens correlates with geographic range size. Our main findings are: (1) these three rare species generally have lower genetic diversity than more abundant oaks; (2) in some cases, small population size and geographic range correlate with genetic diversity and differentiation; and (3) genetic diversity currently protected in botanic gardens is inadequately predicted by geographic range size and number of samples preserved, suggesting non-random sampling of populations for conservation collections. Our results highlight that most populations of these three rare oaks have managed to avoid severe genetic erosion, but their small size will likely necessitate genetic management going forward.

Keywords: conservation biology; fragmentation; botanic gardens; EST; inbreeding; heterozygosity; microsatellites; population genetics; ex situ

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1. Introduction

Genetic diversity is a critical resource for species to adapt to future challenges including pests and diseases, climate change, and other environmental changes. To conserve and sustainably manage genetic diversity, it is important to understand the distribution and amount of genetic diversity present in situ, and to identify the key factors shaping that genetic diversity [1–3]. While genetic diversity has been assessed in hundreds of rare

species globally, there are few comparative, multispecies studies of the influence of range size, environmental, and demographic variables on genetic diversity in rare species. Previous studies have shown that, on average, species with larger range sizes (and thus, more populations, larger populations, and lower genetic drift) have higher genetic diversity [4–6]. Additionally, small local populations whose effective size (N_e) is <500 individuals are generally at risk of genetic drift and loss of adaptive potential; and even smaller populations (<50 individuals) experience rapid genetic erosion and inbreeding problems [7]. Additionally, environmental factors may influence genetic diversity at certain loci through selection by climate or habitat [8,9], or may influence genetic diversity genome-wide through drift as population sizes are reduced or fluctuate in size [10].

It is equally important to understand how well genetic diversity is conserved ex situ. The amount of genetic diversity conserved ex situ may be correlated with some of the same factors impacting genetic diversity in situ (e.g., population size and geographic range size). Species with larger geographic ranges may require more plants ex situ to fully safeguard genetic diversity [11,12], but there are few cases empirically testing this [13]. Determining how many plants are needed to conserve genetic diversity is important to meet global, national, and institutional commitments on conservation. For instance, the Convention on Biological Diversity's Global Strategy for Plant Conservation (GSPC) has created guidelines for crops, crop wild relatives, and other economically important species that calls for "at least 75% of threatened plant species in ex situ collections, preferably in the country of origin", and for "70% of genetic diversity" to be conserved, by 2020 [14]. The GSPC also stipulates that collections be "accessible, backed up, and genetically representative". Collections that meet these requirements provide insurance against extinction, support in situ conservation (e.g., supplementation or reintroduction), and provide material for conservation-relevant research [1,15,16]. Safeguarding exceptional species is especially challenging [17], as they cannot be kept in traditional seed banks but must be kept ex situ in living collections (i.e., of mature individuals rather than seeds). Exceptional species include many common trees such as magnolias, oaks, cycads, and others.

This paper focuses on the tree genus, *Quercus* (oaks), for which there are currently 112 oak species threatened globally under the International Union for Conservation of Nature (IUCN) threat categories vulnerable, endangered, or critically endangered [18] with 17 of those in the United States [19]. Unfortunately, acorns are generally recalcitrant (they cannot be stored in conventional seed banks [20]) and are challenging for tissue culture and cryopreservation [21], making oaks an exceptional species. Conservation through living collections in botanic gardens and arboreta is currently the principal ex situ conservation option for threatened oak species [22,23].

Oaks have significant ecological [24], economic [25], and cultural [26] importance, while facing increasing conservation threats including habitat loss, invasive species, shifting climates, and pests/pathogens, such as sudden oak death [27]. For example, oaks support huge numbers of other species that depend on them for mast or forage [28], and many oak species are used for timber or non-timber forest products [29,30]. Oaks also feature incredible ecological breadth, having diversified into many habitats through adaptations in leaf traits, phenology, habit, water use, and other traits [31,32]. Identifying and quantifying the factors influencing genetic diversity in situ and ex situ is especially important for speciose genera like oaks, that has 450 species estimated worldwide. Characterizing correlates of genetic diversity in a few well-studied species within the oak phylogeny could serve as the foundation for predictive models of the distribution of genetic diversity. Such models should make it possible to manage genetic diversity in a wide range of species, even when data are not available. Discovering the "rules" underlying the distribution of genetic diversity in situ and ex situ can lead to more efficient conservation.

We focus on three threatened oak species that occur in the Southeastern United States, a hotspot for genetic and species richness for plant taxa, including oak species (Figure A1). This region is of particular conservation interest in light of numerous threats including land use change, increasing drought and high temperatures, changing fire regimes, invasive

species, urban and suburban sprawl, and extreme weather events [27]. Our focal species for this study—*Quercus boyntonii* Beadle (critically endangered [19]), *Q. oglethorpensis* W.H. Duncan (endangered [19]), and *Q. georgiana* M.A. Curtis (endangered [19])—fit all three categories of rare as outlined by Rabinowitz [33]; small range sizes, high habitat specificity, and low abundance (Table A1). Each species has less than 2000 mature individuals remaining, which are distributed in a few fragmented populations, facing the aforementioned threats. All species are considered habitat specialists (*Q. boyntonii* on sandstone rock outcrops and steep hillsides, *Q. georgiana* on granite rock outcrops, and *Q. oglethorpensis* on poorly drained marshlands or creek bottoms, Table 1), but have differing geographic distributions and habitat requirements (Table 1, Figure 1).

Table 1. Overview and comparison of each of the three species in this study showing geographic range, area of occupancy (AOO), extent of occurrence (EOO), number of adults estimated in the wild, and current population trends. Data compiled from IUCN Red List of Threatened Species.

Species	IUCN Listing; Habitat and Geographic Range	AOO (km^2)	EOO (km^2)	Number of Adult Trees	Population Trend
Quercus boyntonii	Critically Endangered; restricted occurrence in Alabama, restricted to sandstone outcrops and glades, and steep dry hillsides	24	4157	50–200	Stable
Quercus georgiana	Endangered; restricted to isolated granite outcrops and flat-rocks in the Piedmont Plateau of the Southeastern United States.	72–272	16,570–21,600	Unknown	Decreasing
Quercus oglethorpensis	Endangered; disjunct distribution; small clusters of localities in Louisiana, Mississippi, and Alabama, and a more extensive distribution from Northeast Georgia into South Carolina	180–3000	130,000	1000	Decreasing

In these three oak species, we ask whether range size, population size, and environmental variables correlate to genetic diversity within and among populations in situ. We then ask to what degree genetic diversity is preserved in botanic gardens and if this correlates to geographic range size. Specifically, we aim to:

- Assess levels of genetic diversity and differentiation in our focal species and compare them to other rare and common oak species (hypothesis: common oak species have more genetic diversity);
- Determine if genetic diversity and differentiation correlate with the relative range size of the three oak species (hypothesis: larger range size correlates to higher genetic diversity and higher genetic structure)
- Determine if genetic diversity and differentiation are correlated with demographic (e.g., population size) and/or environmental variables (hypothesis: lower genetic diversity in smaller populations; genetic diversity correlates with environmental variables);
- Quantify the amount of genetic diversity of each species that is conserved ex situ and determine whether this is correlated with features of those species (hypothesis: genetic diversity should be predicted by both the number of plants ex situ and the commonness of the species).

With 31% of the United States' oak species now considered of conservation concern, results from this study should assist in assessing genetic diversity of the many other threatened oak species and designing management strategies for them, especially those for which genetic diversity is not available [18].

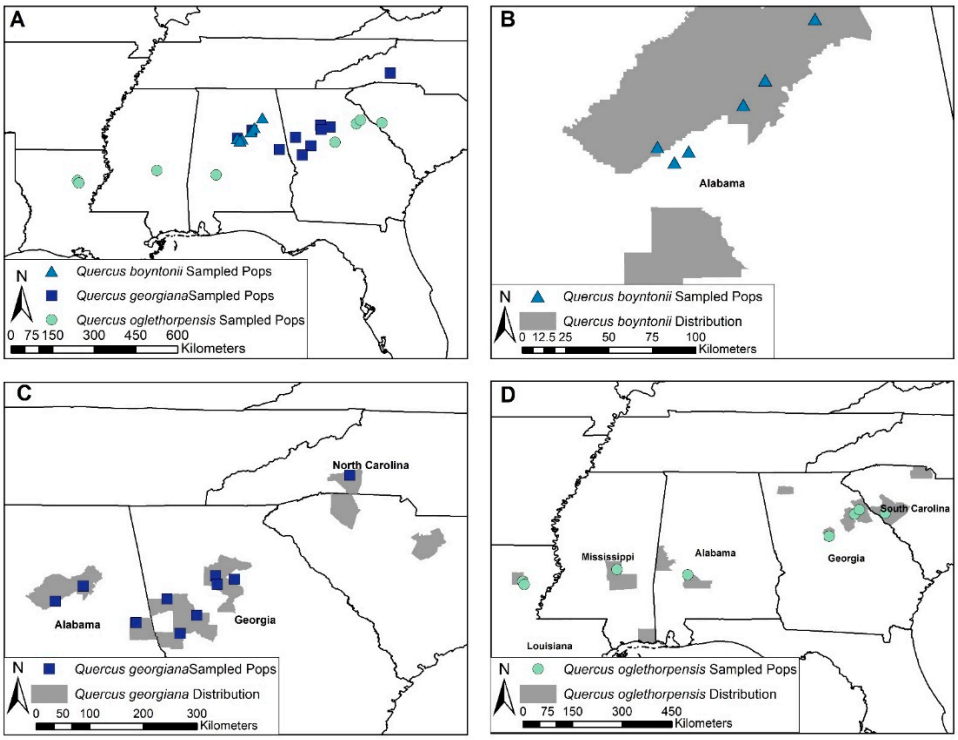

Figure 1. Sampled locations in relation to species geographic range; note that most extant occurrences were sampled. Range is based on county-level occurrences and is derived from USDA PLANTS. Panel (**A**) shows the sampling distribution of all three oak species included in the study; (**B**) depicts the known distribution of *Q. boyntonii* and sampled locations; (**C**) depicts the known distribution of *Q. georgiana* and sampled locations; (**D**) depicts the known distribution of *Q. oglethropensis* and sampled locations.

2. Materials and Methods

2.1. Study Species, Sampling, and Genotyping

These data were collected and used for a previous study answering different questions/in a different application [13]. Previously the data, along with genetic data of eight additional taxa of woody plant, were used to examine genetic diversity ex situ compared to in situ. We briefly described sampling and genotyping methods here for each focal species; complete collection and genotyping methods can be found in Hoban et al. 2020 [13]. For each species, we collected in situ samples from as many known populations as possible, and selected only trees representative of typical leaf morphologies for each focal species to avoid any possible hybrids. However, it is possible some hybrids were sampled because gene flow among oak species can occur (see Discussion). To find ex situ samples, we used Botanic Gardens Conservation International PlantSearch (https://members.bgci.org/data_tools/plantsearch, accessed on 2017), a global database of more than 1000 botanic gardens and their collections.

Quercus boyntonii (Alabama sandstone post oak) is endemic to Alabama (USA.), although historical records say that it formerly grew in Texas [34]. It is a shrub or small tree, sometimes reaching a height of 6 m, but usually smaller. *Q. boyntonii* was sampled ex situ from 16 botanic gardens and arboreta that have *Q. boyntonii* in their collections, totaling 87 individuals. In situ individuals were sampled in natural preserves, private property, and suburban parks. In situ population sizes ranged from fewer than 10 to more than 100 trees. Occurrences of the species are patchy, coinciding with suitable remnant habitat: sandstone outcrops, ridges, and slopes. We sampled 246 in situ samples (227 included in final analysis after clones were removed). In situ samples were collected during May 2017, and ex situ samples were collected between April and September 2017. Due to the patchiness of habitat, occurrence, and wind pollination it is challenging to delimit

strict "populations". For these analyses, we used 8 km distance to delineate populations in instances of continuous distributions. We genotyped all individuals using 11 neutral microsatellites from previous studies in oaks. Extraction, testing of the larger panel of markers from which our microsatellites were drawn, and genotyping are discussed in detail in Hoban et al. 2020 [13].

Quercus georgiana (Georgia oak) is native to the Southeastern United States, mainly in Northern Georgia, but with additional populations in Alabama, North Carolina, and South Carolina. It grows on dry granite and sandstone outcrops of slopes of hills at 50–500 meters' altitude [35]. *Quercus georgiana* is a small tree, often shrubby in the wild, growing to 8–15 m tall. *Quercus georgiana* was sampled from nine populations across the known range of the species through the use of herbarium records, collection data from botanic garden records, and USDA PLANTS Database (USDA 2012). All sampled populations were separated by at least 15 km. A total of 226 samples (223 were retained with sufficient genetic data) were sampled in June 2011. At least 24 individual trees were randomly sampled from each site, and sampled plants were at least five meters apart. Seventeen botanical institutions in the United States, France, and Belgium shared samples, totaling 36 individuals. Eight nuclear and 11 expressed sequence tag (EST) microsatellite markers were used for genotyping, following extraction and genotyping methods detailed in Hoban et al. 2020 [13]. We expect EST microsatellites to have lower polymorphism information content as they are associated with transcribed regions of DNA [36]. Nuclear and EST markers were assessed separately for analysis.

Quercus oglethorpensis (Oglethorpe oak) is a long-lived woody plant endemic to the Southeastern United States. Extant and largely fragmented wild populations are documented in South Carolina, Georgia, Alabama, Mississippi, and Louisiana. *Q. oglethorpensis* has a disjunct distribution across its range, with smaller clusters of localities in Northeast Louisiana, Southeast Mississippi, and Southwest Alabama, and a more extensive and well-known distribution from Northeast Georgia across the border into South Carolina. It grows to up to 25 m in height, and has leaves that are flat, narrowly-elliptical and usually without lobes. We prioritized sites with the most up-to-date occurrence data that was gathered in July 2015 during a germplasm collection effort [37]. We included additional sites not visited during the collection effort so that the greatest geographic distribution could be sampled. Sampled populations were separated by at least 9 km. Eight in situ populations were visited for a total of 191 samples (187 were retained with sufficient genetic data). Ex situ samples were collected from 145 trees, representing 16 botanic gardens around the world. All samples were genotyped with 10 nuclear microsatellite markers following extraction and genotyping methods in Hoban et al. 2020 [13].

2.2. Analysis: Basic Statistics

We used the R v 3.6.3 (R Core Team, Vienna, Austria) package adegenet version 2.1.2 to convert genepop files to genind and genpop formats. We used the R package poppr version 2.8.3 [38] to identify potential clones (and to remove clones/duplicate genotypes before any other calculations), expected heterozygosity, number of alleles, and allelic richness; hierfstat version 0.4.22 [39] to calculate pairwise population F_{ST} values; diveRsity version 1.9.9 [40] to calculate observed heterozygosity and inbreeding coefficient (F_{IS}); and Demerelate version 0.9.3 to calculate measures of relatedness [39–44]. We also tested for signatures of recent bottlenecks using the heterozygote excess test in the BOTTLENECK software [45] with both the infinite allele model and the two phase model and the mode shift test. We performed an ANOVA with species as the factor and population as the unit of analysis, to test for differences among species in the main summary statistics. For this and subsequent tests we only used the nuclear SSRs because EST-SSRs have much lower heterozygosity and allelic richness and we only had them for one species. We also tested for isolation by distance with linear regression of genetic distance (F_{ST}) on geographic distance among populations. All analysis scripts are available at https://github.com/smhoban/SE_oaks_genetics (accessed on 15 February 2021).

2.3. Influence of Environment on Genetic Diversity and Differentiation

We obtained 19 standard bioclimatic variables from WorldClim 2.0 at a resolution of 2.5 min [46]. To determine if there is a relationship between local climatic variables and population-level genetics, for each species, we performed ordinary least squares linear regression [47] of each climatic variable on each of four basic population genetic summary statistics that we may expect to respond to local climate: expected heterozygosity, allelic richness, F_{ST}, and relatedness. All analysis scripts are available at https://github.com/smhoban/SE_oaks_genetics (accessed on 15 February 2021).

2.4. Influence of Local Population Size

Following previous work [48–51], we calculated the percentage of genetic diversity conserved as the proportion of extant in situ alleles preserved in ex situ collections. We focused on alleles existing in the wild; we did not count alleles existing only in botanic garden collections. These data were previously presented in Hoban et al. 2020 [13] but were analyzed in a different context: comparing genetic diversity in ex situ collections among different genera and without regard to species range sizes.

3. Results

3.1. Basic Results

Genetic summary statistics for all three species include: *N* of samples genotyped, genetic diversity, measured as expected heterozygosity (H_e) and allelic richness (A_r), genetic differentiation (pairwise F_{ST}) and relatedness (R), and estimated population size (Table 2). We only present one relatedness estimator [43], but the patterns were similar for all three measures tested. No populations showed significant bottleneck signatures using the heterozygote excess test and the two-phase model, although one population of each species did show a signature of a bottleneck using the heterozygote excess test and the infinite allele model. The smallest population of *Q. oglethorpensis* showed a "mode shift", though no heterozygote excess. For *Q. georgiana* a bottleneck signature was observed for five populations (half of the populations) but only for the EST-SSRs. No bottleneck was detected for *Q. boyntonii* or *Q. georgiana* with neutral microsatellites. *Q. oglethorpensis*, the largest-ranged species we sampled, and was the only species which showed significant isolation by distance.

Comparing these three rare species to a set of other *Quercus* studies, we found that the rare oaks in this study had among the lowest heterozygosity, and that *Q. oglethorpensis* had an exceptionally high inbreeding coefficient (F_{IS}, Table A1).

Table 2. Summary statistics for each population and the average across populations. Reported is the population name (Pop name), the state the population is located (State, specific locality data is not provided given the rarity of the species), the number of samples (*N* samples genotyped) and number of unique multilocus genotypes (unique MLG), the expected heterozygosity (H_{exp}), allelic richness (A_r), mean pairwise F_{ST}, relatedness (Rel), and estimated number of trees based on direct observations in the field (Pop size est.).

Species	Pop Name	State	*N* Samples Genotyped (Unique MLG)	H_{exp}	A_r	Mean Pairwise F_{ST}	Rel	Pop Size est.
Q. boyntonii	IMLS032	AL	14 (12)	0.581	5.02	0.023	−0.014	20
	IMLS048	AL	17 (15)	0.605	5.45	0.029	0.234	30
	IMLS068	AL	22 (22)	0.605	4.84	0.027	0.024	25
	IMLS138	AL	12 (11)	0.593	3.51	0.043	0.013	5
	IMLS280	AL	83 (76)	0.642	5.85	0.015	0.05	165
	IMLS244	AL	60 (60)	0.63	4.78	0.023	0.134	150
	IMLS307	AL	30 (30)	0.651	6.02	0.025	−0.013	70
	AVERAGE	–	34 (32)	0.615	5.07	0.026	0.061	66

Table 2. *Cont.*

Species	Pop Name	State	*N* Samples Genotyped (Unique MLG)	H_{exp}	A_r	Mean Pairwise F_{ST}	Rel	Pop Size est.
Q. georgiana	MR24	AL	24	0.738	7.355	0.051	0.031	24
	EDEN32	AL	25	0.624	7.739	0.056	−0.056	50
	Pen32	AL	26	0.736	7.506	0.062	0.156	50
	CB32	GA	24	0.709	6.827	0.049	0.075	100
	DK32	GA	25	0.677	7.503	0.046	0.123	100
	CR31	GA	24	0.677	6.656	0.056	0.069	50
	SM32	GA	26	0.732	8.114	0.042	0.167	100
	am29	GA	25	0.803	9.706	0.039	0.131	100
	WGHerb	GA	25	0.769	8.993	0.044	0.415	30
	COP-9	NC	26	0.588	5.03	0.074	−0.043	200
	AVERAGE	–	25	0.705	7.54	0.052	0.107	80
Q. oglethorpensis	RMS-9	LA	14	0.645	5.9	0.069	0.017	200
	BIE-3-7	MS	33	0.694	6.03	0.052	−0.025	50
	CAT-9	AL	27	0.639	6.25	0.054	0.075	60
	MOT-9	GA	31	0.653	5.56	0.073	0.172	500
	BUF-9	GA	29	0.62	5.75	0.059	0.008	50
	SUM-9	SC	28	0.648	6.06	0.055	0.028	40
	AVERAGE	–	27	0.65	5.93	0.06	0.046	150

3.2. Genetic Diversity and Range Size for Our Three Rare Oaks

Range size shows some relationship to heterozygosity and allelic richness, in that *Q. boyntonii* (the most geographically restricted species) had the lowest heterozygosity and allelic richness (Figure 2). However, *Q. georgiana* had the highest heterozygosity even though its range size was moderate. Range size strongly related to genetic differentiation as measured by F_{ST}. All ANOVA test comparisons were significant except *Q. georgiana*, *Q. oglethorpensis* for allelic richness and *Q. boyntonii*, *Q. oglethorpensis* for allelic richness and expected heterozygosity.

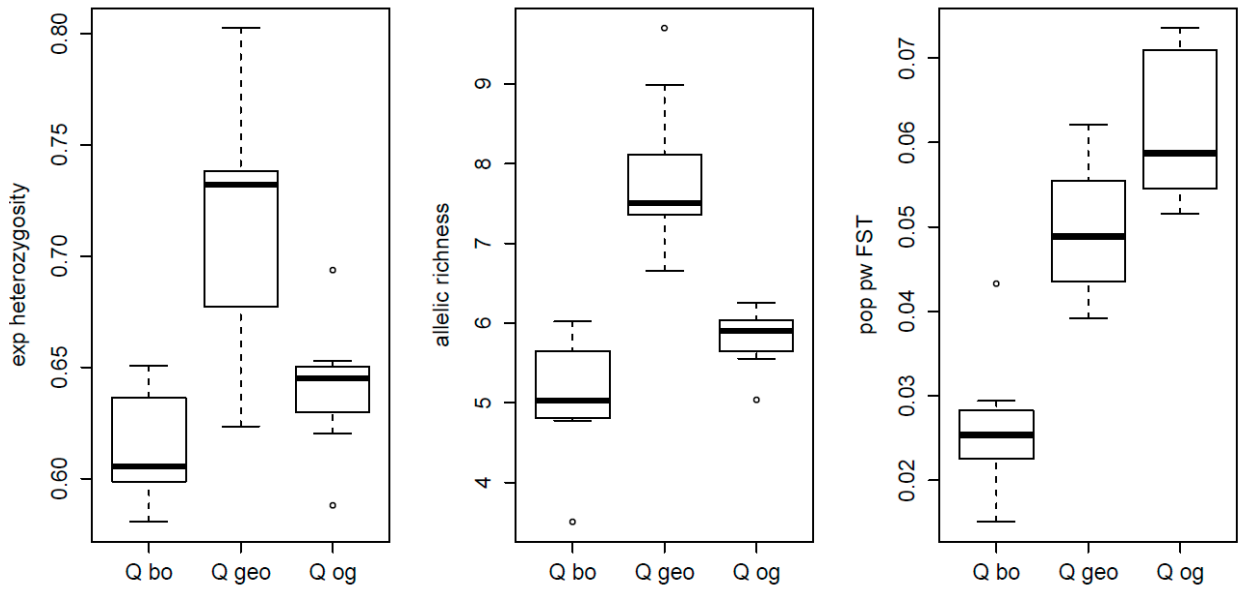

Figure 2. Species genetic diversity boxplots across populations comparing expected heterozygosity (exp heterozygosity), allelic richness, and F_{ST}. (pop pw F_{ST}) Species are arranged by range size, with the smallest range on the left (*Q. boyntonii*, "Q bo"), followed by *Q. georgiana* ("Q geo"), and the largest species range on the right (*Q. oglethorpensis*, "Q og"). Only nuclear SSRs are included in this analysis.

3.3. Genetic Diversity and Range Size within Each Species

Genetic diversity and differentiation statistics are presented in Appendix B for populations above and below N_c of 50 individuals. For two species, *Q. boyntonii* and *Q. georgiana*, the trends were as predicted, with allelic richness and heterozygosity generally higher in larger populations and F_{ST} generally lower for larger populations (Figure A2). Though statistically significant *p*-value differences were observed in only a few comparisons, all other comparisons were not significantly different (Figure A2). Additionally, in these two species, relatedness generally showed no difference. For the third species, *Q. oglethorpensis*, the opposite pattern was observed, with lower genetic diversity, higher differentiation and higher relatedness in larger populations.

3.4. Genetic Diversity and Environment

Genetic diversity and differentiation were not related to climate variables for *Q. georgiana* and *Q. oglethorpensis* when testing all 19 bioclimatic variables at 2.5 min—none were significant after correcting for multiple testing.

3.5. Genetic Diversity in Ex Situ Collections

The percentage of genetic diversity currently preserved in ex situ collections is shown in Table 3. The percentage was not clearly related to species range size or to the number of ex situ samples; although *Q. boyntonii* had the smallest range and moderate number of samples, it had the lowest genetic diversity ex situ. Note that EST diversity was not conserved (Figure A3).

Table 3. Percent allele capture in ex situ collections. The percent of alleles conserved in ex situ collections, for different allele frequency categories, for each species. Allele frequency categories are: all (all alleles); very common alleles (>10%); common alleles (>5%); low (<10% and >1%); and rare (<1%). For rare alleles and all alleles, two results are presented, percentage captured when all alleles including those with fewer than two occurrences are included (complete data), and when alleles with one or two occurrences are excluded (reduced data, shown in parentheses).

			Allele Frequency Category (%)				
Species	***N* Plants Ex Situ**	**Geographic Range Size**	**All**	**Very Common**	**Common**	**Low Frequency**	**Rare**
Q. oglethorpensis	145	large	78 (94)	100	100	97	37 (67)
Q. georgiana (EST-SSR)	36	medium	61 (68)	100	85	51	33 (33)
Q. georgiana (nSSR)	36	medium	69 (76)	100	100	75	35 (41)
Q. boyntonii	77	small	60 (70)	100	100	66	32 (29)

3.6. Other Observations

We only identified clones in *Quercus boyntonii.* For this species we often observed small "rings" or clusters of stems, sometimes 5 or more meters across. We found 12 pairs of clones, which werealways were adjacent individuals, either stems sampled immediately next to each other or within a few meters.

As expected, we found that EST-SSR markers had lower diversity than nuclear SSR markers, with heterozygosity and the number of alleles being 19% and 14% lower on average, respectively.

4. Discussion

Our study tested the influence of range size, environmental and demographic variables on genetic diversity, and differentiation in three rare oak species. Our main findings are as follows. (1) These three rare species generally have lower genetic diversity than more common oaks previously studied, and range size relates strongly to genetic differentiation but less strongly to genetic diversity. (2) In spite of relatively small numbers of populations

available, due to the rarity of these species, we found that in some cases small population size and geographic range may correlate with some metrics of genetic diversity and differentiation. (3) We also found that genetic diversity currently conserved varies among species of comparable geographic range size and numbers of samples preserved. Thus, our study supports the idea that "rarity" and collection history are not sufficient to explain genetic diversity in ex situ collections: the amount of genetic diversity preserved is also a function of intrinsic biology, demography, or life histories that vary independently of rarity.

We first present our observations in the context of rare and common species in the genus. Many population genetic studies have been performed in *Quercus* [52,53]. Genetic diversity is often summarized using allelic richness and heterozygosity. Expected heterozygosity was lower in our study (less than 0.65 for most populations, and a mean of 0.641 for *Q. oglethorpensis*, 0.615 for *Q. boyntonii*, and 0.72 for *Q. georgiana*) than was observed in other oaks, which typically had heterozygosities between 0.7 and 0.9 (Table A2). However, some common oaks were observed with lower genetic diversity (e.g., *Q. phillyreoides*, H_e = 0.535) and some rare oaks were observed with higher genetic diversity (e.g., *Q. pacifica* (H_e = 0.851) and *Q. hinckleyi* (H_e = 0.853) as estimated using microsatellites. Some oaks were postulated to be naturally rare (e.g., *Q. boyntonii*), others were more likely to be rare due to human disturbance (e.g., *Q. arkansana*), and others were increasing in rarity for a long time (e.g., *Q. hinckleyi*) [27]. Due to the relatively long-lived nature of most oak species (100+ years), it is possible that recently rare oaks may take a long time to show the subsequent genetic impacts of a drop in population size and narrowing ranges that are associated with their increasing rarity. This form of "extinction debt" has been shown in simulations [12,54], while more naturally rare oaks would not be expected to show such genetic impacts. The relatively low genetic diversity in the species we studied may be due to relatively low population sizes over multiple generations.

Comparing genetic diversity statistics for these three species with different range sizes we see that *Q. boyntonii* has lowest heterozygosity and allelic richness as expected based on small range and highest endangerment status. However, *Q. oglethorpensis* and *Q. georgiana* had relatively equal allelic richness, and *Q. georgiana* had the highest heterozygosity even though its range size was moderate. It is not surprising that overall range size was only a moderate predictor of genetic diversity, as it is the local effective population size that influences retention of genetic diversity within populations (see next section). The paucity of bottleneck signatures may suggest the species have not suffered bottlenecks, or that bottleneck signatures have not had time to develop (as in other species with known, recent population collapses, e.g., *Juglans cinerea*, [55]); bottleneck tests are unreliable for recent, moderate, or gradual bottlenecks [54].

On the other hand, F_{ST} is related to species range size for these three species: the smallest-range species (*Q. boyntonii*) had lowest F_{ST}, and the species with the largest range size and most general habitat preference (*Q. oglethorpensis*) had highest the F_{ST} (Figure 2). This conforms to population genetic theory regarding isolation by distance, whereby populations of a large range species have the most distance among them, and genetic distance is known to increase with geographic distance. Thus, in our study the influence of range size was much more apparent on among population genetic differentiation than on within population genetic diversity. Of course range size is not the only predictor of F_{ST}, factors such as connectivity can also be used to predict F_{ST}. For example, wide ranging oak species with high numbers of populations, and thus high gene flow, can show low F_{ST} (e.g., in *Q. macrocarpa* [56]).

According to conservation genetic theory we would expect that populations near or below a population size of 50 individuals would be subject to strong genetic drift. The exact threshold for a population to rapidly suffer detrimental genetic consequences has been hotly debated [7,57,58], but here we focused on 50 individuals. For our study we would predict lower allelic richness and heterozygosity, and high F_{ST} and relatedness in such populations. We see this predicted pattern in *Q. georgiana* and *Q. boyntonii*, though comparisons were significant or nearly so only for F_{ST} in *Q. georgiana* (all loci *t* test 0.055,

Wilcox 0.063; ESTs *t* test 0.04, Wilcox 0.063) and heterozygosity for *Q. boyntonii* (*t* test 0.003, Wilcox 0.057). The relatively low number of significant values emphasizes the small number of replicate populations (inherent in rare species) and the fact that for very recently reduced populations, genetic diversity impacts may not yet have accumulated [55].

Interestingly, for *Q. oglethorpensis* all statistics are in the opposite direction of what might be predicted based on a large population size (higher F_{ST}, higher relatedness, lower heterozygosity, and lower allelic richness). It is not clear why *Q. oglethorpensis* shows this pattern. This could be a result of fragmentation coupled with the fact that *Q. oglethorpensis* grows predominantly as a subcanopy tree [59]. Although not well-studied in wind pollinated trees, subcanopy habit could possibly limit pollen dispersal [60]. However, this pattern would be consistent with recent expansions or founding populations, which would result in moderate population size but reduced genetic diversity and increased F_{ST}.

There was no relationship between environmental variables and genetic statistics. It is possible that for these species, neutral genetic diversity is more influenced by current population sizes, which may be impacted by processes other than environment, such as land development, loss of habitat, etc. It is also possible that neutral genetic diversity and demography are influenced by environment but at fine spatial scales and/or along unmeasured environmental axes. Useful future work will be to create ecological niche models for each species to test for the impact of habitat suitability/probability of occurrence in relation to genetic diversity [61–63]. All three species are habitat specialists with typically very restricted populations.

Although the very common and just common alleles are preserved well in ex situ collections, low frequency and rare alleles are not conserved well, and overall, only a moderate amount of genetic diversity is preserved ex situ, between 60 and 78% for these species assuming all alleles are considered (68–94% if the rarest alleles are dropped). Previous modeling work suggests that the species with the largest range and highest F_{ST} would require the most samples [11,12]. *Quercus oglethorpensis* is preserved extremely well, at 78%, even though it has the largest range; it does have the most individuals ex situ. Less of the genetic variation of *Q. boyntonii* is conserved than of *Q. georgiana*, even though *Q. boyntonii* has the smaller range and about twice the number of individuals ex situ. Other studies of the genetic diversity conserved ex situ have primarily been species specific and we are only aware of a few attempts to determine if genetic diversity ex situ correlates to range size. In the plant genus: *Zelkova*, Christe et al. [64] found that a small-range endemic was less well conserved than a larger-range species. Several reasons can explain their similar findings: for the rare species, collectors may have revisited a single accessible site for seed collection, even though it occurred across high topographic and ecological diversity, while for the common species collectors in multiple countries had visited numerous populations. In other words, accessibility and availability of sampling are important to consider.

Although more than 3000 botanical institutions maintain more than 100,000 globally threatened species ex situ [65], the conservation value of these collections is unclear. Most taxa are held in a small number of collections, usually with a small number of inadequately documented accessions [66,67]. While some collections maintain relatively high levels of genetic diversity [68,69], research on the genetic representativeness of species in living collections is sparse. Our results emphasize that the genetic diversity conserved in collections is not only a function of the number of samples conserved, nor simply a function of the species inherent characteristics such as range size. Rather, the amount of genetic diversity conserved is likely a function of the interaction number of samples, range size, and collection strategy (such as which populations are visited, the spatial sampling within populations, the number of maternal plants collected from, etc.) [51,70]. While *Q. oglethorpensis* is conserved quite well, *Q. boyntonii* and *Q. georgiana* may need more individuals sampled to better represent in situ diversity.

Caveats

We used microsatellites because they are an affordable method to achieve an understanding of genetic diversity and structure. We recognize that increased resolution could be obtained with next generation sequencing techniques [71,72]. It is known that microsatellites that are developed in one species and applied in a different species can show reduced genetic diversity due to PCR amplification failure caused by mutations in primer binding sites. The markers applied to our species were all developed from other species of oaks, but were developed in *Quercus* subgenus *Quercus* sections to which they were applied (red oak markers from Section *Lobatae* for the one red oak species, and white oak markers from Section *Quercus* for the two white oak species). However, this is also the case for nearly all microsatellite studies of oaks: the majority of microsatellites were developed in European white oaks and then applied in diverse species (Table A2). It is also known that microsatellites are susceptible to ascertainment bias, such that the investigator will select markers that are polymorphic in a small sample of test individuals, such that less polymorphic markers are not included in the study. We did not have an a priori expectation that the patterns we saw between species were due to this reason, as this should apply to all oak species using these markers.

Other caveats involve the populations we studied. There are likely some populations of these species that we are unaware of, and we sometimes were not able to collect all of the individuals within a population. Moreover, genetic diversity in some populations may reflect gene flow from other species. It is known that gene flow among related oak species does occur, often at low levels and that hybridization may be even higher in species that have low population numbers due to the phenomenon of pollen swamping, where heterospecific pollen may far outnumber conspecific pollen [73,74]. For instance, in the extremely rare *Q. hinckleyi*, hybrids have been identified with genetic markers [52]. We did attempt to only sample individuals consistent with the phenotype of the target species. Of course, any of these caveats would obscure the patterns that we were testing for, and it is possible that if such caveats could be taken into account (for example identifying and removing all hybrids), the patterns we found here might be stronger.

5. Conclusions and Conservation Implications

We found that genetic diversity and differentiation were influenced by both population size and range size, but that patterns did not perfectly accord to predictions. This emphasizes stochastic processes and the influence of multiple factors on genetic diversity we see today (time, human influence, and population recovery). We also found that genetic diversity conserved ex situ was not well predicted by species geographic range size or number of samples, in contrast to theoretical predictions, and that two species need more samples ex situ. The overall low genetic diversity in these three rare oaks relative to more common oaks suggest that genetic diversity may also be low in other threatened oak species, a supposition to be tested by analyzing several more threatened oaks. We note that *Q. oglethorpensis*, in spite of its wide geographic range, had lower allelic richness and heterozygosity than might be expected—nearly as low as the critically endangered and small range *Q. boyntonii*—and thus might already be suffering genetic erosion in its isolated populations.

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Data Availability Statement: The data and code for analyses used in this study are openly available on the Github repository: https://github.com/smhoban/SE_oaks_genetics (accessed on 15 February 2021).

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Appendix A

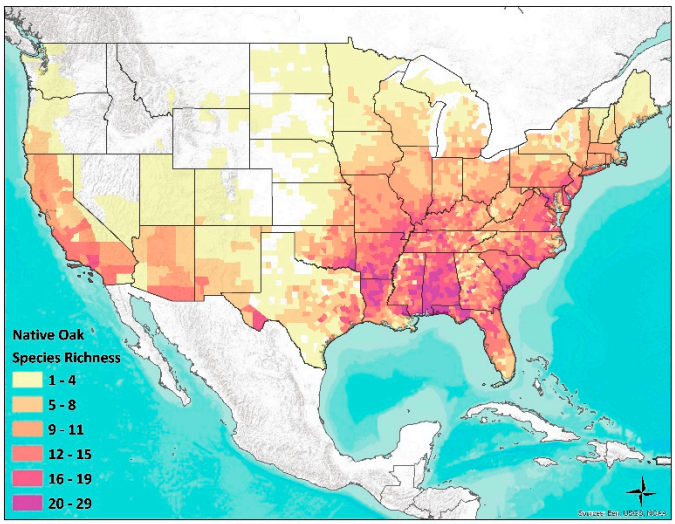

Figure A1. Native U.S. oak species richness by county. County level distribution data from USDA PLANTS and Biota of North America Program (BONAP) were combined to estimate species richness.

Table A1. The matrix of rarity as presented by Rabinowitz mapped with three rare oaks (*Q. boyntonii*, *Q. georgiana*, and *Q. oglethorpensis*) and three oaks which are common by two measures (geographic range and habitat) but rare in abundance (*Q. hemisphaerica*, *Q. incana*, and *Q. laevis*). The three rare species group together in the same rarity ranking, while the three common oaks group together in a different rarity ranking. Bold font indicates species is in the subgenus *Erythrobalanus* (red oak), regular font indicates species is in the subgenus *Leucobalanus* (white oak).

Geographic Range	Large		Small	
Habitat Specificity	Wide	Narrow	Wide	Narrow
Local Population Size				
Large, Dominate somewhere				
Small, non-dominant	***Q. hemisphaerica*** ***Q. incana*** ***Q. laevis***			*Q. boyntonii* ***Q. georgiana*** *Q. oglethorpensis*

Table A2. Literature review and comparison of previously published population genetic studies of oak species and species accessed in this study.

Species	Section	IUCN Status	AOO	EOO	# of Loci	# of Pops	Sample Size (*N*)	F_{ST}	G_{ST}	Mean Number of Alleles	AR	H_o	H_e	F_{IS}	Citation
Quercus phillyraeoides	*Cerris*	*NEN*	NR	NR	11	24	536	0.097	0.09	10.45	4.47	0.51	0.54	0.079	[75]
Quercus georgiana	*Lobatae*	*EN*	272	21,600	8	9	224	0.049	NR	8.03	7.82	0.72	0.69	0.019	-
Quercus rubra	*Lobatae*	*LC*	NR	4,150,115	10	23	980	0.044	NR	NR	5.5– 12.14	NR	NR	NR	[76]
Quercus berberidifolia	*Quercus*	*LC*	NR	250,000	8	2	60	NR	NR	18.6	5.72	0.85	0.88	0.039	[52]
Quercus boyntonii	*Quercus*	*CR*	24	4,157	9	7	238	0.026	NR	7.24	5.07	0.62	0.55	0.061	-
Quercus dumosa	*Quercus*	*EN*	620	12,500	8	2	24	NR	NR	11.1	4.9	0.77	0.8	0.029	[77]
Quercus hinckleyi	*Quercus*	*CR*	30	380	8	4	123	NR	0.03	13.06	5.15–14.73	0.81	0.85	0.036	[52]
Quercus lobata Née	*Quercus*	*NT*	NR	280,000	8	12	270	0.064	NR	8.12	NR	0.8	0.7	-0.196	[78]
Quercus macrocarpa	*Quercus*	*LC*	NR	4056,799	5	14	480	0.027	NR	11.184	NR	0.92	0.86	NR	[79,80]
Quercus oglethorpensis	*Quercus*	*EN*	3000	130,000	9	7	188	0.062	NR	6.81	5.8	0.64	0.48	0.234	-
Quercus pacifica	*Quercus*	*EN*	NR	3800	8	3	133	0.233	NR	15.8	5.54	0.81	0.85	0.05	[77]
Quercus chrysolepis	*Protobalanus*	*LC*	NR	920,000	8	7	100	NR	NR	16.4	4.63	0.71	0.83	0.146	[53]
Quercus tomentella	*Protobalanus*	*EN*	250	43,500	8	6	345	NR	NR	16.3	3.63	0.56	0.76	0.262	[53]
AVERAGE of previously studied species			300	1,079,677	8	10	305	0.093	0.06	13.45	4.82	0.75	0.79	0.056	
AVERAGE of all species			699	822,738	8	9	285	0.075	0.06	11.92	5.29	0.72	0.73	0.069	

Definitions: (F_{ST}): fixation index; (G_{ST}): Nei's estimation F_{ST} generalized for multiple alleles; (AR): Allelic richness; (H_O): observed heterozygosity; (H_E): expected heterozygosity; (F_{IS}): inbreeding coefficient; (NR): Not Reported. The IUCN status abbreviations are as follows: (NE): Not Evaluated; (LC): Least Concern; (NT): Near Threatened; (EN): Endangered; (CR): Critically Endangered.

Appendix B

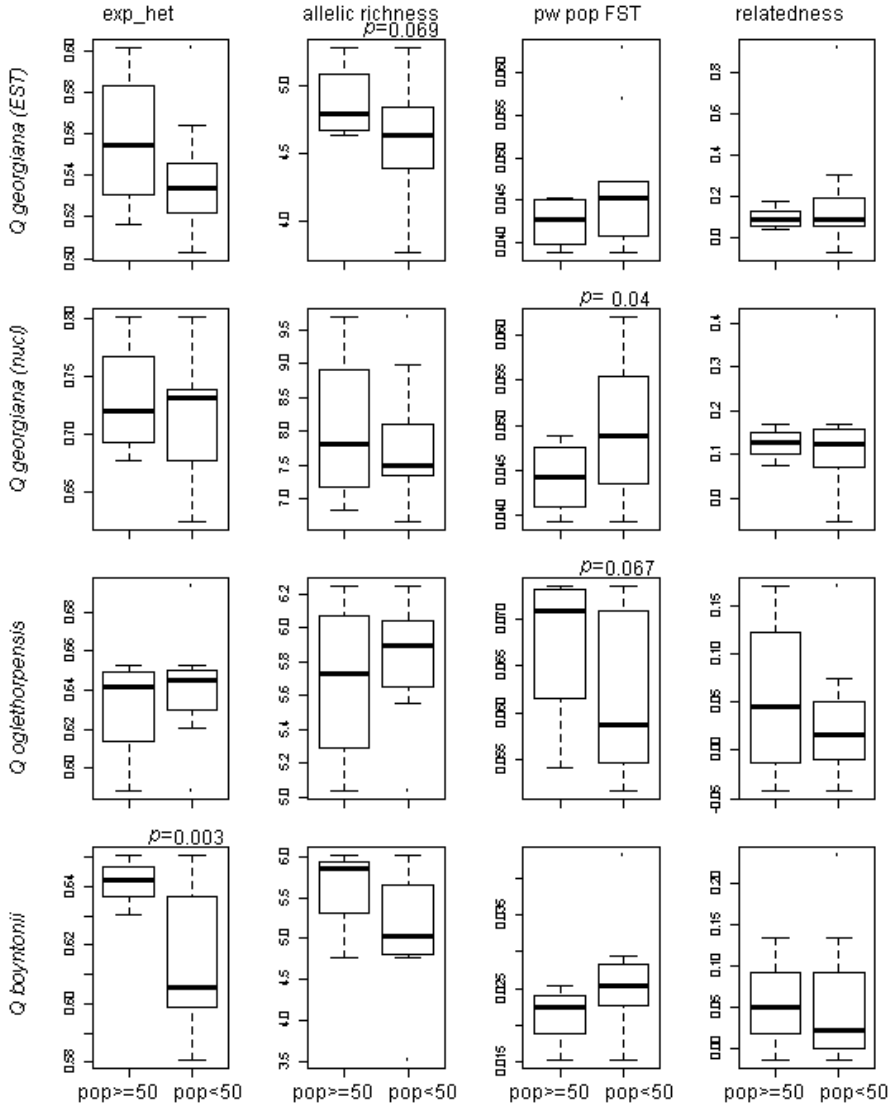

Figure A2. Box and whisker plots for each species by genetic summary. Each column of graphs represents different genetic summary statistics: (exp_het): expected heterozygosity; allelic richness; (pw pop FST): Pairwire population F_{ST}; and relatedness. Within each graph, the boxplot on the right represents populations with greater than or equal to 50 individuals (pop >= 50) and less than 50 individuals (pop < 50).

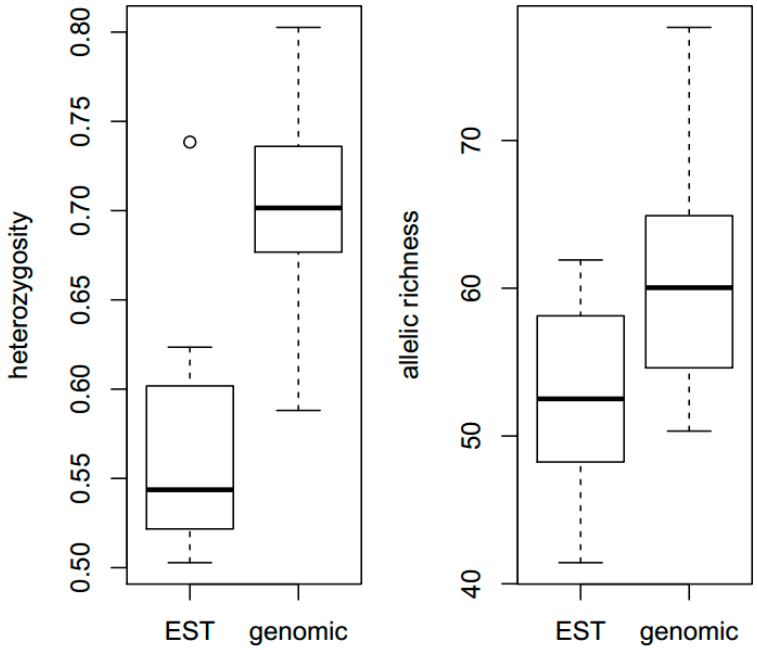

Figure A3. Box and whisker plots comparing heterozygosity and allelic richness between EST and genetic markers for the same species/samples.

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