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POPULATION GENETIC STRUCTURE IN *NOLINA BRITTONIANA* (AGAVACEAE), A PLANT ENDEMIC TO THE CENTRAL RIDGES OF FLORIDA

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ABSTRACT - *Nolina brittoniana* is endemic to the central ridges of peninsular Florida. Its scrub and sandhill habitats have suffered extensive anthropogenic modification. Analysis of isozymes from populations throughout its range revealed less genetic variation than generally reported for endemic plants. Populations were well differentiated, with significant clines in allele frequency along the north-south axis of distribution. Pair-wise F-statistics calculated at four levels of population geographic substructure revealed that current and inferred historical habitat patches had similar genetic structure. We found no evidence of recent bottlenecks or changes in genetic structure due to habitat loss and fragmentation, consistent with populations having always been small, isolated and low density. Our data support preservation of populations from throughout the species' range to meet conservation objectives.

INTRODUCTION

Nolina brittoniana Nash (Agavaceae) is a rare plant restricted to fire-maintained scrub and related habitats on the central ridges of the Florida Peninsula. It principally occurs on the Lake Wales Ridge, with scattered populations north and east onto the Orlando Ridge, west onto the Winter Haven Ridge, and a historical disjunct site in Hernando County (Florida Natural Areas Inventory 1990, Fig. 1). These ridges are regions of high endemism (Dobson et al. 1997), presumably due to persistent isolation of a relictual ancient dune flora (Watts and Hansen 1994). Some ridges have been continuously vegetated for over 2 million years (Watts and Hansen 1994). They are currently home to more than 20 federally listed endangered plants, dozens of endemic invertebrates, and 2 endemic vertebrates (Christman and Judd 1990, Deyrup and Franz 1994).

N. brittoniana is a mostly dioecious perennial herb with the potential for long distance gene flow via both pollen and seeds. It is pollinated by a wide variety of insects, including bees, dipterans, and butterflies (Menges, unpublished data); seeds have a primary dispersal distance of ca. 6.5m (Menges et al. 1998). Plants are widely scattered within populations of 20-50 individuals (median population size = 35). *N.*

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Figure 1. *Nolina brittoniana* distribution on Florida's Lake Wales Ridge (shaded) and nearby ridges, with collection sites and known populations. A single outlying population to the west is not shown.

brittoniana can resprout following fire and has relatively stable population numbers and low annual turnover (Slapcinsky et al. 1998).

The combined threats of habitat loss and fire suppression in scrub have reduced and fragmented already spatially-limited *N. brittoniana* populations, and likely have reduced connectivity via gene flow. Small, isolated populations are highly susceptible to extinction from stochastic events, and may suffer from the negative effects of inbreeding and genetic drift (Barrett and Kohn 1991, Ellstrand and Elam 1993, Oostermeijer et al. 1995), further imperiling population persistence. However, some studies have not found the predicted links among population size, genetic variation and fitness (e.g., Lammi et al. 1999, Ouborg and van Treuren 1995), suggesting the relationships among these factors may be complex, species-specific, or slow to develop.

We studied genetic variation using isozymes in 48 populations of *N. brittoniana* from throughout the species' range. We also conducted pairwise analysis of F-statistics to look for patterns of genetic structure at different spatial scales (all populations vs. populations clustered by geographic region) and by aggregating populations into current and historical habitat patches. If fragmentation has had an impact on genetic structure, the effect likely would be an increase in population differentiation as remaining populations are more isolated and have less inter-population gene flow (Wallace 2002). Our goal was to document patterns of genetic variation and to use this information for management and site acquisition recommendations.

METHODS

We collected leaf pieces from up to 30 plants (range = 13–30, mean = 27) in 48 populations (defined here as aggregates of plants separated by at least 50 m) for genetic analysis. Because *N. brittoniana* can reproduce clonally, we sampled rosette clumps (= individuals) separated from other clumps by at least 1 m. Preliminary studies showed that such plants did indeed have different isozyme genotypes and that rosettes from within a clump had the same genotype.

Leaves were wrapped in paper towels, placed in plastic bags, and shipped to the lab within 24 hr, where they were processed for horizontal starch gel electrophoresis. We extracted tissue using a modified Wendel's sorghum buffer (Morden et al. 1987). Phosphoglucosomerase (*PGI*, Enzyme Commission designation 5.3.1.9), isocitrate dehydrogenase (*IDH*, 1.1.1.41) and alcohol dehydrogenase (*ADH*, 1.1.1.1) were run on tris-citrate pH 7.2 (Kephart 1990). Menadione reductase (*MNR*, 1.6.99.-), triosephosphate isomerase (*TPI*, 5.3.1.1) and peroxidase (*PER*, 1.11.1.7) were run on a lithium hydroxide buffer system (Heywood 1980). Malate dehydrogenase (*MDH*, 1.1.1.37), aldolase (*ALD*, 4.1.2.13), 6-phosphogluconate dehydrogenase (*6PGD*; 1.1.1.44)

and *PGI* were run on a morpholine citrate buffer (Clayton and Tretiak 1972) at pH 6.1. Allele frequency data by population are available at www.butler.edu/herbarium or from the lead author.

Data were analyzed using GDA software of Lewis and Zaykin (1999) and Biosys-1 software of Swofford and Selander (1989), which calculated descriptive genetic statistics (percentage of polymorphic loci, number of alleles per locus, and observed and expected heterozygosity) for populations and for the species as a whole. All polymorphic loci were examined by population (5 loci $\times$ 48 populations for a total of 240 cases) for deviations from Hardy-Weinberg equilibrium (HWE), using standard goodness-of-fit chi-square tests. Both programs were used to calculate *F* statistics (Weir and Cockerham 1984; Wright 1951, 1978) to partition genetic variation. The amount of differentiation between populations is presented both as F_{ST} (calculated by Biosys-1) and θ_p (calculated by GDA) to allow comparison with published values for other species.

Pair-wise genetic similarities between populations were calculated using Nei's unbiased genetic identity (Nei 1978). Interpopulation gene flow (Nm) was estimated from population differentiation data with $Nm = ((1/F_{ST}) - 1)/4$ (Wright 1951). Clines in allele distributions were examined using rank order correlations between allele frequencies and north-south position of populations.

Allele frequency data were used to detect recent (in the past 2–4 generations) reductions in effective population size using the program BOTTLENECK (Piry et al. 1999). Recently bottlenecked populations have an excess of heterozygosity relative to that expected based on the number of alleles since alleles are expected to be lost more quickly than heterozygosity.

In order to detect the signature of pre-fragmentation genetic structure to look for this predicted increase, we grouped extant populations into historical habitat patches based on suitable soil type, clustering populations based on historic contiguity, rather than present-day proximity. Although we can never know the exact pre-fragmentation genetic structure, if there is a lag time between changes in geographic distribution, such as modern habitat losses or fragmentation, and genetic equilibrium brought about through recombination and drift as some have argued (Schmidt and Jensen 2000), our approach allows us to exploit this lag to look back in time.

Analysis of population structure was conducted using Wright's *F* statistics (Wright 1978) at four levels of population aggregation. Populations ($n = 48$) were our sampling units. The next two levels of population aggregation were based on the distribution of suitable soil types and current and inferred historical scrub habitat. Current patches ($n = 39$) combined all sampled populations that occupy the same patch of contiguous soil type currently supporting scrub habitats. Current habitat was

assessed by examining aerial photographs. In contrast, historic patches were inferred from pre-settlement distribution of suitable soil types, combined soil patches that were once contiguous, but that are now separated by agricultural or urban areas. Suitable soils were defined by assessing the current distribution of *N. brittoniana* relative to soil type from printed and digital maps (Menges et al. 2000). This analysis combined sampled populations into 18 inferred historic patches. The last category, region ($n = 3$), corresponded to the northern (Lake and Orange Counties), central (Polk and northern Highlands Counties) and southern (southern Highlands County) regions of *N. brittoniana*'s distribution. This category represented the largest breaks in the species' distribution.

RESULTS

Fifteen putative loci were clearly and consistently resolved for the 9 enzyme systems assayed for *N. brittoniana*. Ten loci were monomorphic: *Mdh-1* and *Mdh-2*, *Per-1*, *Mnr-1* and *Mnr-2*, *Tpi-1* and *Tpi-2*, *Idh-1* and *Idh-2*, and *Ald-1*. Only five were polymorphic at the species level.

Two loci, *6Pgd-1* and *6Pgd-2*, had two alleles each. Each allele was fixed in some populations and occurred at about equal frequency in others. *Adh-1* had three alleles; *Adh-1-b* was the most common in all populations, with two additional rare, lower frequency alleles sometimes present (range 0.02–0.21).

Pgi-1 had 5 alleles. Four (*a, b, c, d*) were common and occurred in high frequencies (> 0.50 for *b, c, d* and > 0.25 for *a*) in some populations. Allele *e* was rare in frequency and distribution, found in only 6 of the 48 or 12.5% of populations sampled. This allele occurred at a frequency of 0.20 in one population but otherwise it did not occur at greater than 0.07.

The putative *Pgi-2* locus possessed three alleles. This slower migrating *PGI* locus did not migrate past the origin but could be scored based on the banding pattern of its interlocus heterodimers formed with *Pgi-1*, an unusual phenomenon that has been reported at least once before (e.g., Chase et al. 1995 for *Cordia allodora*; Boraginaceae). *Pgi-2-b* was the most common allele, occurring at a frequency of > 0.84 in every population.

N. brittoniana had low values for species- and population-level genetic diversity. Percentages of polymorphic loci, average numbers of alleles per locus, and expected heterozygosities were lower than is commonly reported for plants (Table 1), including plants with limited geographic distribution (Hamrick and Godt 1989). For each measure, every *N. brittoniana* population had less genetic variation than plants in general (Table 1). Only 10.4% of populations had a larger number for percent of polymorphic loci than the average reported by Hamrick and Godt (1989) for a survey of endemic plants; only 20.8 had larger values for expected heterozygosity.

N. brittoniana populations had relatively high genetic similarity. The mean of Nei's genetic identity for pairwise comparison of populations was 0.95 (range 0.85–1.00). However, the proportion of variation present among populations was relatively large in comparison to that within populations; θ_p (equivalent to Wright's F_{ST} [Wright 1951]) was 0.36 and mean F_{ST} across all loci in all populations for the species, was 0.39 (Table 1).

We found considerable between locus heterogeneity. The *6Pgd-1* locus, with a θ_p value of 0.633, almost twice the mean of all loci, contributed greatly to the high degree of differentiation between populations (Table 2). F statistics further reveal positive (0.138) values for F_{IS} (fixation indices of individuals relative to subpopulations, Table 2), indicative of mild inbreeding. Gene flow, Nm , estimated from F_{ST} , was 0.38 migrants per generation.

Each of the five variable loci deviated from HWE in at least one population. Non-equilibrium frequencies were found in *Pgi-1* in 13 (27%), *Pgi-2* in 2 (4%), *6Pgd-1* and *6Pgd-2* each in 9 (19%), and *Adh-1* in 1 (2%) of the 48 populations sampled. More than half (25 or 52%) of

Table 1. Comparison of isozyme variation in *Nolina brittoniana* with values from a survey of plant species ($n = 473$) and endemic plant species ($n = 81$, from Hamrick and Godt 1989). Numbers in parentheses are ranges. θ_p and F_{ST} = the amount of differentiation among subpopulations, calculated by the software GDA and Biosys-1, respectively. $f(F_{IS})$ = the fixation indices of individuals relative to subpopulations. $F(F_{IT})$ = the fixation indices of individuals relative to the total population (see Wright 1978).

	<i>N. brittoniana</i>	All plants	Endemic spp.
Mean no. plants/locus/population	25.3 (13–30)	-	-
No. loci	15	16.5	17.8
Species level polymorphic loci (%)	33.3	50.5	40.0
Population level polymorphic loci (%)	18 (7–27)	34.2	26.3
Mean alleles/locus	1.27 (1.07–1.40)	1.53	1.39
Alleles/polymorphic locus	2.50 (2.0–4.0)	-	-
Expected heterozygosity	0.070 (0.027–0.117)	0.113	0.063
Observed heterozygosity	0.062 (0.020–0.122)	-	-
θ_p	0.36	-	-
F_{ST}	0.39	0.22	0.25
$f(F_{IS})$	0.14	-	-
$F(F_{IT})$	0.45	-	-

Table 2. F statistics by locus for 48 populations of *N. brittoniana*, with means and 95% confidence intervals (CI).

Locus	$f(F_{IS})$	$F(F_{IT})$	$\theta_p(F_{ST})$
<i>Pgi-1</i>	0.007	0.262	0.201
<i>Pgi-2</i>	0.179	0.216	0.044
<i>6Pgd-1</i>	0.283	0.767	0.633
<i>6pgd-2</i>	0.157	0.467	0.367
<i>Adh-1</i>	0.184	0.270	0.106
Mean	0.138	0.451	0.363
CI	0.084–0.263	0.257–0.693	0.178–0.583

the populations sampled possessed at least one locus that was not in HWE. Of the 34 cases of non-equilibrium frequencies, 29 had heterozygote deficits, whereas the five cases of heterozygote excess occurred in three populations.

We found several clines in *N. brittoniana* allele frequencies. Significant rank correlations (Kendall's tau) were found between north-south position of populations and the frequency of the *PGL-1a* ($\tau = 0.39$, $p < 0.001$), *PGL-1e* ($\tau = 0.02$, $p < 0.05$) and *6PGDa* ($\tau = 0.26$, $p < 0.010$) alleles.

The program BOTTLENECK detected no significant excess of heterozygosity in any of the study populations, indicating they have not experienced recent reductions in effective population size or genetic bottlenecks.

Pair-wise *F* statistics were calculated for four levels of population geographic substructure (Table 3). Most values showed appreciable differentiation between the levels compared. The greatest amount of genetic differentiation was found among populations in relation to the total sample (0.392). That is, nearly 40% of genetic diversity occurred among populations, a significant amount. When compared to the total, populations clustered into extant (0.367) and historic patches (0.289) showed similarly strong differentiation, reflecting similar genetic structure. Very little differentiation occurred among populations within extant patches (0.041). The low differentiation between the region and total levels (0.046) reflected that most of the structuring occurred on a much smaller than regional scale. Extant populations within their respective historical patches were less differentiated, and therefore more similar, than were populations to the total (0.110 vs. 0.392).

DISCUSSION

Where a plant fits on the genetically diverse–genetically depauperate continuum is influenced by the many-faceted features of its life history and

Table 3. Pair-wise *F*-statistics (Wright 1978), combined across loci ($n = 5$). Larger values reflect greater differentiation between levels compared.

Comparison (n)	F-value
Population (48) - Total	0.392
Population (48) - Region (3)	0.364
Population (48) - Historic patch (18)	0.146
Population (48) - Extant patch (39)	0.041
Extant patch (39) - Total	0.367
Extant patch (39) - Region (3)	0.336
Extant patch (39) - Historic patch (18)	0.110
Historic patch (18) - Total	0.289
Historic patch (18) - Region (3)	0.255
Region (3) - Total	0.046

habitat. *N. brittoniana* had low levels of genetic variation despite possessing several life history characteristics that are generally associated with relatively high genetic variation (Hamrick et al. 1991): a subdioecious gender system with nearly obligate outcrossing by pollinators capable of interpopulation flight (Menges et al. 2000), stable demography (Slapcinsky et al. 1998), and longevity (> 10 years, Menges et al. 1998).

However, there are other characteristics of *N. brittoniana* that may lead to the erosion of genetic variation. Population sizes are small (generally < 50 plants) (Menges et al. 1998) and historical gene flow, estimated from F_{ST} (at the population level), is less than one individual per generation ($Nm = 0.38$). *N. brittoniana* is also a narrow endemic, nearly entirely restricted to the Lake Wales Ridge. These traits may promote the loss of genetic variation through the combined effects of inbreeding and drift (Barrett and Kohn 1991). We did find evidence of mild inbreeding in *N. brittoniana*, based on positive F_{IS} values.

Effects of small population sizes can be compounded in species like *N. brittoniana* where effective population size may be much lower than actual population size. That is, a population may have the genetic characteristics of a population of much smaller number if gender is skewed in favor of one sex or the other. In an extreme case in a related species, a population composed entirely of androecious plants was observed in the California chaparral native *Nolina bigelovii* (Torr.) S. Wats (Bauder 1993). This population has an effective population size of zero. In addition, many *N. brittoniana* plants are non-reproductive in any given year, with numbers of flowering plants decreasing quickly following an initial post-fire pulse in flowering (Menges et al. 1998). Effective population size would therefore be much smaller than actual census numbers for *N. brittoniana*, perhaps contributing to the low levels of genetic variation found in this study.

Phylogenetic history can also have an important influence on current genetic variation. In a recent review, Gitzendanner and Soltis (2000) found levels of diversity between rare and widespread congeners to be highly correlated. We do not know what levels of genetic variation are in other, more widespread species of *Nolina*; low levels of variation may be characteristic of the genus.

Although absolute levels of genetic variation are low in *N. brittoniana* when compared with other endemic plants in general, the species is more genetically diverse than most other endemics restricted to the Lake Wales Ridge. Menges et al. (2000) surveyed seven species of scrub endemics with a range of life history characteristics and found that *N. brittoniana* had the second highest level of genetic diversity. Only *Liatris ohlingerae* (Blake) BL Robins, an outcrossing, self-incompatible, perennial with fairly stable demography, was more genetically diverse. Comparable levels of genetic variation to those reported here

for *N. brittoniana* have been reported for an endangered clonal shrub of the Lake Wales Ridge, *Ziziphus celata* Judd & Hall (mean expected heterozygosity = 0.08) (Godt et al. 1997). However, Lewis and Crawford (1995) found more variation in the extremely geographically restricted Lake Wales endemic herb, *Polygonella myriophylla* (Small) Horton. In their study, 42% of loci were polymorphic and the mean gene diversity within the populations examined was 0.17.

Our isozyme data show mild inbreeding in *N. brittoniana*, based on F_{IS} . Within populations, random mating was evident, because most of the loci were in HWE (85.9%, 206 of 240 of cases). The large percentage was consistent with *N. brittoniana*'s outcrossing breeding system. Yet, this left 14.1% of cases deviating from HWE. Loci with non-equilibrium frequencies usually have positive fixation indices, indicating a deficit of heterozygotes, consistent with randomly mating subpopulations among which matings may be infrequent (aka the Wahlund Effect, Wahlund 1928) (Williamson and Werth 1999).

Our results are consistent with the possibility that populations of *N. brittoniana* have always been scattered, small and low-density and that the current genetic structure and low levels of genetic variation are not the result of anthropomorphic influences. We found no indication of recent reductions in effective population size. Further, the presence of up to 5 alleles at a locus in *N. brittoniana*, with most alleles being present at < 10% or > 90% frequency, also suggests the populations have not been subject to recent bottlenecks (Barrett and Kohn 1991 and papers reviewed therein). Our analysis of populations aggregated into historical and current habitat patches revealed similar patterns of differentiation. A higher F_{ST} for the current patch to total comparison than the historical patch to total would have indicated changes in genetic structure as a result of recent habitat fragmentation. These conclusions suggest *N. brittoniana* is a good candidate for long term conservation through the protection of extant habitat with only minor additional intervention.

Geographic distribution of alleles can be used to suggest critical sites for conservation (Frankel et al. 1995). We detected no unique alleles in *N. brittoniana* but did find some suggestion of genetic structure based on clines in allele frequencies. Spatial structure in isozyme alleles may parallel spatial structure in alleles directly linked to fitness and therefore be of conservation interest. A similar pattern of north-south variation occurs in another central ridge endemic plant, *Warea carteri* (Evans et al. 2000), but not in three other recently studied Lake Wales Ridge endemics (Dolan et al. 1999). Other researchers have documented genetic differences among populations of scrub endemic plants occurring in different patches (Evans et al. 2000, Lewis and Crawford 1995, MacDonald and Hamrick 1996) and have called for the preservation of multiple patches to maintain species' integrity and evo-

lutionary flexibility. Likewise, a study of *Sceloporus woodi*, an endemic Florida scrub lizard, found a very strong geographic pattern of mitochondrial DNA variation (Clark et al. 1999). Strong patterns have also been demonstrated in Florida Scrub-jays (MacDonald et al. 1999).

Our results show that populations of *N. brittoniana* should be protected on numerous sites along the north-south axis of the species' distribution to best conserve extant genetic variation. We further suggest that management practices that include prescribed burning to maintain or increase effective population size may be essential for the maintenance of genetic variation in *N. brittoniana*.

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