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RESEARCH ARTICLE

Spatial genetic structure of *Arundinaria appalachiana* (hill cane), an upland bamboo species endemic to the southern Appalachian Mountains, USA

Jonathan P. Evans[1] | **Ashley B. Morris**[2]

[1]Department of Biology, University of the South, Sewanee, Tennessee 37383, USA

[2]Department of Biology, Furman University, Greenville, South Carolina 29613, USA

Correspondence
Jonathan P. Evans, Department of Biology, University of the South, Sewanee, Tennessee 37383, USA.
Email: jon.evans@sewanee.edu

Abstract

Premise: Long-lived clonal plants that rarely or never reproduce sexually raise important questions about persistence, genetic diversity, and extinction risk. *Arundinaria appalachiana*, an upland bamboo endemic to the southern Appalachian Mountains, has never been observed to flower. We investigated the spatial genetic structure and demography of this species to assess how it persists and to inform its conservation status.

Methods: We used genotype-by-sequencing of microsatellite loci to characterize clonal structure in two populations separated by 19 km on the southern Cumberland Plateau in Tennessee, USA. Rhizome networks were mapped over 8 yr to estimate rates of clonal expansion and infer genet age. Ramet density was monitored annually to detect demographic changes.

Results: Both populations were dominated by a few large, ancient genets; the largest covered >30 ha and was estimated to be >1700 yr old. Clonal diversity was low, and no flowering was observed during the 9 yr study. Rhizome mapping showed slow expansion (0.12–0.30 m yr^{-1}) and local fragmentation. Ramet density declined significantly in one population, while remaining stable in the other.

Conclusions: *Arundinaria appalachiana* persists through clonal growth alone, forming extensive, ancient genets in the absence of sexual reproduction. This clonal longevity enables persistence, but the lack of genetic renewal raises concerns about long-term viability in changing environments. Our findings highlight the importance of understanding spatial genetic structure in clonal plant populations and its consequences for conservation planning.

KEYWORDS

clonal diversity, clone size, Cumberland Plateau, genet age, genotype-by-sequencing, microsatellites, ramet density, rhizome mapping

Many plant species persist for decades or even centuries through clonal growth, with little or no contribution from sexual reproduction (Harper and White, 1974). In such systems, vegetative proliferation maintains population structure and allows long-term survival of established genets (deWitte and Stöcklin, 2010). However, the absence of genetic recombination may constrain adaptive potential and reduce genetic diversity, increasing susceptibility to environmental change, disease, and demographic decline (Ellstrand and Roose, 1987; Honnay and Bossuyt, 2005). Somatic mutation and epigenetic variation may introduce some level of genetic differentiation (Dodd and Douhovnikoff, 2016), but these processes are unlikely to fully compensate for the loss of sexual reproduction. As a result, understanding the ecological and genetic consequences of prolonged clonality is essential for assessing extinction risk and informing conservation strategies for rare or declining species (Funk et al., 2012).

Bamboo are a diverse subfamily of rhizomatous grasses (>1600 species) that manifest an unusual clonal biology in that populations are composed of long-lived genets that episodically flower, set seed, then die, often synchronized at the landscape level (Janzen, 1976). The extended period of clonal expansion between flowering events can range from 30 yr to 150 yr (Franklin, 2004; Hodkinson et al., 2010), resulting in genets covering large areas of a landscape (10–20,000 ha; Abe and Shibata, 2012). The semelparous,

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synchronized mass flowering behavior of bamboo is believed to be controlled by a linear, endogenous clock that is immune to environmental influences (Janzen, 1976; Franklin, 2004; Abe and Shibata, 2012). Genet death following mass flowering is thought to result from the exhaustion of rhizome reserves (Janzen, 1976). Seeds are passively dispersed at the base of the dead parents and exhibit no dormancy mechanisms (Keeley and Bond, 1999).

Reproductive behavior in bamboo creates populations with non-overlapping generations of long-lived genets, composed of single, distinct cohorts. This has unique consequences for the spatial genetic structure of bamboo populations over time. Bamboo species exhibiting leptomorphic rhizome growth (length of internodes is greater than width) can proliferate ramets across a large area of habitat, often in discrete patches, creating three possible spatial genetic structures within populations: (1) each patch consisting of multiple, intermingled genets (Isagi et al., 2004; Franklin et al., 2008); (2) each patch consisting of one unique genet distinct from the next patch (Suyama et al., 2000; Mathews et al., 2009); or (3) a highly expansive genet extending across the landscape, fragmenting into many discrete genetically identical patches over time (Isagi et al., 2016). It is likely that spatial genetic structure in bamboo populations is linked to the length of time between flowering events and the age of cohorts. Genets that live longer have greater opportunity to expand clone size, and genet mortality is higher closer to the time of seedling establishment (Ma et al., 2013).

Arundinaria appalachiana Triplett, Weakley & L.G. Clark (hill cane, Poaceae) is a recently described (Triplett et al., 2006) leptomorphic bamboo species that is endemic to the southern Appalachian Mountains in the southeastern United States. It is currently known to occur in <50 counties in Alabama, Georgia, North Carolina, South Carolina, and Tennessee (Barger et al., 2018). Prior to 2006, it was not distinguished from its two North American congeners (*A. gigantea* and *A. tecta*), and little is known about its life history or ecology (Triplett et al., 2006). While flowering intervals have been reported to range from 20 yr to 50 yr in *A. gigantea* populations and from 4 yr to 7 yr in *A. tecta*, no *A. appalachiana* population has ever been observed to flower (Triplett et al., 2010). This lack of evidence for sexual reproduction has also been associated with the recently described *A. alabamensis* (Triplett, 2023). *Arundinaria* populations in North America have declined dramatically over the past century as a result of habitat loss, overgrazing, and fire suppression. Large monodominant patches of cane (called canebrakes) were once believed to have been maintained by an intermediate disturbance regime of fire and windthrow that promoted clonal growth in a positive feedback loop. Greenberg and Collins (2021) have speculated that, as with *A. gigantea* and *A. tecta*, fire was needed to maintain *A. appalachiana* populations. However, Oakman et al. (2021) showed that *A. appalachiana* abundance was actually lower in burn treatments than in control plots.

There is speculation that uniform stands of *Arundinaria* may represent single genetic individuals (Platt and Brantley 1997). Both Mathews et al. (2009) and Rajewski (2015) found that stands of *A. gigantea* generally contained one dominant genet—although additional, less frequent, genets were also commingled. While Triplett et al. (2006) speculated that clones of *A. appalachiana* are slow-growing and may persist for centuries, no studies have been published on the population ecology of this species.

Our research examined the spatial genetic structure of *A. appalachiana* populations within two watersheds on the southern Cumberland Plateau in Tennessee, where the species is distributed in discrete patches of ramets along streams and adjacent slopes. We used a genotype-by-sequencing data set of microsatellite loci to determine clonal and population genetic structure in these two watersheds. This analysis allowed us to estimate the distribution, size, and age of genets within the two populations. We also mapped *A. appalachiana* rhizome networks to determine the size and change in spatial position of integrated clonal fragments over time. The rate of clonal spread, combined with genetically determined clone size, was used to estimate genet age. Finally, we examined the change in ramet density within patches across our two populations and determined whether this varied as a function of genetic identity.

MATERIALS AND METHODS

Study areas

We studied two distinct populations of *A. appalachiana* located ~19 km apart on the surface of the southern Cumberland Plateau (Figure 1). One population was located on the Sewanee Domain, the campus of the University of the South in Sewanee, Tennessee (hereafter "Domain"; 35.216167 N, 85.955483 W), and the other within Franklin State Forest (hereafter "Franklin"; N35.073, W85.8456). Drainages within the study area create a subtle, variable topography of intermittent streams. Derived from underlying sandstone, the soils have a very high sand content that makes them nutrient poor (with low ion exchange capacity), drought prone, and highly acidic (Mays, 1991; Evans et al., 2016). The vegetation of both study sites was typical of the mature upland forest habitat of the Cumberland Plateau surface. Oaks (*Quercus alba*, *Q. coccinea*, *Q. montana*, and *Q. velutina*) and hickories (*Carya glabra*, *C. pallida*, and *C. tomentosa*) dominate the canopy, along with *Acer rubrum*, *Nyssa sylvatica*, and *Oxydendrum arboreum*. There is a distinct understory layer of shrubs and small trees, including *Sassafras albidum*, *Rhododendron* spp., *Vaccinium* spp., and *Viburnum acerifolium* (Ramseur and Kelly, 1981; Evans et al., 2016, 2019).

The two study sites have similar land-use history. Each site was subject to selective timber removal in the late 1800s and early to mid-1900s, but the original soil structure was retained, since neither site was subject to agricultural conversion (Cowan, 1970; Evans et al., 2016). Although the history of fire disturbance is incomplete, it is believed that the Plateau upland forest had a long association with

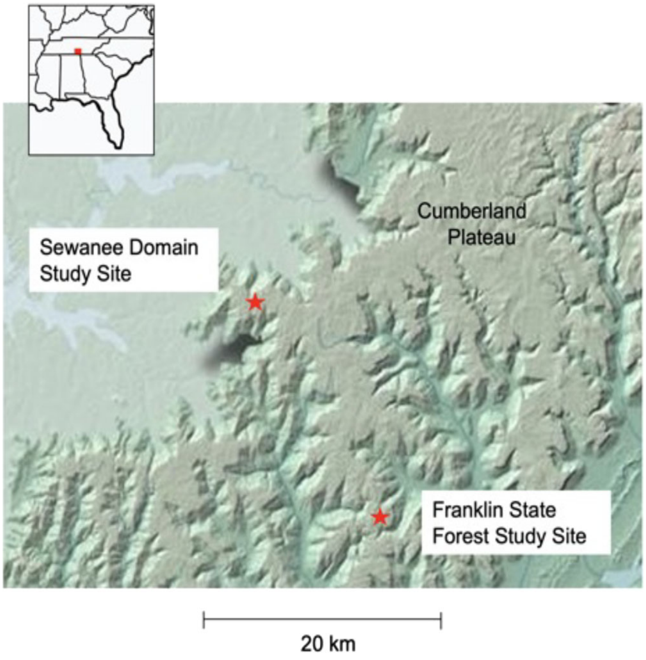

FIGURE 1 Locations of study sites on the southern Cumberland Plateau of Tennessee, USA.

anthropogenically derived fires, the frequency of which increased with the arrival of European settlers in the mid-1800s. There has been no recorded history of fire in either study area since the 1950s (Evans et al., 2016; Tennessee Division of Forestry, unpublished data).

Clonal fragment mapping

Within the Domain study site, six *A. appalachiana* patches were randomly selected from a population of patches identified in 2013. A patch is a discrete clump of ramets (culms) isolated by at least 1–2 m from the next nearest such clump. Each of these patches was excavated and mapped in 2014 and remapped 3 yr and 8 yr later, in 2017 and 2022. The rhizome structure of each patch was mapped by placing a 500 cm^2 circle plot in the area of highest ramet density near the center of the patch. Starting from this circle plot, all of the rhizomes within the circle plot were uncovered and mapped to their full extent. Locations of all living ramets along the uncovered rhizome networks were also mapped. Like Tomimatsu et al. (2020), we took care not to disturb the roots, rhizomes, or ramets while uncovering and mapping. In 2014, there was one intact clonal fragment associated with each of the six plots.

Comparison of rhizome maps for each plot over time (in 2014, 2017, and 2022) allowed us to determine the following: total rhizome length (total linear extent of living rhizome material that composed the fragment), total number of ramets per clonal fragment, rhizome persistence, and degree of rhizome fragmentation. By analyzing spatial change in the mapped rhizome networks, we were able to determine the extent of rhizome extension away from a 2014 point of origin for each clonal fragment over the course of the 8 yr study. From this snapshot in time, we calculated an annual rate of radial clonal expansion, which we used in our calculation of genet age (see below).

Spatial genetic structure

Within each study site, we used a nested hierarchical sampling scheme to examine the spatial genetic structure of *A. appalachiana* populations within patches, among patches, and within different drainages. We sampled multiple drainages in a watershed and multiple watersheds. Within each study site, we initially located all *A. appalachiana* patches, then chose discrete geographic clusters in which all patches were sampled.

At the Domain study site, we sampled 56 patches that were distributed across five geographic clusters located within three separate watersheds spanning an area of ~100 ha (see Figure 3). We randomly sampled three (or more) individual ramets at each of 48 of these patches. All patches used in the rhizome mapping study and the ramet density study were included in this group.

At the Franklin study site, we sampled 73 patches located within four geographic clusters spanning two distinct watersheds over an area of ~50 ha (see Figure 4). We randomly sampled three individual ramets at each of 42 of these patches. DNA was extracted from green leaves clipped from ramets in the field and placed in silica gel packets.

Tissue disruption and genomic DNA extraction

Tissue disruption of silica-dried leaf material was done using a Qiagen TissueLyser II (Qiagen, Germantown, Maryland, USA). We assessed the results of disruption with varying steel bead size (5 mm or 7 mm), number of beads per tube (one or two), and disruption time (20 s, 45 s, 60 s). We found that using two 7 mm beads per tube with a disruption time of 60 s at 25 Hz provided the most complete disruption of *Arundinaria* dried leaf tissue. We assessed the results of genomic DNA extraction with two kits: Qiagen DNeasy Plant Mini Kit (Qiagen) and Thermo Scientific GeneJET Plant Genomic DNA Purification Kit (Thermo Scientific, Waltham, Massachusetts, USA). We found that the Thermo Scientific GeneJET Plant Genomic DNA Purification Kit provided greater genomic DNA yield as visualized on a 2% agarose gel with Ethidium Bromide stain included. As a result, all subsequent extractions were performed using this kit.

A total of 327 individuals were included in genetic work, five of which were run for outgroup comparison: *Arundinaria tecta* (Dauphin Island, Alabama [AL]; coll. J.K. Triplett), *A. alabamensis* (Camp Sequoyah, Delta, AL; coll. J.K. Triplett); and three samples of *A. appalachiana* from outside of our study area (Desoto State Park, Laurel Creek, AL; Desoto State Park, West Fork, AL; Desoto Scout Trail, AL). The remaining 322 individuals of *A. appalachiana* were sampled at the Domain ($n = 159$) and Franklin ($n = 163$) study sites (both in Franklin County, Tennessee).

Development and genotyping of microsatellite loci

Genomic DNA of one sample of *A. appalachiana* was submitted to Steve Bogdanowicz in the Evolutionary Genetics Core Facility (EGCF) at Cornell University (Ithaca, New York, USA) to generate and sequence an enriched genomic library using an Illumina MiSeq platform (Illumina, San Diego, California, USA) to target tetrameric microsatellite repeats (Andrés and Bogdanowicz, 2012). EGCF developed >3000 possible primer pairs, which were identified in msatcommander version 1.0.3 (Rozen and Skaletsky, 2000; Faircloth, 2008). We then coordinated with Steve Bogdanowicz and Jose Andrés of HerediTec (Ithaca, New York, USA) to select, optimize, amplify, and sequence ≤60 microsatellite loci for ≤384 individuals of *Arundinaria* (details below) in an Illumina MiSeq run (2 × 250 bp) following the protocols outlined in D'Aloia et al. (2017). In this case, alleles were determined by unique sequences at a given locus, rather than by fragment analysis. A custom Perl script was used to assign MiSeq reads to the appropriate microsatellite locus and sampled individual. As described in D'Aloia et al. (2017), this script (1) trims adapters and low-quality reads, (2) creates contigs from overlapping reads (for paired-end sequencing), (3) identifies reads corresponding to each locus, (4) collapses identical reads for each individual, and (5) identifies the top two haplotypes for individuals at all loci (i.e., their diploid genotypes). HerediTec then provided us with the raw data reads and a genotype file consisting of called haplotypes and frequencies for each locus per sample, with missing data trimmed out (<20% missing samples for each locus, and <20% missing loci for each sample). It is important to note that *A. appalachiana* is likely an allotetraploid, with 2n = 48 (cytotypes AABB; J. Triplett, personal communication). It is possible, then, that primers for a given microsatellite locus may amplify both parental homologous loci, resulting in the possibility of amplifying alleles from either (or both) subgenomic loci. Although traditional population genetic analyses would require a clear understanding of these patterns, assessment of clonal structure should not be affected by this (P. Soltis, personal communication). Therefore, we treated all individuals as diploids in our analyses.

In an effort to limit any erroneous calls in the data set, we marked any genotype for which either allele had <10 reads as missing, and individual samples were removed from the data matrix if they were missing data at ≥10% of the included loci. Individual loci were removed from the data matrix if those loci exhibited consistently low read counts (<10 per allele) across individuals, or if they were missing data at ≥10% of the included loci.

Summary statistics

For the following analyses, we used package poppr (Kamvar et al., 2014, 2015) in R version 4.3.2 (R Core Team, 2023), with code coming from the "Primer in Population Genetics and Genomics in R" (https://grunwaldlab.github.io/Population_Genetics_in_R/index.html; last accessed 27 February 2024). In absence of the outgroup samples, we determined the number of alleles, allelic diversity (Simpson's Index, 1-D), expected heterozygosity (Nei, 1978), and evenness per locus. To assess whether the 51 microsatellite loci included here were sufficient to distinguish among genets, we constructed a genotype accumulation curve. This approach determines the number of unique multilocus genotypes (MLGs) determined for all possible combinations of 1-*n* loci, where *n* is the total number of loci sampled. When a sufficient number of loci have been sampled to identify all unique MLGs in the data set, the genotype accumulation curve will approach an asymptote.

Clonal assignment

For the assignment of individuals to clones, GenoDive version 3.06 (Meirmans and Van Tienderen, 2004) uses a genetic distance matrix determined by the user to assign individuals to multilocus genotypes (MLGs). We generated a genetic distance matrix among individuals using the infinite allele model (IAM; missing data not counted), given that alleles in this data set were called on the basis of nucleotide sequence rather than repeat size and that allele identity in our data matrix is based on the order in which they were discovered by the automated calling script, such that identity does not imply degree of relatedness. To determine an appropriate genetic distance threshold for identifying unique genets, we used the IAM genetic distance matrix to compare distances among individuals known to be interconnected ramets of the same genet on the basis of excavation studies. For this comparison, we excavated clusters of three ramets (i.e., a triad) included in the ecological and genetic studies presented here. We were able to confirm connectivity within three triads and one additional pair of ramets. Genetic distances among connected ramets ranged from two to five; therefore, we selected a minimum genetic distance threshold of 5 for clonal assignment. For comparison, we also include results from clonal assignment based on genetic distance thresholds of 0, 4, 5, 6, and 7.

Within the Domain site and again for the Franklin site, we determined the largest genet by ramet count (largest *G*) and calculated the proportion distinguishable (PD; Ellstrand and Roose, 1987), which is defined as the ratio of genets (*G*) to ramets (*N*). As PD approaches a value of 1.00, high levels of sexual reproduction are inferred; as PD approaches a value of 0.00, high levels of asexual reproduction are inferred. Clones were mapped within each watershed using QGIS version 3.30.3 (QGIS.org, 2023), with clonal assignment at a genetic distance threshold of 5 indicated by unique color. Distance between members of the same genet was measured using the measure line tool within QGIS. Including the outgroup samples, a dendrogram with bootstrap support (BS) was generated using the "aboot" function. Tree type was neighbor-joining (NJ) using Nei's genetic distance and generating 1000 bootstrap replicates, showing bootstrap values ≥ 75%.

Estimation of genet size and age

Since it is generally impossible to directly determine the starting point of genet growth and the extent of its expansion from that point, genet size can instead be conservatively estimated by enclosing all known genet-specific samples within a polygon and then determining the centroid of that polygon. One half the distance separating the two furthest points on the edge of the polygon provides an estimate of the maximum radius of the genet, assuming equidistant growth in all directions. Genet age can then be estimated by dividing the genet radius by the annual rate of radial clonal expansion (Centenaro et al., 2023), which we determined from our 8 yr clonal mapping study (see above). We applied this method to the genets that we identified at the two study sites. This indirect estimate of genet age has been used in numerous studies to determine the age of clones for a variety of plants (deWitte and Stöcklin, 2010; Centenaro et al., 2023). However, we recognize that it is only an approximation, since it assumes a linear relationship between genet age and size, when radial growth rate is actually more likely to vary over the life span of a genet (deWitte and Stöcklin, 2010). Also, without sampling every individual ramet in a population, it is not possible to determine the full size of a genet (Arnaud-Haond et al., 2012).

Change in ramet density

At each study site, we tracked mean ramet density over time within a select number of the patches that were hierarchically sampled as part of the spatial genetic structure analysis (described above). Six blocks of four patches each were located in different sub-drainages within the Domain study site. Four blocks of five to nine patches each were located in different sub-drainages at the Franklin site. We used permanently marked, 1-m-diameter circular plots that were established within the center of patches. All ramets within the circular plot were counted at each sampling period. The purpose of these plots was to contrast ramet density within patches at the two sites and to assess the stability of ramet density within these populations. By October, no new ramets are being produced in *A. appalachiana*; therefore, annual censuses were completed in October of each year from 2014 through 2022, for a total of 8 yr at the Domain site. At the Franklin site, plots were censused in October 2020 and again in October 2022.

A repeated-measures analysis of variance (ANOVA) was used to determine the effect of time on ramet density in plots at the Domain site from 2014 to 2022, with a two-way ANOVA used to examine the interaction between block and year ($n = 24$ plots). A Wilcoxon signed-rank test was used to examine the change in ramet density at the Franklin site between 2020 and 2022 ($n = 28$ plots). The difference in ramet density between the two study sites in 2022 was determined using a Mann-Whitney *U*-test, and one-way ANOVAs were used to test the effect of genet variation on ramet density at each of the study sites. All statistical tests were conducted using R version 4.3.2 (R Core Team, 2023).

RESULTS

Clonal fragment mapping

Within each of the six patches used for clonal fragment mapping, plots were centered on one large, intact clonal fragment that was identified and mapped in 2014. The average clonal fragment in 2014 consisted of 5.9 m of rhizome that interconnected an average of 17 total ramets (Table 1). Of the six mapped clonal fragments, only four showed continued growth between 2014 and 2017. The two fragments that failed to grow had lost >90% of their living rhizome by 2017 and were completely dead by 2022. The four remaining clonal fragments displayed continuous rhizome growth over the 8 yr (see Figure 2A–D), with new ramets being produced between mapping intervals and a high level of rhizome persistence. In 2017, the average clonal fragment consisted of 6.1 m (total length) of rhizome, interconnecting a total of 13 ramets. In 2022, the average size was 5.6 m of rhizome length, interconnecting 30 ramets (Table 1). Over the 8 yr, two of these four clonal fragments stayed connected as one intact rhizome structure (Figure 2A, C), whereas the other two had each severed into two parts (Figure 2B, D). On average, 64% (range: 12%–89%) of the new rhizome material produced by these clonal fragments in 2017 was alive 5 yr later (in 2022). The average annual rate of clonal expansion over the 8 yr period was determined to be 0.21 m yr^{-1} (range: 0.12–0.30 m yr^{-1}).

Spatial genetic structure

Microsatellite loci summary statistics

Locus and primer information are provided in Appendix S1, as are summary statistics for number of alleles, allelic

TABLE 1 Average total rhizome length and average number of ramets per fragment within *Arundinaria* patches excavated and mapped in 2014, 2017, and 2022 at the Domain study site in Sewanee, Tennessee. Data range in parentheses.

Year	Total rhizome length (m)	Total ramet number
2014 ($n = 6$)	5.9 (2.3–13.0)	17 (4–29)
2017 ($n = 4$)	6.1 (3.3–11.7)	13 (11–16)
2022 ($n = 4$)	5.6 (1.2–13.2)	30 (8–82)

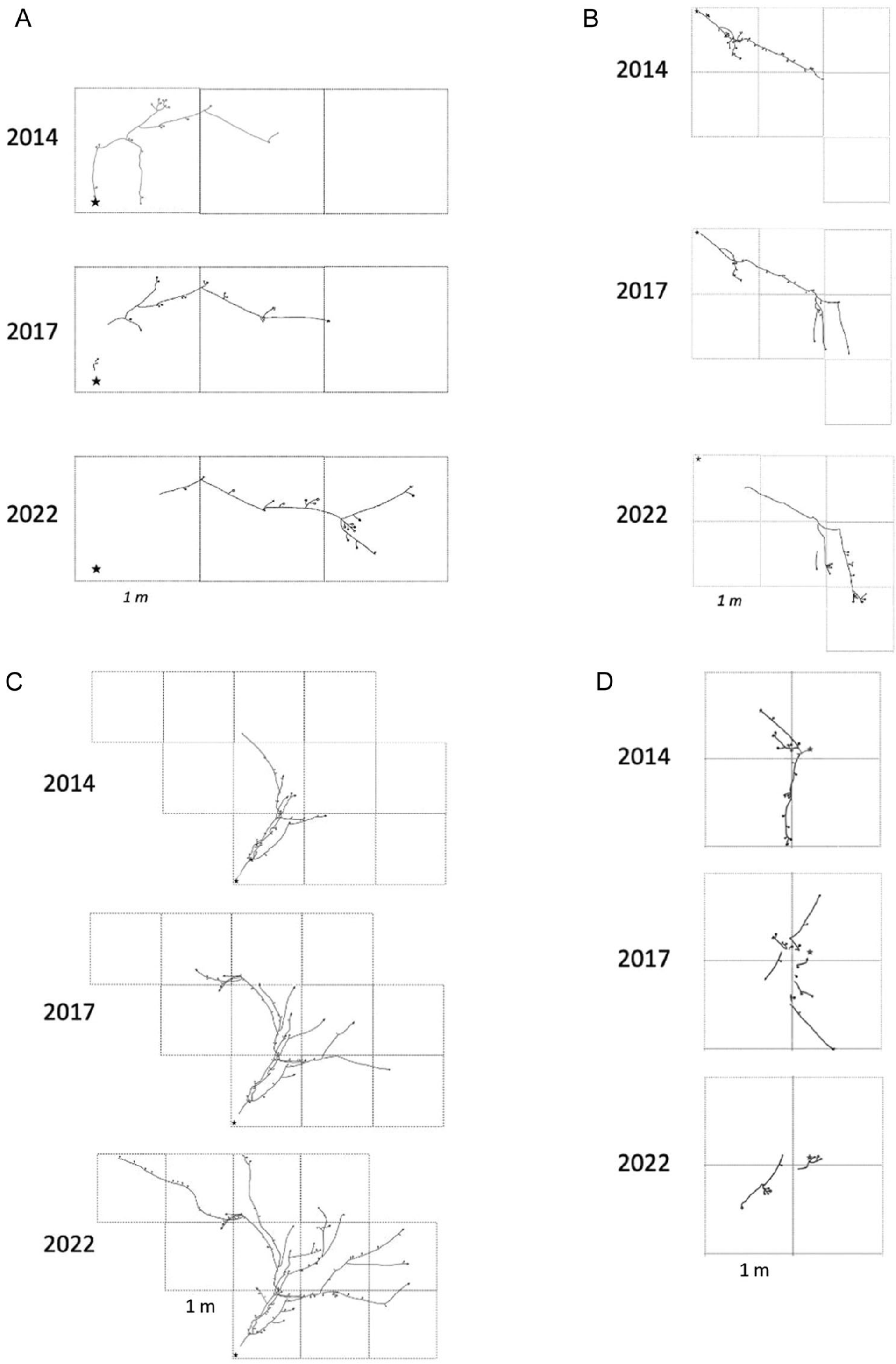

FIGURE 2 Maps of the rhizome systems associated with *Arundinaria appalachiana* clonal fragments excavated in 2014, 2017, and 2022. Star indicates point of origin of living rhizome in 2014.

diversity, expected heterozygosity, and evenness per locus for the 322 individuals of *A. appalachiana* sampled at the Domain and Franklin study sites. Number of alleles per locus varied from a minimum of two to a maximum of 16 (mean number of alleles over all loci = 5.86). Allelic diversity ranged from 0.64 to 0.83 (mean over all loci = 0.64). Expected heterozygosity ranged from 0.38 to 0.84 (mean over all loci = 0.64). Evenness ranged from 0.59 to 1.00 (mean over all loci = 0.84). The genotype accumulation curve (Appendix S1) shows an asymptotic curve at ~47 loci, indicating sufficient resolution to distinguish among multilocus genotypes in the present data set.

Clonal assignment

Clonal assignment is summarized in Table 2, including number of unique genets (*G*), largest *G*, and PD at genetic distance thresholds of 0, 4, 5, 6, and 7 for each watershed (outgroup samples are not included). There were no shared genotypes across sites at any of these thresholds, and each of the outgroup individuals provided by J.K. Triplett exhibited a unique genotype regardless of threshold used. Among the thresholds presented, 4 and 5 were the two values observed among connected ramets in the excavation study, such that these values can be considered a minimum genetic distance (conservative estimate) defining clonal connections in this system. At these distances (4 and 5), there were 17 and 10 genets, respectively, identified at the Domain study site (163 sampled ramets). Largest *G* was 142 and 151, respectively; PD was 0.10 and 0.06, respectively. At the Franklin site, there were 36 and 24 genets, respectively (159 ramets sampled). Largest *G* was 84 and 98, respectively; PD was 0.23 and 0.15, respectively. The NJ dendrogram (not shown), provided strong support for a close alliance among JTA201, JTA301, and JTA401 (100% BS), with JTA501 being sister to JTT1 (99% BS). Domain samples clustered together, and Franklin samples clustered together (100% BS, respectively). Clustering within watersheds was largely based on triads and close geographic proximity, but there were almost no nodes supported by ≥70% BS.

Estimation of genet size and age

Using threshold 5, the largest genet at the Domain study site encompassed 151 (93%) of the 163 sampled points. These points were distributed in geographic clusters 2–5 (Figure 3). A polygon encompassing all of these points had an area of ~31 ha. The largest genet at the Franklin study site encompassed 98 of 159 sampled points. These points were distributed in geographic cluster 2 (Figure 4). A polygon encompassing all these points had an area of ~12 ha. Genet age was estimated using the mean yearly radial spread of 0.12–0.30 m yr^{-1} (see above), with the assumption that the genet grew centrifugally from its centroid along the major axis (Reusch et al., 1999). Based on a genetic distance threshold of 5, the greatest distance measured between two members of the same genet was 1075 m at the Domain site and 866 m at the Franklin site. Using one-half these distances as the radius, we determined the age of the largest genet: 1792–4479 yr within the Domain population and 1443–3608 yr within the Franklin population. These age ranges are based on the minimum and maximum rates of clonal expansion reported earlier.

TABLE 2 Clonal assignment of *Arundinaria* sampled at the two study sites in Tennessee, based on genetic distance thresholds (T) of 0 and 4–7. Largest genet by ramet count [*G*] in square brackets; proportion distinguishable (number of genets/number of ramets; Ellstrand and Roose, 1987) in parentheses.

		Number of unique genets identified				
Sampling location	***n***	**T 0**	**T 4**	**T 5**	**T 6**	**T 7**
Sewanee Domain	163	153	17	10	5	4
		[5]	[142]	[151]	[155]	[156]
		(0.94)	(0.10)	(0.06)	(0.03)	(0.02)
Franklin State Forest	159	157	36	24	10	3
		[2]	[84]	[98]	[105]	[113]
		(0.99)	(0.23)	(0.15)	(0.06)	(0.02)
Overall	321	310	53	34	15	7
		(0.97)	(0.17)	(0.11)	(0.05)	(0.02)

Change in ramet density

From the genetic analysis above, we determined that two genets were represented in this analysis of ramet density at the two sites. Five of the six blocks at the Domain site represented the same genet, while three of the four blocks at the Franklin site represented the same genet (Figure 4). Genet was not a significant predictor of ramet density at the Domain site (linear mixed-effects model, $\beta = -1.775$, $p = 0.706$, $n = 24$) or at the Franklin site ($\beta = -10.251$, $p = 0.251$, $n = 28$).

Ramet density at the Domain site exhibited a significant decline over time (Figure 5; Friedman test, $\chi^2 = 73.89$, $p < 0.001$, $n = 24$), with ramet density in 2022 significantly reduced when compared to 2022 (Wilcoxon signed-rank test, $W = 13.0$, $p < 0.001$, $n = 24$). When analyzed at the block level, all blocks at the Domain site exhibited a significant decrease (Friedman test by block, $p < 0.05$), except in one block ("Dom4"). In the five blocks that showed a decline, only 15% of plots (3 of 20) contained ramets by 2022. The "Dom4" block did not show a statistically significant decline ($p = 0.332$), suggesting localized variation in ramet decline within the largest genet (Figure 5).

In contrast to the Domain site, ramet density at the Franklin site did not change significantly over time (Figure 5; Friedman test, $p > 0.05$, $n = 28$), with ramet density in 2022 not significantly different from that in 2020 (Wilcoxon signed-rank test, $W = 77$, $p = 0.239$, $n = 28$). Plots at the Franklin site had significantly higher ramet density than even the "Dom4" block of the Domain site (Mann-Whitney *U*-test, $U = 88.5$, $p = 0.034$), suggesting that the overall ramet mortality observed at the Domain site has resulted in lower ramet densities, even in the least-affected area.

No flowering occurred in any of the plots at either study site over the course of the 8 yr study.

DISCUSSION

Our findings provide novel ecological and genetic data for *A. appalachiana*, a bamboo species endemic to the southern Appalachian region. We assessed the spatial genetic

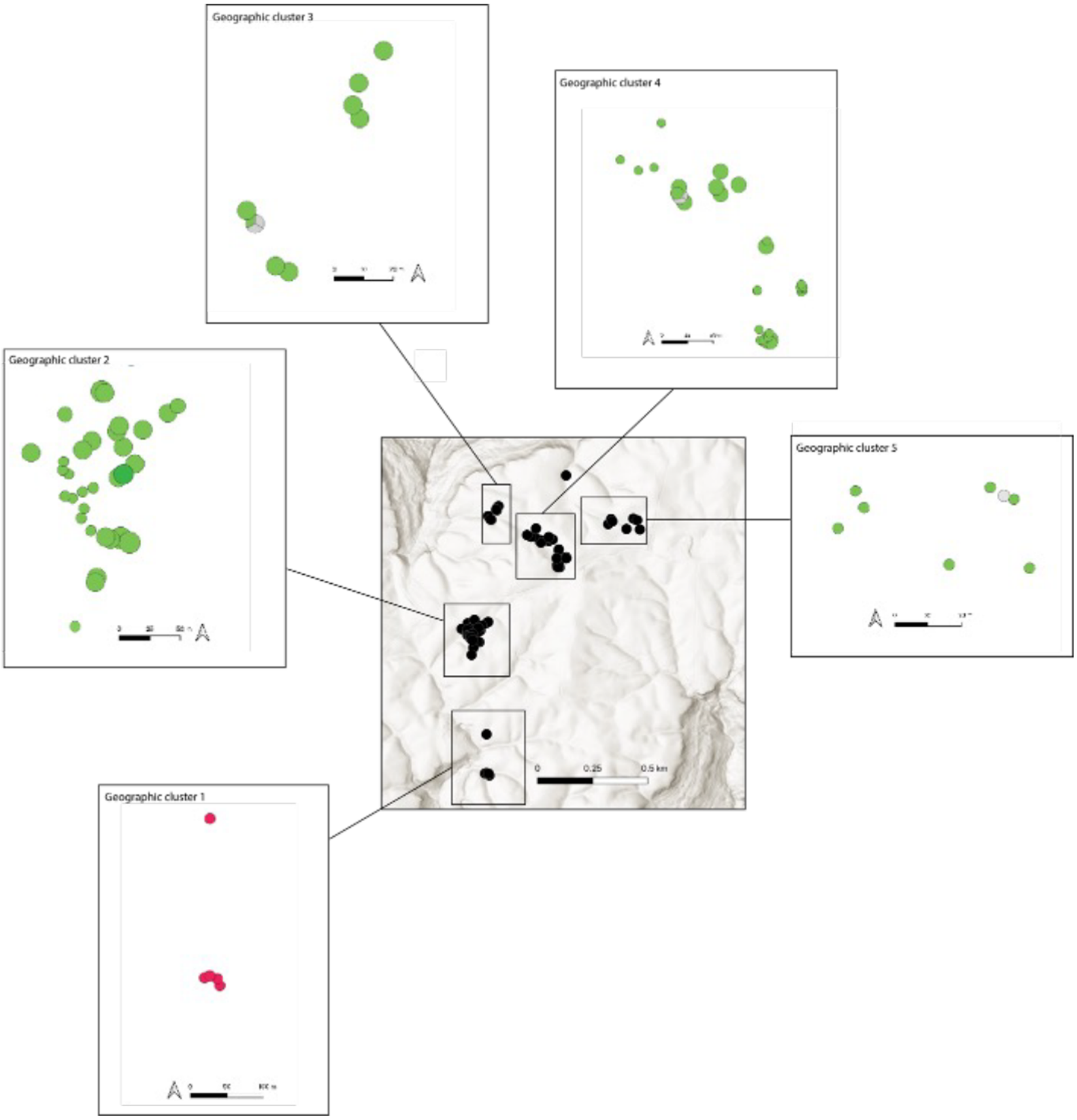

FIGURE 3 Map of unique *Arundinaria appalachiana* clones documented within the Sewanee Domain watershed. Clonal assignment is based on a genetic distance threshold of 5; circle size indicates number of individuals sampled in that area. Color coding is used to highlight larger clones; infrequent genotypes were coded gray.

structure and demographic patterns of two populations on the southern Cumberland Plateau, and our results revealed large, old genets that exhibited predominantly non-overlapping distributions across the landscape. These genets, expressed as discrete patches of rhizome fragments and ramets, were spatially clustered along stream drainages and on adjacent slopes, suggesting long-term expansion along drainage networks. Over time, patches shifted position through gradual rhizome growth, fragmentation, and senescence.

Fragmentation of rhizome networks was observed, potentially impairing physiological integration and increasing vulnerability to environmental stress (Evans, 1992). Previous work in *A. gigantea* has shown that intact rhizome networks enhance persistence under canopy cover (Gagnon et al., 2007), while fragmentation correlates with increased patch mortality (Gagnon et al., 2013). Although the extent of physiological integration in *A. appalachiana* remains unknown, other bamboos have demonstrated resource sharing among ramets (Li et al., 2000; Saitoh et al., 2002; Lu and Franklin, 2022), suggesting that similar mechanisms may operate in this species. Long-term monitoring revealed a decline in patch number and ramet density at the Domain site over the past two decades (Figure 6). This decline occurred in the absence of major disturbances but coincided with multiple severe droughts. Rhizome fragmentation and aging may have contributed to this observed mortality. By contrast, ramet density remained stable at the

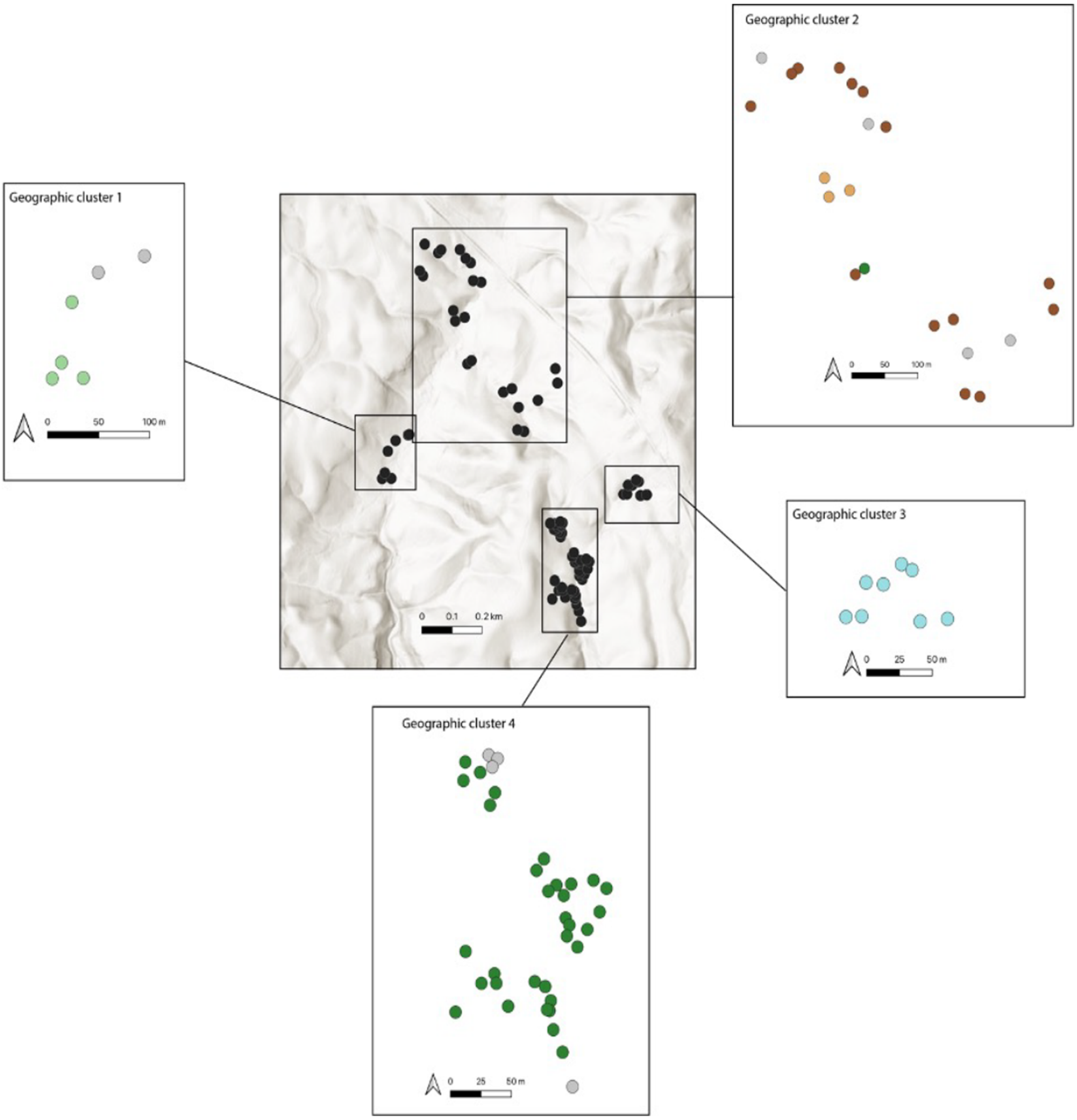

FIGURE 4 Map of unique *Arundinaria appalachiana* clones documented within the Franklin State Forest watershed. Clonal assignment is based on a genetic distance threshold of 5; circles represent triads sampled at that location. Color coding is used to highlight larger clones; infrequent genotypes were coded gray.

Franklin site, indicating potential site-specific differences in genet health or environmental stressors.

As a monocarpic, semelparous species with no known seed bank, *A. appalachiana* populations represent single-age cohorts established during a past flowering event. The estimated ages of the largest genets (>1000 yr) suggest extremely long flowering intervals compared with related taxa (*A. gigantea*, 20–50 yr; *A. tecta*, 4–7 yr). The complete absence of observed flowering in this species (Triplett et al., 2010) raises the possibility of reproductive failure, potentially due to the accumulation of somatic mutations (Honnay and Bossuyt, 2005). In the absence of sexual reproduction, *A. appalachiana* may exhibit remnant population dynamics (deWitte and Stöcklin, 2010), persisting through clonal propagation despite environmental challenges.

We estimated genet age using radial clonal expansion rates and genet size, acknowledging that this approach assumes constant growth rates that likely vary with environmental history (deWitte and Stöcklin, 2010; Centenaro et al., 2023). Historical land use, such as grazing and selective logging, may have facilitated faster rhizome spread in the past (Evans et al., 2016). Nonetheless, the clonal expansion rates observed here are consistent with those reported for other temperate bamboos (Suyama et al., 2000).

Most clumps at our study sites were composed of a single genet, though some contained rare co-occurring

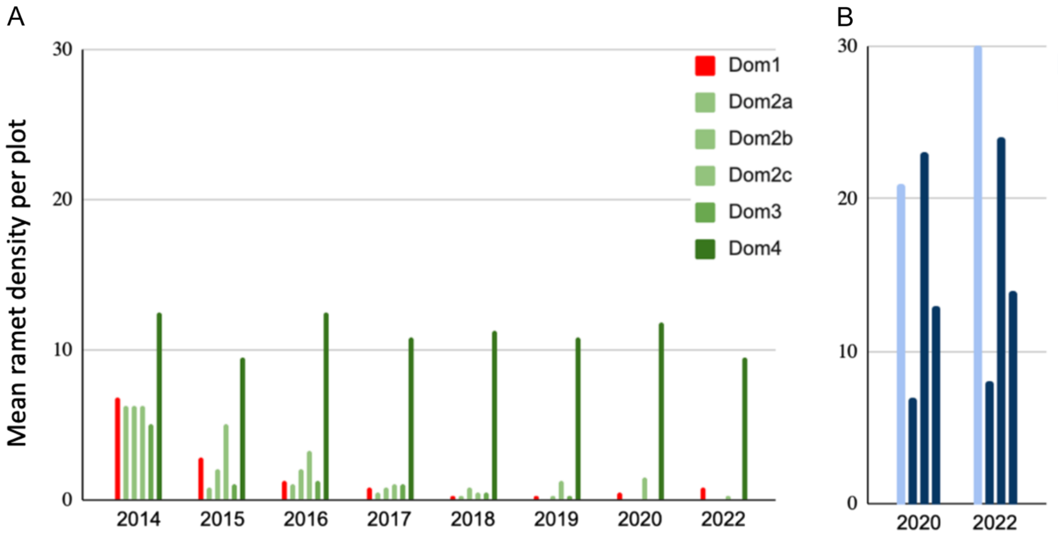

FIGURE 5 Change in mean ramet density of *Arundinaria appalachiana* per patch over time at the two study sites: (**A**) Sewanee Domain and (**B**) Franklin State Forest. Each bar represents a geographic cluster or subcluster (**A**–**C**) of hill cane patches. Two genotypes were represented at each study site: Sewanee Domain (Dom)—red (Dom1) and green (Dom2-4); Franklin State Forest (FSF)—light blue (FSF3) and indigo (FSF4).

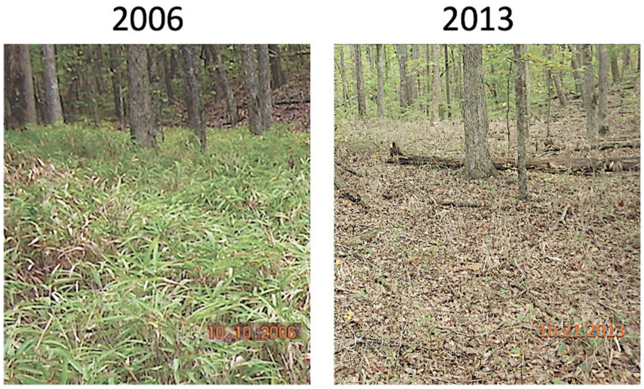

FIGURE 6 Major decrease in ramet density between 2006 and 2013 within a large patch of *Arundinaria appalachiana* within geographic cluster 2 at the Sewanee Domain study site. By 2022, ramets had mostly disappeared from this location.

genets. This pattern is consistent with leptomorphic rhizome growth and has been observed in other bamboo species (Isagi et al., 2004; Franklin et al., 2008). The few dominant genets likely represent survivors of an initially diverse cohort that experienced strong seedling mortality. Limited seed dispersal in *A. appalachiana* further suggests that extant genets may be closely related.

Our study confirms that *A. appalachiana* persists in both open and closed canopy conditions, indicating habitat generalism with respect to light availability (Szakacs et al., 2022). Although our data were collected at high spatial resolution within a limited geographic range, the patterns observed here warrant broader investigation across the species' distribution. Since *A. appalachiana* was not recognized as a distinct species until 2006, we have no specific records of its historical extent. However, canebrakes—dense, monodominant stands of *Arundinaria* species—once occupied large areas of the southeastern United States (Noss et al., 1995) and have declined by >98% due to land conversion, overgrazing, and altered disturbance regimes (Platt and Brantley, 1997; Gagnon and Platt, 2008).

In theory, clonal species can respond to environmental change through migration, adaptation, or persistence (Cunze et al., 2013; Amor et al., 2020; Pazzaglia et al., 2021). However, in *A. appalachiana*, migration is limited by lack of seed dispersal, and adaptive evolution may be hindered by extremely long generation times. If sexual reproduction has essentially been lost, genetic diversity can be maintained only through somatic mutation or epigenetic mechanisms (Dodd and Douhovnikoff, 2016; Dong et al., 2019). *Arundinaria appalachiana* exemplifies the duality associated with clonal longevity—enabling millennial persistence while restricting evolutionary flexibility (Tepedino, 2012).

Arundinaria appalachiana exhibits numerous traits associated with extinction risk: narrow geographic distribution, small effective population sizes, habitat specificity, poor dispersal, and no documented sexual reproduction. While long-term clonal expansion allows persistence and prolongs survival in the absence of new genet production, the lack of sexual reproduction and the age of ancient genets suggests that the persisting remnant populations of this species are facing sexual extinction and perhaps exhibiting an overall extinction debt (Honnay and Bossuyt, 2005). For these reasons, *A. appalachiana* should be considered a high-priority species for conservation (Triplett et al., 2006, 2010). However, the conservation of clonal plant species can be challenging. It remains unclear whether conservation units should be defined at the level of genets or ramets (Funk et al., 2012). Genets more accurately represent the evolutionary units that contribute to effective population size and adaptive potential (Tepedino, 2012), but their assessment is difficult and expensive. Ramet counts have a practical advantage but tend to overestimate population viability (Fox et al., 2024; Fox and Morris 2025). Additionally, for *Arundinaria*, genetic augmentation is constrained by the absence of seed production and difficulties in rhizome propagation (Platt and Brantley, 1993).

CONCLUSIONS

This study provides the first detailed assessment of spatial genetic structure and clonal demography in *A. appalachiana*, an upland bamboo endemic to the southern Appalachian region of the southeastern United States. Across two populations on the southern Cumberland Plateau in Tennessee, we found that a few exceptionally large and ancient genets dominate local stands, some extending over tens of hectares and persisting for millennia without evidence of sexual reproduction. These results demonstrate that *A. appalachiana* persists through extremely slow clonal expansion and remarkable genet longevity, a strategy that ensures persistence in stable environments but severely limits opportunities for genetic renewal or adaptation.

From a conservation perspective, *A. appalachiana* exemplifies a relictual species at risk of genetic and demographic erosion. Its survival likely hinges on the endurance of a few long-lived genets and the protection of habitats that sustain them. Conservation efforts should prioritize the preservation of remaining populations and the microhabitats that support their rhizome networks, maintaining favorable hydrological and microclimatic conditions and protecting the potential for rare reproductive events that could restore genetic diversity. These findings underscore the importance of incorporating clonal structure, life history strategy, and geographic context into conservation planning for narrowly endemic, long-lived plant species.

AUTHOR CONTRIBUTIONS

J.P.E.: conceptualization, field methodology and analyses, funding acquisition, supervision, visualization, and writing; A.B.M.: conceptualization, genetic methodology and analyses, visualization, and writing.

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DATA AVAILABILITY STATEMENT

Data from this study are available at Figshare (https://figshare.com/account/mycontent/projects/267821).

ORCID

Jonathan P. Evans https://orcid.org/0000-0002-2550-0031

REFERENCES

Abe, Y., and S. Shibata. 2012. Spatial and temporal flowering patterns of the monocarpic dwarf bamboo *Sasa veitchii* var. *hirsuta*. *Ecological Research* 27: 625–632.

Amor, M. D., J. C. Johnson, and E. A. James. 2020. Identification of clonemates and genetic lineages using next-generation sequencing (ddRADseq) guides conservation of a rare species, *Bossiaea vombata* (Fabaceae). *Perspectives in Plant Ecology, Evolution and Systematics* 45: 125544.

Andrés, J.A., and S.M. Bogdanowicz. 2012. Isolating microsatellite loci: looking back, looking ahead. *In* V. Orgogozo, and M. Rockman [eds.], Molecular methods for evolutionary genetics: Methods in molecular biology, 772. Humana Press. Totowa, New Jersey, USA.

Arnaud-Haond, S., C. M. Duarte, E. Diaz-Almela, N. Marbà, T. Sintes, and E. A. Serrão. 2012. Implications of extreme life span in clonal organisms: millenary clones in meadows of the threatened seagrass, *Posidonia oceanica*. *PLoS One* 7: e30454.

Barger, T. W., C. T. Taylor, A. S. Peters, B. D. Holt, J. K. Triplett, and J. K. England. 2018. The vascular flora of the Freedom Hills Forever Wild tract, Colbert County, AL. *Castanea* 83: 305–322.

Centenaro, G., A. Petraglia, M. Carbognani, A. Piotti, C. Hudek, U. Büntgen, and A. Crivellaro. 2023. The oldest known clones of *Salix herbacea* growing in the Northern Apennines, Italy are at least 2000 years old. *American Journal of Botany* 110: e16243.

Cowan, W.F. 1970. A management plan for Franklin State Forest. Tennessee Division of Forestry, Nashville, Tennessee, USA.

Cunze, S., F. Heydel, and O. Tackenberg. 2013. Are plant species able to keep pace with the rapidly changing climate? *PLoS One* 8: e67909.

D'Aloia, C., S. M. Bogdanowicz, R. G. Harrison, and P. M. Buston. 2017. Cryptic genetic diversity and spatial patterns of admixture within Belizean marine reserves. *Conservation Genetics* 18: 211-223.

deWitte, L. C., and J. Stöcklin. 2010. Longevity of clonal plants: why it matters and how to measure it. *Annals of Botany* 106: 859–870.

Dodd, R. S., and V. Douhovnikoff. 2016. Adjusting to global change through clonal growth and epigenetic variation. *Frontiers in Ecology and Evolution* 4: 86.

Dong, B.-C., P. Alpert, and F.-H. Yu. 2019. Transgenerational effects of herbivory and soil nutrients transmitted via vegetative reproduction in the clonal plant, *Alternanthera philoxeroides*. *Perspectives in Plant Ecology, Evolution and Systematics* 41: 125498.

Ellstrand, N. C. and M. L. Roose. 1987. Patterns of genotypic diversity of clonal plant species. *American Journal of Botany* 74: 123-131.

Evans, J. P. 1992. The effect of local resource availability and clonal integration on ramet functional morphology in *Hydrocotyle bonariensis*. *Oecologia* 89: 265–276.

Evans, J. P., C. A. Oldfield, M. P. Priestley, Y. M. Gottfried, L. D. Estes, A. Sidik, and G. S. Ramseur. 2016. The vascular flora of the University of the South, Sewanee, Tennessee. *Castanea* 81: 206–236.

Evans, J. P., C. A. Oldfield, and J. L. Reid. 2019. Differential resistance to tree species loss between two dominant communities in a resilient southeastern landscape. *Natural Areas Journal* 39: 182.

Faircloth, B. C. 2008. Msatcommander: Detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources* 8.: Detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources* 8.1: 92–94.

Fox, S., L. Eberth, J. B. Floyd, C. Pederson, L. Stafford, N. Tillmanns, L. Vodo, and A. B. Morris. 2024. The importance of understanding clonal structure for species listing and recovery: case studies from the rare oconee bells (*Shortia brevistyla* and *Shortia galacifolia*; Diapensiaceae) and the federally endangered bunched arrowhead (*Sagittaria fasciculata*; Alismataceae). *Conservation Genetics* 25: 1–18.

Fox, S., and A. B. Morris. 2025. Clonal reproduction and hydrochory drive colony and population structure in a narrowly endemic and endangered emergent aquatic plant (*Sagittaria fasciculata*: Alismataceae). *Conservation Genetics* 26: 573–589.

Franklin, D. C. 2004. Synchrony and asynchrony: observations and hypotheses for the flowering wave in a long-lived semelparous bamboo. *Journal of Biogeography* 31: 773–786.

Franklin, D. C., S. Kaneko, N. Yamasaki, and Y. Isagi. 2008. Short Communication. Some wild bamboo clumps contain more than one genet. *Australian Journal of Botany* 56: 433.

Funk, W. C., J. K. McKay, P. A. Hohenlohe, and F. W. Allendorf. 2012. Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution* 27: 489–496.

Gagnon, P. R., H. A. Passmore, and W. J. Platt. 2013. Multi-year Salutary effects of windstorm and fire on river cane. *Fire Ecology* 9: 55–65.

Gagnon, P. R., and W. J. Platt. 2008. Reproductive and seedling ecology of a semelparous native bamboo (*Arundinaria gigantea*, Poaceae). *The Journal of the Torrey Botanical Society* 135: 309–316.

Gagnon, P. R., W. J. Platt, and E. B. Moser. 2007. Response of a native bamboo [*Arundinaria gigantea* (Walt.) Muhl.] in a wind-disturbed forest. *Forest Ecology and Management* 241: 288–294.

Greenberg, C., and B. Collins [eds.]. 2021. Fire ecology and management: past, present, and future of U.S. forested ecosystems. Springer, Cham, Switzerland.

Harper, J.L., and J. White. 1974. The demography of plants. *Annual Review of Ecology and Systematics* 5: 419–463.

Hodkinson, T. R., G. N. Chonghaile, S. Sungkaew, M. W. Chase, N. Salamin, and C. M. A. Stapleton. 2010. Phylogenetic analyses of plastid and nuclear DNA sequences indicate a rapid late Miocene radiation of the temperate bamboo tribe Arundinarieae (Poaceae, Bambusoideae). *Plant Ecology & Diversity* 3: 109–120.

Honnay, O., and B. Bossuyt. 2005. Prolonged clonal growth: escape route or route to extinction? *Oikos* 108: 427–432.

Isagi, Y., T. Oda, K. Fukushima, C. Lian, M. Yokogawa, and S. Kaneko. 2016. Predominance of a single clone of the most widely distributed bamboo species *Phyllostachys edulis* in East Asia. *Journal of Plant Research* 129: 21–27.

Isagi, Y., K. Shimada, H. Kushima, N.Tanaka, A. Nagao, T. Ishikawa, H. Onodera, and S. Watanabe. 2004. Clonal structure and flowering traits of a bamboo [*Phyllostachys pubescens* (Mazel) Ohwi] stand grown from a simultaneous flowering as revealed by AFLP analysis. *Molecular Ecology* 13: 2017–2021.

Janzen, D. H. 1976. Why bamboos wait so long to flower. *Annual Review of Ecology and Systematics* 7: 347–391.

Kamvar, Z. N., C. B. Jonah, and N. J. Grünwald. 2015. Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Frontiers in Genetics* 6: 208.

Kamvar, Z. N., J. F. Tabima, and N. J. Grünwald. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2: e281.

Keeley, J. E., and W. J. Bond. 1999. Mast flowering and semelparity in bamboos: The bamboo fire cycle hypothesis. *The American Naturalist* 154: 383–391.

Li, R., M. Werger, H. de Kroon, H. During, and Z. Zhong. 2000. Interactions between shoot age structure, nutrient availability and physiological integration in the giant bamboo *Phyllostachys pubescens*. *Plant Biology* 2: 437–446.

Lu, Z., and S. B. Franklin. 2022. Clonal integration and regeneration in bamboo *Bashania fargesii*. *Forest Ecology and Management* 523: 120504.

Ma, Q., H. Song, S. Zhou, W. Yang, D. Li, and J. Chen. 2013. Genetic structure in dwarf bamboo (*Bashania fangiana*), clonal populations with different genet ages. *PLoS One* 8: e78784.

Mathews, K. G., J. Huguelet, M. Lanning, T. Wilson, and R. Young. 2009. Clonal diversity of *Arundinaria gigantea* (Poaceae; Bambusoideae) in western North Carolina and its relationship to sexual reproduction: an assessment using AFLP fingerprints. *Castanea* 74:213–233.

Mays, P. A., J. M. Kelley, and D. A. Lietzke. 1991. Soils of the Camp Branch and Cross Creek experimental watersheds. Research report 91-21. Department of Plant and Soil Science, Agricultural Experiment Station, University of Tennessee, Knoxville, USA.

Meirmans, P. G., and P. H. Van Tienderen. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* 4: 792–794.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.

Noss, R.F., E.T. LaRoe, and J.M. Scott. 1995. Endangered ecosystems of the United States, a preliminary assessment of loss and degradation. US Department of the Interior, National Biological Service.

Oakman, E. C., D. L. Hagan, T. A. Waldrop, and K. Barrett. 2021. Understory community shifts in response to repeated fire and fire surrogate treatments in the southern Appalachian Mountains, USA. *Fire Ecology* 17: 1–15.

Pazzaglia, J., T. B. H. Reusch, A. Terlizzi, L. Marín-Guirao, and G. Procaccini. 2021. Phenotypic plasticity under rapid global changes: The intrinsic force for future seagrasses survival. *Evolutionary Applications* 14: 1181–1201.

Platt, S. G., and C. G. Brantley. 1993. Switchcane: propagation and establishment in the southeastern United States. *Restoration & Management Notes* 11: 134–137.

Platt, S. G., and C. G. Brantley. 1997. Canebrakes: an ecological and historical perspective. *Castanea* 62: 8–21.

QGIS.org. 2023. QGIS Geographic Information System. QGIS Association. Website: http://www.qgis.org.

R Core Team. 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website: https://www.R-project.org/.

Rajewski, A. C. 2015. Micropropagation and evaluation of the genetic population structure of river cane *Arundinaria gigantea*, a species suitable for riparian restoration. M.S. thesis, University of Georgia, Athens, Georgia, USA.

Ramseur, G., and J. Kelly. 1981. Forest characterization and biomass estimates for two sites on the Cumberland Plateau. *Journal of the Tennessee Academy of Sciences* 56: 99–104.

Reusch, T. B. H., W. T. Stam, and J. L. Olsen. 1999. Size and estimated age of genets in eelgrass, *Zostera marina*, assessed with microsatellite markers. *Marine Biology* 133: 519–525.

Rozen S., and H. Skaletsky. 2000. Primer 3 on the WWW for general users and for biologist programmers. In S. Krawetz and S. Misener [eds.], Bioinformatics methods and protocols: Methods in molecular biology, 365–386. Humana Press, Totowa, New Jersey.

Saitoh, T., K. Seiwa, and A. Nishiwaki. 2002. Importance of physiological integration of dwarf bamboo to persistence in forest understory: a field experiment. *Journal of Ecology* 90: 78–85.

Suyama, Y., K. Obayashi, and I. Hayashi. 2000. Clonal structure in a dwarf bamboo (*Sasa senanensis*) population inferred from amplified fragment length polymorphism (AFLP) fingerprints. *Molecular Ecology* 9: 901–906.

Szakacs, A., A. Krings, and T. R. Wentworth. 2022. Shade-tolerance classification of the upland herbaceous flora of the Carolina and Virginia Piedmont. *American Midland Naturalist* 187: 113–147.

Tepedino, V. J. 2012. Overestimating population sizes of rare conal plants. *Conservation Biology* 26: 945–947.

Tomimatsu, H., A. Matsuo, Y. Kaneko, E. Kudo, R. Taniguchi, T. Saitoh, Y. Suyama, and A. Makita. 2020. Spatial genet dynamics of a dwarf bamboo: Clonal expansion into shaded forest understory contributes to regeneration after an episodic die-off. *Plant Species Biology* 35: 185–196.

Triplett, J. 2023. Tallapoosa Cane (*Arundinaria alabamensis*), a new species of temperate bamboo (Poaceae: Bambusoideae) from East Central Alabama. *Phytotaxa* 600: 153–168.

Triplett, J. K., K. A. Oltrogge, and L. G. Clark. 2010. Phylogenetic relationships and natural hybridization among the North American woody bamboos (Poaceae: Bambusoideae: Arundinaria). *American Journal of Botany* 97: 471–492.

Triplett, J. K., A. S. Weakley, and L. G. Clark. 2006. Hill cane (*Arundinaria appalachiana*), a new species of bamboo (Poaceae: Bambusoideae) from the southern Appalachian Mountains. *SIDA, Contributions to Botany* 22: 79–95.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Primers used to amplify 51 novel nuclear microsatellite loci in Arundinaria appalachiana.

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