Isolated coastal populations of *Tilia americana* var. *caroliniana* persist long-term through vegetative growth[1]

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PREMISE OF THE STUDY: Sprouting in woody plant species allows for the long-term persistence of small, isolated populations experiencing changing environments and can preserve genetic diversity in these populations despite the infrequent recruitment of sexually produced individuals. We examined demographic data collected over a 10-yr period for *Tilia americana* var. *caroliniana* populations in the context of genetic structure as an empirical case study of this concept.

METHODS: Two back-barrier islands on the Georgia coast of the United States were completely censused for *Tilia americana* var. *caroliniana*. Recruitment, growth, and mortality of all stems were tracked over 10 yr. All genets were genotyped using eight nuclear microsatellite loci to assess population genetic structure among sampled stems and among populations in the region.

KEY RESULTS: The two island populations differed in their ability to establish seedlings despite having similar patterns in flowering frequency. Seedling mortality was high throughout the 10 yr, and cycling of ramets within genets was common. Long-term recruitment in this system appears to be primarily a result of vegetative growth via basal sprouts. Genetic structure was limited, both between islands and among populations in the region.

CONCLUSIONS: Long-lived woody species that persist by vegetative reproduction may unexpectedly influence regional forest responses to climate change, particularly on the trailing edge of a species' distribution.

  KEY WORDS clonal reproduction; demography; Malvaceae; microsatellites; persistence; sea level rise

Long-lived plant species that persist through repeated vegetative sprouting and the proliferation of ramets are able to tolerate long periods of little or no recruitment of sexually produced individuals (Bond and Midgley, 2001; Clarke et al., 2010). Sprouting has the potential to not only extend the life of a genetic individual, but it may also preserve genetic diversity in small, isolated populations (Eriksson, 1996; Steinger et al., 1996; Premoli and Steinke, 2008). Habitat fragmentation leads to decreased gene flow among isolated populations and can contribute to an erosion of genetic diversity (Lande, 1988), which in turn limits the adaptive response of these populations to changing environments (Jump and Penuelas, 2005). Species dependent primarily on outcrossing for the maintenance of

populations are particularly vulnerable to the genetic impacts associated with habitat fragmentation (Honnay and Jacquemyn, 2007). However, sprouting may negate these impacts over long periods of time allowing for the preservation of ancient, fine-scale genetic structure despite restricted population size, isolation, and infrequent recruitment of sexually produced individuals (Eriksson, 1996; Premoli and Steinke, 2008).

During the Pleistocene and through the Holocene, woody plant species along the southeastern coast of North America underwent major changes in distribution as a result of habitat fragmentation and disappearance caused by changes in sea level. Fluctuations in sea level caused populations to become isolated then reconnected again over time. Since the end of the Pleistocene, forest stands on the barrier and back-barrier islands of the southeastern North American coast have undergone periods of isolation from each other and from mainland forests as a result of changing sea level (Turck, 2011). From 12,000 to 4500 yr BP, the back-barrier islands and the mainland were one contiguous forested landmass, where the barrier and back-barrier islands represented areas of higher

topographic relief in an upland setting (Turck, 2011; Napolitano, 2012). From 4500 to 4000 BP, sea level was within 1 m below the present, and islands were isolated by marsh and water. From 4000 to 2700 BP, sea level fell, and islands were once more connected as part of an upland forest. Between 2700 and 2400 BP, sea level rose rapidly, and since 2400 BP, back-barrier islands have been isolated by water (Turck, 2011). Over this span of time, tree species with restricted dispersal capabilities that became isolated on these islands would have gone through population bottlenecks and a loss of genetic diversity and possible extinction when faced with changing climatic conditions. Multistemmed, basal-sprouting tree species, however, had the potential to persist through changing environmental conditions without depending on the recruitment of sexually produced individuals for the maintenance of populations. Genetic individuals in these populations can be maintained over long periods through continual stem replacement.

American basswood, *Tilia americana* L. s.l. (Malvaceae) is a common component of eastern North American forests known for its distinctive bracteate infructescence and its ability to persist through basal sprouts (Crow, 1990). Intraspecific taxonomic delimitation within this group has been highly debated (for review, see McCarthy, 2012). Weakley (2015) currently recognizes three varieties within *T. americana* based on morphology and geography: *T. americana* var. *americana* L. (northern basswood), *T. americana* var. *heterophylla* (Vent.) Loudon (mountain basswood), and *T. americana* var. *caroliniana* (Mill.) Castigl. (southern basswood).

The subject of the current work is *T. americana* var. *caroliniana*, which is known from the outer coastal plain of southeastern North America and is often associated with shell deposits, middens, or other limestone substrates (Weakley, 2015). It may be found on back-barrier islands (small islands or hammocks between larger barrier islands and the mainland), and it is known to be a prolific basal sprouter through the production of lignotubers (Del Tredici, 2001; Pigott, 2012). Pollen records indicate that *Tilia* has been a component of coastal forests since the Pleistocene (Booth et al., 1999). Given the propensity of basswood species for calcium-rich, high pH soils (Whitaker et al., 2004; Pigott, 2012), it is likely that shell middens (shell refuse mounds), first established by Native Americans 4500 BP (Turck, 2011; Napolitano, 2012), would become areas that promoted *Tilia* populations on back-barrier islands. This relationship between *Tilia* populations and shell-midden-influenced soils has been demonstrated on back-barrier islands along the coasts of North Carolina, South Carolina, and Georgia (Whitaker et al., 2004; Pigott, 2012). Given the limited ability of *Tilia* to disperse by seed (Johnson, 1988; Hewitt and Kellman, 2002) and the large expanses of salt marsh and tidal creeks that separate the islands, it is possible that small *Tilia* populations on these islands have been isolated from each other for the last 2400 yr due to sea-level rise (Napolitano, 2012).

In this study, we examined the maintenance of *T. americana* var. *caroliniana* populations on two isolated back-barrier islands in Liberty County, Georgia, located directly west of St. Catherine's Island. Over 10 yr, we contrasted seedling recruitment and basal sprouting as mechanisms contributing to the density of genetic individuals on each island. We also compared the genetic diversity of these two populations and contrasted them with other *T. americana* var. *caroliniana* populations in the region. We hypothesized that *T. americana* var. *caroliniana* persists on these islands solely as a function of basal sprouting, allowing for the long-term maintenance of genetic individuals. We also hypothesized that the limited turnover of adult genetic individuals coupled with the failure to recruit new genetic individuals has slowed genetic divergence among populations. We predicted that this combination of factors would result in an overall lack of genetic structure among coastal *T. americana* var. *caroliniana* populations.

MATERIALS AND METHODS

Study site and population mapping—The populations of *T. americana* var. *caroliniana* surveyed in this study were located on two back-barrier islands, Bull (31.627138, −81.198386) and Moss (31.626551, −81.206482) Islands, located west of St. Catherines Island along the Georgia coast (Fig. 1). The islands consist of a small supratidal, forested hammock surrounded by salt marsh. Shell middens can be found on the edges of the hammock. Napolitano (2012) found that the center portion of each forested hammock was cleared for sea cotton in the late 1700s. Cotton rows are still visible on both islands. Cotton farming was abandoned in 1811 following a major hurricane event that flooded the islands (Napolitano, 2012). A maritime forest dominated by *Quercus virginiana* Mill. and *Juniperus virginiana* L. subsequently became established on both islands. Other common tree species on these islands currently include *Magnolia virginiana* L., *Prunus caroliniana* (Mill.) Aiton, *Pinus taeda* L., and *Sabal palmetto* (Walter) Lodd. ex Schult. & Schult.f.

In 1999, all adult and juvenile *T. americana* var. *caroliniana* (Mill.) Castigl. genets on Bull Island and Moss Island were located and mapped. An adult genet consisted of a main (largest) stem and associated basal sprouts, which is the typical habit of *T. americana* (Pigott, 2012). Juvenile genets consisted of a single stem and were less than 0.5 m in height. The *T. americana* var. *caroliniana* population on Bull Island was restricted to the northwestern edge of the island, occupying less than approximately 3.6% of the island's 8.18 ha (Fig. 1). Spatially separated from the Bull Island population by 900 m, a distance physiographically composed of two equal spans of salt marsh and a tidal channel, the *T. americana* var. *caroliniana* population of Moss Island was restricted to the northeastern edge of the island and encompassed an area less than approximately 6.1% of the island's 4.95 ha (Fig. 1). In an inventory of the flora of Saint Catherines Island and other back-barrier islands in its vicinity, no additional *T. americana* var. *caroliniana* populations were found within a 4.5 km radius of either island (Coile and Jones, 1988; J. Evans, University of the South-Sewanee, unpublished data).

Demography—The recruitment, growth, and mortality of all juvenile and adult genets in each of the two sampled populations were tracked over 10 yr. Demographic data were initially recorded at both sites in 1999 and then re-censused in mid-June in 2003, 2006 (Bull Island only), and 2008. For each adult genet, we recorded either height (if less than 1.5 m) or stem diameter at breast height (BH = 1.4 m) of the largest stem and for each of its associated sprouts. For each juvenile genet, we recorded its height in centimeters and determined its age based on the number of stem growth increments between bud scars. All juvenile genets were marked with numbered aluminum tags. Subsequent re-censusing of marked juveniles confirmed the use of bud scars as an accurate determinant of age. In 2008, the reproductive status of adult genets was determined based on the presence/absence of flowers.

Genetic analyses—Total genomic DNA was acquired from Diane McCarthy (Colorado State University, Fort Collins, Colorado,
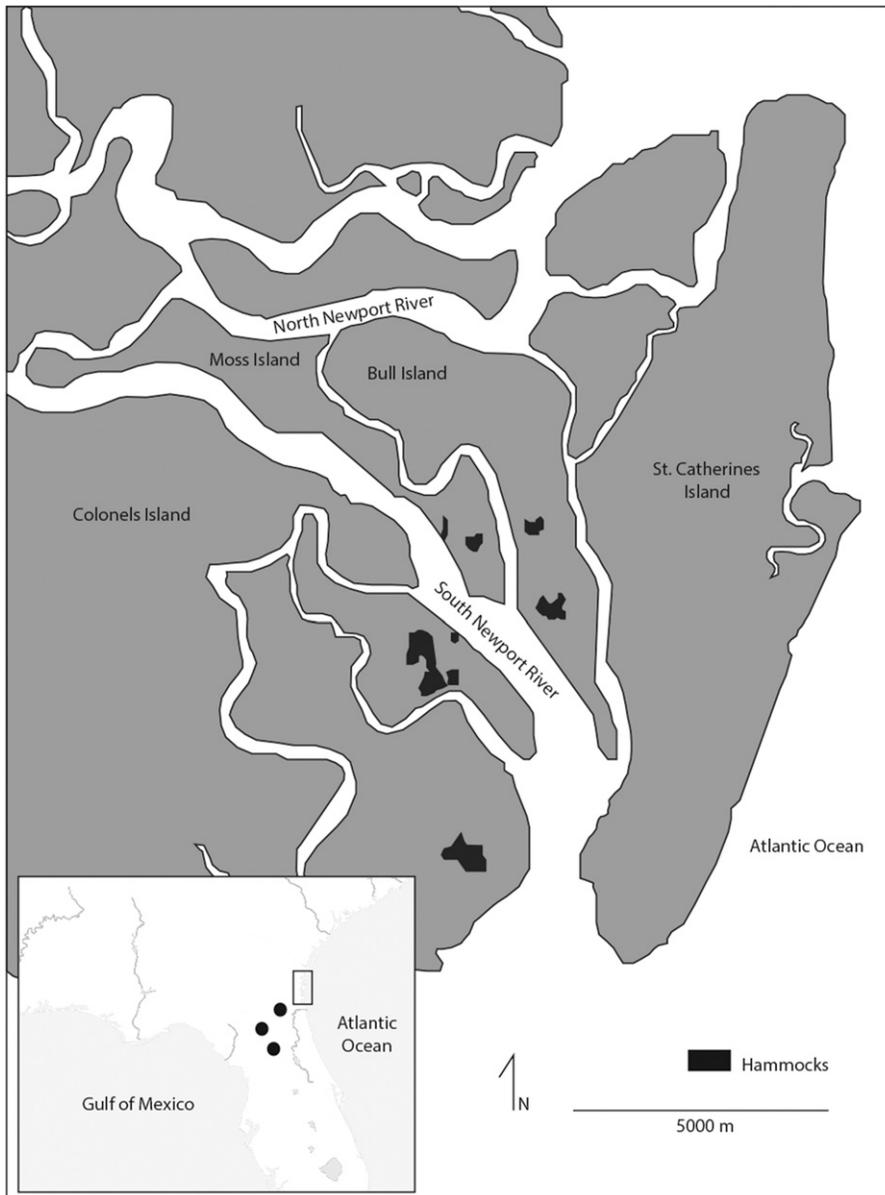
FIGURE 1 Areas sampled for *Tilia americana* var. *caroliniana* in the current study. Inset shows broader region of southeastern North America; box indicates area of focus in larger map; dots indicate additional areas sampled for genetic analyses (from north to south): Crooked River State Park (Georgia), Suwanee County (Florida), and Mill Creek Preserve (Florida). Demographic work was completed on the upland hammocks of Bull and Moss Islands (larger map) on the coast of Georgia, just west of St. Catherines Island. Figure modified from Napolitano (2012).

USA) for a total of 70 individuals representing five collection localities (including Bull and Moss Islands) (Fig. 1). McCarthy treated all collections of *Tilia* from across the North American range as *T. americana* and did not recognize distinct subspecies or other taxa (McCarthy, 2012; also found at http://hdl.handle.net/10027/9498). However, collection localities included in this study are consistent with the expected range for *T. americana* var. *caroliniana*. Collection localities included Crooked River State Park, Camden County, Georgia (GA) (*N* = 15), Bull Island, Liberty County, GA (*N* = 22), Moss Island, Liberty County, GA (*N* = 19), Mill Creek Preserve, Alachua County, Florida (FL) (*N* = 6), and a ravine in Suwannee

County, FL (*N* = 8). McCarthy extracted total genomic DNA from leaf material using a protocol developed for the Malvaceae. Samples from Bull and Moss Islands represented all adult genets known from those populations, while samples collected from other sites represent limited within-site collections for phylogeographic work (McCarthy, 2012). All samples included here were previously sequenced for the chloroplast DNA noncoding regions *rpL32-trnL* and *ndhF-rpL32* by McCarthy (2012). No sequence data were analyzed for the current study.

Thirteen nuclear microsatellite loci developed for *Tilia platyphyllos* were previously shown to amplify successfully in two accessions of *T. americana* (Phuekvilai and Wolff, 2013). Eight of these were used in the current study, following the methods summarized in Appendix S1 (see Supplemental Data with the online version of this article). A resampling procedure implemented in the program GEN-CLONE v. 2.0 (Arnaud-Haond and Belkhir, 2007) was used to determine that genetic resolution was sufficient to determine the multilocus genotypes (MLGs) for the current data set. Clonal assignment was determined using a range of thresholds (1-step to 15-step) under the stepwise mutation model as implemented in the program GENODIVE 2.0b23 (Meirmans and Tienderen, 2004). This assessment was done to estimate the potential of genotyping error or somatic mutation influencing genotyping results. A test using corrected Nei's diversity index was also performed to determine the probability that shared genotypes were a consequence of clonality rather than a function of sexual reproduction involving closely related individuals (Gómez and Carvalho, 2000). Clonal richness (*R*) was calculated as $(G – 1)/(N – 1)$ where *G* is the number of genotypes and *N* is the number of individual samples. *R* is a modification of proportion distinguishable (PD; Ellstrand and Roose, 1987) that accounts for small sample sizes (Dorken and Eckert, 2001).

Genetic data were analyzed using the software package GenAlEx 6.5 (Peakall and Smouse, 2006, 2012). Summary statistics calculated for each locus over all populations included number of alleles (N_a), allele size range in base pairs, observed heterozygosity (H_o), expected heterozygosity (H_e), F_{IS}, and F_{ST}. Summary statistics calculated for each population over all loci included number of alleles (N_a), effective number of alleles (N_e), number of private alleles, observed heterozygosity (H_o), expected heterozygosity (H_e), and the fixation index (*F*). Deviation from Hardy–Weinberg equilibrium (HWE) was tested for each locus by population. Pairwise population F_{ST} values were used to compare relatedness among populations. Analysis of molecular variance (AMOVA) was performed using a matrix of pairwise F_{ST} values. Comparisons were

made among populations, among individuals, and within individuals. Principal coordinate analysis (PCoA) was performed using an individual genetic distance matrix to visually assess major patterns among all individuals sampled. Bayesian clustering of individuals by genotype was performed as implemented in the software package STRUCTURE 2.3.4 (Pritchard et al., 2000; Falush et al., 2003) following the recommendations of Porras-Hurtado et al. (2013). Fifty iterations were performed of 500,000 Markov chain Monte Carlo (MCMC) replicates following a burn-in period of 100,000 for each K, varying from 1 to 6 using the admixture model and allele frequencies correlated. The estimated value of K was determined following the method of Evanno et al. (2005) using Structure Harvester (Earl and vonHoldt, 2012) to estimate the number of populations in our study based on ΔK.

RESULTS

Genet demography—Between 1999 and 2008, the adult population of basswoods on Bull Island and Moss Island decreased in size as a result of both adult genet mortality and the complete lack of recruitment of new adult genets from the pool of juvenile genets (Table 1, Fig. 2). The number of adult genets declined from 31 to 22 on Bull Island and from 23 to 19 on Moss Island. There was no significant relationship between maximum DBH of genets in 1999 and likelihood of mortality by 2008 ($\chi^2 = 0.21$, df = 1, $p = 0.643$), nor was absence of sprouts in 1999 a significant predictor of genet mortality ($\chi^2 = 2.01$, df = 1, $p = 0.157$). In 2002 and 2006, many of the living basswood ramets on Bull Island were damaged by *Sphyrapicus varius varius* L. (yellow-bellied sapsucker). In 2002, sapsucker-damaged basswood genets had a greater mean number of sprouts than genets lacking such damage ($\chi^2 = 4.61$, df = 1, $p = 0.032$). By 2006, 41% of adult genets on Bull Island showed sapsucker damage in the form of holes that girdled stems, and 33% of adult genets that had died by 2006 had manifest serious sapsucker damage to one or more ramets.

In 2008, the only year in which flowering was recorded, 91% of adult genets were flowering on Bull Island, and 84% were flowering on Moss Island. On Bull Island, this high level of reproductive activity also translated to increasing density of juvenile genets over the study period (43 to 144 between 2002 and 2008) as a result of successful seedling establishment. All originally tagged and newly established juvenile genets on Bull Island were located within 15 m of an adult genet, with more than 65% of juveniles located either under or directly adjacent to the canopy of an adult genet. Of the original cohort of 43 juvenile genets tagged in 1999, only one persisted throughout the study period of 10 yr. No other juvenile genet was found to live longer than 9 yr, and no juvenile of any cohort was able to grow taller than 0.2 m (including the 10-yr-old individual).

Interestingly, the high frequency of flowering on Moss Island as recorded in 2008 did not translate to high fecundity during the study. There were no juvenile genets identified on Moss Island at the time of the initial census in 1999, and no seedlings were established during the study.

During the 10-yr study, the percentage of adult genets with sprouts was consistently higher on Moss Island (95% in 2008) than on Bull Island (55% in 2008; Table 1). On both islands in 2008, there was an average of approximately six sprouts per genet, with small sprouts (<1.5 m tall) composing a greater proportion of the genet clump than large sprouts (>1.5 m tall; Table 1). Of the adult genets alive in 2008, 9% on Bull Island and 28% on Moss Island had lost the largest stem originally recorded in each clump in 1999, and each of these genets had transitioned to a new dominant stem that was smaller in size. This transitioning of stems contributed to the fact that the average maximum DBH associated with an adult genet in both populations remained constant over the 10-yr period (Table 1).

Genetic analyses—A total of 70 individuals from five populations were assessed for genetic variation using eight nuclear microsatellite loci. Summary statistics are provided in Table 2 and Appendix S2 (see online Supplemental Data). Of these, three samples were removed from analyses due to poor genotype profiles, likely from lower DNA quality, for a total of 67 individuals in the current study. The previously determined cpDNA haplotypes for these individuals are included in Table 3. All loci were polymorphic, with the number of alleles ranging from 8 to 16 per locus across all individuals sampled. One locus (*Tc6*) exhibited deviation from HWE in all but one population (Moss Island), while several other loci deviated from HWE in only one or two populations (online Appendix S3).

The resampling procedure of Arnaud-Haond and Belkhir (2007) indicated that five loci were sufficient to determine all MLGs for the current data set; all statistics were calculated based on eight loci. Clonal assignment identified 61 unique genets among the 70 individuals genotyped. These results were not affected by implementing different mutation thresholds until reaching a 14-step threshold. The probability of finding the observed clonal diversity under random mating using corrected Nei's diversity index was significant overall ($p = 0.001$). Individual sites with significant values for this measure ($p = 0.001$) included Bull Island and Mill Creek (Alachua County, FL), while the remaining sites of Crooked River (Camden County, GA), Moss Island, and Suwanee County, FL, were not significant ($p = 1.00$). Clonal richness is summarized in Table 3. Of the 21 individuals sampled at Bull Island, 17 represented unique genets ($R = 0.80$). At Mill Creek, three of five sampled individuals represented unique genets ($R = 0.50$). At the remaining sites, all sampled individuals represented unique genets ($R = 1.00$). No clonal genotypes were shared across sites.

TABLE 1. Comparison of adult genet characteristics of *Tilia americana* var. *caroliniana* from two isolated populations on Bull and Moss Islands in Liberty County, Georgia, presented as mean and standard deviation (in parentheses) at the start and end of the 10-yr study.

Study site	Max stem diam. (cm) within genet (±SD)		No. small sprouts/genet (±SD)		No. large sprouts/genet (±SD)		Total no. sprouts/genet (±SD)		Genets (%) with sprouts	
	1999	2008	1999	2008	1999	2008	1999	2008	1999	2008
Bull Island	11.3 (5.3)	11.6 (5.1)	1.5 (2.7)	5.3 (10.6)	0.9 (1.5)	0.8 (1.2)	2.4 (3.2)	5.8 (11.1)	67.7	54.5
Moss Island	9.9 (4.6)	10.1 (5.2)	3.2 (2.7)	3.6 (6.9)	2.3 (2.6)	1.9 (1.5)	5.6 (4.1)	5.5 (7.1)	87.0	94.7
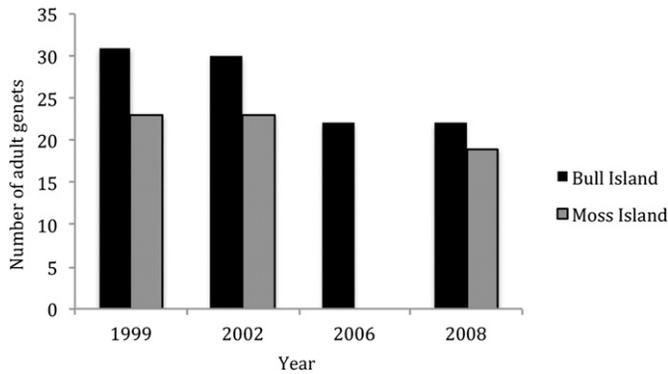
FIGURE 2 Change in total number of adult genets in each island population of *Tilia americana* var. *caroliniana* from coastal Georgia during the 10-yr study. No data were collected from Moss Island in 2006.

Genetic structure among populations was limited, as indicated by multiple analyses. Pairwise population F_{ST} values varied from 0.031 to 0.099 (Table 4), with the Crooked River population exhibiting the greatest differentiation, followed by Moss Island. The vast majority of variation was within individuals (92%), while only 8% of variation was observed among populations, based on our AMOVA results (online Appendix S4). PCoA revealed that 35.13% of the observed variation was explained by the first three axes combined (online Appendix S5). Bayesian clustering analysis failed to converge on a consistent selection of K (data not shown), suggesting limited or no true structure among individuals sampled. Furthermore, evaluation of bar plots indicated that samples were evenly distributed across K populations for each selection, another indication that true structure was not detected.

DISCUSSION

Clonality is well recognized as a mechanism for long-term regional persistence, but estimating the age of clones is a significant challenge (de Witte and Stöcklin, 2010). In some taxa, clonal growth patterns allow researchers to associate annual growth rate with clone age (de Witte and Stöcklin, 2010; Takahashi et al., 2011). However, most tree species are sprouters, rather than being truly clonal (i.e., capable of vegetative spreading), making it even more difficult to quantify the longevity of a genet (Bond and Midgley, 2001). In the current study, historical, demographic, and genetic data were combined to assess long-term persistence in coastal *Tilia americana* var. *caroliniana* populations on Bull and Moss Islands, Georgia.

During the 10-yr study, adult *Tilia americana* var. *caroliniana* genets in both populations were maintained through the continual

production of stem sprouts. Given the indeterminate flux of stems following the loss of the original stem within a genet clump, it is not possible to determine the age of the genets. However, it has been suggested that genets of *Tilia cordata*, growing at the northern limits of its range in Europe, have survived by sprouting for more than 5000 yr (reviewed by Bond and Midgley, 2003). *Tilia cordata* has a similar multistemmed growth form to that of *T. americana*. *Tilia americana* produces a belowground lignotuber, a site for the production and storage of suppressed buds, the storage of carbohydrates, and the production of adventitious roots, all of which facilitate basal sprouting, particularly following traumatic injury (Del Tredici, 2001). Lignotubers have often been described as an adaption to fire-prone environments, but studies have also documented their importance in the absence of fire and presence of other disturbances (Mesléard and Lepart, 1989; Bond and Midgley, 2003).

Tilia americana var. *caroliniana* populations in this study were located on the edges of both islands growing on soil dominated by shell midden material (Napolitano, 2012). *Tilia americana* var. *caroliniana* may have been eliminated from the rest of each island when it was cleared for cotton production in the late 1700s. Its limited dispersal ability may have restricted its spread into these formerly cleared fields. Rackham (1986) reported that *T. cordata* growing in ancient woodlands in Suffolk, England, failed to colonize adjacent secondary woodlands that were established in the 1600s. Similarly, Hewitt and Kellman (2002) found that *T. americana* growing in a mature hardwood forest in southern Ontario failed to colonize adjacent pine plantations beyond 25 m. The seed bracts are considered to function in wind dispersal of the fruits. Jones (1968) however, observed that the fruits were never dispersed far from the parent trees, and often, the fruits fell off leaving the bracts hanging on the trees. The majority of *Tilia* seeds fall less than two tree lengths from the parent as a result of gravity and limited wind dispersal (Anderson, 1976; Crow, 1990; Pigott, 2012). The tight clustering of *Tilia americana* var. *caroliniana* seedlings around the parent trees within the Bull Island population attests to limited dispersal ability.

The genetic data presented here suggest that genetic structure is limited, both between islands and among populations across the broader sampled region. In the context of the current study, limited genetic differentiation among Bull and Moss Islands and the mainland sites for both chloroplast sequence data (McCarthy, 2012) and nuclear microsatellite data (present study) would suggest long-term gene flow among sites that would limit genetic isolation. However, geographic isolation of Bull and Moss Islands from other populations of *T. americana* would suggest limited opportunities for gene flow. This species does not self nor is it apomictic. Given that seed set in *T. americana* is strictly through insect pollination (Crow, 1990), the 900 m of marsh and water between the islands likely isolates the populations from each other and any more distantly located populations (although this supposition has not explicitly been tested). The results of the demographic work presented here support the idea of long-term persistence through sprouting, which would be expected to maintain a relictual genetic connection to mainland sites in absence of recent gene flow.

One unexpected finding in the current study was the occurrence of multiramet genets separated by a considerable distance. At Bull Island,

TABLE 2. Summary statistics for five populations of *Tilia americana* var. *caroliniana* based on 67 individuals from Georgia and Florida averaged across eight nuclear microsatellite loci. All loci were previously published by Phuekvilai and Wolff (2013).

Population	N_a	N_e	H_o	H_e	F
Crooked River, GA	8.500 (1.052)	5.257 (0.750)	0.733 (0.052)	0.781 (0.030)	0.058 (0.060)
Bull Island, GA	7.500 (0.378)	4.100 (0.314)	0.714 (0.018)	0.743 (0.026)	0.028 (0.047)
Moss Island, GA	5.375 (0.680)	3.263 (0.490)	0.799 (0.074)	0.641 (0.053)	−0.243 (0.071)
Mill Creek, FL	4.625 (0.420)	3.593 (0.464)	0.800 (0.065)	0.678 (0.054)	−0.183 (0.035)
Suwanee Co., FL	5.500 (0.567)	4.189 (0.721)	0.766 (0.107)	0.683 (0.072)	−0.094 (0.082)

Notes: All values are means (with standard error) across all loci; N_a = number of alleles; N_e = effective number of alleles; H_o = observed heterozygosity; H_e = expected heterozygosity; F = fixation index.

TABLE 3. Clonal structure among 67 individuals from five sampling localities of *Tilia americana* var. *caroliniana* in southeastern North America based on eight nuclear microsatellite loci.

Site	N	G	R	cpDNA haplotypes (McCarthy, 2012)
Bull Island, GA	21	17	0.80	A
Moss Island, GA	18	18	1.00	A, B
Crooked River, GA	15	15	1.00	A, D, H
Mill Creek, FL	5	3	0.50	A
Suwanee County, FL	8	8	1.00	H

Notes: N = sample size; *G* = number of unique genets; *R* = clonal richness as calculated by (*G* − 1)/(*N* − 1). *R* is a modification of proportion distinguishable (PD; Ellstrand and Roose, 1987) that accounts for small sample sizes (Dorken and Eckert, 2001).

four two-ramet genets were identified, with each pair being separated by several meters. All analyses indicate that this finding is not due to genotyping error, nor is it due to lack of genetic resolution. Given that sprouting in *T. americana* results from a lignotuber, sprouts are expected to be limited to the base of the tree. As such, sprouts are not expected to be found at any distance from the tree as might be seen in species in which root suckering is prevalent (e.g., *Fagus grandifolia*). One possible explanation for this finding could be the formation of adventitious rooting from a snapped or fallen tree (Koop, 1987). Koop (1987, p. 105) indicated that *T. cordata* may experience "reiterative trunk suckers" when the parent tree has experienced disturbance, including damage that "results in partial uprooting or breaking of the stem". While this region of the Atlantic Coast has not experienced hurricane activity in recent years, at least three major hurricanes made direct hits on or near Savannah in the late 1800s, which would be within the potential life span of a clonally persistent individual. Other studies of forest recovery following hurricane activity in Puerto Rico (Walker, 1991) and Jamaica (Bellingham et al., 1994) have indicated that sprouting from the trunks of both snapped and uprooted trees of many species was a common occurrence, although longevity of those sprouts was not followed. Zywiec and Holeska (2012) followed sprout demographics in *Sorbus aucuparia* for 12 yr, finding that individuals within the species had different capacities for sprouting and sprout replacement. Differential sprouting capacities appear to be at play for *T. americana* var. *caroliniana* on Bull and Moss Islands.

Our data show that long-term persistence of genets in *T. americana* var. *caroliniana* populations on isolated back-barrier islands is dependent on continued basal sprouting and not recruitment of new sexually reproduced genets. Germination of *Tilia americana* seeds is delayed (2–5 yr) and generally low regardless of seedbed conditions (Crow, 1990). Parthenocarpy and insect predation limit seed viability in *T. americana*, with one study finding only 2% seed viability among fruit present in the forest litter (Godman and Mattson, 1976). Seedlings require shade for initial establishment, but then require canopy gaps for continued growth and recruitment as saplings (Crow, 1990). Variable seed production, limited seed viability, and poor seedling

TABLE 4. Population pairwise F_{ST} values for five populations of *Tilia americana* var. *caroliniana* from Georgia and Florida.

Population	Crooked River	Bull Island	Moss Island	Mill Creek	Suwanee Co.
Crooked River, GA	0.000				
Bull Island, GA	0.058	0.000			
Moss Island, GA	0.099	0.055	0.000		
Mill Creek, FL	0.081	0.031	0.045	0.000	
Suwanee Co., FL	0.067	0.049	0.084	0.044	0.000

success all likely contribute to the restricted recruitment of new genets. Although seedlings did become established in one of the populations, no seedlings survived long enough to be recruited into a larger size class during the 10 yr. While conditions on both islands during the study did not allow for new genet recruitment, it is possible that "windows of opportunity" (Eriksson and Fröborg, 1996) occurring intermittently over the past centuries (potentially associated with unique disturbance events) have facilitated a low level of genet recruitment into the population. Even an extremely low rate of genet input into a long-lived clonal population can result in the maintenance of genetic diversity over long periods (Eriksson and Fröborg, 1996; Persson and Gustavsson, 2001; Franks et al., 2004).

The back-barrier populations of *T. americana* in Georgia can be considered part of the trailing edge of the distribution of this species in North America. Populations in the trailing edge can be characterized as declining in size and number and generally increasing in interpopulation distances (Hampe and Petit, 2005; Levin, 2012). As noted by Davis and Shaw (2001), for tree species whose ranges are expected to shift in response to climate change, trailing edge populations are more dependent on local genetic variation to feed evolutionary adaptation in absence of new sources of variation, which equates to slower rates of adaptive change and higher rates of mortality than might be expected in interior populations (Levin, 2012). Alternatively, phenotypic plasticity of plant functional traits is expected to improve the ability of trailing edge populations to respond relatively quickly to such changes (Nicotra et al., 2010). In general, long-lived species are expected to be more resistant to the potential negative impacts of climate change than are short-lived species (Morris et al., 2008). Trailing-edge disequilibrium has been described as the unexpected persistence of a species or community type in light of environmental change, and the intensity of this response can be heightened by the presence of clonal individuals (Svenning and Sandel, 2013). With the trailing-edge equilibrium in mind, the results of the current study on *T. americana* var. *caroliniana* suggest that the role of clonal persistence in response to anticipated climate change warrants further investigation because it may have significant as yet unrecognized impacts on ecosystem disequilibrium of temperate forested systems.

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