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SHORT COMMUNICATION

Frequent fire protects shortleaf pine (*Pinus echinata*) from introgression by loblolly pine (*P. taeda*)

John F. Stewart · Rodney E. Will · Kevin M. Robertson · C. Dana Nelson

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Abstract Across much of the globe, fire is a major disturbance agent of forest and grassland communities. The removal of fire from previously fire-maintained ecosystems, which has occurred in many areas, changes species composition, favoring later less fire tolerant species over fire-adapted ones. A recent measured increase in the rate of hybridization between the fire-adapted shortleaf pine (*Pinus echinata*) and less fire-adapted loblolly pine (*P. taeda*) suggests that introgression may be an emerging threat to shortleaf pine as a genetically distinct species. We used 25 microsatellite markers on seedlings and saplings to test how the use of frequent fire affects the survival of hybrids between the two species by contrasting species makeup and hybridity in regularly burned areas (every 2 years) to that in neighboring unburned areas, both with mixed canopies of loblolly pine, shortleaf pine, and hybrids. The results show that frequent prescribed fire selects against loblolly pine and hybrids, restoring the community to one dominated by shortleaf pine. These results are the first to indicate that frequent fire can resist introgression between two co-occurring native species and that fire exclusion as a land management policy may be having unrecognized deleterious effects on the genetic integrity of species previously isolated from one another based on fire tolerance.

Keywords Shortleaf pine · Introgression · Fire ecology · Ecological genetics · Silviculture

J. F. Stewart · R. E. Will (✉)
Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK, USA
e-mail: rodney.will@okstate.edu

K. M. Robertson
Tall Timbers Research Station and Land Conservancy, Tallahassee, FL, USA

C. D. Nelson
United States Department of Agriculture Forest Service, Southern Research Station, Southern Institute of Forest Genetics, Saucier, MS, USA

Introduction

The closely related shortleaf pine (*Pinus echinata*) and loblolly pine (*P. taeda*) have shared a sympatric range throughout most of the Southeastern United States for the last 14,000 years (Schmidtling 2007). Historically, shortleaf pine had twice the range of loblolly pine and was more often found on drier sites with a frequent fire regime compared to the faster growing loblolly pine which preferred more mesic sites with less frequent fire (Mattoon 1915; Mohr 1897). Currently, the shortleaf pine forest type occupies only 1.9 million ha across the Southeastern United States and is continuing to decline (South and Buckner 2003). Government policy in the twentieth century led to widespread fire suppression and reduction of intentional burning, changing the species makeup of forests and altering the natural selection pressures on all community constituents (Van Wagtendock 2007). Fire exclusion has contributed to reduced abundance of shortleaf pine (Guyette et al. 2007). In contrast, loblolly pine forests have increased from 12 million ha since the 1950s to approximately 16 million ha today due primarily to plantation establishment.

Recent evidence suggests that the rate of hybridization between the loblolly pine and shortleaf pine has dramatically increased since the 1950s, from 4.5 to 27.3 % in loblolly pine stands and from 3.3 to 45.7 % in shortleaf pine stands, when current seedling populations were

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compared to adult trees (Stewart et al. 2012). Introgression is a threat to many species, since it can lead to genomic extinction (Allendorf et al. 2001). The recent increase in hybridization could be due to factors such as wide-scale planting of loblolly pine (often with non-local seed sources), climate change, and habitat fragmentation (Tauer et al. 2012). Importantly, increased survival of hybrid seedlings could be a result of wide-scale fire exclusion, because shortleaf pine seedlings more vigorously sprout after top kill by burning than loblolly pine seedlings (Williams 1998). In addition to a greater inherent capacity to sprout, shortleaf pine produces a basal crook, a 2–7 cm horizontal segment of stem lying on the ground that contains dormant buds. The basal crook is often covered by duff and soil which protects dormant buds from fire. Will et al. (2013) found that artificially produced shortleaf pine × loblolly pine F1 hybrid seedlings exhibit rapid growth like loblolly pine, have sprouting capacity intermediate to parental species following top kill, and possess an intermediate crook that results in an exposed stem segment extending from the soil surface at an approximately 45° angle.

To test the hypothesis that fire selects against hybrids and therefore limit introgression, we determined whether prescribed fire favors shortleaf pine regeneration compared to loblolly pine and hybrids in mixed species stands. We estimated the genomic proportion of the regeneration component (i.e., seedlings and saplings) in four pairs of adjacent stands that were burned every 2 years and non-burned stands (no fire for previous 4–5 decades) growing under a mixed overstory of shortleaf-loblolly pine at Tall Timbers Research Station and Land Conservancy (TTRSLC, Tallahassee, FL, USA). Relatively higher occurrence of hybrids favoring a loblolly pine genomic component in non-burned stands versus burned stands would indicate that fire exclusion is leading to increasing introgression of loblolly pine into shortleaf pine and that prescribed fire can be used as a tool to conserve the genetic integrity of shortleaf pine.

Materials and methods

The TTRSLC (30°40′N, 84°14′W) occupies 1619 ha dominated by open-canopy, upland pine communities of primarily loblolly pine and shortleaf pine with herb and shrub surface vegetation arising from agricultural fields abandoned between the late 1800s and 1930s as well as native shortleaf pine areas (Reid et al. 2012). Research plots on previously (but not currently) burned areas have been fire-excluded since 1960 (Stoddard Fire Plots) or 1966 (NB66) and consequently have become dominated by deciduous broadleaf trees in addition to pines. Currently burned areas are have been burned at 2 year fire return intervals during the past 3–4 decades, and prior to that were burned annually since agricultural abandonment in the 1920s or before. Fires are applied from late February to early April (dormant to growing season transition) with a combination of backing fire, flanking fire, and strip head fire ignition techniques. Fuel and fire behavior measurements on TTRSLC report an average of 6 Mg ha^{-1} of fuel are consumed during burns, with an average head fire flame length of 0.9 m, rate of spread of 0.06 m min^{-1}, and fireline intensity of 473 kJ s^{-1} m^{-1}.

In November 2012, we collected foliage samples from seedlings and saplings (ranged between 9 and 106 cm tall) within four pairs of adjacent non-burned and burned stands. We sampled either all seedling/saplings within a stand or seedlings/saplings along a randomly located transect to avoid bias (total number of stands was eight; four pairs of burned and non-burned stands). Non-burned stands ranged in size from 0.24 to 1.30 ha, and burned stands ranged from 0.47 to 1.53 ha (Table 1). Two of the paired stands were along different edges of a large non-burned area (NB66, 9.2 ha). Overstory trees ranged up to ca. 150 years. Basal areas ranged from 5.4 and 8.8 m^2 ha^{-1} in burned stands and from 17.5 to 33.1 m^2 ha^{-1} in non-burned stands, and hardwoods were totally excluded from burned stands (Table 1). Of the 309 hardwood trees measured, 185 were *Quercus nigra*, 65 were *Prunus serotina*, 27 were *Liquidambar styraciflua*, 16 were *Quercus hemispherica,* 7 were *Quercus virginiana,* 4 were *Quercus phellos,* 3 were *Quercus falcata,* and 1 was *Carya tomentosa.* In October 2013, we measured the overstory pine population trees with diameter 20 cm or more at 1.4 m height), and we identified tree species by morphological characteristics.

We collected foliage from 12 to 14 randomly selected trees in each stand. DNA samples from seedlings and overstory trees were extracted from the foliage samples using the DNeasy Kit (Qiagen, Valencia, CA). Q_{Lob} (the approximate loblolly pine genomic proportion) was determined for each tree using genotypic data from 25 microsatellite markers from Stewart et al. (2012) using Structure 2.3.4 (Pritchard et al. 2000). We used allele calls from Stewart et al. (2012) to calibrate the new allele calls to existing data prior to the structure analysis and calculated the Q_{Lob} mean and standard deviation for each stand and tested for significance differences among stands with ANOVA using a randomized complete block design ($n = 4$).

Results

Fire highly affected species distribution of seedlings/saplings (Fig. 1; Table 2). On all sites, seedlings/saplings from

Table 1 Overstory tree (>20 cm DBH) and stand attributes for the burned and non-burned sites at Tall Timbers, FL

Area	Treatment	Stand size (ha)	Species	Trees	Density (trees ha^{-1})	DBH ± S.E. (cm)	Basal area (m^2 ha^{-1})
1	Burn	0.47	PIEC	6	12.8	42.8 ± 3.8	1.91
			PITA	13	27.7	55.0 ± 3.4	6.86
1	No-burn	0.25	PIEC	5	20.4	36.2 ± 4.1	2.20
			PITA	18	73.3	48.2 ± 2.9	14.21
			HRWD	34	138.5	26.4 ± 0.8	7.82
2	Burn	0.74	PIEC	23	31.0	44.7 ± 3.0	5.34
			PITA	5	6.7	59.2 ± 9.6	2.05
2	No-burn	0.25	PIEC	18	73.3	40.3 ± 2.3	9.86
			PITA	11	44.8	38.2 ± 5.6	6.24
			HRWD	35	142.5	30.7 ± 2.1	12.26
3a	Burn	1.53	PIEC	53	34.7	40.6 ± 2.0	5.06
			PITA	27	17.7	47.4 ± 3.2	3.50
3a	No-burn	1.30	PIEC	40	30.8	42.6 ± 2.6	5.00
			PITA	21	16.2	54.4 ± 4.0	4.17
			HRWD	169	130.4	27.2 ± 0.7	8.33
3b	Burn	0.77	PIEC	10	13.0	49.9 ± 5.1	2.77
			PITA	6	7.8	65.4 ± 3.2	2.65
3b	No-burn	0.68	PIEC	80	118.1	38.2 ± 1.3	14.66
			PITA	68	100.4	36.1 ± 0.9	10.73
			HRWD	71	104.4	29.4 ± 1.0	7.71

PIEC shortleaf pine, *Pinus echinata*; *PITA* loblolly pine, *Pinus taeda*; *HRWD* hardwood species

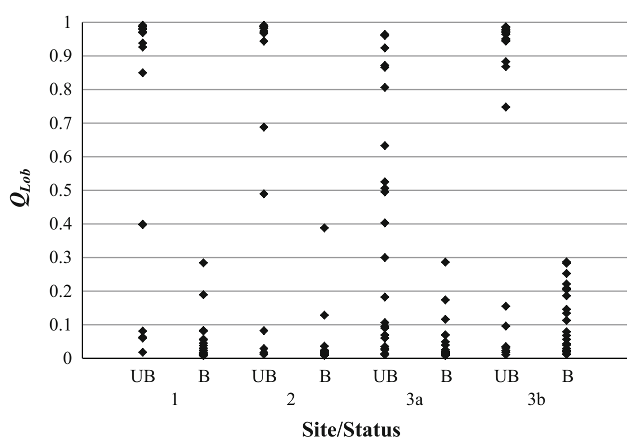

Fig. 1 The species status (Q_{Lob} or genomic composition) of seedlings/saplings from the unburned (UB) and burned (B) stands of the studied sites (1, 2, 3a, and 3b). While hybrid and loblolly pine seedlings/saplings (intermediate and large Q_{Lob} values) are distributed throughout the unburned areas, they are selected against in the burned areas

the non-burned stands were a mixture of shortleaf pine, loblolly pine, and hybrids similar to the mixture of overstory trees (Fig. 2). However, in the fire-treated stands loblolly pines were absent, and hybrids were less common. The mean Q_{Lob} value for seedlings/saplings from

Table 2 The distribution (%) of seedlings/saplings in the unburned (UB) areas was bimodal and included many hybrids, but the distribution of seedlings/saplings in the burned (B) stands was concentrated as shortleaf pine-identified

Site/status	1 UB	1 B	2 UB	2 B	3a UB	3a B	3b UB	3b B
Number of trees	17	25	21	25	24	24	24	24
LL[a]	47	0	62	0	8	0	42	0
LLBC3[a]	12	0	5	0	4	0	17	0
LLBC2[a]	6	0	0	0	8	0	8	0
LLBC1[a]	0	0	5	0	8	0	4	0
F1[a]	12	0	5	4	17	0	0	0
SLBC1[a]	0	8	0	0	4	4	0	25
SLBC2[a]	0	0	0	4	13	8	8	17
SLBC3[a]	18	16	5	0	13	13	0	13
SL[a]	6	76	19	92	25	75	21	46

[a] LL indicates loblolly pine identification (Q_{Lob} = 0.953–1), while LLBC1–3 identifications indicate hybrids backcrossed into loblolly pine 1–3 times (Q_{Lob} = 0.625–0.813, Q_{Lob} = 0.813–0.906, and Q_{Lob} = 0.906–0.953, respectively). Likewise, SL indicates shortleaf pine identification (Q_{Lob} = 0–0.047), and SLBC1–3 indicate degrees of backcrossing (Q_{Lob} = 0.188–0.375, Q_{Lob} = 0.094–0.188, Q_{Lob} = 0.047–0.094, respectively) of hybrids into shortleaf pine. F1 refers to saplings identified as first-generation shortleaf pine/loblolly pine hybrids (Q_{Lob} = 0.375–0.625). The pedigree history of each sapling may be more complex than what is indicated in the table

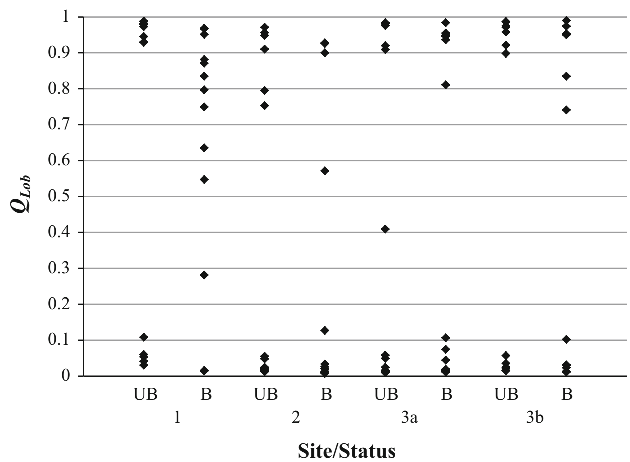

Fig. 2 The species status of the overstory trees in the unburned (UB) and burned (B) stands of the studied sites 1, 2, 3a, and 3b. The overstory trees were generally a mix of loblolly pine, shortleaf pine, and their hybrids, indicating that the genetic background was not biased for any species in either fire treatment. Individuals are plotted on their sites (1, 2, 3a, and 3b) and treatment (UB or B) status

non-burned stands was significantly higher (0.614) than those in burned stands (0.056; $P = 0.006$). Because most of the species identities of the seedlings/saplings from burned stands were shortleaf pine, the standard deviations of Q_{Lob} values calculated from seedlings in burned stands (0.075) were much smaller than for seedlings/saplings from non-burned stands (0.398; $P < 0.0001$) which contained a mixture of shortleaf pine, loblolly pine, and their hybrids.

In contrast, the identities of overstory trees were a mixture of shortleaf pine, loblolly pine, and hybrids (Fig. 2) regardless of burn regime. There were no significant differences between trees from burned versus non-burned stands for the mean Q_{Lob} values (0.478 versus 0.431, respectively; $P = 0.36$) and the standard deviations (0.458 versus 0.480, respectively; $P = 0.14$). Thus, the local genetic source for seed on the sites was well-mixed between shortleaf pine and loblolly pine. Overall, 10 % of trees were identified as F1 or first generation backcrosses, though most of those were from one study site (Table 2).

Discussion

To our knowledge, this is the first study to show that fire selects against the hybrids of two plant species and thereby maintains genetic distinctions between co-occurring, interbreeding species. While it was known that fire favors shortleaf pine seedlings over loblolly pine seedlings (Williams 1998), the selectivity of fire at the genetic level is a new insight. Our results are consistent with the strong correlation between fire frequency and shortleaf pine dominance during the 1700–1800s (Batek et al. 1999). Frequent (2–3 years interval), low-intensity fires started by local inhabitants and lightning were once widespread throughout the Southeastern United States and in shortleaf pine ecosystems in particular (Guyette et al. 2006).

Our results have broad implications for shortleaf pine forest conservation, which is an emerging concern. They indicate that prescribed fire can be used to return the fire-dependent shortleaf pine to dominance within its historic locations and maintain the genetic integrity of shortleaf pine as a species in the Southeastern United States. Loblolly pine and shortleaf pines are most likely to survive fire if they are greater than 2.4 m tall or 3.8 cm in ground line diameter (Cain and Shelton 2002). Dey and Hartman (2005) indicate ground line diameter of greater than 4 cm for 50 % survival and 10 cm for 90 % survival of shortleaf pine. Therefore, the potential to favor shortleaf pine using prescribed fire is only efficacious during the seedling and sapling stages. The presence of loblolly pine and hybrids in the overstory at TTRSLC indicates periods of fire exclusion in the past that allowed for their establishment and is a testament to their persistence and potential lingering effects on population genetics once they develop bark thick enough to tolerate surface fire.

While the role of fire in maintaining grasslands, savannas, and woodlands by elimination of fire-sensitive species is well known, the role of fire in preventing introgression through selective mortality is virtually unstudied. Typically, introgression occurs when non-natives invade and interbreed with related native species, but in this case, there is evidence that the loss of an important selective pressure contributed to the process of displacing non-hybrid individuals. By reducing the abundance of pure shortleaf pine, hybridization with loblolly pine may reduce the resilience of the southern pine forest in the United States to drought and wildfire. We present one case study, but there are likely other examples of hybrid survival leading to introgression that are occurring due to fire exclusion as previous barriers that separated habitats of related species break down. Widespread hybridization may present major conservation concerns that we do not yet appreciate. Further studies such as this may emphasize the need to reintroduce historical fire regimes to restore and maintain the genetic integrity of native plant populations.

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