Genetic characterization of *Liriodendron* seed orchards with EST-SSR markers

Xinfu Zhang1, Alanna Carlson1, Zhenkun Tian2, Margaret Staton3, Scott E. Schlarbaum4, John E. Carlson5 and Haiying Liang1*

Abstract

*Liriodendron tulipifera* L., is a wide-spread, fast-growing pioneering tree species native to eastern North America. Commonly known as yellow-poplar, tulip tree, or tulip-poplar, the species is valued, both ecologically and economically. It is perhaps the most commonly used utility hardwood in the USA, and is planted widely for reforestation and, in varietal forms, as an ornamental. Although most seedlings used for reforestation today derive from collections in natural populations, two known seed orchards, established from plus-tree selections, i.e. superior phenotypes, in the 1960’s and 1970’s have been used for local and regional planting needs in Tennessee and South Carolina. However, very little is known about the population genetics of yellow-poplar nor the genetic composition of the existing seed orchards. In this study, 194 grafted yellow-poplar trees from a Clemson, SC orchard and a Knoxville, TN orchard were genetically characterized with 15 simple sequence repeat (SSR) markers developed from expressed sequence tags (ESTs). Of the 15 EST-SSR markers, 14 had a polymorphic information content (PIC) of at least 0.5. There was no significant difference between the Clemson and Knoxville orchards in average effective number of alleles (5.93 vs 3.95), observed and expected heterozygosity (H<sub>O</sub>: 0.64 vs 0.58; H<sub>E</sub>: 0.74 vs 0.70), Nei’s expected heterozygosity (0.74 vs 0.58), or Shannon’s Information index (1.84 vs 1.51). The larger Clemson orchard exhibited a significantly greater number of observed alleles than the Knoxville orchard (15.3 vs 7.4). Overall, substantial genetic diversity is captured in the Clemson and Knoxville orchards.

Keywords: Genetic diversity, seed orchard, SSR markers, species

Introduction

*Liriodendron tulipifera* L., commonly known as yellow-poplar or tulip-poplar, is a wide-spread, fast-growing pioneering hardwood species of considerable economic value in the forests of eastern North America. Yellow-poplar is distributed predominantly east of the Mississippi River from the gulf coast to southern Canada (28° to 43° north latitude) [35]. According to the forest inventory analysis [11], as surveyed from 2006-2012, the total saw log volume of *L. tulipifera* on timberland in the United States was 25.9 billion cubic feet, with the majority (65%) located in the southeastern United States. The species is shade intolerant and highly competitive, growing faster than *Acer rubrum* L. (red maple) and *Quercus rubra* L. (northern red oak) seedlings under a variety of silvicultural understory treatments (Beckage and Clark 2003). Yellow-poplar is often seen as a pioneering species in old fields. As a component of 16 forest cover types, this species’ degree of dominance has created differentiation between the ecological communities [46]. In addition, yellow-poplar is valued as a nectar source for honey production, as a source of wildlife food (mast), and as a large shade tree in urban plantings [3]. The wood of yellow-poplar is used in a diverse range of products, such as in furniture, pallets and framing construction as well as pulp [12,41]). Chemical extracts from yellow-poplar wood or leaves have proven useful,
such as sesquiterpenes which have an anti-tumor effect and antifeeding for herbivores [27], and antimicrobial alkaloids [2].

*L. tulipifera* has been cultivated since 1663 [5] and is currently widely planted in eastern forests. Although seed orchards have been established to meet local or regional planting needs in the U.S.A. [6,36], genetic diversity of *Liriodendron* seed orchards in relation to natural stands has not been studied. Because seed orchards is the bridge between breeding and silvicultural activities, genetic diversity of tree seeds orchards determines the genetic quality of future forest stands and forms the basis for further improving the management of genetic resources and for the genetic modification of cultivars to meet new environmental challenges. Thus, the lacking information limits utilization of these *Liriodendron* orchards in a tree improvement program.

The primary goal of our study was to determine the genetic composition and diversity in two *Liriodendron* seed orchards in the southeastern USA. The orchard residing in Knoxville, Tennessee, was established in 1966 and contains 100 grafted ramets, representing 31 genotypes or clones. The Clemson orchard in South Carolina was established in 1976 by grafting multiple ramets of 150 plus trees selected from throughout the 17,500-acre Clemson Experimental Forest by Dr. Roland E. Schoenike (http://www.clemson.edu/trails/history/schoenike.html#top). Seeds from this orchard have been used for reforestation efforts for a number of years. Currently there are 165 surviving trees in the Clemson orchard. Besides *L. tulipifera*, the only other *Liriodendron* species is *Liriodendron chinense*, which is native to China and Vietnam.

Although the two species separated 10–16 million years ago [32], they are quite similar morphologically and are cross fertile [26,34], and the hybrids exhibit heterosis [31,39]. Because the incomplete records suggest that the Clemson orchard may contain *L. chinense* or hybrids, we first used the sequence of a chloroplast gene, *maturase K* (*matK*), to discriminate the two *Liriodendron* species and their hybrids. Then we investigated the genetic diversity and allele richness among selections of this unique native species in each orchard as a first step toward contrasting orchard-produced seedling diversity with natural diversity. We chose simple sequence repeat (SSR) markers (also called microsatellites) in the study, because SSR markers are co-dominant, easily reproduced and scored, highly polymorphic, abundant through the genome, and have higher information content than isoenzyme and dominant markers [45].

### Materials and methods

#### Plant materials and DNA isolation

Fresh leaves of all *Liriodendron* trees (165) from the Clemson seed orchard and 31 trees from the Knoxville seed orchard were collected in the spring of 2013 and stored in plastic bags at -80°C prior to DNA isolation. All these trees represented different clones as validated by the SSR markers used in this study. Leaves from a *Liriodendron tulipifera* tree (accession number 70921 H) from the US National Arboretum (collected by Kevin Conrad) were also included in the study. Total genomic DNA was isolated from leaves using a CTAB protocol as described in [16] and suspended in TE buffer (Tris base 6.1g/L, EDTA 0.37 g/L, pH 8). The quality and concentrations of genomic DNA from individual plants were determined with a NanoDrop 3300 (Thermo Scientific, Wilmington, Delaware, USA) and by electrophoresis on 0.8% agarose gels.

#### Distinguishing between two *Liriodendron* species based on *maturase K* sequence

The record of the 165 surviving *Liriodendron* trees in the Clemson orchard is not complete. Therefore, the sequence of a chloroplast gene, *maturase K* (*matK*) was used to discriminate between the species/hybrids. The *matK* sequence was amplified with forward (5'-CGATCTATTATTCTTATTCC-3') and reverse primers (5'-TCTAGCACACGAAAGTCGAAGT-3') in a 12.5-μl reaction containing 6.875 uL ddH2O, 1 uL MgCl2 (25 mM), 0.5 uL forward primer (10uM), 0.5 uL reverse primer (10uM), 0.25 uL dNTPs (10 mM each), 0.25 uL BSA (0.8ug/μL), 0.125 uL Taq Pololymerase (5u/μL), 0.5 uL DNA (~20ng/μL), 2.50 uL 5X PCR buffer (-Mg).

The conditions for polymerase chain reactions (PCR) were as follows: 5 minutes of initial denaturation at 94°C, 35 cycles of touch-down PCR with 30 seconds of denaturation at 94°C, 30 seconds of annealing at 60-50°C (first cycle 60°, then each subsequent cycle 1°C lower than the previous until 51°C annealing temperature, followed by 25 cycles each with a 50°C annealing temperature), and 3 minutes of extension at 72°C, and a final extension at 72°C for 10 minutes. Before being sequenced with 1 ul of 10 uM forward or reverse primer, PCR products were cleaned with ExoAP mix (89 uL H2O+ 10 uL 5000U/mL Antarctic Phosphatase +1 uL 20000U/mL Exonuclease I) for 30 minutes in a reaction containing 1 ul of PCR product and 1uL of ExoAP mix, followed by a heat inactivation step at 80°C for 15min. An 834 bp-segment of maturase K gene from each tree was used for alignment with MUSCLE and curated with Gblocks, and a phylogenetic tree was built with maximum likelihood (PhyML) (http://www.phylogeny.fr/) [7].

The maturase K gene sequence of *L. tulipifera* (GI: 5731451), *L. chinense* (GI: 7239759), and a hybrid (GI: 389955358) available in GenBank were included in the analysis.

#### *L. tulipifera* EST-SSR markers, PCR amplification, and allele sizing

Twenty simple sequence repeat (SSR) markers (also called microsatellites) were used to investigate the genetic composition of the *Liriodendron* seed orchards. These markers included seven Expressed Sequenced Tags (EST)-SSR markers (LT002, LT015, LT021, LT086, LT096, LT131, LT157) previously characterized by electrophoresis on 8% polyacrylamide gels [42] and thirteen new markers (LTCU19, LTCU40, LTCU51, LTCU53, LTCU125, LTCU139, LTCU142, LTCU143,
The two species are similar morphologically, except that...
| Marker name | Repeat motif | Forward primer sequence (5’-3’) | Reverse primer sequence (5’-3’) | Expected size (bp) | Stuttering | Annealing temperature °C |
|-------------|-------------|---------------------------------|---------------------------------|-------------------|------------|--------------------------|
| LT002       | (GCA)8      | CTTACCACCACGCA ATACCTA          | TCTCGTGCGCTGAAGAT ATG           | 189               | N          | 59                       |
| LT015       | (CCGAAC)5   | TCCGTATCTCTCTCTCAA             | CIAGACAGGTGCTCGG ATAC          | 110               | N          | 59                       |
| LT021       | (TTC)8      | CAAATACCAATGCA CACCCG           | ACGCATCCTCTCTCAC TAC           | 180               | N          | 57                       |
| LT086       | (CTT)10     | AAGCAGAGCTTCC CACTGGA           | GAACGAAACCTA ACACA            | 274               | N          | 55                       |
| LT096       | (CT)20      | TGCAACCTAAACAA ATGTCGA          | TGAAGAGCAACCAAG AATGA         | 272               | N          | 55                       |
| LT131       | (AC)22      | GCAGCATTCCTCTC ATATTCT          | TTGCAGTGTAGCTATT GATG         | 240               | Y          | 55                       |
| LT157       | (TTC)6      | AGTTGCCCTTTAGC TCTTTT          | GCCACAGAGTTTTGGAG AGTA        | 222               | Y          | 55                       |
| LTCU19      | (AG)10      | GTGAATGCAAAG GCAGGT             | AAAAAAAAGCAAACG AAGGG        | 183               | N          | 57                       |
| LTCU40      | (ATG)8      | TTGGTAGTAAATGCA TCCAAAG         | GAAGCCCtTGCAAGAT GCA         | 181               | Y          | 55                       |
| LTCU51      | (CT)18      | ATACCCATCTCTTCT CATGGGC         | AACCCATCCACCAT CCA           | 198               | N          | 55                       |
| LTCU53      | (TG)14      | CGGATCTTTCTCTGT TCCATGC         | AAGAGATTGCAAGAG GCAGA        | 223               | N          | 55                       |
| LTCU125     | (TC)8       | CGAAAGACATTCCC CATCCA           | CCATTCAATCCACAG CCA          | 205               | N          | 55                       |
| LTCU159     | (TCT)10     | GAATAACCGCTCT TTTTTGA          | AAGCAAAGTTGCAGGA GAAGA       | 164               | Y          | 55                       |
| LTCU142     | (AAT)8      | TGGTGCATATGGG CTTGAAA          | TATCTCCCCGAGCTTCT CTTT        | 171               | Y          | 55                       |
| LTCU143     | (TG)13      | AAAAAATGCTAATC CAATACTTTCTG    | TATCCAAACGTACC CATT          | 160               | N          | 55                       |
| LTCU145     | (GA)18      | TGGAAGTCCACAT GATTTG           | GCCTAGGAGTGTGTT TGG           | 157               | N          | 55                       |
| LTCU150     | (TC)10      | TCTTCAAACCAAG GCAGGTG          | GCACCTACATCTCTCTCa CCA        | 167               | N          | 55                       |
| LTCU151     | (TC)11      | TGAGGTGACTTGG GCTTCTG          | GCCCgATGTTAAAA TGGA          | 189               | N          | 55                       |
| LTCU152     | (CA)17      | CATCCAAATGCAG CAGAAAT          | ATTCCTACCTCGGTTGA ACAC       | 177               | N          | 55                       |
| LTCU154     | (CT)10      | GATGAAGGAAATG TCTATATTGCTGA    | CCAGCCAAGAAGA AATGG          | 156               | N          | 55                       |

*Y: Yes; N: No.*
The observed and expected heterozygosities (Ho and He) ranged from 0.17 to 0.89 and from 0.19 to 0.93, with averages of 0.62 and 0.74, respectively. The polymorphic information content (PIC) ranged from 0.17 to 0.92, with an average of 0.71. Overall, 14 of the 15 markers had a PIC ≥ 0.5.

Many genomic resources, such as expressed sequence tag (EST) databases [15, 22, 23] and genomic DNA libraries [24], have been developed for L. tulipifera. Through these resources, several thousand putative SSR markers have been identified by in silico mining. However, only 345 L. tulipifera SSR markers have been tested for polymorphism by polyacrylamide denaturing gels [42, 43]. Compared to other species, Liriodendron has lacked development of polymorphic and informative SSR markers.

As a result, no genetic linkage maps of Liriodendron have been reported. This is in contrast with the species’ ecological and economic value and phylogenetic position as a basal angiosperm.
### Table 3. Genetic variation at 15 EST-SSR loci characterized in the clemson orchard.

| Locus  | Sample size | Na | Ne  | Obs_Hom | Obs_Het | Exp_Hom | Exp_Het | Nei's I |
|--------|-------------|----|-----|---------|---------|----------|---------|--------|
| LT002  | 326         | 6.00 | 3.16 | 0.28    | 0.72    | 0.31     | 0.69    | 0.68   |
| LT015  | 324         | 8.00 | 2.30 | 0.46    | 0.54    | 0.43     | 0.57    | 0.57   |
| LT021  | 324         | 6.00 | 1.99 | 0.54    | 0.46    | 0.50     | 0.50    | 0.50   |
| LT086  | 318         | 9.00 | 1.84 | 0.69    | 0.31    | 0.54     | 0.46    | 0.46   |
| LT096  | 308         | 18.00 | 3.75 | 0.38    | 0.62    | 0.26     | 0.74    | 0.73   |
| LTCU19 | 326         | 15.00 | 3.20 | 0.40    | 0.60    | 0.31     | 0.69    | 0.69   |
| LTCU51 | 324         | 18.00 | 7.74 | 0.26    | 0.74    | 0.13     | 0.87    | 0.87   |
| LTCU53 | 310         | 13.00 | 4.84 | 0.30    | 0.70    | 0.20     | 0.80    | 0.79   |
| LTCU125| 308         | 26.00 | 11.46| 0.05    | 0.95    | 0.08     | 0.92    | 0.91   |
| LTCU143| 306         | 14.00 | 4.84 | 0.23    | 0.77    | 0.20     | 0.80    | 0.79   |
| LTCU145| 324         | 12.00 | 7.49 | 0.17    | 0.83    | 0.13     | 0.87    | 0.87   |
| LTCU150| 322         | 14.00 | 2.90 | 0.42    | 0.58    | 0.34     | 0.66    | 0.66   |
| LTCU151| 294         | 9.00  | 2.99 | 0.54    | 0.46    | 0.33     | 0.67    | 0.67   |
| LTCU152| 258         | 25.00 | 15.89| 0.33    | 0.67    | 0.06     | 0.94    | 0.94   |
| LTCU154| 302         | 37.00 | 14.49| 0.28    | 0.72    | 0.07     | 0.93    | 0.93   |
| Mean   | 312         | 15.33 | 5.93 | 0.36    | 0.64    | 0.26     | 0.74    | 0.74   |
| St. Dev.| --         | 8.53  | 4.59 | 0.16    | 0.16    | 0.15     | 0.15    | 0.15   |

Na: Observed number of alleles. Ne: Effective number of alleles (Kimura and Crow 1964). Obs_Hom/Obs_Het: Observed homozygosity/heterozygosity. Exp_Het/Exp_Het: expected homozygosity/heterozygosity (Levene 1949). Nei's (1973) expected heterozygosity. I=Shannon's Information index (Lewontin 1972). St. Dev.: Standard deviation.

### Table 4. Genetic variation at 15 EST-SSR loci characterized in the knoxville orchard.

| Locus  | Sample size | Na | Ne  | Obs_Hom | Obs_Het | Exp_Hom | Exp_Het | Nei's I |
|--------|-------------|----|-----|---------|---------|----------|---------|--------|
| LT002  | 62          | 5  | 3.65 | 0.32    | 0.68    | 0.26     | 0.74    | 0.73   |
| LT015  | 60          | 5  | 3.38 | 0.43    | 0.57    | 0.28     | 0.72    | 0.7    |
| LT021  | 62          | 2  | 1.17 | 0.84    | 0.16    | 0.85     | 0.15    | 0.15   |
| LT086  | 62          | 6  | 2.81 | 0.35    | 0.65    | 0.35     | 0.65    | 0.64   |
| LT096  | 62          | 10 | 4.75 | 0.32    | 0.68    | 0.2     | 0.8     | 0.79   |
| LTCU19 | 62          | 9  | 3.59 | 0.52    | 0.48    | 0.27     | 0.73    | 0.72   |
| LTCU51 | 60          | 12 | 5.84 | 0.27    | 0.73    | 0.16     | 0.84    | 0.83   |
| LTCU53 | 62          | 6  | 2.77 | 0.74    | 0.26    | 0.35     | 0.65    | 0.64   |
| LTCU125| 60          | 15 | 6.14 | 0.3    | 0.7     | 0.15     | 0.85    | 0.84   |
| LTCU143| 60          | 8  | 5.26 | 0.33    | 0.67    | 0.18     | 0.82    | 0.81   |
| LTCU145| 60          | 8  | 4.64 | 0.1    | 0.9     | 0.2     | 0.8     | 0.78   |
| LTCU150| 62          | 5  | 3.29 | 0.68    | 0.32    | 0.29     | 0.71    | 0.7    |
| LTCU151| 56          | 3  | 2.26 | 0.39    | 0.61    | 0.43     | 0.57    | 0.56   |
| LTCU152| 62          | 9  | 5.88 | 0.42    | 0.58    | 0.16     | 0.84    | 0.83   |
| LTCU154| 58          | 8  | 3.83 | 0.24    | 0.76    | 0.25     | 0.75    | 0.74   |
| Mean   | 61          | 7.4 | 3.95 | 0.42    | 0.58    | 0.29     | 0.70    | 0.58   |
| St. Dev.| --         | 3.4 | 1.44 | 0.2    | 0.20    | 0.17     | 0.17    | 0.16   |

Na: Observed number of alleles. Ne: Effective number of alleles (Kimura and Crow 1964). Obs_Hom/Obs_Het: Observed homozygosity/heterozygosity. Exp_Het/Exp_Het: expected homozygosity/heterozygosity (Levene 1949). Nei's (1973) expected heterozygosity. I=Shannon's Information index (Lewontin 1972). St. Dev.: Standard deviation.
US orchards had slightly lower values of average Ho and He, with 0.64 (Ho) and 0.74 (He) in the Clemson orchard and 0.58 (Ho) and 0.70 (He) in the Knoxville orchard. These values are comparable to those reported in a *L. chinense* cultivated population in China, which had a Ho and He of 0.48 and 0.74 [43]. Other forest tree species have similar heterozygosities as well, for example, a *Pinus merkusii* parental and seedling populations had a He of 0.55 and 0.49, respectively [10, 30]. Reported 0.48 (Ho) and 0.63 (He) in a white spruce plantation and 0.49 (Ho) and 0.63 (He) in a white spruce improvement selection population. It is noteworthy that genetic diversity of natural *L. tulipifera* populations has been reported [e.g., 18,32]. However these studies utilized either allozymes or amplified fragment length polymorphism (AFLP) markers, which usually have lower information content than SSR markers. None of the reported expected heterozygosities from these studies exceeded 0.29. Overall, substantial genetic diversity is captured in the Clemson and Knoxville seed orchards.

**Conclusion**

The data obtained in this study will be useful in future applications such as prediction of genetic gain and gene diversity in the seed orchards. Nei’s genetic distance between the two orchards was 0.39, which was the lowest among all comparisons (Table 5). The *L. chinense* and *L. tulipifera* trees from the National Arboretum exhibited the largest genetic distance (1.17). The two orchards and the *L. tulipifera* sample from the US National Arboretum grouped together in the UPGMA dendrogram. The genetic distance of the hybrids in the Clemson orchard was closest to the Clemson orchard (0.50), followed by the Knoxville orchard (0.80) and *L. chinense* from the National Arboretum (0.88), and then by the *L. tulipifera* from the National Arboretum (1.17) (Figure 2). With a widespread range of distribution, *L. tulipifera* has adapted to many different ecological conditions and is one of the species becoming increasingly dominant in forests due to its quick respond to increases in light to the forest floor and rapid initial growth rate [8]. Its increasingly important roles in forestry and wood products is making studying *Liriodendron* of great interest. Our study provides a first look at the genetic diversity and allele richness among selections of this unique native species, and provides a foundation for further genetic and breeding exploration. The polymorphic markers developed in this study will serve as a resource enabling the future study of population dynamics and adaptive variation in *Liriodendron*.

**Additional files**

- Supplementary Table S1
- Supplementary Table S2
- Supplementary figure S1
- Supplementary figure S2
- Supplementary figure S3

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

| Authors’ contributions          | XZ | AC | ZT | MS | SES | JEC | HL |
|----------------------------------|----|----|----|----|-----|-----|----|
| Research concept and design      | ✓  | ✓  | ✓  | ✓  | ✓   | ✓   | ✓  |
| Collection and/or assembly of data| ✓  | ✓  | ✓  | ✓  | ✓   | ✓   | ✓  |
| Data analysis and interpretation | ✓  | ✓  | ✓  | ✓  | ✓   | ✓   | ✓  |
| Writing the article              | ✓  | ✓  | ✓  | ✓  | ✓   | ✓   | ✓  |
| Critical revision of the article | ✓  | ✓  | ✓  | ✓  | ✓   | ✓   | ✓  |
| Final approval of article        | ✓  | ✓  | ✓  | ✓  | ✓   | ✓   | ✓  |
| Statistical analysis             | ✓  | ✓  | ✓  | ✓  | ✓   | ✓   | ✓  |

**Acknowledgement**

The authors thank Nick Wheeler for his guidance and thorough review of the manuscript. The funding for the study came from the NSF Plant Genome Research program (NSF 1025974) and National Institute of Food and Agriculture, USDA SC-1700449 with a Clemson University Experiment Station technical contribution number of 6215).

**Publication history**

Editor: Shouan Zhang, University of Florida, USA.
Received: 21-Jan-2015 Revised: 24-Feb-2015
Accepted: 17-Mar-2015 Published: 27-Mar-2015

**References**

1. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W and Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997; 25:3389-402. | Article | PubMed Abstract | PubMed Full Text
2. Bae K and Byun J. Screening of leaves of higher plants for antibacterial action. Kor J Pharmacogn. 1987; 8:1.
3. Beck DE. *Liriodendron tulipifera* L. Yellow-poplar. In Silvics of North

---

**Table 5. Nei’s (1978) unbiased identity (above diagonal) and distance (below diagonal).**

| Pop ID  | Clemson | Knoxville | NA *L. chinense* | Hybrids in Clemson | NA *L. chinense* |
|---------|---------|----------|-----------------|-------------------|-----------------|
| Clemson | --      | 0.6792   | 0.6234          | 0.6051            | 0.4051          |
| Knoxville | 0.3869 | --       | 0.4648          | 0.4484            | 0.3495          |
| NA *L. tulipifera* | 0.4725 | 0.7662 | --              | 0.3608            | 0.3097          |
| Hybrids in clemson | 0.5025 | 0.8021 | 1.0195          | --                | 0.4138          |
| NA *L. chinense* | 0.9037 | 1.0513 | 1.1721          | 0.8823            | --              |
4. Beckage B and Clark JS. Seedling survival and growth of three forest tree species: The role of spatial heterogeneity. Ecology. 2003; 84:1849-1861. | Article

20. Bonner FT and Russell TE. Liriodendron tulipifera L. Yellow-poplar. In Schopmeyer, CS (Tech. Coord.), Seeds of woody plants of the United States. USDA For Agric Handb. 1974; 450:508-511.

6. Cech F, Brown J and Weingartner D. Wind damage to a yellow-poplar seed orchard. Tree Planters’ Notes. 1976; 27:3-4.

7. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM and Gascuel O. Phylogeny, fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 2008; 36:W465-9. | Article | PubMed Abstract | PubMed Full Text

8. Dyer JM. Using witness trees to assess forest change in southeastern Ohio. Can J Bot. 2003; 71:1708-1718. | Article

9. Ellis JR and Burke JM. EST-SSRs as a resource for population genetic analyses. Heredity (Edinb). 2007; 99:125-32. | Article | PubMed

10. Fageria MS and Rajora OP. Effects of silvicultural practices on genetic diversity and population structure of white spruce in Saskatchewan. Tree Genoma Genome. 2014; 10:287-296. | Article

11. Forest Service. Forest Inventory and Analysis National Program, The United State Department of Agriculture. FIADB 5.1.6 ed. 2014. | Website

12. Han Y, Chagne D, Gasic K, Rikkerink EH, Beever JE, Gardiner SE and Korban SS. BAC-end sequence-based SNPs and Binning mapping for rapid integration of physical and genetic maps in apple. Genomics. 2009; 93:282-8. | Article | PubMed

13. Hernandez R, Davalos JF, Sonti SS, Kim Y and Moody RC. Strength and stiffness of reinforced yellow-poplar glued laminated beams. Res. Pop. PFL-RP-U.S. Department of Agriculture, Forest Service, Forest Products Research Laboratory, Madison, WI. 1997. | Pdf

14. Huang MR and Chen DM. An isozyme analysis of tulip-tree hybrids (Liriodendron chinense X L. tulipifera). J Naniong For Univ. 1979; 1:156-158. | Article

15. Jin H, Do J, Moon D, Noh EW, Kim W and Kwon M. EST analysis of functional genes associated with cell wall biosynthesis and modification in the secondary xylem of the yellow poplar (Liriodendron tulipifera) stem during early stage of tension wood formation. Planta. 2011; 234:959-77. | Article | PubMed

16. Kimura M and Crow JF. The measurement of effective population number. Evolution. 1964; 18:279-288. | Article

17. Kobayashi N, Hirahara T, Katsuyama H, Handa T and Takayanagi K. A simple and efficient DNA extraction method for plants, especially woody plants. Plant Tissue Culture Biotechnol. 1998; 4:76-80. | Article

18. Kovach KE. Assessment of genetic variation of Acer rubrum L. and Liriodendron tulipifera L. populations in unmanaged forests of the Southeast United States. 2009. | Article

19. Levene H. On a matching problem arising in genetics. Ann Math Stat. 1949; 20:91-94. | Article

20. Lewontin RC. The apportionment of human diversity. Evol Biol. 1972; 6:381-398. | Pf

21. L. Iizawa and Y. Sumitani. RAPD markers used for the hybrid identification and parents choice in Liriodendron. Sci Silv Sinic. 2002; 38:169-174. | Article

22. Liang H, Ayampongayam S, Wickett N, Barakat A, Xu Y, Landherr L, Ralph P, Xu T, Schlarbaum SE, Leebens-Mack JH and dePamphilis CW. Generation of a large-scale genomic resource for functional and comparative genomics in Liriodendron. Tree Gen Genom. 2011; 7:941-954. | Article

23. Liang H, Carlson JE, Leebens-Mack JH, Wall PK, Mueller LA, Buzgo M, Landherr LH, Hu Y, Dioreto DS, Iltu DC, Field D, Tanksley SD, Ma H and dePamphilis CW. A 378 n EST Database for Liriodendron tulipifera L. floral buds: the first EST resource for functional and comparative genomics in Liriodendron. Tree Gen Genom. 2008; 4:419-433. | Article

24. Liang H, Feng E, Tomkins J, Arumuganathan K, Zhao S, Luo M, Kudrina D, Wing R, Banks J, dePamphilis C, Mandoli D, Schlarbaum S and Carlson JE. Development of a BAC library resource for yellow poplar (Liriodendron tulipifera) and the identification of genomic regions associated with flower development and lignin biosynthesis. Tree Genet Genomes. 2007; 3:215-225. | Article

25. Marshall TC, Slate J, Kruuk LE and Pemberton JM. Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol. 1998; 7:639-55. | Article | PubMed

26. Merkle SA, Hoey MT, Watson-Pauley BA and Schlarbaum SE. Propagation of Liriodendron hybrids via somatic embryogenesis. Plant Cell Tissue Organ Cult. 1993; 34:191-198. | Article

27. Moon MK, Oh HM, Kwon BM, Baek NI, Kim SH, Kim JS and Kim DK. Farne-syl protein transferase and tumor cell growth inhibitory activities of lipiferolide isolated from Liriodendron tulipifera. Arch Pharm Res. 2007; 30:299-302. | Article | PubMed

28. Nei M. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci U S A. 1973; 70:3231-3. | PubMed Abstract | PubMed Full Text

29. Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 1978; 89:583-90. | Article | PubMed Abstract | PubMed Full Text

30. Nei M, Zhong Y, Kajinagish IIG, Saito Y, Tsuda Y and Ide Y. Genetic diversity of parental and offspring populations in a Pinus merkusii seedling seed orchard detected by microsatellite markers. Bulletin of the Tokyo University Forest, the Tokyo University Forests. 2007; 118:1-14. | Pdf

31. Parks CR, Miller NG, Wendel JF and McDougal KM. Genetic divergence within the genus Liriodendron (Magnoliaceae). Ann Miss Bot Gard. 1983; 70:658-666. | Article

32. Parks CR and Wendel JF. Molecular divergence between Asian and North American species of Liriodendron (Magnoliaceae) with implications for interpretation of fossil floras. Am J Bot. 1990; 77:1234-1256. | Article

33. Raymond M and Rousset F. GENEPOP (version 1.2): population genetics software for exact tests and ecumens. J Heredity. 1995; 86:248-249. | Article

34. Santamour FS. Interspecific hybrids in Liriodendron and their chemical verification. For Sci. 1972; 18:233-236. | Article

35. Sewell MM, Parks CR and Chase MW. Intraspecific chloroplast DNA variation and biogeography of North American Liriodendron L. (Magno-liaeae). Evolution. 1996; 50:1147-1154. | Article

36. Thor E. Tree Breeding at the University of Tennessee, 1959-1976. Univ Tenn Ag Exp Stn Bull, 1976; 48. | Article

37. Turmel M, Ottis C and Lemieux C. The chloroplastic genome sequence of Chara vulgaris sheds new light into the closest green algal relatives of land plants. Mol Biol Evol. 2006; 23:1324-38. | Article | PubMed

38. van Oosterhout C, Hutchinson WF, Wills DPM and Shipley P. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 2004; 4:535-538. | Article

39. Wang Z. Utilization and species hybridization in Liriodendron. Chinese Forestry Press, Beijing. 2005.

40. Wicke S and Quandt D. Universal primers for the amplification of the plastid trnk/matK region in land plants. Anales Jard Bot Madrid. 2009; 66:285-288. | Article

41. Williams RS and Feist WC. Durability of yellow-poplar and sweetgum and service life of finishes after long-term exposure. Forest Products J. 2004; 54:96-101. | Article

42. Xu M, Sun Y and Li H. EST-SSRs development and paternity analysis for Liriodendron spp. New Forests. 2010; 40:361-382. | Article

43. Yang AH, Zhang JJ, Tian H and Yao XH. Characterization of 39 novel EST-SSR markers for Liriodendron tulipifera and cross-species amplification in L. chinense (Magnoliaceae). Am J Bot. 2012; 99:e460-4. | Article | PubMed

44. Yu JK, La Rota M, Kantety RV and Sorrells ME. EST derived SSR markers for comparative mapping in wheat and rice. Mol Genet Genomics. 2004; 271:742-51. | Article | PubMed

45. Zane L, Bargelloni L and Patarnello T. Strategies for microsatellite isolation: a review. Mol Ecol. 2002; 11:1-16. | Article | PubMed

46. Zhang LJ, Oswald BP and Green TH. Relationships between overstory species and community classification of the Sipsey Wilderness, Ala-
47. Zhang H, Li H, Xu M and Feng Y. Identification of *Liriodendron tulipifera*, *Liriodendron chinense* and hybrid *Liriodendron* using species-specific SSR markers. *Sci Silv Sinic*. 2012; 46:36-39.

**Citation:**
Zhang X, Carlson A, Tian Z, Staton M, Schlarbaum SE, Carlson JE and Liang H. Genetic characterization of *Liriodendron* seed orchards with EST-SSR markers. *J Plant Sci Mol Breed*. 2015; 4:1.
http://dx.doi.org/10.7243/2050-2389-4-1