

LOW VARIABILITY OF DNA FINGERPRINTS OF TEXAS SNOWBELLS: CONSERVATION IMPLICATIONS

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ABSTRACT

Texas snowbells (*Styrax platanifolius* var. *texanus*) is one of the most threatened native Texas plants. A preliminary study using DNA fingerprinting (RAPDs) was performed on plants from three natural populations. Almost no genetic variation was found, either within or between these three populations. Implications for conservation are discussed. *Phytologia* 93(2):198-202 (August 1, 2011)

KEY WORDS: *Styrax platanifolius* var. *texanus*, Texas snowbells, RAPDs, conservation.

Texas snowbells (*Styrax platanifolius* Engelm. ex Torr. var. *texanus* (Cory) B. L. Turner) is a shrub or small tree that grows out of crevices on steep limestone bluffs, rock ledges, or cliff faces along creeks. It can also grow in the dry gravel of streambeds. The flowers are clustered at the end of the branches and hang upside down. The flowers dangle and look like small white bells, thus the common name, snowbells. Texas snowbells are endemic to an area of a 64 km radius within the southwestern Edwards Plateau of Texas. All but two populations are on private land. The natural populations are represented by 15 sites, varying from one mature plant to about 350 individuals (seedlings, juveniles, and reproductive plants). The sizes of these sites range from a few square meters to about 100 acres. Texas

snowbells are in cultivation at the San Antonio Botanical Center and in seed storage at the Wildflower Center in Austin (as part of their Center for Plant Conservation collection). Several dozen new populations representing hundreds of individuals have been reintroduced on private lands through the efforts of J. David Bamberger, his staff, and volunteers.

Fritsch (1996) examined 24 isozymes from 36 individuals of *S. platanifolius* in west Texas and found low levels of variation both within and among three populations. He concluded that there was no evidence of polyploidy and the gene flow between populations appeared to be high (but with a reservation that the methods may not be accurate). Later, Fritsch (1997) recognized three subspecies (*stellatus*, *texanus*, and *youngiae*), these subsequently treated as varieties by Turner and Nesom (2000). Fritsch (2001) further examined the phylogeny of *Styrax* using nrDNA and cpDNA data and found no differences between *S. platanifolius* var. *mollis*, var. *stellatus* and var. *texanus*.

The purpose of this paper is to report on a preliminary study of variation within and between three natural populations of Texas snowbells using RAPDs (Random Amplified Polymorphic DNAs).

MATERIALS AND METHODS

Specimens collected: leaves were collected from 10 trees in three populations of var. *texanus* by J. Poole (10 Nov 2003: C1-C10 (= lab # *Adams 10091-10101*), Corbin property, sse of Dolan Falls, Val Verde Co., Texas; DF2, 31, 41, 45, 108a, 111, 123, 129, 141, 154, 164 (= lab # *Adams 10101-10111*), Dolan Falls Preserve, Val Verde Co., Texas; GV1, 6, 7, 7-3, 8, 17, 20, 25, 25a (= lab # *Adams 10112-10120*), Greenwood Valley, Ranch, Edwards Co., Texas.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA). The RAPD analyses follow that of Adams and Demeke (1993). Sixteen ten-mer primers were

purchased from the University of British Columbia (5'-3'): 116, 153, 184, 204, 212, 218 239, 244, 250, 265, 338, 347, 375, 389, 413, 431.

PCR stock solutions (Taq, primer, buffer) were made in bulk so that all the PCR reaction tubes for a primer were prepared using the same bulk stock. This is a critical factor for minimizing variation in band intensities from sample to sample (see Adams, Flournoy and Pandey, 1998, for protocols to minimize PCR band variation). PCR was performed in a volume of 15 μ l containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.0 mM MgCl₂, and 0.1% Triton X-100, 0.2 mM of each dNTPs, 0.36 μ M primers, 0.3 ng genomic DNA, 15 ng BSA and 0.6 unit of Taq DNA polymerase (Promega). A negative control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). Samples were run in duplicate to insure reproducibility (Adams, Flournoy and Pandey, 1998). A temperature profile was obtained for each well of the thermocycler to be sure that no variation existed among wells in the heating cooling block. The thermal cycle used was: 94°C (1.5 min) for initial strand separation, then 40 cycles of 40°C (2 min), 72°C (2 min), 91°C (1 min). Two additional steps were used: 40°C (2 min) and 72°C (5 min) for final extension. The temperature inside a PCR tube with 15 μ l buffer was monitored with a temperature probe for each step for each of the 40 cycles (Adams, Flournoy and Pandey, 1998) to insure that each cycle met temperature specifications and that each PCR run was exactly the same. Amplification products were analyzed by electrophoresis on 1.5% agarose gels, 75V, 55 min, and detected by staining with ethidium bromide. The gels were photographed over UV light using Polaroid film 667 and scanned to digital images. The digital images were size normalized in reference to pGem® DNA size markers before band scoring.

RESULTS AND DISCUSSION

The 16 primers utilized resulted in 120 bands. In general, there was very little if any variation among individuals or between populations. Figure 1 shows bands for primers 218, 244, and 431. Notice that most of the individuals have identical DNA bands. RAPD 244 reveals 5 mutations scattered among the 3 populations. The other

13 primers resulted in similar patterns with little or no variation among individuals. Fritsch (1996) found very low diversity in isozymes in *S. platanifolius* populations in west Texas and concluded they had undergone a genetic bottleneck. The present preliminary data seems to confirm his data.

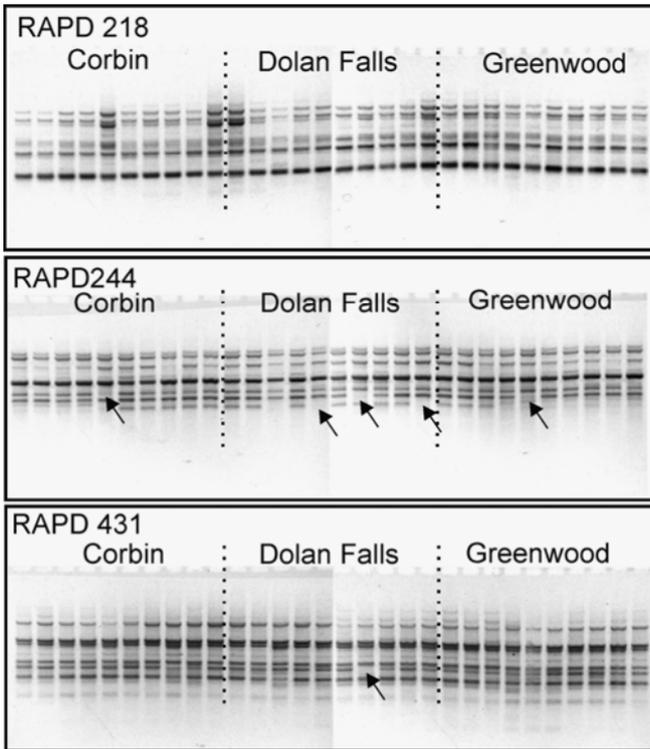


Figure 1. Banding patterns for individuals from three populations for three RAPD primers. The arrows indicate missing bands.

CONCLUSIONS

The present data, although preliminary, are concordant with the isozyme data (Fritsch, 1996) that there is very little genetic variation among Texas snowbells. It appears that conservation of several natural

populations will not conserve genetic variation. However, maintaining several natural populations guards against a catastrophic extinction of Texas snowbells and might lead to the accumulations of genetic mutations in the future to diversify the genetic base.

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