Molecular Confirmation of Intergeneric Hybrid $\times$ *Chitalpa tashkentensis* (Bignoniaceae)

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Abstract. Intergeneric hybrids are generally rare; nevertheless, such putative hybrids need confirmation from other lines of evidence besides morphological intermediacy. In this study the authors used DNA sequences of nuclear and chloroplast genes to determine the hybrid identity of $\times$ *Chitalpa*. Their results confirm that both $\times$ *Chitalpa tashkentensis* 'Pink Dawn' and $\times$ *C. tashkentensis* 'Morning Cloud' are the result of an *Chilopsis linearis* $\times$ *Catalpa* sp. cross. However, *Catalpa bignonioides* does not seem to have participated in the cross, as speculated before. Different species of *Catalpa* may have been used as the paternal parent. $\times$ *C. tashkentensis* 'Morning Cloud' is the result of the *C. linearis* $\times$ *C. speciosa* cross, whereas the paternal parent of $\times$ *C. tashkentensis* 'Pink Dawn' may be a hybrid plant of *C. ovata* and *C. speciosa*.

Hybridization has played an important role in the evolution and diversification of plants and some animals (Hilu, 1993; Rieseberg, 1995). However, it is generally a rare event that two genera hybridize naturally producing fertile offspring (Stebbins, 1985). Many people, nevertheless, have attempted to cross closely related genera hoping to generate descendants with ideal horticultural and economical traits. Most attempts have ended with frustration whereas a few have succeeded. $\times$ *Chitalpa* appears to be one of the success stories. The hybrid adopts the best features of both parents and has the potential to become an important landscaping plant (Elias, 1991; Elias and Wisura, 1991).

In the 1960s at the Uzbek Academy of Sciences Botanical Garden in Tashkent of the Republic of Uzbekistan, Nikolai Fyodorovich Rusanov studied the phenology of *Catalpa* L. (Rusanov, 1964, 1969) and conducted a series of crossing experiments using the monotypic *Chilopsis* D. Don and four species of *Catalpa*, *C. bignonioides* Walt., *C. speciosa* Ward., *C. ovata* G. Don, and one unidentified species (Rusanov, 1964). Several plants were obtained from the experiments. As expected, these plants are morphologically intermedi-

ate between *Catalpa* and *Chilopsis*, and the offspring was reproductively sterile. Softwood cuttings, therefore, have been used to propagate the hybrid plants (Rusanov, 1976). The putative hybrid plants flower from late spring to early fall and grow well in many landscaping situations in numerous cities in Uzbekistan (Rusanov, 1982).

To evaluate the landscaping potential of the putative hybrid in North America, Robert Hebb of New York Botanical Garden brought cuttings of two clones to North America in 1977 from the Uzbek Academy of Sciences Botanical Garden in Tashkent (Bob Hebb, pers. comm.). The cuttings were rooted and grown at the Cary Arboretum in Millbrook, N.Y., and the resulting plants began to flower by the third growing season, showing two color forms. In 1982, the rooted cuttings of each of the color forms were distributed to many botanical gardens (Hebb, 1982). According to Elias and Wisura (1991), all living plants of this intergeneric hybrid in North America have originated from this single introduction and distribution. The hybrid was not officially described until 1991, when Elias and Wisura named it as $\times$ *Chitalpa tashkentensis*. They also described two cultivars for the two flower color forms. $\times$ *C. tashkentensis* 'Pink Dawn' is the smaller of the two in stature and has spreading growth habit and light-pink flowers. Elias and Wisura (1991) considered it as resulting from the cross between *Chilopsis linearis* $\times$ *Catalpa* sp. $\times$ *C. tashkentensis* 'Morning Cloud', however, is more upright and has a vigorous growth habit with very pale pink

to white flowers, and is probably the result of the cross of *Catalpa* sp. $\times$ *C. linearis* (Elias and Wisura, 1991). These conclusions were mainly based on morphological grounds, and the true identity of these cultivars remains to be tested using other lines of evidence.

In plants, nuclear, chloroplast, and mitochondrial genomes have different patterns of inheritance. Nuclear genes are biparentally inherited, whereas both chloroplast and mitochondrial genes are inherited through the maternal parent in most angiosperms including Bignoniaceae (Corriveau and Coleman, 1988). Thus we expect to see nuclear genes of both parents in the hybrid, whereas the hybrid is expected to have chloroplast genes only from the maternal parent. The differential patterns of inheritance provide a convincing way of determining the parentage of hybrid species. In this study we compared nucleotide sequences of nuclear and chloroplast genes, 1) to test the hybrid identity of $\times$ *C. tashkentensis*, and 2) to provide insights into the parentage of the two cultivars.

Materials and Methods

Plant material

Fourteen samples were used in this study representing three outgroup species (*Kigelia africana* Benth., *Tecoma stans* Juss., and *Campsis radicans* Seem.), *C. linearis* Sweet, eight species of *Catalpa*, and the two cultivars of $\times$ *Chitalpa* (Table 1). Outgroup selection was based on previous phylogenetic analyses of Bignoniaceae, indicating that these genera are closely related to but outside the clade of *Chilopsis* and *Catalpa* (Spangler and Olmstead, 1999).

Molecular techniques

DNAs were extracted from silica gel dried or fresh leaf tissue using a Qiagen DNeasy Plant Mini Kit (cat. no. 69104, Germantown, Md.). The nuclear ribosomal DNA internal transcribe spacers (nrDNA ITS) region was amplified using primers ITS4 (White et al., 1990) and ITSLeu (Baum et al., 1998). A 25- μ L polymerase chain reaction (PCR) included 2.5 μ L *Taq* polymerase buffer (10 $\times$), 4 μ L dNTP (2.5 mM), 2 μ L MgCl₂ (25 mM), 1 μ L of each primer (10 μ M), 0.2 μ L *Taq* polymerase (5 U- μ L⁻¹), 50 to 100 ng DNA, 2 μ L dimethyl sulfoxide, and an appropriate amount of sterilized water. The thermocycler program consisted of the following steps: a hot start of 94 °C for 3 min, 35 cycles of 94 °C for 1 min, 55 °C for 2 min, and 72 °C for 1 min. The final cycle was followed by an additional 7-min extension at 72 °C. The amplified products were purified using a Qiagen Gel Purification Kit (Santa Clarita, Calif.).

The 3' portion of the *ndhF* gene was amplified in an MJ-PT200 Thermocycler (MJ Research, Watertown, Mass.) using primers *ndhF*972F and 2210R (Olmstead and Sweere, 1994). A 25- μ L reaction contained 50 to 100 ng genomic DNA, 4 μ L dNTPs (2.5 mM), 3 μ L MgCl₂, 2.5 μ L *Taq* polymerase buffer (10 $\times$), 0.3 μ L *Taq* polymerase (5 U- μ L⁻¹), 1 μ L of each primer (10 μ M),

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Table 1. Accessions used in this study.

Taxon	DNA accession	Source ^a	Genbank accession	
			ITS	<i>ndhF</i>
<i>Kigelia africana</i> Benth.	—	—	AY178638 ^y	AF102632 ^y
<i>Tecoma stans</i> Juss.	—	—	AY178636 ^y	AF130145 ^y
<i>Campsis radicans</i> Seem.	4199	AA 142–83E	DQ515201	AF102626 ^y
<i>Chilopsis linearis</i> (Cavanilles) Sweet	4196	Genhua Niu	AY178657 ^y	DQ515218–DQ515221
<i>Catalpa longissima</i> (Jacq.) Dum.-Cours.	3181	FG 961378 ^y A	AY486294	DQ411414
<i>C. macrocarpa</i> (A. Rich.) Ekam ex Urban	3182	FG 66346 ^y A	AY486295	DQ515213–DQ515214
<i>C. duclouxii</i> G. Don	3233	AA 642–94A	AY486296	DQ411418
<i>C. bungei</i> C.A. Mey	3221	AA 12927A	AY486299	DQ411410
<i>C. fargesii</i> Bureau	3211	AA 12222B	AY486300	DQ411411, DQ515215
<i>C. ovata</i> G. Don	3212	AA 516–87A	AY486302	DQ411412, DQ515216
<i>C. speciosa</i> Warder ex Engelm.	3230	AA 131–54A	AY486307	DQ411407, DQ515217
<i>C. bignonioides</i> Walter	3237	AA 592–60C	AY486308	DQ411416
× <i>Chitalpa tashkentensis</i> 'Pink Dawn'				
Elias & Wisura	4244	AA 226–2004	DQ515202–DQ515205	DQ515222–DQ515224
× <i>C. tashkentensis</i> 'Morning Cloud'				
Elias & Wisura	4330	AA 227–2004	DQ515206–DQ515212	DQ515225–DQ515229

^aVouchers are deposited in AA (Arnold Arboretum) and FG (Fairchild Garden).

^ySequences obtained from the GenBank.

and sterilized water. The PCR program consisted of a 3-min hot start at 94 °C and 35 cycles of 1-min denaturing at 94 °C, 1.5 min annealing at 46 to 50 °C, and 2 min of extension at 72 °C. The cycles were followed by an additional 7-min extension at 72 °C.

Cloning of PCR products of the ITS and *ndhF* regions was done using a standard p^{GEM} T Easy Vector System (Promega, Madison, Wis.). Repeated PCR and sequencing reactions were conducted to detect PCR errors that may have led to sequence variation. Clones and PCR products were sequenced using the Dideoxy Terminator Chemistry with an ABI BigDye Cycle Sequencing Ready Reaction kit. Sequences were analyzed using an ABI 3100 or 3730 Genetic Analyzer, and were edited using Sequencher (version 4.1; GeneCode Inc., Ann Arbor, Mich.).

Phylogenetic analyses

Sequences were readily aligned by sight. Gaps were treated as missing data. Characters were equally weighted and their states were unordered. Phylogenetic analyses were conducted using both maximum parsimony (MP) and maximum likelihood (ML) methods. Parsimony analyses were conducted in PAUP* (version 4.0b10) (Swofford, 2002) using heuristic tree search with the following options: tree bisection reconnection branch swap, deepest descent off, Multrees on, and 1000 random sequence addition with 10 trees held for each replicate. Likelihood hierarchical ratio tests were used to select the best-fit model of evolution for the data sets using the Modeltest program (Posada and Crandall, 1998). Then, the model parameters estimated in Modeltest were applied to the ML analyses using PAUP*. Bootstrap analyses of 100 replicates were used to estimate support for individual branches and the tree search options were as in parsimony analyses.

Results

Sequence lengths of the nrDNA ITS region including the 5.8S gene ranged from 627 base pairs (bp) in ×*Chitalpa* 'Pink Dawn' to 639 bp in *Campsis radicans*. They were

627 to 629 bp in ×*Chitalpa* 'Pink Dawn' and 629 to 630 bp in ×*Chitalpa* 'Morning Cloud'. The alignment resulted in a data set of 651 sites, 210 of which were variable and 121 were parsimony informative. Parsimony analyses of the ITS data set generated four trees of 337 steps [consistency index (CI) = 0.83, retention index (RI) = 0.88]. Fig. 1 is the strict consensus tree. Sequences of ×*Chitalpa* 'Pink Dawn' were in three positions of the tree and were clustered with those of *C. ovata*, *C. speciosa*, and *C. linearis*. Sequences of ×*Chitalpa* 'Morning Cloud' were allied with *C. speciosa* and *C. linearis*.

Modeltest selected the GTR+Γ model as the best-fit model of evolution of the nrDNA ITS. The estimated parameters were as follows: base frequencies of A = 0.1924, C = 0.3217, G = 0.3048, and T = 0.1811; substitution rate matrix = 1.0000, 2.0623, 1.0000, 1.0000, 4.6985; Γ = 0.3474; and Pinvar = 0. ML analyses produced a single tree (−Ln

likelihood = 2537.1464) with identical topology as the MP consensus tree (Fig. 1).

The amplified portion of the chloroplast *ndhF* gene was from 1081 to 1086 bp in length, and the aligned data set contained 1086 sites, 150 of which were variable and 54 were parsimony informative. Parsimony analyses of the *ndhF* data set produced 118 trees of 163 steps (CI = 0.94, RI = 0.96). The strict consensus tree is shown (Fig. 2). All *ndhF* gene sequences from both ×*Chitalpa* 'Pink Dawn' and ×*Chitalpa* 'Morning Cloud' were allied with those of *C. linearis*. Modeltest analyses suggested that the best-fit model was the HKY model with the estimated base frequencies of A = 0.2908, C = 0.1683, G = 0.1784, T = 0.3625, and the transition-to-transversion ratio of 1.22. ML analyses with these parameters generated a single tree with the likelihood score of 2551.1994. The tree topology was identical to the MP consensus tree.

Fig. 1. Strict consensus of four trees of 337 steps based on parsimony analyses of nrDNA ITS sequences (CI = 0.83, RI = 0.88). Numbers above and below branches are bootstrap support and the number of shared base substitutions respectively. Numbers and letters following taxon names are DNA accessions and clone numbers as shown in Table 1.

Fig. 2. Strict consensus of 118 trees of 163 steps based on parsimony analyses of chloroplast *ndhF* gene sequences (CI = 0.94, RI = 0.96). Numbers above and below branches are bootstrap support and the number of shared base substitutions respectively. Numbers and letters following taxon names are DNA accessions and clone numbers as shown in Table 1.

Discussion

xChitalpa tashkentensis is an intergeneric hybrid with *Chilopsis* as the maternal parent

xChitalpa tashkentensis is intermediate between *Catalpa* and *C. linearis* in general morphology. This is especially true in leaf morphology and flower color. Leaves of *Chilopsis* are narrowly lanceolate without lobes, whereas leaves of *Catalpa* are broadly ovate with or without lobes. Leaves of *xChitalpa tashkentensis* stand in between with broadly lanceolate leaves. Similarly, flowers of *xChitalpa tashkentensis* are intermediate between *Catalpa* and *Chilopsis*. Flowers of *Catalpa* range from white in *C. speciosa* and *C. bignonioides* to creamy yellow in *C. ovata* and to purple in *C. bungei*, *C. duclouxii*, and *C. fargesii*. Flowers of *Chilopsis* are pink to lavender. Flowers of *xChitalpa tashkentensis* are pink, pale pink, and white. *Catalpa* species flower from late May to early June, whereas flowers of *Chilopsis* open from late spring to the fall. *xChitalpa tashkentensis* is similar in floral phenology to *Chilopsis* (Elias and Wisura, 1991).

Because the nuclear nrDNA ITS is biparentally inherited, genes of both parents are expected to exist in the hybrid. In the ITS tree (Fig. 1), some sequences of *xChitalpa tashkentensis* 'Pink Dawn' and 'Morning Cloud' are grouped with those of *Chilopsis*, whereas others are with those of *Catalpa*. Therefore, our results unequivocally support the hypothesis that *xChitalpa tashkentensis* is derived from a successful artificial hybridization of *Chilopsis* and *Catalpa* (Rusanov, 1964; Elias and Wisura, 1991).

Chloroplast genes in Bignoniaceae are inherited maternally (Corriveau and Coleman, 1988), thus the hybrid should have chloroplast genes only from the maternal parent. In the *ndhF* tree (Fig. 2), all sequences of *xChitalpa tashkentensis* are grouped with *Chilopsis*, indicating that *Chilopsis* is the maternal parent for both *xChitalpa tashkentensis* 'Pink Dawn' and 'Morning Cloud'. Our results, therefore, support the speculation that *xChitalpa tashkentensis* 'Pink Dawn' is the result of the *C. linearis* $\times$ *Catalpa* sp. cross (Elias and Wisura 1991). However, our sequence data do not support the suggestion that *xChitalpa tashkentensis* 'Morning Cloud' is the reciprocal cross (i.e., *Catalpa* sp. $\times$ *C. linearis*) (Hebb, 1982; Elias and Wisura, 1991).

Catalpa speciosa and *C. ovata* may be involved in the hybridization

It has been speculated that *C. bignonioides* might be involved in the hybridization (Hebb, 1982). However, neither *xChitalpa tashkentensis* 'Pink Dawn' nor *xChitalpa tashkentensis* 'Morning Cloud' shows nrDNA ITS sequences of *C. bignonioides* (Fig. 1). Instead, *xChitalpa tashkentensis* 'Pink Dawn' has sequences from both *C. ovata* and *C. speciosa*. This indicates that a hybrid of the latter two species (*Catalpa* $\times$ *galleana*) was probably used as pollen source for the artificial crosses. Hybrids of *C. ovata* and *C. speciosa* have long been known to grow faster and produce more seeds than either parent (Duffield and Snyder, 1958; Jones and Filley, 1920; Sargent, 1889). Sequences of *xChitalpa tashkentensis* 'Morning Cloud' are clustered with *C. speciosa* (Fig. 1), suggesting that this cultivar

may result from the crosses between *Chilopsis* and *Catalpa speciosa*.

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