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BRIEF COMMUNICATION

SPECIES-SPECIFIC SSR ALLELES FOR STUDIES OF HYBRID CATTAILS (*TYPHA LATIFOLIA* × *T. ANGUSTIFOLIA*; TYPHACEAE) IN NORTH AMERICA[1]

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- *Premise*: Studies of hybridizing species are facilitated by the availability of species-specific molecular markers for identifying early- and later-generation hybrids. Cattails are a dominant feature of wetland communities, and a better understanding of the prevalence of hybrids is needed to assess the ecological and evolutionary effects of hybridization. Hybridization between *Typha angustifolia* and *T. latifolia* produce long-lived clones, known as *Typha* ×*glauca*, which are considered to be invasive. Although morphological variation in cattails makes it difficult to recognize early- and later-generation hybrids, several dominant, species-specific RAPD markers are available. Our goal was to find codominant, species-specific markers with greater polymorphism than RAPDs, to identify later-generation hybrids more efficiently.
- *Methods*: We screened nine SSR (simple sequence repeat) loci that were described from populations in Ukraine, and we surveyed 31 cattail populations from the upper Midwest and eastern USA.
- *Key results*: Seven SSR loci distinguished the parent taxa and were consistent with known species-specific RAPD markers, allowing easier detection of backcrossing. We used linear discriminant analysis to show that F_1 hybrid phenotypes were intermediate between the parent taxa, while those of backcrossed plants overlapped with the hybrids and their parents. Log(leaf length/leaf width), spike gap length, spike length, and stem diameter explained much of the variation among groups.
- *Conclusions*: We provide the first documentation of backcrossed plants in hybridizing cattail populations in Michigan. The diagnostic SSR loci we identified should be extremely useful for examining the evolutionary and ecology interactions of hybridizing cattails in North America.

Key words: cattail; gene flow; hybrid; introgression; invasive species; molecular markers; morphological traits; species-specific markers; STRUCTURE; *Typha*; wetlands.

Interest in the ecology and evolution of hybridizing species has heightened in recent years, in part due to the availability of molecular methods for identifying early- and advanced-generation hybrids. In some cases, hybridization may serve as a stimulus for the evolution of greater invasiveness and/or the disappearance of a parental gene pool (e.g., Rhymer and Simberloff, 1996; Arnold, 1997; Ellstrand and Schierenbeck, 2000; Petit et al., 2004; Hegde et al., 2006). Several studies have shown that hybrid taxa can be superior to their parents in terms of survival, fecundity, or clonal growth rates (e.g., Vila and D'Antonio, 1998; Campbell et al., 2006; Lavergne and Molofsky, 2007). Here, we studied *Typha angustifolia* L., *T. latifolia* L., and their hybrid derivatives, which often occur as large F_1 clones (Kuehn et al., 1999). Our goal was to identify species-specific SSR (simple sequence repeat) DNA alleles to facilitate further studies of the population genetics and ecology of hybrid cattail populations in North America.

Cattails (*Typha* spp.) are an important component of wetland vegetation on several continents. In North America, *T. latifolia* (broad-leaf cattail) is a native species that is common throughout much of the United States and southern Canada (Smith, 2000). *Typha angustifolia* (narrow-leaf cattail) is often considered to have been introduced from Europe (Stuckey and Salamon,

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TABLE 1. Collection Group 1 with location, year sampled, and numbers of genetically unique clones of *T. angustifolia*, *T. latifolia*, and F_1 hybrids. Species identifications and F_1 hybrid status were determined using 8-11 species-specific RAPD markers.

State	Site	Latitude, Longitude	Year	*T. angustifolia*	*T. latifolia*	F_1 hybrid
Parent species						
Maryland	Assateague Island National Seashore	38.195, −75.158	2005	9		
New York	Fire Island National Seashore	40.623, −73.218	2005	3		
Connecticut	Connecticut River near Old Saybrook	41.273, - 72.358	2005	24		
Minnesota 1	Cruiser Lake, Voyageurs National Park	48.498, −86.926	2005		13	
Minnesota 2	Jorgens Lake, Voyageurs National Park	48.483, −92.853	2005		5	
Minnesota 3	Little Shoepack Lake, Voyageurs National Park	48.487, −92.896	2005		7	
Minnesota 4	Peary Lake, Voyageurs National Park	48.522, −92.774	2005		4	
Minnesota 5	Quarterline Lake, Voyageurs National Park	48.477, −92.852	2005		5	
Minnesota 6	Ryan Lake, Voyageurs National Park	48.515, −92.706	2005		9	
Minnesota 7	Wyapka Lake, Voyageurs National Park	48.395, −92.708	2005		11	
F_1 hybrids						
Indiana	Cowles Bog, Indiana Dunes National Lakeshore	41.649, −87.086	2004			13
Wisconsin	St. Croix Falls and Wolf Lake, St. Croix National Scenic Riverway	45.413, −92.652 45.614, −92.655	2004			10
Minnesota 8	Sphunge Island and Cranberry Bay, Voyageurs National Park	48.442, −93.012 48.580, −93.050	2004			19
Total clones				36	54	42

1987), although recent palynological studies show that it may have occurred in brackish habitats in southern New England prior to European settlement (Carmichael, 1980; Pederson et al., 2005; Shih and Finkelstein, 2008). This species is now widespread across the eastern range of *T. latifolia* and in California. It has been described as an invasive species due to its rapidly spreading range and its ability to dominate wetland habitats (e.g., Grace and Harrison, 1986; Galatowitsch et al., 1999; Smith, 2000). Both species are clonal, self-compatible, and wind-pollinated, with chromosome numbers of $2n = 30$ (Grace and Harrison, 1986; Smith, 1967). Reproductive shoots are monoecious and protogynous, which facilitates outcrossing (Smith, 2000). *Typha angustifolia* often flowers earlier than *T. latifolia* where the two species co-occur (Selbo and Snow, 2004), but overlapping flowering times can lead to hybridization.

The hybrid between *T. latifolia* and *T. angustifolia* is known as *T.* ×*glauca* [Godr.], as is the hybrid between southern *T. domingensis* and *T. angustifolia* (Smith, 1967). The taxonomic status of *T.* ×*glauca* has long been disputed (see Kuehn et al., 1999), and relatively little is known about its ecological or evolutionary effects in *Typha* populations (but see Woo and Zedler, 2002; Angeloni et al., 2006; Boers et al., 2007; Farrer and Goldberg, 2009; Tuchman et al., 2009; Travis et al., 2010). *Typha* ×*glauca* is reported to be mostly sterile, with vigorous clonal growth (Smith, 2000). Hybrids may be even more invasive than *T. angustifolia* (Galatowitsch et al., 1999), but this has not been tested using common garden experiments and known genotypes.

Hybrid cattails are difficult to identify because considerable phenotypic variation occurs within each parent species (e.g., Kuehn and White, 1999; Kuehn et al., 1999; and references therein). To date, the most extensive investigation of species-specific genetic markers in cattails is by Kuehn et al. (1999), who used 10 primers to identify 30 RAPD fragments that were fixed and species-specific in North American populations of *T. angustifolia* and *T. latifolia*. Because RAPD markers have only two alleles at each locus (the presence or absence of an amplified DNA fragment), data from many loci are needed to identify backcrossed or advanced-generation hybrids. In contrast, codominant SSR loci (also known as microsatellites) can be highly polymorphic and are often preferable to RAPDs for population genetic analyses (e.g., Selkoe and Toonen, 2006).

Tsyusko-Omeltchenko et al. (2003) identified 11 dinucleotide SSR loci from *T. angustifolia* in Ukraine and used these loci in population genetic studies of *T. angustifolia* and *T. latifolia* (Tsyusko et al., 2005). Their analyses suggest that *T. angustifolia* and *T. latifolia* may be fixed for alternate alleles of the SSR loci, but this conclusion was not tested explicitly, nor is it certain that results from Ukraine can be extrapolated to other continents. Here, we screened nine of the Ukraine SSR loci and found seven that distinguish between *T. angustifolia* and *T. latifolia* in North America, consistent with species-specific RAPD markers. Samples from Michigan included the parent species, F_1 hybrids, and introgressed individuals. We used these samples to examine the extent to which morphological characteristics of the parent taxa, F_1 hybrids, backcrossed plants, and advanced-generation plants overlap and to determine which morphological characters contribute most to differences among these groups.

MATERIALS AND METHODS

Collections—Two groups of collections were used. Collection Group 1 (Table 1) includes samples from sites in Minnesota, Maryland, New York, and Connecticut that were systematically sampled for cattails that resembled either *T. latifolia* or *T. angustifolia*, based on visual observations of morphological traits (not measured). Samples from these populations were used to identify species-specific SSR markers. Individual samples were separated by at least 10 m where possible to minimize repeated sampling within clones. Visual identifications of *T. latifolia* and *T. angustifolia* were confirmed using RAPD markers as described below. Collection Group 1 also includes samples from Minnesota, Wisconsin, and Indiana that were identified as F_1 hybrids in a previous study (Travis et al., 2010) (Table 1). These samples were used to test for a similar F_1 designation using species-specific SSR markers.

Plants in Collection Group 2 were obtained from 18 populations in Michigan and were chosen to represent several categories of plants—typical *T. latifolia*, typical *T. angustifolia*, and intermediate morphological phenotypes that appeared to be hybrids (Table 2). We included plants that were intermediate between the hybrids and each parent species with the aim of including backcrossed plants and advanced-generation hybrids. Morphological measurements were obtained to examine the correspondence between morphology and hybrid

TABLE 2. Collection Group 2 with locations of 18 Michigan populations, subpopulations (areas), and year sampled for DNA and morphological measurements of *Typha angustifolia* (A), *T. latifolia* (L), F_1 hybrids, advanced-generation hybrids, backcrosses to *T. angustifolia* (BA), and backcrosses to *T. latifolia* (BL), as determined by species-specific SSR alleles (see text). Several samples were from nonreproductive plants and were not included in the discrimination analysis of morphological characteristics (Fig.1). Samples of *T. angustifolia* and *T. latifolia* with RAPD data are included in Table 3.

Location	Latitude, Longitude	Year	Number of clones					
			A	L	F_1 hybrid	Adv hybrid	BA	BL
Alanson	45.472, −84.832	2004, 2005	3	8				
Barbeau	46.164, −84.217	2006	1		1			
Carp Lake, Cecil Bay	45.747, −84.801	2005	1				1	
Cheboygan, population 1	45.657, −84.475	2004, 2005	8	9	7	2	8	
Cheboygan, population 2	45.598, −84.551	2005	3		1			
Chippewa County, Gogomain River	46.147, −84.093	2006	1				2	
Cheboygan, Duncan Bay pop., area 1	45.653, −84.458	2005			1			
Cheboygan, Duncan Bay pop., area 2	45.652, −84.464	2005						1
Cheboygan, Duncan Bay pop., area 3	45.653, −84.459	2005		1				
Cheboygan, Grass Bay pop., area 1	45.654, −84.374	2005	1	1				
Cheboygan, Grass Bay pop., area 2	45.656, −84.377	2005	1					
Cheboygan, Grass Bay pop., area 3	45.656, −84.377	2005	1					
Indian River	45.421, −84.551	2007	3				1	
Brimley, Bay Mills Rd.	46.425, −84.606	2006		2				
Dollar Settlement, Pendills Creek	46.446, −84.794	2006		1				
Cedarville	45.637, −84.947	2005	1	1				1
Munuscong, population 1	46.205, −84.268	2006	1		1			
Munuscong, population 2	46.187, −84.317	2006	1	1	1			
St. Ignace, Point La Barb pop., area 1	45.847, −84.734	2005	1		2			1
St. Ignace Point La Barb pop., area 2	45.848, −84.735	2005						1
St. Ignace Point La Barb pop., area 3	45.841, −84.749	2005	1					
St. Ignace Point La Barb pop., area 4	45.841, −84.748	2005	1		3			
St. Ignace Point La Barb pop., area 5	45.840, −84.750	2005			1			
Cedarville, Prentis Bay	45.990, −84.228	2006	1		2			
Pellston	45.622, −84.782	2005		1				
Moran, Summerby Swamp	45.969, −84.793	2005	1	1			1	
Total clones per group			**31**	**26**	**20**	**2**	**13**	**4**

status, as determined by molecular markers. Habitats included coastal marshes of Lake Huron, Lake Michigan, and Lake Superior, roadside ditches, inland lakes, and open and wooded marshes. For each individual, we measured stem height (height from the soil surface to the tip of the longest leaf), leaf width midway up the longest leaf, length of the longest leaf, and stem diameter 5 cm above the soil. We also measured three reproductive traits: length of the gap between female and male parts of the flowering spike, and the width and length of the female part of the spike.

Molecular markers—DNA was extracted from 3-cm^2 leaf tissue samples using standard protocols (e.g., Tsyusko-Omeltchenko et al., 2003). SSR genotypes were determined using nine loci developed from *T. angustifolia* by Tsyusko-Omeltchenko et al. (2003); these were labeled TA 3, 5, 7, 8, 13, 16, 19, 20, and 21 (locus 21 was used with Collection Group 2 only). PCR amplifications followed recommended protocols (Tsyusko-Omeltchenko et al., 2003). We used the SSR results to identify unique clones within each population by matching alleles among ramets and deleting data from identical, multilocus genotypes in the same population. Following methods in Kuehn et al. (1999), we also genotyped samples using 8–11 species-specific RAPD fragments (from primers A2, A8, K6) for Collection Group 1, and three species-specific RAPD markers (from primer A2) for a subset of plants in Collection Group 2.

To screen for species-specific SSR alleles, we used samples of each parent species in Collection Group 1 after confirming visual identifications by using RAPD markers. Single-locus F_{ST} values (Wright, 1951; Weir and Cockerman, 1984) were calculated for SSR loci using the software GENETIX 4.04 (Belkhir, et al., 2004) to measure interspecific genetic variation among the "pure" populations of *T. angustifolia* and *T. latifolia* in Table 1. Six SSR loci had F_{ST} values >0.44, which indicates highly differentiated populations.

The software STRUCTURE 2.3.3 (Pritchard, et al., 2000) was used to determine if the six selected SSR loci could distinguish between the two parent species using apparently pure samples (based on RAPD markers) from Collection Group 1 (Table 1). STRUCTURE was run by varying the number of clusters (K) from 1 to 10 (where 10 represented the total number of collection sites). The most likely number of genetic clusters in the data was subsequently estimated using the ad hoc statistic ΔK (Evanno et al., 2005). This statistic is based on the second order rate of change of the posterior probability of data with respect to K values. Because this approach provided strong evidence for $K = 2$ highly differentiated populations, consistent with two distinct species, two more STRUCTURE analyses were performed based on SSR loci at $K = 2$ to detect hybridization between *T. angustifolia* and *T. latifolia*. For each STRUCTURE analysis, the basic admixture model with unlinked loci, correlated allele frequencies, and with no a priori information about site of origin was used. Each K was run five times with a burn-in length of 100 000 and a postburning data collection length of 10^6.

We used RAPD-identified samples from the "pure" parent genotypes to determine which individual SSR alleles at each locus were most useful for distinguishing the parent species from Collection Groups 1 and 2. Although only three RAPD markers were used for Collection Group 2, these samples represented typical phenotypes of the two species and had SSR alleles that were consistent with results from Collection Group 1. We also used the species-specific RAPD markers to identify a seventh diagnostic SSR locus (TA 21) and to confirm that F_1 hybrids with one diagnostic RAPD marker from each parent species also had one diagnostic SSR allele from each parent, as expected. Overall, SSR and RAPD analyses of different samples were run in two laboratories, and the diagnostic alleles for each species were consistent between research groups.

Morphometric analyses of parent and hybrid taxa—The seven species-specific SSR loci were used to designate plants from Collection Group 2 as *T. latifolia*, *T. angustifolia*, F_1 hybrids, or backcrossed/advanced-generation hybrids. Plants with a complete set of species-specific SSR alleles from each parent were designated as F_1 hybrids. Plants with a mixture of hybrid and pure alleles favoring one parent species were designated as backcrosses to that parent, and those with an inconsistent pattern of parental alleles were classified as advanced-generation hybrids. For an unknown reason, five individuals that were classified as pure *T. angustifolia* based on the SSR alleles were identified as hybrids using RAPD markers. This discrepancy strongly suggests a clerical error in the laboratory, so these samples were not considered further.

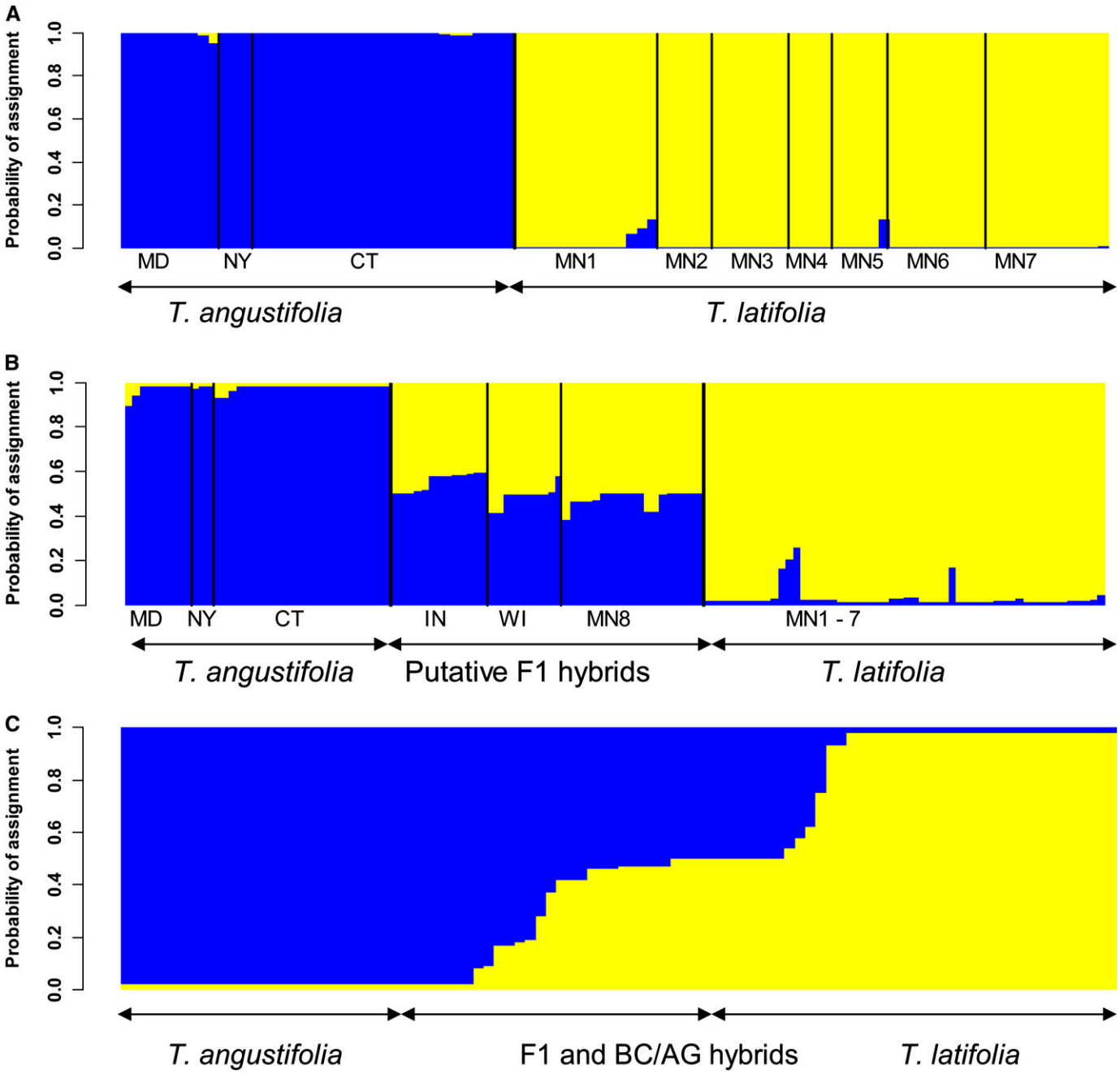

Fig. 1. Barplots of STRUCTURE analyses for (A) RAPD-identified *T. angustifolia* and *T. latifolia* from Collection Group 1; (B) RAPD-identified *T. angustifolia*, *T. latifolia*, and putative F_1 hybrids from additional Collection Group 1 sites in Indiana, Wisconsin, and Minnesota; and (C) mixed populations in Michigan (Collection Group 2) that included advanced-generation/backcrossed individuals (AG/BC). Each bar represents a single individual. Locations sampled are listed in Tables 1 and 2. Six SSR loci were used in these STRUCTURE analyses (TA 21 was not included).

Because individual stems exhibited a range of sizes, we first examined linear and nonlinear allometric relationships of morphological traits in *T. latifolia*, *T. angustifolia*, and F_1 hybrids using the length of the longest leaf as our index of stem size. We found strong allometric relationships between this index and leaf width and stem diameter, but not with reproductive traits, so we used the following ratios in analyses: log(leaf width/leaf length) and log(stem diameter/leaf length), in addition to spike length, spike width, and spike gap length.

We performed linear discriminant analyses following Leps and Smilauer (2003) to predict classification of each cattail individual into six prespecified groups (*T. angustifolia*, *T. latifolia*, F_1 hybrids, backcrosses to each parent, and advanced-generation hybrids). Linear discriminant analysis is a multivariate classification method that allows us to find scores of linear combinations of the explanatory variables (morphological traits) that optimally separate the a priori defined groups. We used forward selection to compare the discrimination ability and significance of each trait independently. Then we selected the trait with the highest discrimination power and calculated the discrimination power of the remaining traits after removing effects of the first selected trait. This process was continued until all traits with significant additional contributions to discrimination among groups were selected. At each step, the significance of the marginal effects of each individual trait was tested by a nonparametric Monte Carlo permutation test (CANOCO ver. 4.5; Ter Braak and Smilauer, 1998).

TABLE 3. Distribution frequency of SSR alleles in clones that were identified as either *Typha latifolia* (L) or *Typha angustifolia* (A) using RAPD markers. Shading indicates fragment sizes that were designated as *T. latifolia* (pink, underlined), *T. angustifolia* (blue), or uncertain (no shading); boldface with green shading indicates exceptions for these designations. Collection sites are shown in Tables 1 and 2.

	TA 3 locus			TA 5 locus			TA 7 locus			TA 8 locus			TA 16 locus			TA 20 locus			TA 21 locus		
	Allele			Allele			Allele			Allele			Allele			Allele			Allele		
	Size (bp)	L	A	Size (bp)	L	A	Size (bp)	L	A	Size (bp)	L	A	Size (bp)	L	A	Size (bp)	L	A	Size (bp)	L	A
	174	40	0	276	4	0	176	5	0	267	18	0	167	15	0	91	84	0	278	26	0
	176	57	0	278	26	0	182	4	2	269	25	0	177	2	0	93	52	**2**	280	0	14
	178	9	0	280	80	0	186	3	6	271	85	0	179	80	1	99	0	15			
	180	18	**1**	282	2	0	188	10	0	273	0	2	181	1	5	101	0	65			
	210	**6**	56	286	0	14	190	108	0	275	**2**	44	191	0	1	103	0	2			
	216	0	9	288	0	31	192	6	0	287	0	9	193	0	70						
				290	0	8	196	0	68	289	0	19	195	0	9						
				292	0	1	210	0	8	291	0	12									
				294	0	26															
Total no. of clones		65	33		56	40		68	42		65	42		49	43		68	42		13	7
Group 1		52	26		43	33		55	35		52	35		36	36		55	35		0	0
Group 2		13	7		13	7		13	7		13	7		13	7		13	7		13	7

RESULTS AND DISCUSSION

SSR loci for identifying early- and late-generation hybrids—Two SSR loci did not prove useful for identifying hybrids but were appropriate for identifying clones. These were TA 13, which had a relatively low F_{ST} value and was not consistent with species-specific RAPD markers, and TA 19, which had a null allele for *T. latifolia*. Data from these two loci are not considered further.

The multilocus STRUCTURE analysis for RAPD-identified samples from *T. angustifolia* and *T. latifolia* gave very clear results (Fig. 1A). Five independent runs for each value of K between $K = 1$ and $K = 10$ showed that the peak value of ΔK was at $K = 2$, suggesting two genetic clusters that represent *T. latifolia* and *T. angustifolia*. When the results are plotted with $K = 2$, each population is correctly assigned to the expected species based on morphological traits, and a few individuals show evidence of possible hybridization (Fig. 1A). In a second analysis, RAPD-identified F_1 hybrids showed the expected admixture pattern relative to the parent species (Fig. 1B). Adding the F_1 data to the previous analysis caused slight changes in admixture patterns within *T. angustifolia* and *T. latifolia*, but the two parent species are clearly distinguishable. A third analysis using Collection Group 2 from Michigan confirmed that these samples included the parent species, F_1 hybrids, and also backcrossed/advanced generation clones (Fig. 1C). In this case, SSR data were used without preassigning samples to species and hybrid groups using RAPD markers or phenotypic traits. Most of the backcrossed/advanced generation plants were more similar to *T. angustifolia*, while a few were more similar to
T. latifolia. This pattern suggests that crosses between F_1 hybrids and each parent species are possible. In summary, the SSR loci we identified can be used to test for hybridization and introgression between cattail species using multilocus data in STRUCTURE.

STRUCTURE does not require data from species-specific loci (Pritchard et al., 2000). To determine which alleles at each locus were species-specific, we examined the SSR alleles that characterize individuals of each RAPD-identified parent plant in Collection Groups 1 and 2. The seven SSR loci had alleles that were species-specific, or nearly so, for the sampled populations (Table 3). SSR allele fragments were larger in the species that was used to develop these markers, *T. angustifolia* (Tsyusko-Omeltchenko et al., 2003). Ellegren et al. (1995) also reported larger allele fragments of SSR loci from the species for which they were first obtained.

All alleles of SSR loci TA 5 and TA 21 were completely nonoverlapping for the parent species (Table 3). For TA 7, allele sizes 182 and 186 were not definitively species-specific. For the other alleles and loci in Table 3, SSR data that did not match the RAPD-based identifications perfectly were rare and probably represent backcrossed genotypes.

In addition, nearly all of the putative F_1 hybrids in Fig. 1B had one SSR allele from each parent species across the seven loci, as expected. For this analysis, we used samples with data for 8–11 RAPD markers to identify 42 F_1 hybrids, which then were screened at six SSR loci. These clones exhibited the expected pattern of having one SSR allele from each parent species, with 14 exceptions in 504 locus/clone combinations, seven of which involved alleles that were not clearly species-specific. The expected pattern of having one SSR allele from each parent species was also seen in all 23 of the F_1 hybrids from Michigan that were identified using RAPD markers.

Phenotypic characteristics of F_1 hybrids and advanced-generation hybrids—Collection Group 2 included intermediate morphological phenotypes from populations in Michigan. Linear discriminant analyses of morphological data from these samples show expected patterns for SSR-identified genotype groups (Fig. 2A, B). The two parent taxa represent very distinct groups, and F_1 hybrids form a fairly distinct third group that is intermediate between them (Fig. 2A, B). This analysis suggests that researchers who are familiar with morphological variation in *Typha* may be able to identify these three groups in the field. However, accurate field identification will be more challenging if plants are sampled randomly or if introgression has occurred. Backcrossed plants were generally intermediate between hybrids

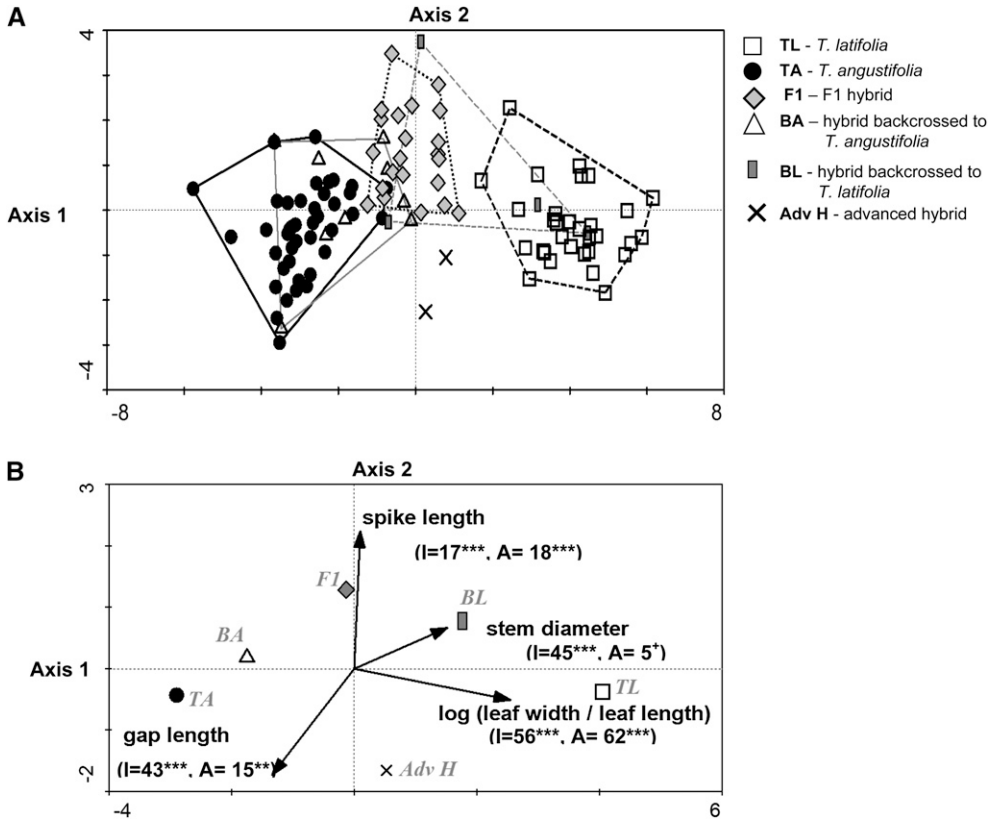

Fig. 2. (A) Diagram showing the first two discrimination axes in a linear discriminant analysis for six SSR-identified groups from populations in Michigan (Collection Group 2): *T. angustifolia* ($N = 36$), *T. latifolia* ($N = 29$), F_1 hybrids ($N = 21$), advanced-generation hybrids ($N = 2$), backcrosses to *T. angustifolia* (BA, $N = 9$), and backcrosses to *T. latifolia* (BL, $N = 4$). Plants of each group are enclosed in an envelope that defines the range of the group's morphological traits. (B) The same analysis as in (A) showing the centroids of each group and arrows indicating the discrimination power of morphological traits with significant additive discrimination ability. Traits with longer arrows have larger discrimination power (I: independent discrimination ability, A: additive discrimination ability). The direction of the arrow is the direction of the effect in the multivariate space and indicates that the trait helps discriminate among groups on that axis. The significance of the marginal effects of each trait was obtained by a nonparametric Monte Carlo permutation test with 999 permutations: *** $P = 0.001$, ** $P \leq 0.01$, * $P < 0.05$ and $^{+}P < 0.1$.

and parent taxa and could also be confused with F_1 hybrids (Fig. 2A). F_1 hybrid phenotypes were somewhat closer to those of *T. angustifolia* than *T. latifolia*, perhaps reflecting the fact that F_1 hybrids are reported to have the maternal cytotype of *T. angustifolia* (Kuehn et al., 1999).

The most useful character for discriminating among parental and F_1 hybrids was the log of the ratio of leaf width to leaf length (Fig. 2B). This ratio is an allometric trait not included in a very similar study by Kuehn and White (1999). Thus, because taller plants of all groups have wider leaves, it is important to incorporate variation in size when using leaf width to distinguish these taxa. After the discrimination ability of log(leaf width/leaf length) was accounted for, spike length, spike gap length (the gap between male and female flowers), and stem diameter also provided significant and substantial additive discrimination ability among parental species and hybrids (Fig. 2B).

Utility of SSR markers—These SSR markers should be useful for identifying hybrids and introgressed individuals in North American cattail populations because (1) our samples were collected over a broad geographic area (31 populations in six states), (2) most F_1 hybrid individuals had one diagnostic SSR allele from each parent, as expected, (3) putative hybrids and hybrid derivatives had intermediate phenotypes, as expected, and (4) analyses carried out in separate laboratories gave consistent results. Further analyses of the genetics of hybridizing populations could be carried out using cytoplasmic markers to determine the maternal parents of naturally occurring progeny from interspecific crosses (e.g., Kuehn et al., 1999; Zhang et al., 2008).

In conclusion, a new contribution of this study was determining which SSR markers from a study in the Ukraine are species-specific in North America—seven such markers were identified. Also, to our knowledge, this paper is the first to describe the occurrence of backcrossed generations in hybridizing cattail populations in Michigan. Our results and those of Travis et al. (2010) show that hybrid populations can include substantial numbers of backcrossed genotypes, contrary to earlier descriptions of F_1 populations by Kuehn et al. (1999). Thus, the availability of diagnostic SSR markers supplements earlier work involving RAPD markers and facilitates further studies of the population genetics and ecological effects of hybridizing cattail populations. Because *T. angustifolia* has recently expanded its range over much of North America, frequently coming into contact with *T. latifolia*, it will be interesting to examine the abundance and relative invasiveness of hybrid lineages, as well as the persistence/survival of the native *T. latifolia*.

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