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The potential for hybridization between *Typha angustifolia* and *Typha latifolia* in a constructed wetland

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Abstract

Three *Typha* taxa are recognized in the central USA: native *Typha latifolia* (broad-leaved cattail), the invasive *Typha angustifolia* (narrow-leaved cattail), and a hybrid between the two species, *Typha* × *glauca*. Previous authors have suggested that interspecific hybridization is common in cattails. In a 6-year-old constructed wetland in central Ohio, USA, we found that *T. angustifolia* began flowering about 2 weeks earlier than *T. latifolia*, with female flowers opening several days earlier than male flowers. *T. angustifolia* shoots were 15 times more abundant and twice as dense as those of *T. latifolia*. Male flowers of *T. angustifolia* far outnumbered the male flowers of *T. latifolia* when the latter species began flowering, such that interspecific pollination was likely during the short period of overlap. DNA markers (RAPDs) were used to screen for hybrids. These markers corresponded well to other species-specific traits, such as pollen type (monads versus tetrads) and the presence or absence of a gap in the floral spike. We found no putative hybrids based on surveys involving molecular and/or morphological traits. Thus, we did not detect any gene flow between the cattail species, despite opportunities for cross-pollination and F_1 seedling establishment.

Keywords: Hybridization; Invasive; RAPDs; *Typha angustifolia*; *Typha latifolia*

1. Introduction

The occurrence of hybridization between native and non-native species has increased as invasive species have expanded their ranges worldwide (e.g., Ayres et al., 1999).

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Hybridization between native and non-native species can compromise biodiversity through several mechanisms, including the potential for the new hybrid to be more invasive than its parental species (Arnold, 1997; Ellstrand and Schierenbeck, 2000; Moody and Les, 2002). Hybridization also has the potential to degrade species diversity by "swamping out" native genotypes (Rhymer and Simberloff, 1996; Wolf et al., 2001). Displacement of native genotypes through genetic assimilation is often difficult to detect because of the morphological similarities among hybridizing species and their progeny (e.g., Ayres et al., 1999). In most cases, little is known about the impacts of hybrids on native species (Carr, 2001).

In the northern USA and southern Canada, similarities among three taxa of cattail (Typhaceae) have led to difficulties in identification and lack of knowledge concerning inter-taxon gene flow and hybrid frequencies. *Typha latifolia* L. (broad-leaved cattail), a native species, is common in wetlands throughout much of the United States (Smith, 2000). *Typha angustifolia* L. (narrow-leaved cattail), thought to be introduced from Europe (Stuckey and Salamon, 1987), occurs in the northeastern range of *T. latifolia* and is considered an invasive species due to its rapidly spreading range and its ability to successfully establish monospecific stands that displace native plants (Grace and Harrison, 1986). Both species are clonal, self-compatible, and wind pollinated (Grace and Harrison, 1986). Reproductive shoots are monoecious, producing female flowers prior to male flowers, a trait which could facilitate outcrossing and hybridization (Smith, 2000).

The hybrid between these species, *Typha* × *glauca*, is considered common in regions where both the species occur and may invade areas not previously inhabited by the parental species, such as eutrophic and disturbed habitats with unstable water levels (Smith, 1987). Galatowitsch et al. (1999) described the "rapid expansion of hybrid populations" and noted evidence that hybrids occur "wherever the parental species are sympatric". Likewise, Smith (2000) stated that "hybrid seedlings are likely wherever the two species form mixed stands and bare wet soil is available for seed germination and establishment".

The species status of *Typha* × *glauca* has long been disputed and relatively little is known about the genetic variation, taxonomy, ecology, or evolutionary implications of this plant (Les and Philbrick, 1993). Most recently, Kuehn et al. (1999) identified species-specific randomly amplified polymorphic DNA (RAPD) markers to examine hybridization between *T. angustifolia* and *T. latifolia*. All of the markers were clearly associated with either *T. angustifolia* or *T. latifolia*, which can be difficult to distinguish unless pollen and stigmas are present. *T. angustifolia* typically sheds pollen as monads, *T. latifolia* sheds its pollen as tetrads, and F_1 hybrids between the two species have a combination of pollen in tetrads, dyads, and monads (Smith, 2000; Selbo, 2002). Spike gap distance is a fairly diagnostic trait in that *T. angustifolia* typically has a gap between the staminate and pistillate flowers and *T. latifolia* does not (Smith, 2000; Kuehn and White, 1999). Kuehn and White (1999) found that morphological characters were correlated with species-specific RAPD markers in 90% of the cases examined. Because of the variability of many morphological traits, DNA markers are considered to be more reliable for identifying cattail species, their hybrids, or backcrossed progeny.

Kuehn et al. (1999) found that all of the putative hybrids had a full complement of DNA markers from both parental species, indicating that they were F_1 plants. The lack of intermediate marker frequencies suggests that F_1 hybrids between *T. angustifolia* and *T. latifolia* may be vigorous but highly sterile, which is consistent with the previous studies

of artificially produced F_1 hybrids (Smith, 1987). Smith (2000) noted that "unfortunately, (*Typha* × *glauca*) has been treated as a distinct species by many authors", rather than a sterile F_1 hybrid.

A better understanding of the potential for *T. angustifolia* and *T. latifolia* to hybridize is useful to predict the evolutionary future of these species. Colonization of created wetlands represents a unique opportunity for studying early establishment and distribution of cattails. New wetlands have ample habitat for the seedlings to establish and spread, thus allowing a hybrid seed source the opportunity to gain ground. Young plants produce multiple rhizomes and typically flower in their second year (Yeo, 1964). The goals of this study were to (1) document relative abundances of native and non-native cattail in a 6-year-old constructed wetland, (2) quantify the overlap in flowering time and the potential for hybridization between *T. angustifolia* and *T. latifolia*, and (3) search for hybrids using both morphological and molecular methods.

2. Materials and methods

2.1. Study site

This research was carried out at the Olentangy River Wetlands Research Park (ORWRP) at The Ohio State University, Columbus, Ohio. Two similar 1 ha basins were created in the spring of 1994 (Mitsch et al., 1998). The wetland is fed by a river water delivery system that maintains natural hydroperiods by matching the pumping protocol to the flow of the river (Mitsch et al., 1998). The wetland basin we examined (known as wetland 2) was colonized naturally (Svengsouk and Mitsch, 2001). The cattail composition of the wetland has not been studied previously, but reports indicate that stands of both *T. angustifolia* and *T. latifolia* were present in 1995 (Mitsch et al., 1998).

2.2. Relative abundance of Typha spp.

Cattail species were identified based on characteristics of the leaves and flowering spikes, unless indicated otherwise below. To estimate the relative abundance of each cattail species, we used aerial photography, a survey map, and reconnaissance on the ground. Total cattail cover was discernible from the aerial photos taken in July, 2000 and was recorded on a detailed survey map. Stands of the less common *T. latifolia* were mapped based on a careful observation along a network of boardwalks within the wetland and by walking around the edge of the wetland during May–July, 2000. Mixed patches of both species were uncommon and were assigned to the more dominant species in the patch. We used the vegetation map to estimate the number of square meters occupied by each species.

To document the differences in shoot densities between *T. angustifolia* and *T. latifolia*, we counted the number of shoots per square meter at six sites in central Ohio where both species were present, including the ORWRP in September, 2000. Three monospecific 1 m^2 plots were chosen per species at each site except for the ORWRP, where we used eight plots per species. All vegetative and reproductive shoots were counted within each plot. To estimate the relative abundance of each cattail species at the ORWRP, we multiplied the

average number of shoots per square meter for each species by the total number of square meters occupied.

2.3. Overlap in flowering time

To assess overlap in flowering time between *T. angustifolia* and *T. latifolia*, we designated 10 plots measuring 0.5 m × 2 m along the boardwalks at the ORWRP in May, 2000. Each plot was chosen to contain both species, based on leaf and spike characteristics. From May 24 to July 27, we recorded flowering times several times a week by counting all shoots and noting whether female or male flowers were present.

2.4. Occurrence of intermediate forms

An extensive survey was conducted throughout the ORWRP and the surrounding area but no obvious hybrids were found based on morphological traits. To characterize variation in morphological traits within species, approximately 50 flowering shoots from each putative species were flagged in June, 2000. We recorded spike gap length (distance between pistillate and staminate flowers), leaf width, and pistillate spike width and length for each labeled shoot. Differences in the morphological measurements between the species were compared using *t*-tests.

Pollen samples from 21 putative *T. angustifolia* and 18 putative *T. latifolia* plants were stained (Alexander, 1969) and the presence of monads, tetrads, or a combination of pollen types (indicative of F_1 hybrids) was recorded. To check for cryptic hybridization and introgression, plants were also screened for RADP markers based on methods in Kuehn et al. (1999). We used primer OPA-02 (TGCCGAGCTG, Operon Technologies Inc.), which revealed two species-specific markers for *T. angustifolia* and three for *T. latifolia*. We collected leaf tissue samples from the 50 flagged plants of each species and extracted DNA from a subset of individuals ($N = 81$). RAPD products were separated by electrophoresis on 1.2% agarose for 1 h at 90 V. Gels were stained with ethidium bromide and visualized under UV light. Molecular weights of amplification products were estimated using a 1-kb ladder and visually compared to results reported by Kuehn et al. (1999). We screened each plant sample for the five species-specific markers at least twice. Different gels from the same plant sample showed consistent banding patterns.

3. Results

The non-native cattail, *T. angustifolia*, was more abundant and more dense than the native *T. latifolia* (Tables 1 and 2). *T. angustifolia* colonized eight times more area than *T. latifolia* in the 6 years since the wetland was created. Moreover, *T. angustifolia* produced nearly twice as many shoots per square meter, such that it was ∼15 times more abundant than *T. latifolia* based on total shoot densities (Table 2). The tendency for *T. angustifolia* to occur at much higher shoot densities than *T. latifolia* was observed at three out of five additional sites in central Ohio (Table 1). Across all six sites, density differences between species were significant at $P < 0.001$ (ANOVA). Similar differences in abundance are likely to be found

Table 1
Shoot density (number of shoots per square meter) for *T. angustifolia* and *T. latifolia* at sites in central Ohio

	Mean		S.E.	
	T. angustifolia	*T. latifolia*	*T. angustifolia*	*T. latifolia*
West Columbus (N40:05:05, W82:51:11)	57.7	18.3	10.2	1.5
East Columbus (N40:00:52, W83:0211)	26.7	20.0	4.4	3.2
Powell (N40:10:14, W83:03:25)	34.7	22.0	9.5	4.0
Granville (N40:04:36, W82:31:22)	43.3	19.0	5.0	0.6
New Albany (N40:05:15, W82:49:25)	34.7	33.7	5.7	0.3
ORWRP (N40:01:13, W83:01:10)	32.5	16.6	1.3	0.8
All	37.0	20.5	2.7	1.3

Means are based on three plots per site except for the Olentangy River Wetlands Research Park, where $N = 8$. Differences in density between cattail species was significant at $P < 0.001$ (ANOVA). Latitude and longitude for each location are listed.

at other sites as well, but could depend on factors such as proximity of seed sources, timing of disturbances, and variation in water depth.

Typha angustifolia began flowering about 2 weeks prior to *T. latifolia*, but we observed a small amount of overlap in flowering times (Fig. 1). Both cattail species are protogynous, with female flowers opening first on the lower end of the spike and male flowers releasing pollen after the female flowers begin to senesce. Thus, self-pollination within spikes is unlikely to occur. Selfing between different flowering shoots in the same clone is possible, but we did not obtain data on the degree to which female and male flowering times are synchronized within clones. In the earlier *T. angustifolia*, the peaks for male and female flower production were more staggered than in *T. latifolia* (Fig. 1). By the time *T. latifolia* began to flower, only a small fraction of *T. angustifolia* plants were still producing pollen, and even fewer had receptive stigmas. Because the earlier *T. angustifolia* was more common, it seems likely that stigmas of *T. latifolia* could receive pollen from *T. angustifolia* during the first 2 weeks of the total flowering period (∼8 weeks) of *T. latifolia* when pistillate flowers were receptive.

Despite the potential for interspecific pollination, we found no evidence that hybrids or introgressed plants had colonized the wetland. The RAPD primer produced two species-specific bands for *T. angustifolia* and three species-specific bands for *T. latifolia*, as reported in Kuehn et al. (1999), and no hybrids were detected in our analysis of DNA from 81 individuals. RAPD markers corresponded well with morphological markers in all but six individuals (Table 3). Using RAPD markers to identify species, we found that two *T. angustifolia* shoots displayed a wider leaf width and one displayed the absence of a spike

Table 2
Estimated densities of *T. angustifolia* and *T. latifolia* at the ORWRP in 2000

	Total area (m^2)	Estimated number of shoots	Percentage of total cattail shoots	Percentage area dominated by cattails
T. angustifolia	2647	86025	94	88
T. latifolia	350	5820	6	12

Values are based on vegetation mapping and shoot densities in Table 1.

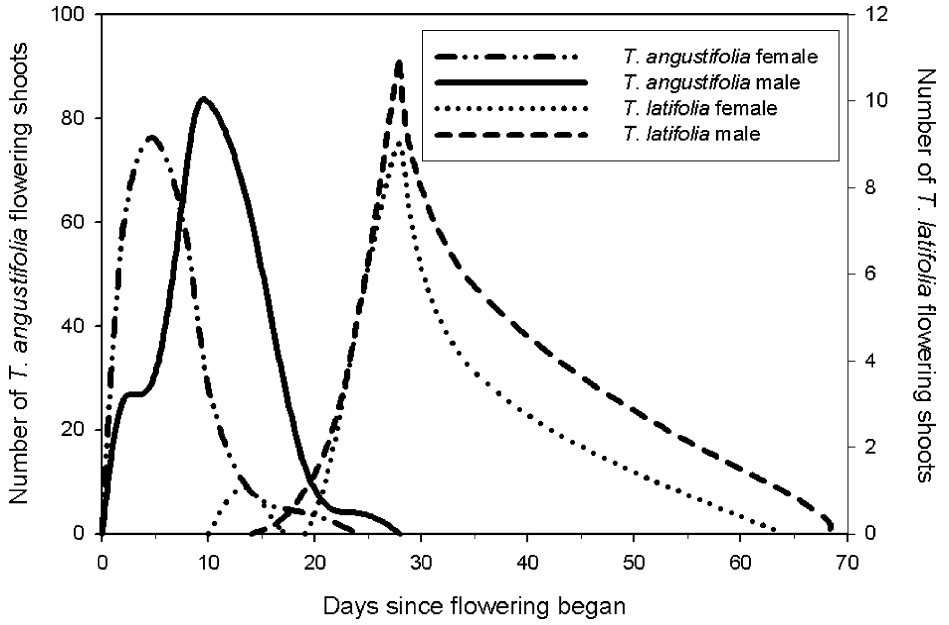

Fig. 1. Flowering times of *T. angustifolia* and *T. latifolia* at the Olentangy River Wetlands Research Park, Columbus, Ohio. Female indicates when stigmas were receptive. Male indicates when anthers were shedding pollen. Day 0 indicates May 24, 2000; day 68 indicates July 27, 2000.

gap, while one *T. latifolia* had a narrow leaf width and two displayed a spike gap. Therefore, based on DNA markers, the presence of a spike gap was a species-specific character in 96% of the individuals we examined ($N = 81$). In *T. angustifolia*, the average length of the gap between the female and male portions of the spike was 2.6 cm ($N = 42$, range = 0–9.5,

Table 3
Comparison of morphological and molecular traits in Typha plants grouped according to RAPD marker type

Parameter	RAPD marker type	
	T. angustifolia	*T. latifolia*
Pollen		
Tetrad	0	18
Monad	18	0
Other	0	0
Spike gap		
Present	41	2
Absent	1	37
Leaf width		
<13 mm	40	1
≥13 mm	2	38

The number of plants in each category is listed. *T. angustifolia* had two diagnostic RAPD markers, while *T. latifolia* had three; none of the individuals sampled had markers from both species.

S.E. = 0.26). Pollen from *T. angustifolia* plants ($N = 18$) was consistently shed as monads, while tetrads were shed from *T. latifolia* plants ($N = 18$). No mixtures of pollen type were observed. In a larger sample of plants, some of which lacked data on DNA markers, we found significant differences between species in leaf width and pistillate spike width but not spike length (Selbo, 2002).

4. Discussion

This study documents the extent to which the non-native *T. angustifolia* outnumbered the native *T. latifolia* in a 6-year-old, naturally colonized wetland. Combining data on shoot density and area colonized, *T. angustifolia* was ~15 times more abundant than *T. latifolia*. Other cattail populations near this site were also dominated by *T. angustifolia* (Selbo, 2002). Theoretically, the much greater abundance of *T. angustifolia* could allow massive pollen flow to usurp stigmas and ovules of the native *T. latifolia*. However, the degree to which this occurs depends on the amount of overlap in flowering times.

To our knowledge, this study represents the first documentation of flowering phenology in *T. angustifolia* and *T. latifolia*. We found considerable temporal displacement in flowering times, such that the peak flowering of *T. angustifolia* occurred 15–20 days earlier than that of *T. latifolia*. This indicates that the proportion of female flowers that could receive heterospecific pollen is quite small, being restricted to the short interval when the "outliers" of each species flower simultaneously. The period of overlap was further reduced by the fact that the earlier flowering species, *T. angustifolia*, was strongly protogynous. Individuals of *T. latifolia* that flowered during the first 2 weeks of the 8 week flowering period were surrounded by staminate spikes of the dominant *T. angustifolia*. Conspecific pollen was available after the first few days, but the far greater abundance of heterospecific pollen could foster hybridization, with *T. latifolia* as the likely maternal parent. However, Kuehn et al. (1999) found that all of the hybrids they tested had a maternally inherited DNA marker from *T. angustifolia*. Their findings may be related to the fact that artificial crosses between these species are most successful when *T. angustifolia* is used as the maternal parent (Lee, 1975; Smith, 1967). Based on the flowering phenologies we observed, interspecific crosses in this direction seem unlikely.

Although interspecific pollination is theoretically possible, we did not detect any hybrid cattails at the ORWRP or other mixed species stands in central Ohio. The sample sizes for our DNA and pollen type surveys were modest ($N = 81$, 39, respectively), but extensive searches for plants with intermediate morphological characteristics did not reveal any evidence of hybrids. Although it is preferable to rely on DNA, pollen type, and stigma width to identify hybrids (Kuehn and White, 1999), many previous investigators found *Typha* × *glauca* populations using other morphological traits (e.g., Smith, 1987 and references within).

If hybrids are absent, as we suggest, a major reason could be that *T. latifolia* is relatively rare and few of its stigmas are receptive when *T. angustifolia* pollen is abundant. Hybridization in the opposite direction seems unlikely because the vast majority of *T. angustifolia* stigmas were no longer receptive when male flowers of the less common *T. latifolia* began shedding pollen. Even if interspecific pollination occurred, pollen effectiveness, pollen

competitive ability, and/or embryo abortion might further reduce the proportion of hybrid seeds. Mechanisms for complete or partial reproductive isolation have rarely been studied in Typha spp., but *T. angustifolia* has been shown to be more successful as the maternal parent than the reciprocal cross (Smith, 1967).

Another explanation for the lack of hybrids at this site could be that the populations are no more than 6 years old and more time is needed for them to occur and produce clones with flowering shoots. Under optimal conditions, *T. latifolia* can produce as many as 30 vegetative shoots during its first year, but 2 years of growth may be required before flowering shoots are formed (Yeo, 1964). Comparable data have not been reported for *T. angustifolia* or F_1 hybrids between *T. angustifolia* and *T. latifolia*. However, given that *Typha* × *glauca* is known for its vigorous growth, we expect that flowering shoots could be produced in the second year. Lack of uncolonized substrate could prevent the establishment of late-arriving hybrids, but gaps in the vegetation are common at our site due to herbivory by muskrats (Selbo, 2002). It is also possible that hybrids once existed at the wetlands and did not survive, or that ecological conditions were not conducive to hybrid establishment.

Once F_1 hybrids become established at a given site, they can form large stands by means of vegetative reproduction, but they are unlikely to produce seeds or backcross with the parental species. Early work on *Typha* × *glauca* showed that F_1 plants are highly sterile (Marsh, 1962; Smith, 1967). Furthermore, no backcrossed individuals were found in populations with *Typha* × *glauca* that were surveyed by Kuehn et al. (1999). If this finding is borne out by more extensive surveys, it may be misleading to classify *Typha* × *glauca* as a widespread, invasive species that occurs throughout the range of its two parental species (as in Galatowitsch et al., 1999). In addition, if hybridization is rare and introgression is absent or extremely uncommon, we can conclude that *T. latifolia* is unlikely to be threatened by genetic assimilation by the non-native *T. angustifolia*. F_1 hybrids may only be common where *T. latifolia* is much more abundant relative to *T. angustifolia*. In this scenario *T. angustifolia* can be the maternal parent and viable hybrid seed can potentially be produced. Further studies of these species would be useful, especially studies that include diagnostic DNA markers to test for cryptic gene flow between the species and the studies that document flowering periods in other regions where the parental species co-occur. In the meantime, concerns about the potential invasiveness of *Typha* × *glauca* should focus on its occurrence and competitive ability rather than the unlikely scenario that the genome of *T. latifolia* will be displaced by hybridization with *T. angustifolia*.

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