# Decrypting the invasion of non-native cattails (*Typha* spp.) in the Fraser River Estuary, British Columbia using morphological and microsatellite analyses

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# Abstract

Hybrid cattail (*Typha × glauca*), a hybrid between native *T. latifolia* and introduced *T. angustifolia*, has been recently identified through macroscopic traits in the Fraser River Estuary (FRE), Canada. This detection represents a significant new focus of invasion for this taxon in North America. *T. × glauca* has been referred to as a cryptic invasive species in the FRE due to its resemblance to other *Typha* taxa, and uncertainty around the reliability of identification based on field characters alone has slowed their detection and management. To test the accuracy of identifications based on morphology, we used molecular tools to evaluate 46 samples identified using morphology in the field as follows: 15 *T. angustifolia*, 15 *T*. × *glauca*, and 16 *T. latifolia*. Taxa were identified in the field across three populations. We used microsatellite markers and admixture analyses to verify our field identifications and found them to be 100% accurate. All 16 field-identified *T. latifolia* and 14 out of 15 *T. angustifolia* were pure genotypes. One *T. angustifolia* showed possible evidence of introgression. Eleven out of 15 *T. × glauca* were clear F1s, whereas three showed possible, but relatively weak, evidence of backcrossing and one may represent a potential F2. Hybrid samples displayed heterosis in three of the six traits measured, and strict intermediacy in the others, providing further evidence that most hybrids are F1s. This study assists in the regional monitoring and management of *Typha* by providing the first genetic evidence of *T. × glauca* in British Columbia, and a morphological methodfor “decrypting” this invasion within the FRE.

**Keywords:** *Typha*, cattail, invasive species, heterosis, hybridization

# Introduction

Estuaries represent the highly productive interface between marine, terrestrial, and freshwater environments, possessing high biodiversity and offering numerous ecological services (Barbier et al. 2011). These ecosystems, particularly those situated near human settlements, are vulnerable to plant species invasions due to a constant flux of non-native propagules from human habituated areas, and ongoing disturbances that favour the colonization, establishment, and dominance of non-native species, which concentrate within these landscape “sinks” (Zedler and Kercher 2004). Once established, invasive species can reduce the resilience and function of estuarine ecosystems, impacting the numerous ecological services they provide (Levin and Crooks 2011; Hensel et al. 2021; Tait et al. 2023). The Fraser River Estuary (FRE) is the largest and most productive estuary in Pacific Canada. The estuary is home to over 100 at-risk species (Kehoe et al. 2021), supports large numbers of Pacific salmon (Chalifour et al. 2019), and continues to provide a critical stopover point for migratory birds along the Pacific Flyway (Butler and Campbell 1987). As with many estuaries in the Pacific Northwest (Brophy et al. 2019), habitat loss has been significant in the FRE, with an estimated 10–20% of wetland habitats still intact in the Fraser River floodplain (Kistritz and Scott 1992; Finn et al. 2021). Most of the remaining wetlands in the FRE are now protected (Stewart et al. 2023), however degradation from abiotic and biotic stressors, including invasive plants, is ongoing (Grout et al. 1997; Lee 2021; Stewart et al. 2024). The importance and the vulnerability of these ecosystems require management that can detect and respond to new and emerging species invasions effectively.

*Typha* are rhizomatous, herbaceous perennial plants that can only survive in wetland conditions (Lichvar et al. 2016), and can dominate these habitats due to their robust size, dispersal ability, and rapid growth rate (Bansal et al. 2019). *Typha × glauca* Godr., originating *in situ* as the cross between parental native *Typha latifolia* L.and non-native *Typha angustifolia* L., is considered a problematic wetland invasive species in many regions of North America. Originally restricted to the eastern seaboard, *T. angustifolia* expanded northward and westward over the 20th century and now occurs in nine Canadian provinces and 42 continental states. This expansion was likely driven at regional scales by wetland disturbances, and the construction of habitat corridors such as canals, ditches, and railways (Finkelstein 2003), while long-distance dispersal has been facilitated by the horticultural trade and waterbirds (Ciotir and Freeland 2016; Tóth et al. 2023). In areas such as the FRE where *T. angustifolia* and *T. latifolia* are sympatric, hybridization may occur (Smith 1967). *Typha × glauca* frequently dominates wetlands where it occurs, as a result of robust growth (Zapfe and Freeland 2015), nutrient appropriation (Larkin et al. 2012), leaf litter accumulation (Farrer and Goldberg 2009; Szabo et al. 2018; Stewart 2021), and allelopathy (Szabo et al. 2018). Resulting changes associated with these invasions are numerous, and include losses in plant species diversity (Farrer and Goldberg 2009; Stewart 2021), and reduced habitat usage by wildlife (Kantrud 1986; Cooper et al. 2008) and macroinvertebrates (Lawrence et al. 2016; Lee 2021). When dominant, *T*.× *glauca* can also alter the nutrient processes of wetlands by amplifying internal nutrient cycling (Currie et al. 2014), promoting sedimentation and nutrient deposition (Woo and Zedler 2002), enhancing denitrification (Lishawa et al. 2014), and accumulating leaf litter, which can increase soil nitrogen mineralization rates (Farrer and Goldberg 2009).

Invasive *Typha × glauca* was recently documented in the FRE for the first time by Stewart et al. (2023), who estimated that *T. angustifolia* and *T. × glauca* together occupy an estimated 50 ha or 4% of remaining tidal marsh habitat in the FRE, and that *T. × glauca* is the more abundant taxon. The authors of Stewart et al. (2023) referred to *T. × glauca* as a “cryptic” invasive species (Morais and Reichard 2018), speculating that its detection was impeded by local taxonomic confusion and morphological similarities among local *Typha* taxa, which hindered field identification. For example, local herbarium records and identification guides assigned the name *Typha latifolia* forma *ambigua* (Sonder) to plants with hybrid traits for decades. The prevention of further expansion of *T. × glauca* invasions is therefore dependent on the ability of scientists, professionals, and the public to readily identify *T. × glauca*, thus facilitating the timely detection and cost-effective management of newly established populations (Ahmed et al. 2022). Microsatellites are a reliable tool for differentiating *Typha* taxa (Snow et al. 2010; Travis et al. 2010; Kirk et al. 2011; Geddes et al. 2021). However, molecular tools are largely inaccessible to the public and practitioners, possibly stymieing their ability to detect and respond to invasions in a timely manner. Ideally detection would be possible through use of morphological traits, paired with verification using genetic markers.

Previous studies have investigated the use of both macroscopic and microscopic traits in differentiating *Typha* elsewhere in North America, with varying success. Microscopic characteristics have often been considered more reliable than macroscopic traits, as they are assumed to have less selective pressure and be less plastic (Kuehn and White 1999). Such features include pollen shape (Finkelstein 2003; Marburger 2013) and stigma width (Kuehn and White 1999). Despite these benefits, floristic microscopic traits may be limited in application by seasonality. For example, pollen is only available for a short period each growing season, and stigmas are only measurable before they wither after pollination (Wasko et al. 2022). Additionally, the use of microscopic traits is frequently limited to individuals who have access to the necessary equipment, expertise, or resources, and may further delay *T*.× *glauca* detection and management (Ohsowski et al. 2024).

Macroscopic identification may offer a practical approach for identifying *T. × glauca* in some instances, particularly if the invasion is relatively recent and hybrids are F1 or have limited introgression. Macroscopically, *T. angustifolia* is usually distinguishable from native *T. latifolia* in having narrower leaves, thinner inflorescences, and typically possesses a prominent gap between staminate and pistillate flowers (Kuehn and White 1999; Pieper et al. 2017; Geddes et al. 2021; Tangen et al. 2022; Ohsowski et al. 2024). Identification of hybrid *T. × glauca* is more confounding, because they may have traits that are intermediate or overlap with parental species in features such as leaf width, or spike gap (Kirk et al. 2011; Tangen et al. 2022). In contrast, ramet height and spike length are larger than either parent, likely due to heterosis (Kuehn and White 1999; Zapfe and Freeland 2015). Due to their overlap with parental species, most studies have found macroscopic traits to be unreliable in identifying *T. × glauca*, particularly in areas with long hybridization periods, as backcrossing may further diminish the morphological distinctiveness of the hybrid (Pieper et al. 2017; Geddes et al. 2021; Tangen et al. 2022). Recent studies have made progress in using macroscopic traits, however. For example, Ohsowski et al. (2024) found that two field measurements, leaf counts and longest leaf, were reliable at different differentiating *T. × glauca*, its parental species, and advanced generation hybrids in Great Lakes wetlands. Wasko et al. (2022) found that identifications based on the mean leaf-apex angle for all leaves on a plant were also reliable. The authors of Wasko et al. (2022) advised caution in applying their findings to other regions due to variability in *T. × glauca* genetics. To our knowledge, no evaluation of morphological traits has occurred in the Pacific Northwest of North America, nor within the unique environmental conditions of an estuary.

This study aimed to increase our knowledge of cattail genetics and assess the value of macroscopic traits to detect *T.* *×* *glauca* in the FRE. In so doing, this work attempts to “decrypt” this cryptic invasion, by offering information that will enhance awareness and management actions in the region. Through morphological and microsatellite analyses we asked: (1) do molecular markers confirm the presence of *T.* *× glauca* in the FRE, and (2) can macroscopic traits be used to differentiate hybrids from their parental species at this point in time in the FRE?

# Methods

## Sample Collection

Plant measurements and tissue samples were collected from three brackish tidal marsh sites in the Fraser River Delta in southwest British Columbia (Figure 1): Frenchies Island (49.101o N, 123.112 oW), Sturgeon Bank (49.174o N, 123.202o W), and Iona Island (49.220o N, 123.212o W). These environments experience varying degrees of mixed semi-diurnal tidal inundation and seasonal salinity exposure, depending on location in the estuary and within each respective marsh. The exception is Iona Island, where five of the 15 specimens were collected from the perimeter of a sewage settling pond in the adjacent upland (AI1, LI2, LI5, GI1, GI2; Table S1). The pond differs environmentally in being freshwater, non-tidal, and likely differs in other factors, such as water temperature and nutrient availability.

Sites were selected for their ease of access, and because they contained several discrete patches of presumed *T. angustifolia, T. latifolia,* and *T.* × *glauca.* Taxa were identified by morphology in previous field surveys using a combination of macroscopic traits including plant height, leaf width, spike gap length, pistillate spike width, pistillate spike length, and staminate spike length, which have been shown to be useful at discriminating these taxa previously (Kuehn and White 1999; Kirk et al. 2011; Hitchcock et al. 2018).In most cases, *Typha* taxa occurred in discrete patches ranging in size from an estimated 5 – 800 m2 within the site. To avoid sampling the same clone multiple times, we assumed each discrete patch was a clone, limiting our sampling to one ramet per clone. Samples and measurements were collected between 5–21 July 2020 to ensure flowers and ramets were mature, and to avoid deterioration of staminate flowers, which commences in this region by August. Prior to collection, we made coarse identifications for each plant using the same macroscopic plant traits as our previous field surveys. Five samples of each of the three putative taxa were sampled per site, for a total of 15 samples per site and 45 total samples in total (Supplementary Figures S1 – S3, Table S1). An additional sample (LS6) from Sturgeon Bank possessed traits of both *T. latifolia* and *T. angustifolia*. LS6 was assigned a provisional field identification of *T. latifolia*. A subset of these samples, comprised of one of each presumed taxa per site, and the single confounding specimen, were collected, preserved, and submitted to the University of British Columbia Herbarium (Vouchers V252063–V252072). All leaf measurements were acquired in the field, and flowers were collected and measured within 72 hours (Supplementary Photo S1). Leaf samples for later DNA extraction were collected and stored in envelopes filled with silica gel, initially at room temperature and then at -20 °C.

## Microsatellite and Plastid DNA Amplification

We carried out DNA extraction following a 2% CTAB extraction protocol as outlined in Doyle and Doyle (1987), with minor modifications. Five microsatellites, TA3, TA5, TA7, TA8, TL305, were used for amplification (Tsyusko-Omeltchenko et al. 2003; Freeland et al. 2013). Primers were amplified using the following two-step PCR protocol: step one began with 30 seconds at 95 °C and three-minutes at 95 °C, followed by 9 cycles of 94 °C for 30 seconds, 65 °C for 30 seconds, and 72 °C for 45 seconds, with a temperature decrease of 1.8 °C per cycle. Step two was 29 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 45 seconds, with a final extension of 72 °C for 20 minutes. During amplification, PIGTAILS were included on the reverse primers to reduce the risk of stutter, and a universal M13 sequence was included on the forward to provide fluorescent labelling of PCR fragments (Brownstein et al. 1996; Schuelke 2000). Four chloroplast regions were initially assessed for variation in two to three individuals per taxon. Chloroplast regions were as follows: intron rpS16 using primers from Shaw et al. (2005) and intergenic spacers trnH-psbA, rpS16-trnK, and trnL-trnF. Respective primers for these intragenic spacers were from Taberlet et al. (1991), Sang et al. (1997), Shaw et al. (2005), and Shaw et al. (2007). As all regions gave identical assignment of the hybrid for our sampling, the rpS16-trnK region was chosen to uniquely distinguish the parental plastotypes for all samples. Amplification for the rpS16-trnK region used primer pair rpS16x2F2 (5′-AAA GTG GGT TTT TAT GAT CC-3′) and trnK (UUU)x1 (5′-TTA AAA GCC GAG TAC TCT ACC-3′) following Shaw et al. (2007) and Zhou et al. (2018). The PCR cycles were 4 minutes at 95°C, 35 cycles of 45 seconds at 95 °C, 45 seconds at 50 °C, 90 seconds at 72°C 35 and 10 minutes at 72 °C. Bidirectional Sanger dideoxy sequencing was used to sequence the PCR products. Sequences were aligned and trimmed using the software CodonCode Aligner v.9.0 (CodonCode Corporation, [www.codoncode.com/aligner/](http://www.codoncode.com/aligner/)). In addition to parental confirmation, plastid sequences were used to confirm that no *T. angustifolia* were in fact *Typha domingensis* Pers. *T. domingensis* has not yet been recorded in the FRE but is known to be closely related to *T. angustifolia* (Aleman et al. 2024). Exemplary plastid sequences were compared to those from Genbank using the National Center for Biotechnology Information Basic Local Alignment Search Tool (BLAST; Camacho et al. 2009). The plastid sequences were found to have the highest query cover and percentage identity to the expected plant, either *T. latifolia* or *T. angustifolia*, meaning it is highly unlikely that any sample was *T. domingensis*.

## Microsatellite Analysis

PCR products from microsatellite amplification were first visualized on agarose gels and then sized using an Applied Biosystems™ 3730XL 96-capillary DNA Analyzer with GeneScan™ 500 LIZ® Size Standard. The chromatogram output was analysed using the software GeneMarker (Holland and Parson 2011). GenAlEx was used to calculate allele frequencies for each microsatellite for each species and calculate heterozygosity scores (Peakall and Smouse 2012). First, the software STRUCTURE (v2.3; Pritchard et al. 2000) was used at K=2 to generate ad-mixture scores in order to distinguish the non-admixed species and hybrids. Next, the package HIest was used to generate ancestry scores, values that represent the proportion of an individual’s alleles that came from known *T. angustifolia* alleles, and heterozygosity scores, which were then used to create a triangle plot (v2.0; Fitzpatrick 2012). The package HIestwas also used to calculate the likelihoods for early generation hybrid genotype classes (Fitzpatrick 2012). Finally, Bruvo’s distance between individuals was calculated using the *poppr* package in R (v2.8; Bruvo et al. 2004; Kamvar et al. 2014, 2015). Multidimensional scaling was performed on the distance matrix. The scores from the MDS were also plotted against the morphology MDS scores (see 2.4) to create a scatterplot.

## Morphological Analysis

Analysis of variance (ANOVA) was used as a simple, preliminary statistical test to identify differences in the mean values of six morphological characters between taxa: maximum leaf width, maximum plant height, spike gap length, pistillate length, pistillate width, and staminate length. These data were also analysed using *randomForest* package (v4.6-14; Liaw and Wiener, 2002) in an unsupervised analysis to create a proximity matrix in which every cell contains a measure of similarity or distance between the item in the column and row that corresponds to that cell. A tree number of 1000 was chosen for the analysis using an error x trees plot, which showed that error was minimised at > 200 trees. This proximity matrix was then used for multidimensional scaling (MDS), using both *randomForest* and *vegan* (v2.5; Oksanen et al. 2019) packages. All data analyses were performed using R (v4.0.2; R Core Team 2021).

# Results

## Microsatellite and Plastid Analyses

The number of alleles for each microsatellite marker varied from one to four. *T. angustifolia* samples often had more allelic richness than *T. latifolia*, 2.40 (standard deviation [SD] = 0.51) and 1.20 (SD = 0.20) respectively. TA5 showed the most variation with four possible alleles for *T. angustifolia* and *T. × glauca* and two for *T. latifolia*. Across all markers *T. × glauca* samples contained alleles from both parents and represented the variety of alleles displayed in the parents (Table 1). Based on the Hardy-Weinberg principle, *T. angustifolia* displayed a lower observed heterozygosity than expected (0.19 [SD = 0.08] – 0.39 [SD = 0.12]), and *T. latifolia* showed almost no difference between observed and expected (0.03 [SD = 0.03] – 0.02 [SD = 0.02]). *T.* *× glauca* showed a much higher observed heterozygosity than expected (0.96 [SD = 0.08] – 0.61 [SD = 0.03]).

Results of the microsatellite analysis using program STRUCTURE confirmed that our coarse field identifications of *Typha* at the time of tissue collection were 100% correct. The presumed parental plants all had low amounts of estimated admixture, whereas the presumed hybrids all had admixture of 50% (Figure 2). Note that the parental samples do not show zero admixture, a pattern that is expected as some alleles are shared between parents.

The function HIclass from the package *HIest* used the genetic marker data and parental allele frequencies to calculate the likelihood for each genotype class for each sample (Fitzpatrick 2012). Three potential backcrosses were detected in this analysis, all from Iona Island and one potential F2 from Frenchies Island was identified. These appear in triangle plots as points on the diagonal between parents (Figure 3). Thirteen of the 15 samples identified as *T. × glauca* in the fieldwere classified by HIclass as F1 hybrids, mainly with strong support (LLD: 3.04 – 3.05), although one (GF2) had weak support (LLD: 0.71). The plants classified as backcrosses and F2 (GF3) had only weak support for that assignment (LLD: 0.46 – 0.77, and 0.32), respectively (Supplementary Table S2). Two of these potential backcrosses were to *T. angustifolia* (AI2, GI4), and one was to *T. latifolia* (GI2). Interestingly, HIclass categorized GF2 as an F1 hybrid even though it has a lower heterozygosity score than the majority of *T. × glauca.* Fourteen of the samples identified as *T. angustifolia* in the fieldwere classified as pure *T. angustifolia,* while one sample (AI2) was classified as a backcross to *T. angustifolia*. This sample had a higher ancestry and a lower heterozygosity score than the potential *T. × glauca* backcrosses, so it possibly represents the offspring of a backcross that subsequently went through multiple generations of breeding with *T. angustifolia.* This would likely produce a plant that contains more *T. angustifolia* characteristics than a recent backcross,as seen in this sample. All 16 samples identified as *T. latifolia* in the field were pure *T. latifolia*, including the specimen with confounding morphology (LS6).

The MDS on microsatellite data was able to capture 81.6% of variation in axis 1 and 12% in axis 2 (Figure 4). Some of the samples labelled as backcrosses by HIclass do not cluster as closely as the rest of the samples. AI2 fell closer to the *T. × glauca* samples than the other *T. angustifolia* samples. GF2 is at the *T. angustifolia* side of the periphery of *T. × glauca* samples. GF3, classified as an F2 hybrid by Hiclass, clusters towards the bottom of the *T. × glauca* clump. GI2 does not appear to be any different from the other *T.**×* *glauca* samples. A comparison of MDS plots shows that the results of our morphological analysis, which was identical to those of our initial field identifications based on macroscopic characters (i.e., plant height, leaf width, spike gap length, pistillate spike width, pistillate spike length, staminate spike length) is consistent with the microsatellite identification in MDS plots (Figures 4 & 5).

All four chloroplast regions assessed were invariable within taxa, and all regions indicated *T. × glauca* has an identical plastotype to *T. angustifolia*. All four regions were variable between the parental species with the least variation in the rpS16 intron (1 SNP + 1 indel), followed by the trnL-trnF spacer (2 SNPs + 4 indels), with the most variation found in the trnH-psbA spacer (3 SNPs + 7 indels) and the rpS16-trnK spacer (7 SNPs + 3 indels). In the more extensive survey of all individuals using the rpS16-trnK spacer, the same two plastid haplotypes (plastotypes) were consistently recovered for *T. angustifolia* and *T. latifolia*, while all *T. × glauca* individuals found to have the *T. angustifolia* plastotype (Figure 4). *Typha angustifolia* was therefore consistently the maternal parent of the hybrid at all sites sampled. The rps16-trnK region had the largest number of SNPs separating the species. Exemplar sequences of the four plastid regions for each taxon will be deposited in GenBank following submission of this manuscript.

## Morphological Analysis

Hybrid *T. × glauca* was found to be intermediate in the traits of maximum leaf width, spike gap length, and staminate length. However, the hybrid displayed marked heterosis in pistillate length, pistillate width, and ramet height resulting in means that were higher than either parent (Table 2). The greatest difference between *T. angustifolia* and *T. latifolia* was in spike gap length, which averaged 4.79 cm (SD = 1.14) and 0.25 cm (SD = 0.79) respectively. *T. latifolia* also had a higher average maximum leaf width and slightly higher average pistillate length and staminate length than *T. angustifolia*. An ANOVA found differences in all measured morphological features between taxa: leaf width (*p*<.001), gap length (*p<*.001), pistillate spike length (*p*=.005)*,* pistillate spike width (*p*=.023*),* staminate length (*p*<.001)*,* andramet height (*p*<.001; Table 2). Both the mean decrease Gini and mean decrease accuracy scores of the random forestanalysis show the most to least important characters for distinguishing all taxa are staminate length, pistillate length, ramet height, spike gap length, maximum leaf width, and pistillate width (Supplemental Figure S4).

Results of this morphological analysis are consistent with preliminary coarse field identifications, with the possible exception of AF5, which measured much larger in maximum leaf width, pistillate length, staminate length, and ramet height than average *T. angustifolia*. This may represent a late-stage backcross with *T. × glauca.* The first axis of our proximity matrix accounts for 32% of the morphological variation in our samples, while the second axis accounts for 20.8% (Figure 5). All the taxa separated on the first axis in the order *T. × glauca*, *T. latifolia,* *T. angustifolia,* while on the second axis *T. latifolia* separated from both the others.

# Discussion

## Microsatellite Support of Field Identification

*T. × glauca* has been described as a cryptic invasive species in the FRE, as its morphological similarity with parental *T. latifolia* and *T. angustifolia* likely inhibited its earlier detection and management (Stewart et al. 2023). This study assists in “decrypting” this invasion, by (1) providing the first molecular evidence of *T. × glauca* in British Columbia, and (2) demonstrating the potential usefulness of macroscopic traits in differentiating *Typha* at this point in timein the FRE. Previous research from other regions of North America have found that macroscopic traits are not reliable for differentiating *T. × glauca* from parent species (e.g., Geddes et al. 2021; Tangen et al. 2022), though recent studies have demonstrated their usefulness (Wasko et al. 2022; Ohsowski et al. 2024). The unity of results between our initial coarse field identifications, morphological analyses, and molecular analyses, suggests identification through macroscopic traits may have useful applications in the current genetic and environmental context of the FRE. All of the 16 specimens field identified as *T. latifolia* were identified by HIest as pure *T. latifolia*. Of the 15 field-identified *T. angustifolia* samples, 14 were found to be 100% *T. angustifolia,* and only one was a potential *T. angustifolia* backcross. Finally, 12 of the 15 field-identified *T. × glauca* samples were found to be F1 hybrids of *T. angustifolia* and *T. latifolia,* and the remaining three may be advanced generation hybrids. The usefulness of macroscopic traits in the FRE may be attributed to environmental differences between study areas, as *Typha* morphology can be influenced by factors such as climate, water depth, light availability, salinity, and nutrient availability (e.g., Waters and Shay, 1990; Woo and Zedler, 2002), and to our knowledge this is the first investigation of this kind to occur in the Pacific Northwest or in brackish tidal marsh conditions. Genetic differences may also be a factor, as many comparable studies occurred in Central and Eastern North America, where invasion durations are presumably greater and advanced generation hybrids are more prevalent. The timeline of non-native *Typha* establishment in the FRE is poorly understood, but is likely more recent than many areas of North America. The earliest herbarium records of both *T. angustifolia* and *T. × glauca* occur in eastern North America in the 19th century, and in subsequent years these taxa expanded westward to the Great Lakes and prairie potholes regions (Shih and Finkelstein 2008). In contrast, the disjunct *Typha* populations in FRE appear to be recently established, as there are no herbarium records or local literature documenting *T. angustifolia* in the FRE prior to the 1980s (see Stewart et al. 2023), and the first *T. × glauca* specimens in the FRE were collected in conjunction with this study, in 2020.

## Practical field identification of *Typha* in the FRE

Our findings show that macroscopic traits, if used carefully, are potentially a practical and reliable approach for differentiating between native and non-native *Typha* in the FRE and possibly nearby regions at this point in time. To assist with the regional monitoring of *Typha* invasions,we have used our morphological data to generate a dichotomous key, which was validated by our microsatellite findings, and a related field guide (Supplementary Figure S5). The key should be used as a preliminary tool for early detection, not a proxy or substitute for genetic investigations, and will be most effective when applied (1) to specimens within the same time period as our measurements (July), (2) in similar tidal estuarine environments (so as to minimize genotypic and phenotypic variability), and (3) to representative ramets within a clonal patch, as morphological anomalies frequently occur within *Typha* populations that confound identification:

**1a** Maximum fresh or rehydrated leaf width < 9 mm

**2a**Spike gap length > 35 (32) mm, staminate spike length 10–23 cm, pistillate spike length 6–14 (19) cm, pistillate spike width 5–18 (24) mm, rarely exceeds 20 dm height………….……..……………………………………………...………………………**T.** **angustifolia**

**1b** Maximum fresh or rehydrated leaf width > 9 mm

**3a** Leaf width 13–20 (22) mm, spike gap usually absent or length < 3 (32) mm, staminate spike length 10 (7)–17 (20) cm, pistillate spike length 11 (7)–19 cm, pistillate spike width 1.1 (0.9)–3.3 cm, plant height 18–25 dm ………………………………………………………………………….…………. **T. latifolia**

**3b** Leaf width 10–14 (17) mm, spike gap length 5– 30 (35) mm, staminate spike length > 17 cm, pistillate spike length 15 (12) – 20(30) cm, pistillate spike width 0.8–1.7 (2.5) cm, plant height > 18 dm, can exceed 30 dm……………………………………………………………………..………...**T. × glauca**

## Backcrossing and genetic variation

Past investigations into *Typha* backcrossing have had varied results in North American wetlands. Kuehn et al. (1999) found no evidence of backcrossing in *Typha* samples collected throughout North America, including the FRE, however this could be related to their use of rapid amplifying polymorphic DNA (RAPD) and small sample sizes. Conversely, advanced generation hybrids have been identified in several studies ranging from the Prairie Pothole Region to the Great Lakes, to Atlantic North America, where they have comprised approximately 6–57% of *Typha* collected (Kirk et al. 2011; Geddes et al. 2021; Tangen et al. 2022; Ohsowski et al. 2024). Kirk et al. (2011) found that backcrossed hybrids were primarily detected in Great Lakes wetlands, while other collection areas had few or none, suggesting the abundance of backcrosses may vary between locations, possibly due to differences in hybridization timescales, and the abundance and distribution of parental species. Our HIest analysis classified most hybrid individuals as F1 hybrids, but suggested that one was a putative F2 (GF3) and three were putative backcrosses (AI2, GI2 and GI4). However, the evidence for backcrossing in these individuals is relatively weak and these samples do not appear to cluster differently on our MDS plot. Further molecular data with extra microsatellite loci would be needed to confirm backcrossing with certainty. In addition, one of the *T. angustifolia* samples (AI2) was classified by HIest as having some *T.*× *glauca* ancestry. The plot does show tight clustering of *T. latifolia* samples and a greater spread of the *T. angustifolia* samples.This is likely because 4 of the 5 primers used were optimized for *T. angustifolia* and were therefore more likely to capture more variation between samples of *T. angustifolia.* Future research is needed to determine the presence and precisely quantify the extent of backcrossing in the FRE, using broader sampling and more microsatellite markers.

Raw scores showed that the Iona Island collection site had more genetic variation than the other sites, and it was also the source of the putative backcrosses detected with HIest. One possible explanation for this is that *T. angustifolia* and *T. × glauca* have been present and abundant for longer durations at Iona than the other sites, and *T. angustifolia* may have arrived via multiple introduction events. This would be consistent with local grey literature and early herbarium records, which suggest the earliest detections of *T. angustifolia* in the estuarywere nearby, dating to at least the 1980s (Adams and Williams 2004). Stewart et al. (2023) also found that the largest infestation in the estuary, over three times greater than the next largest patch, is located approximately 500 m to the north, further indicating the duration of their establishment in the vicinity. A second explanation is that unlike Frenchies Island and Sturgeon Bank, Iona Island has been heavily modified and frequently disturbed by human activities since the creation of a sewage treatment plant in the 1950s. Frequent modifications to the site, including upgrades to sewage infrastructure, may have promoted genetic diversity by promoting ongoing recruitment (Pieper et al. 2020).

## Heterosis and Hybrid Breakdown

Hybrids often possess morphological traits that are intermediate to their parental species, or they display heterosis, in which the hybrid is larger than the parents (Hochholdinger and Hoecker 2007). Intermediacy was observed in our *T. × glauca* samples with maximum leaf width, spike gap length, and the staminate length, while heterosis was observed in the pistillate length, pistillate width, and ramet height. These observations mirror other studies, for example Kuehn and White (1999) found that the hybrid was intermediate for all traits but spike length, which was larger than either parent. Others have found that hybrids were larger than either parent, and attributed this to heterosis (Zapfe and Freeland 2015; Tangen et al. 2022). It is likely that the display of heterosis in certain traits is due to the young age of the invasion, resulting in many of the hybrids being F1, but there is still some uncertainty as to why heterosis occurs and why it occurs in conjunction with intermediacy in other traits (Mackay et al. 2021).

Ongoing hybridization of *Typha* may have implications for future ecological behaviour and management of these species in the FRE and warrants further and more rigorous investigation. Hybrid breakdown occurs when the hybrid vigour observed in F1s diminishes with ongoing hybridization. Advanced generation hybrids can therefore be less vigorous and dominant than their parental genotypes in their respective ecosystems (Hochholdinger and Hoecker 2007). In North American *Typha* populations, Bhargav et al. (2022) observed lower plant growth, such as plant height and aboveground biomass, in F2 hybrids than F1s, and attributed this to hybrid breakdown. Though our data are limited, we observed no evidence of hybrid breakdown among our samples, with the ramet heights of presumed backcrosses averaging 240.7 cm (SD = 85.3, *n* = 3) and F1s averaging 219.3 cm (SD = 14.5, *n* = 13). Future genetic studies in the FRE should investigate the degree of *Typha* hybridization occurring, and identify changes in dominance between generations, as this will assist managers in understanding the threat and trajectory of non-native *Typha* genotypes in tidal marshes of the FRE.

## Conclusion

This study highlights the usefulness of macroscopic traits in identifying *T. × glauca* and its parental species at this point in time in the FRE. Molecular data demonstrated that 100% of our 46 field identifications were correct, and also provided the first genetic evidence of hybrid *T. × glauca* in British Columbia, Canada. The dichotomous key generated from these findings may be a useful tool for *Typha* identification in comparable environments with similar timescales of hybridization. Our findings do not negate the need for genetic identification, and is meant to assist researchers and managers in the northwest margins of this North American invasion. A delay in *Typha* identification and management can be detrimental to wetlands, as there is now overwhelming evidence of their multitrophic impacts in North America (Bansal et al. 2019). Within the FRE, *Typha* invasions have been shown to reduce plant community richness (Stewart 2021), lower the abundance of salmon-associated benthic invertebrates (Lee 2021), and threaten the outcome of marsh restoration and creation projects (Stewart et al. 2024). Recent confirmation that *T.* *× glauca* is not only present, but extensive throughout the estuary, raises concerns around how to minimize the impact of this invasion process on culturally and ecologically important species, including juvenile salmonids (Chalifour et al. 2019). Our analysis contributes to future conservation efforts in the region by “decrypting” the invasion of non-native *Typha*, thus allowing for more effective monitoring and detection, and providing a starting place for future morphological and molecular *Typha* research in Western North America.

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# Tables

**Table 1** Primer name and number of alleles observed for each *Typha* taxon.The left column shows the primer name followed by the repeating base pair pattern in parentheses. For each taxon we outline the number of alleles observed for each primer, with length of each allele in parentheses

|  |  |  |  |
| --- | --- | --- | --- |
| Primer Name (repeat motif) | Number of Alleles (allele sizes) | | |
| *T. latifolia* | *T. angustifolia* | *T*. × *glauca* |
| TA3 (AC)12 ... (AG)13 | 1 (203) | 2 (241, 247) | 3 (203, 241, 247) |
| TA5 (AG)21 | 2 (299, 309) | 4 (299, 309, 311, 315) | 4 (299, 309, 311, 315) |
| TA7 (AC)9... (AG)17 | 1 (214) | 1 (220) | 2 (214, 220) |
| TA8 (AC)11 | 1 (293) | 2 (297, 313) | 3 (293, 297, 313) |
| TL305 (CT)6 | 1 (354) | 3 (338, 346, 354) | 3 (338, 346, 354) |

**Table 2** ANOVA results for each macroscopic trait used to differentiate *Typha* taxa in the Fraser River Estuary (n = 46). All units are in centimetres

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Mean ± SD | | |  | |  | |  | |  | |  | |
|  | *T. latifolia* | *T. angustifolia* | *T.* × *glauca* | | df | | SS | | MSE | | F | | *p* value | |
| Leaf width | 1.64 ± 0.24 | 0.68 ± 0.13 | 1.27 ± 0.16 | | 2 | | 7.20 | | 3.60 | | 105.21 | | <.001 | |
| Gap length | 0.25 ± 0.79 | 4.79 ± 1.14 | 1.81 ± 0.93 | | 2 | | 163.06 | | 81.53 | | 88.12 | | <.001 | |
| Pistillate length | 14.19 ± 3.37 | 11.27 ± 3.25 | 16.75 ± 5.82 | | 2 | | 225.57 | | 112.78 | | 6.12 | | .005 | |
| Pistillate width | 1.62 ± 0.70 | 1.03 ± 0.57 | 1.33 ± 0.38 | | 2 | | 2.65 | | 1.33 | | 4.14 | | .023 | |
| Staminate length | 13.76 ± 3.13 | 14.99 ± 3.42 | 22.42 ± 3.25 | | 2 | | 668.74 | | 334.37 | | 31.37 | | <.001 | |
| Ramet height | 197.38 ± 26.13 | 182.87 ± 23.38 | 227.33 ± 31.99 | | 2 | | 15452.00 | | 7726.20 | | 10.31 | | <.001 | |

# Figure Legends

**Fig. 1** General locations of the three sites for *Typha* morphometric measurements and genetic sample collections in the Fraser River Estuary, British Columbia. Locations are displayed with black dots

**Fig. 2** Admixture scores as generated using the program STRUCTURE. This plot confirms the accuracy of field identifications with the putative hybrids (GF1 to GS5), all showing admixture of c. 50%. Note that *T. angustifolia* (AF1 to AS5) and *T. latifolia* (LF1 to LS6) samples do not show 100% *T. angustifolia*/*T. latifolia* DNA respectively as some alleles are shared between parents

**Fig. 3** Triangle plot showing the extent of backcrossing in our samples (n = 46). Samples are labelled according to presumed taxa based on field identification. *T. angustifolia* begin with ‘A’ and are orange, *T.* *× glauca* begin with ‘G’ and are green, and *T. latifolia* begin with ‘L’ and are blue

**Fig. 4** Microsatellite (SSR) MDS ordination of the first two axis based on a Bruvo’s genetic distance matrix. *T. angustifolia* begin with ‘A’ and are orange, *T.* × *glauca* begin with ‘G’ and are green, and *T. latifolia* begin with ‘L’ and are blue. Ellipses represent the 95% confidence intervals for each group. The lack of a visible ellipse around the *T. latifolia* samples is due to the tight clustering resulting in confidence regions that are too narrow to be effectively represented

**Fig. 5** Morphological MDS ordination of the first two axis based on a random forest proximity matrix (see methods for detail). The ordination separates the two parents and hybrids. *T. angustifolia* begin with ‘A’ and are orange, *T. x glauca* begin with ‘G’ and are green, and *T. latifolia* begin with ‘L’ and are blue. Ellipses represent the 95% confidence intervals for each group

# A map of the coast of vancouver Description automatically generatedFigure 1

# A blue and orange graph Description automatically generatedFigure 2

# Figure 3



# Figure 4



# Figure 5



# Statements & Declarations

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# Author Contributions

QCBC and DS contributed to the study conceptualisation. Funding for this research was acquired by QCBC, TGM, and DS. Fieldwork, morphological measurements and relevant data entry were performed by DS with guidance and support from TGM and QCBC. Microsatellite analysis was performed by GB with guidance from DMP and QCBC. Data analysis was performed by GB. The first draft of the manuscript was written by DS and GB, and all other authors provided subsequent editorial guidance. All authors read and approved this final manuscript

# Data Availability

Spatial and morphological field data, R code, and other files pertaining to this study are available at the following GitHub repository: <https://github.com/asarum-ecological/2024_TyphaMorph>.